FINAL REPORT

on

MICROSOMAL AROMATASE PREVALIDATION SUPPLEMENTARY STUDY: TASK 4-ESTABLISH INHIBITION CURVES AND IC_{50}S FOR TWO REFERENCE CHEMICALS TASK 5- COMPARE ESTRONE AND TRITIATED WATER MEASUREMENT METHODS

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Final Task Report

Microsomal Aromatase Prevalidation Supplementary Study

WA 4-10 Task 4.0: Establish Inhibition Curves and IC_{50}s for Two Reference Chemicals and Task 5.0: Compare Estrone and Tritiated Water Measurement Methods

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FINAL REPORT

Title: **Microsomal Aromatase Prevalidation Supplementary Study: WA 4-10 Task 4.0: Establish Inhibition Curves and IC_{50}s For Two Reference Chemicals and Task 5.0: Compare Estrone and Tritiated Water Measurement Methods**

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Quality Assurance Statement

Study Title: Microsomal Aromatase Prevalidation Supplementary Study
WA 4-10, Tasks 4 and 5

Sponsor: Battelle Memorial Institute

Study Code: An04-900

Protocol Number: RTI-900-AN

This study was audited by the Sciences and Engineering – Health Sciences Quality Assurance Unit and the results of the inspections and audits were reported to the task leader 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 Task Leader and Management
Protocol Review	March 12, and 15, 2004	March 15, 2004
Data and Report Audit*	July 14-21, 2004	July 22, 2004

*Sections 2.3 and 3.2 of the Report were not audited (they relate to Protocol Section 5.0 which is exempt from QAPP requirements).

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1.0 OBJECTIVES

The objectives of this Work Assignment are to:

- investigate the high variability found during the conduct of Work Assignment (WA) 2-24 for the study of 4-hydroxyandrostenedione (4-OH ASDN) in the recombinant assay,
- generate complete inhibition curves for econazole and ketoconazole, and
- compare two different methods for measuring aromatase activity. One method measures the amount of tritiated water formed by the aromatization of radiolabeled androstendione (ASDN). The second method measures estrone formation by radioimmunoassay (RIA) .

The purpose of this WA is to perform a series of prevalidation studies to address the problems originally identified from WA 2-24 before beginning the interlaboratory validation studies and to compare two different methods for measuring aromatase activity.

This task report includes data from WA 4-10 Task 4.0: Establish Inhibition Curves and IC_{50}s For Two Reference Chemicals and Task 5.0: Compare Estrone and Tritiated Water Measurement Methods. The experiments reported herein are described in the protocol (RTI-900-AN) Sections 4.0 and 5.0.

2.0 MATERIALS AND METHODS

2.1 Establish Inhibition Curves and IC_{50}s For Two Reference Chemicals

2.1.1 Assay Conduct

The aromatase assay was conducted with both human placental and recombinant microsomes using the optimized conditions determined in WA 2-24 and presented below in Table 1 with the addition of varying concentrations of the inhibitors ketoconazole and econazole. Appropriate controls (defined below) were included with each assay set. The following final concentrations (M) of ketoconazole were chosen for assay in consultation with the Sponsor: (log10 units) -3.1, -4, -5, -5.3, -5.6, -6, -7, -8. Similarly, the following concentrations (M) of

econazole were chosen: (log10 units) -7, -8, -8.6, -9, -9.6, -10, -11, -12. Inhibitor and substrate sources, lot numbers and vehicles are described in the WA 4-10 Task 3 report.

Table 1. Optimized Aromatase Assay Conditions

Assay Factor (units)	Assay Type	
	Human Placental	Human Recombinant
Microsomal Protein (mg/mL)[a]	0.0125	0.004
NADPH (mM)[a]	0.3	0.3
[^{3}H]ASDN (nM)[a]	100	100
Incubation Time (min)	15	15

[a] Final concentrations

A Study (Table 2) was defined as a set of assays using a single microsome type with varying concentrations (in triplicate) of the inhibitor (with appropriate controls) conducted by a single technician on a given day. One econazole and one ketoconazole study using a given microsome type was conducted on a single day. The two Studies conducted on a single day employed the same ASDN, NADPH and microsome solutions. All solutions (except buffer) were prepared fresh each day. Stock solutions of ketoconazole were prepared in ethanol and those of econazole were prepared using DMSO. Stock solutions of inhibitors and all dilutions were prepared fresh each day.

Table 2. Study Designations

	Recombinant Assay	Placental Assay
Econazole	Studies 1, 2, 3	Studies 4, 5, 6
Ketoconazole	Studies 7, 8, 9	Studies 10, 11, 12

Positive and negative control samples (as in WA 2-24) were included for each study. Positive controls contained substrate, NADPH, propylene glycol, buffer, vehicle (20 µL EtOH or DMSO) and microsomes. Negative controls contained all positive control assay components except NADPH. Four positive and four negative controls were included with each Study and

treated the same as the other samples. The control's sets were split so that two tubes (of each positive and negative controls) were run at the beginning and two at the end of each study set.

The assays were performed in 13x100 mm test tubes maintained at 37 ± 1°C in a shaking water bath. Propylene glycol (100 µL), [^{3}H]ASDN, NADPH, inhibitor (at varying concentrations) or vehicle and buffer (0.1 M sodium phosphate buffer, pH 7.4) were combined in the test tubes (total volume 1 mL). The volume of solvent (inhibitor solutions or vehicle) added to each tube was 20 µL (1% of final volume). The tubes were placed at 37 ± 1°C in the water bath for 5 min prior to initiation of the assay by the addition of 1 mL of the diluted microsomal suspension. The total assay volume was 2.0 mL, and the tubes were incubated for 15 min. The incubations were stopped by the addition of methylene chloride (2.0 mL); the tubes were vortex-mixed for ca. 5 s and placed on ice. The tubes were then vortex-mixed an additional 20-25 s. The tubes were centrifuged using a Beckman GS-6R centrifuge with GH-3.8 rotor for 10 min at a setting of 1000 rpm. The methylene chloride layer was removed and discarded; the aqueous layers were extracted again with methylene chloride (2 mL). This extraction procedure was performed one additional time, each time discarding the methylene chloride layer. The aqueous layers were transferred to vials and duplicate aliquots (0.5 mL) were transferred to 20-mL liquid scintillation counting vials. Liquid scintillation cocktail (Ultima Gold, Packard, 10 mL) was added to each counting vial and shaken to mix the solution. The radiochemical content of each aliquot was determined by liquid scintillation spectrometry (LSS).

At the end of the incubation time for the recombinant assay tubes, a 200-µL aliquot (for RIA analysis) of the reaction mixture was removed immediately prior to addition of the CH_2Cl_2. The aliquots for RIA were combined with 20 µL of a 11 µM solution of 4-OH ASDN, capped, vortexed to mix and then placed in the -20 °C freezer. The samples were transferred to the -70 °C freezer after they were frozen for storage until analyzed by RIA. RIA analysis was conducted the day after the ^3H_2O aromatase assay.

2.1.2 Data Analysis

Raw LSS data (CPM) were converted to DPM using Process2 (Version 2.1 09251998) software. DPM data and protein assay data were entered into a validated Excel spreadsheet. The spreadsheet calculated DPM/mL for each aliquot of extracted aqueous incubation mixture and average DPM/mL and total DPM for each aqueous portion (after extraction). Multiplication of the volume (mL) of substrate solution added to the incubation by the substrate solution specific

activity (DPM/mL) yielded the total DPM present in the assay tube at initiation. The total DPM remaining in the aqueous portion after extraction divided by the total DPM present in the assay tube at initiation, times 100 yielded the percent of the substrate that was converted to product. The total DPM remaining in the aqueous portion after extraction was corrected for background by subtracting the average DPM present in the aqueous portion of the negative control tubes (for that day/assay). This corrected DPM was then converted to nmol product formed by dividing by the substrate specific activity (DPM/nmol). The activity of the enzyme reaction was expressed in nmol (mg protein)$^{-1}$min^{-1} and was calculated by dividing the amount of estrogen formed (nmol) by the product of mg microsomal protein used times the incubation time, e.g. 15 min. Average activity in the positive control samples for a given Study was calculated. Percent of control activity remaining in the presence of various inhibitor concentrations was calculated by dividing the aromatase activity at a given concentration by the average positive control activity and multiplying by 100.

IC_{50} was calculated using Prism (Version 3.02) software to fit the percent of control activity and log concentration data to a curve using the following equation:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom})/(1 + 10^{((\text{LogIC}_{50} - X)*\text{HillSlope})})$$

where: X is the logarithm of concentration
Y is the percent activity
Bottom is the lower plateau
Top is the upper plateau

2.1.3 Statistical Analysis

2.1.3.1 Quality control comparisons. For purposes of comparison, the control responses were combined across batches and an analysis of variance was carried out on the data. The factors in the analysis were microsome type, batch, portion of batch (beginning or end) and the interaction between batch and portion of batch. The responses were enzymatic activity. The estimated variance between the two replicate runs/batch was based on the pooled value across the quadruplicate replicate runs in a given Study for a given microsome type. In addition, the data was analyzed by multiple comparisons and graphical analyses.

2.1.3.2 Variability assessment. Differences among microsome types and day-to-day variances within microsome types for enzymatic activity at various concentrations of an inhibitor were estimated. The variance components were inspected across the assays to determine their comparability. Analyses were based on mixed effects analysis of variance models using PROC MIXED or PROC GLM in SAS Version 8.2. Microsome types and inhibitor concentrations were treated as fixed effects; day-to-day variation was treated as a random effect. Variation between replicates within a single assay was examined using Levene's test for homogeneity of variance. Graphical analyses were used to examine the changes in the variances across microsome type, inhibitor type, day and inhibitor concentration.

2.1.3.3 Concentration-response curve fits. Analysis of variance tests were carried out on log10(IC_{50}), slope, and associated within day standard errors. Microsome type, inhibitor type and microsome x inhibitor interaction variation was determined for each of the parameters and was tested for significance.

2.2 Compare Estrone and Tritiated Water Measurement Methods

THIS SECTION IS NOT CONDUCTED OR REPORTED UNDER QAPP REQUIREMENTS (see WA 4-10).

Test kits for the determination of estrone content by radioimmunoassay (RIA) were obtained from Diagnostic Systems, Inc. (Webster, TX). The catalog number of the kits was DSL-8700.

2.2.1 General RIA Assay Procedure

The RIA was conducted as described in the kit insert. Each sample and standard for RIA was run in duplicate or triplicate. Each assay set contained tubes for Total Counts, Non-Specific Binding, Standards (range: 15-2000 pg/mL), two Control Concentrations (35 and 300 pg/mL) and Unknowns.

2.2.2 Preliminary Interference and Accuracy Testing

Preliminary experiments were conducted to test for interference of all assay components with the kit. A mixture of buffer, propylene glycol and NADPH (at concentrations used in the ^3H_2O assay) was prepared and tested for interference with the RIA assay. Other ^3H_2O assay components (microsomes, ASDN, and the inhibitors, ketoconazole and econazole, at the highest concentrations used in the ^3H_2O assay) were added in series to the above mixture and these new samples (see Table 3) were also tested for interference. In Mix 4, the NADPH was omitted in order to simulate the negative control assay conditions.

Table 3. Description of Interference Test Samples

Assay Component	Mix 1	Mix 2	Mix 3	Mix 4	Mix 5	Mix 6
Buffer	X	X	X	X	X	X
Propylene glycol	X	X	X	X	X	X
NADPH	X	X	X		X	X
Microsomes		X	X	X	X	X
ASDN			X	X	X	X
Ketoconazole					X	
Econazole						X

Accuracy testing was conducted by fortifying each of the interference testing mixtures with a known amount of estrone and assaying by RIA.

2.2.3 Preliminary Testing to Ensure Aromatase Activity is Abolished

These experiments were conducted to address the concern that viable protein may remain in samples that were either flash frozen or slow frozen and lead to further generation of product when the samples were thawed at room temperature for RIA. Samples that contained microsomes, substrate and all co-factors were assayed by RIA both with and without prior incubation at 37 °C for 15 min and after either slow or flash freezing and thawing (see Table 4). In Mix 4, the NADPH was omitted in order to simulate the negative control assay conditions.

Table 4. Description of Samples for Investigating Residual Aromatase Activity after Freeze/Thaw

Assay Component	Mix 1	Mix 2	Mix 3	Mix 4	Mix 5
Buffer	X	X	X	X	X
Propylene glycol	X	X	X	X	X
NADPH	X	X	X		X
Microsomes	X	X	X	X	X
ASDN	X	X	X	X	X
Econazole					X
Incubated at 37 °C			X		
Slow Freeze	X		X	X	X
Flash Freeze		X			
Assayed immediately on thawing and again after 15 min at room temp	X	X			X

In subsequent experiments, the ability of 4-OH ASDN to abolish the aromatase reaction (and any interference with RIA) was tested (Table 5). A complete aromatase reaction mixture (microsomes, substrate, co-factors) was prepared on ice. One aliquot (200-µL) of this sample was combined with 4-OH ASDN (final concentration - 1E-6M [abolished 90% of aromatase activity in previous studies]) and one aliquot (200-µL) was combined with 20 µL EtOH. The aromatase reaction mixture was incubated at 37 °C for 15 min and then two aliquots (200-µL) were combined with 4-OH ASDN and two aliquots (200-µL) were combined with EtOH. One of each of the aliquots combined with 4-OH ASDN and EtOH was further incubated at 37 °C for 15 min. Each aliquot was then slow frozen. The next day each sample was thawed and assayed for estrone content by RIA.

Table 5. Description of Samples for Investigating Residual Aromatase Activity in Presence of 4-OH ASDN

Assay Component	Mix 1	Mix 2	Mix 3	Mix 4	Mix 5	Mix 6
Incubated at 37 °C			X	X	X	X
Aliquot added to 4-OH ASDN	X		X		X	
Aliquot added to EtOH		X		X		X
Second incubation at 37 °C					X	X

3.0 RESULTS

3.1 Inhibition Curves and IC_{50}s for Two Reference Chemicals

3.1.1 Detailed Data Tables

The aromatase assay was conducted using each microsome type (placental and recombinant) and the inhibitors ketoconazole and econazole on three days each as described in Section 2.1.1. The detailed results for each study are presented in Appendix A.

3.1.2 Control Sample Analysis

Negative control tubes (containing all assay components except NADPH) served as blanks for the assay. Generally, the aqueous portion of the negative control tubes contained less than 100 DPM/mL after extraction. In a few instances the negative controls contained in the range of 100-400 DPM/mL (see Appendix A) and in one case (Day 3 Placental assay, ketoconazole set, negative control 3, Appendix A, Table A-9) the negative control contained 706 DPM/mL after extraction. Based on our experience and data obtained during the conduct of WA 2-24, the elevated DPM/mL values in these isolated negative control tubes probably indicate a slight cross-contamination of the assay tube with NADPH. All negative control tubes contained radioactivity equivalent to less than 1% conversion of ASDN to estrone. Overall (3 days x 2 inhibitors x 4 negative control replicates; n=24) the mean aromatase activity in the negative control tubes was 0.0000 ± 0.0007 nmol/mg/min and 0.0000 ± 0.0003 nmol/mg/min for the placental and recombinant assays, respectively.

Means and standard deviations (n=2) for the positive control activities are presented in Table 6. Within an assay day, there was generally little variance (<5%) in positive control activities within an inhibitor, however, this variance was as high as 12% in one instance. Also, variance across inhibitors within an assay day was generally < 10%. The positive control samples for each inhibitor contained a different test article vehicle. Therefore, the absence of any marked variance in the positive control activity across inhibitors within an assay day indicates little variance in the effect of the test article vehicles on aromatase activity. The vehicles were EtOH or DMSO, for ketoconazole or econazole, respectively, at 1% total assay volume.

Table 6. Means and Standard Deviations for Positive Control Activities*

Microsome Type	Vehicle†	Control Type	Portion of Batch (Day)	Day 1 Mean	Day 1 Std Dev	Day 2 Mean	Day 2 Std Dev	Day 3 Mean	Day 3 Std Dev
Placental	DMSO	Positive	Begin	0.0608	0.0117	0.0640	0.0010	0.0597	0.0015
		Positive	End	0.0676	0.0005	0.0626	0.0016	0.0569	0.0007
	Mean ± SD by Vehicle and Day (% CV)			0.0642 ± 0.0078 (12.13%)		0.0633 ± 0.0014 (2.16%)		0.0583 ± 0.0018 (3.12%)	
	EtOH	Positive	Begin	0.0721	0.0010	0.0662	0.0002	0.0655	0.0024
		Positive	End	0.0704	0.0000	0.0640	0.0001	0.0603	0.0013
	Mean ± SD by Vehicle and Day (% CV)			0.0713 ± 0.0011 (1.62%)		0.0651 ± 0.0012 (1.91%)		0.0630 ± 0.0034 (5.42%)	
	Mean ± SD by Day (% CV)			0.0677 ± 0.0064 (9.44%)		0.0642 ± 0.0015 (2.39%)		0.0606 ± 0.0035 (5.84%)	
	Overall Placental Positive Control Mean ± SD			0.0642 ± 0.0051 (7.94%)					
Recombinant	DMSO	Positive	Begin	0.3111	0.0148	0.2381	0.0064	0.2623	0.0172
		Positive	End	0.2914	0.0010	0.2227	0.0082	0.2506	0.0043
	Mean ± SD by Vehicle and Day (% CV)			0.3012 ± 0.0142 (4.72%)		0.2304 ± 0.0107 (4.64%)		0.2564 ± 0.0123 (4.78%)	
	EtOH	Positive	Begin	0.3106	0.0200	0.1609[a]	0.1631	0.2910	0.0156
		Positive	End	0.3011	0.0062	0.2414	0.0072	0.2619	0.0003
	Mean ± SD by Vehicle and Day (% CV)			0.3059 ± 0.0133 (4.34%)		0.2011 ± 0.1051 (52.2%)[b] 0.2530 ± 0.0207 (8.19%)		0.2764 ± 0.0191 (6.90%)	
	Mean ± SD by Day (% CV)			0.3036 ± 0.0130 (4.27%)		0.2158 ± 0.0709 (32.9%)[b] 0.2401 ± 0.0186 (7.75%)		0.2664 ± 0.0183 (6.87%)	
	Overall Recombinant Positive Control Mean ± SD			0.2619 ± 0.0551 (21.0%)[b] 0.2713 ± 0.0309 (11.4%)					

* Control activities from the ^3H_2O release assay (nmol/mg/min). N=2

† Control samples run with the econazole assay sets contained DMSO as vehicle control while those run with the ketoconazole assay sets contained EtOH as vehicle control.

[a] The activity values for the recombinant beginning positive controls with EtOH vehicle (ketoconazole set) were: 0.2762 and 0.0455 nmol/mg/min for tubes 1 and 2, respectively.

[b] Because the Tube 2 activity was an outlier from the mean of all four positive control values for this set, it was excluded from the set and a new mean and SD was calculated. The mean and standard deviation shown in the first row includes all data, the second row contains the mean and standard deviation calculated after removal of the data from Day 2, replicate 2 of the EtOH vehicle (ketoconazole) set.

The analysis of variance results for the control samples are presented in Table 7. As expected, there were statistically significant variances in the positive control activities by microsome type. Statistically significant variances were also detected by day within a microsome type. Where statistically significant variances were detected, the mean activities were further examined for variance by least squares pair-wise comparison (Table 8). No significant day-to-day variance was found in the mean placental positive control values. There were significant day-to-day variances of means for the recombinant positive control activities.

Table 7. ANOVA Results for Positive Control Activities

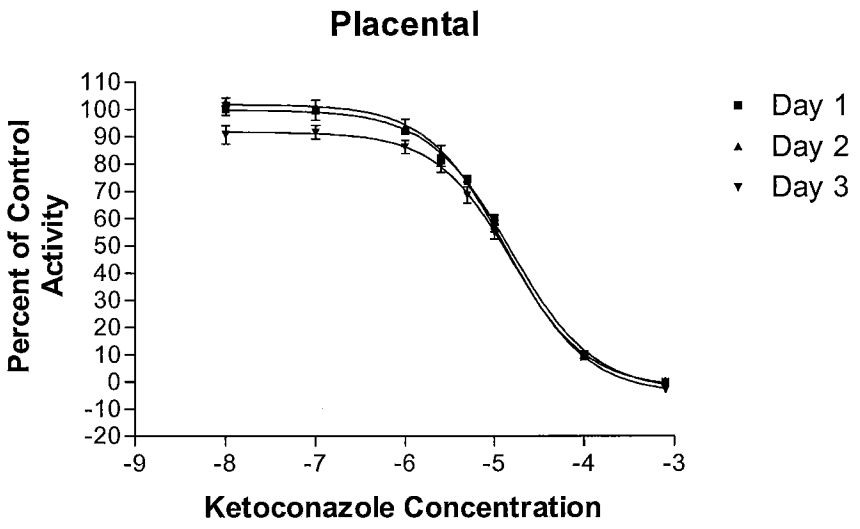
Control Type	Source	DF	Type III SS	Mean Square	F Value	Pr > F
Positive	Type	1	0.4994	0.4994	7338.09	<0.0001
Positive	Inhibitor	1	0.001268	0.001268	18.64	0.0001
Positive	Portion	1	0.001139	0.001139	16.73	0.0002
Positive	Day(Type)	4	0.0144	0.003599	52.88	<0.0001
Positive	Type*Inhibitor	1	0.0004099	0.0004099	6.02	0.019
Positive	Type*Portion	1	0.0009058	0.0009058	13.31	0.0008

Table 8. Least Squares Means Pair-wise Differences and T-Tests

Control	Mean 1 Type	Mean 1 Day	Mean 2 Type	Mean 2 Day	Mean Diff	Tstat	Pvalue
Positive	Placental	1	Placental	2	0.00351	0.8507	0.95562
Positive	Placental	1	Placental	3	0.00712	1.725	0.52473
Positive	Placental	2	Placental	3	0.00361	0.8743	0.95031
Positive	Recombinant	1	Recombinant	2	0.060972	14.231	0.00000
Positive	Recombinant	1	Recombinant	3	0.037139	9.0039	0.00000
Positive	Recombinant	2	Recombinant	3	-0.02383	-5.5627	0.00000

3.1.3 Inhibitor Results

3.1.3.1 Ketoconazole. Aromatase activity was assayed in both microsome types in the presence of varying concentrations of ketoconazole. Each ketoconazole concentration was assayed in triplicate on each of three assay days per microsome type. Graphs of aromatase activity as percent of control activity versus ketoconazole concentration (log(M)) are presented in Figure 1. This data is also presented in Table 9.



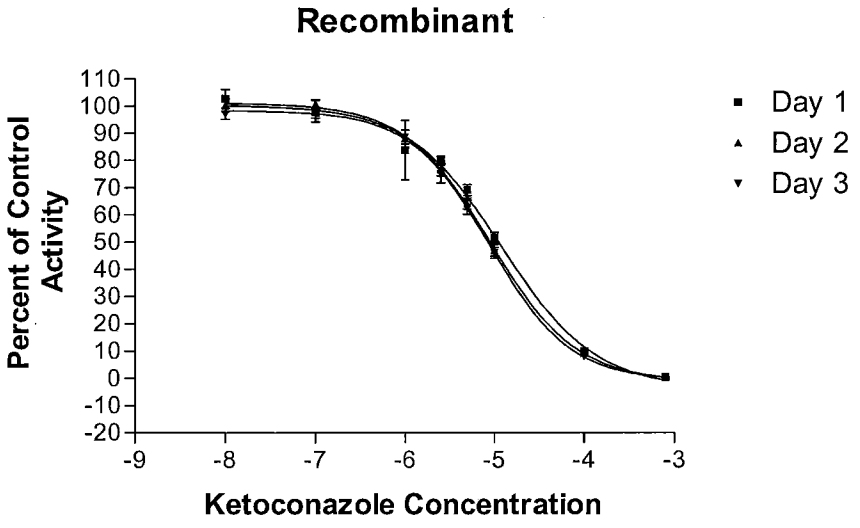
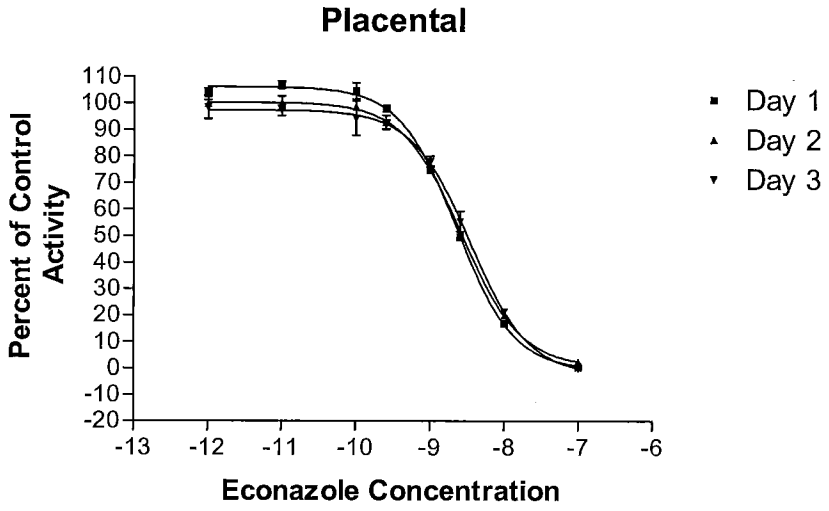


Figure 1. Ketoconazole Inhibition Curves
Concentration units are log (M)

Table 9. Percent of Control Activities for Aromatase Activity in the Presence of Ketoconazole

Concentration Code	Ketoconazole Concentration (Log(M))	Day 1 Replicate 1	Day 1 Replicate 2	Day 1 Replicate 3	Day 2 Replicate 1	Day 2 Replicate 2	Day 2 Replicate 3	Day 3 Replicate 1	Day 3 Replicate 2	Day 3 Replicate 3
		Placental								
1	-3.1	-0.2	-0.1	-0.1	0.2	0.2	0.1	-2.9	-2.8	-2.8
2	-4.0	10.7	10.4	9.4	10.1	8.8	8.8	10.2	10.6	8.3
3	-5.0	60.5	60.6	57.6	58.3	58.5	54.8	57.8	53.0	54.1
4	-5.3	75.0	73.2	75.6	74.2	74.5	72.4	71.8	68.0	65.9
5	-5.6	78.9	82.1	82.6	85.4	85.5	80.7	83.4	77.4	79.2
6	-6.0	91.2	91.8	93.3	95.8	94.5	90.6	88.9	84.5	85.2
7	-7.0	103.7	98.8	96.5	100.9	101.9	99.5	93.4	92.6	88.7
8	-8.0	102.4	99.8	98.0	102.9	103.9	100.9	92.6	92.7	86.8
		Recombinant								
1	-3.1	0.4	0.2	0.3	0.2	0.2	0.3	0.1	0.0	0.1
2	-4.0	10.6	10.5	8.7	9.0	9.7	8.5	8.9	7.8	7.2
3	-5.0	54.0	52.8	47.6	49.9	48.2	42.6	49.7	45.4	42.9
4	-5.3	72.7	68.9	65.7	66.7	65.7	60.0	67.1	63.1	58.0
5	-5.6	81.3	81.6	77.2	81.1	76.0	73.0	80.4	75.4	69.1
6	-6.0	99.6	88.8	63.0	93.1	88.5	82.1	91.5	90.5	83.4
7	-7.0	100.8	97.9	94.0	102.8	101.7	97.3	100.8	103.2	90.2
8	-8.0	107.8	104.0	96.1	102.5	102.4	95.7	97.7	100.2	93.3

3.1.3.2 Econazole. Aromatase activity was assayed in both microsome types in the presence of varying concentrations of econazole. Each econazole concentration was assayed in triplicate on each of three assay days per microsome type. Graphs of aromatase activity as percent of control activity versus econazole concentration (log(M)) are presented in Figure 2. This data is also presented in Table 10.



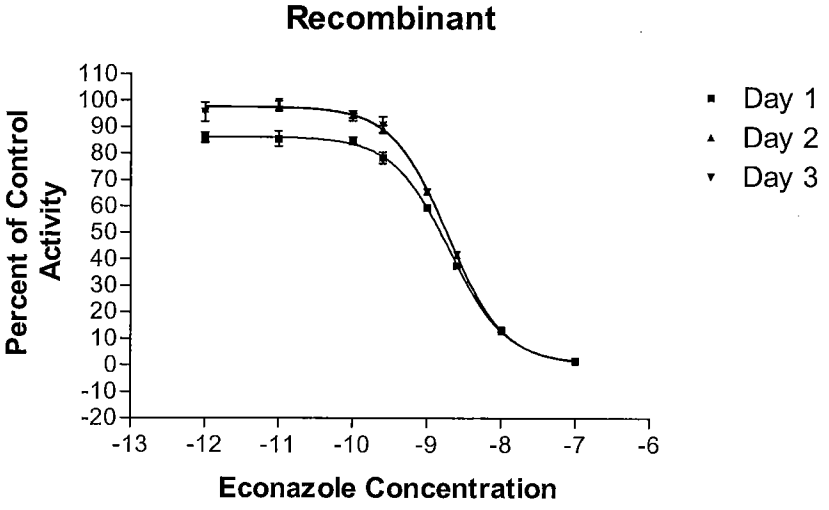


Figure 2. Econazole Inhibition Curves
Concentration units are log(M)

Table 10. Percent of Control Activities for Aromatase Activity in the Presence of Econazole

Concentration Code	Econazole Concentration (Log(M))	Day 1 Rep 1	Day 1 Rep 2	Day 1 Rep 3	Day 2 Rep 1	Day 2 Rep 2	Day 2 Rep 3	Day 3 Rep 1	Day 3 Rep 2	Day 3 Rep 3
		Placental								
1	-7.0	0.7	0.4	0.7	2.3	2.4	2.4	0.1	0.0	-0.2
2	-8.0	17.9	16.8	16.2	19.8	20.1	19.9	21.5	21.2	21.4
3	-8.6	49.4	50.1	49.2	51.6	51.5	51.7	51.1	57.4	57.9
4	-9.0	74.1	75.8	76.0	74.0	75.5	75.2	80.4	77.3	76.5
5	-9.6	97.7	99.2	97.1	94.0	94.5	89.9	93.9	91.1	90.7
6	-10.0	101.3	104.8	107.6	98.6	99.1	98.0	97.4	98.5	86.7
7	-11.0	108.0	106.9	105.4	101.9	100.9	97.2	99.3	97.0	95.3
8	-12.0	105.6	102.4	103.8	101.3	99.0	99.4	103.2	97.8	94.5
		Recombinant								
1	-7.0	1.4	1.5	1.6	1.5	1.6	1.6	1.6	1.5	1.8
2	-8.0	13.1	13.1	13.2	13.6	12.8	12.7	13.7	13.7	13.0
3	-8.6	38.1	38.3	36.3	42.4	41.7	40.6	42.2	41.6	41.7
4	-9.0	61.4	59.5	58.0	67.5	65.8	63.5	64.3	67.2	64.7
5	-9.6	83.4	75.7	76.6	91.4	89.7	86.4	96.4	89.8	85.7
6	-10.0	87.1	84.4	82.6	96.4	92.0	95.7	93.3	96.3	91.5
7	-11.0	90.7	84.6	81.1	101.3	97.5	94.7	101.0	97.4	99.7
8	-12.0	89.9	83.7	84.5	99.0	95.9	95.0	102.2	94.9	89.9

3.1.3.3 Statistical analysis of inhibitor results. Coefficients of variance (CV) for the measured activities of the replicate tubes within a study are presented in Table 11. The samples containing the highest concentration of inhibitor, had very low (or even non-detectable) activities (< 5% of control) which led to much higher CVs than those measured for the other concentrations. When the CVs from those highest inhibitor concentration samples are excluded, the mean CVs across all three assay days for each microsome/inhibitor set were less than 5%, except in the case of the recombinant microsomes/ketoconazole set where the mean CV was 6.9%. The data demonstrate that there is little variance between replicates tubes (at each inhibitor concentration) within a study and across all inhibitor concentrations across three independently conducted assays (days). There was no discernable pattern toward differing variances between microsome types or inhibitors.

Table 11. Coefficients of Variance for Replicates within a Study

Microsome Type	Inhibitor	Concentration Code	Day 1	Day 2	Day 3
Placental	Econazole	1*	28.9	2.1	0.0
		2	5.1	0.9	0.7
		3	0.9	0.1	6.8
		4	1.4	1.1	2.6
		5	1.1	2.7	1.9
		6	3.0	0.6	6.9
		7	1.2	2.5	2.1
		8	1.5	1.2	4.4
	Mean ± SD*		2.3 ± 0.4		
Recombinant	Econazole	1*	5.0	3.3	7.1
		2	0.3	3.5	3.1
		3	2.9	2.1	0.7
		4	2.8	3.1	2.4
		5	5.3	2.8	6.0
		6	2.7	2.5	2.6
		7	5.7	3.4	1.8
		8	3.9	2.2	6.5
	Mean ± SD*		3.2 ± 0.3		
Placental	Ketoconazole	1*	0	55.5	0
		2	7.0	7.8	12.7
		3	2.9	3.6	4.6
		4	1.7	1.6	4.4
		5	2.5	3.3	3.8
		6	1.2	2.8	2.7
		7	3.7	1.2	2.7
		8	2.3	1.5	3.7
	Mean ± SD*		3.7 ± 0.6		

(continued)

Table 11. Coefficients of Variance for Replicates within a Study (continued)

Microsome Type	Inhibitor	Concentration Code	Day 1	Day 2	Day 3
Recombinant	Ketoconazole	1*	40.4	21.3	35.3
		2	10.7	6.7	11.1
		3	6.6	8.2	7.5
		4	5.1	5.6	7.2
		5	3.1	5.3	7.5
		6	22.5	6.3	4.9
		7	3.5	2.9	7.0
		8	5.8	3.9	3.6
	Mean ± SD*		6.9 ± 0.9		

*The activities of all Concentration Code 1 samples were <5% of the positive control activity for their respective set. These very low (in some cases non-detectable) activities typically had much higher variances than the rest of the set. Therefore, all Concentration Code 1 coefficients of variance were excluded from the calculation of Mean and Standard Deviation for each microsome type and inhibitor set.

IC_{50} values for each inhibitor and microsome type are summarized in Table 12. IC_{50} values were similar within an inhibitor and the mean IC_{50} value for the recombinant assay was 69% and 67% of the placental value for econazole and ketoconazole, respectively. Significant day-to-day variance (Table 13) by microsome type was found for both inhibitors. As expected significant variance in activity by microsome type and inhibitor concentration and the interaction between them was detected for both inhibitors (results not shown). Levene's test for homogeneity of variance detected significant variances between replicates within a single assay for inhibitor concentration, day, and all interactions between inhibitor concentration and day for the placental data. Significant differences were also found for all of those parameters in the recombinant assay. The magnitude of these variances appeared to be small compared with the activities, however, and no pattern to the variances was obvious. Therefore, evaluation of weighting schemes for use in the nonlinear curve fitting was not pursued at this time.

Table 12. IC_{50}s by Inhibitor and Microsome Type

Inhibitor	Microsome Type	Study	IC_{50}	IC_{50} Mean ± SD (CV) (by microsome type)
Econazole	Recombinant	1	2.00 nM	
		2	1.89 nM	
		3	1.90 nM	1.93 ± 0.06 nM (3.1%)
	Placental	4	2.27 nM	
		5	2.65 nM	
		6	3.44 nM	2.79 ± 0.60 nM (21.5%)
Ketoconazole	Recombinant	7	12.12 µM	
		8	9.04 µM	
		9	8.98 µM	10.05 ± 1.80 µM (17.0%)
	Placental	10	15.38 µM	
		11	13.16 µM	
		12	16.44 µM	14.99 ± 1.67 µM (11.1%)

Table 13. Tests for Day-to-Day Variability

Inhibitor	Cov Parm	Estimate	Standard Error	Z Value	Pr > |Z|
Ketoconazole	Day(Type[a])	0.0002	0.00012	1.35	0.0892
Ketoconazole	Level[b]*Day(Type[a])	0	0	1.44	0.0747
Ketoconazole	Residual	0.0001	0	6.93	<0.0001
Econazole	Day(Type[a])	0	0.0001	1.35	0.0891
Econazole	Level[b]*Day(Type[a])	0	0	2.61	0.005
Econazole	Residual	0	0	6.93	<0.0001

[a]Type refers to microsome type - recombinant vs. placental
[b]Level refers to inhibitor concentration

Analysis of variance results for log10(IC_{50}), slope, and associated within day standard errors are presented in Tables 14 and 15. Log[IC_{50}] variances were significant for microsome type and inhibitor type. There was a significant variance in slope associated with inhibitor type.

Table 14. ANOVA Results for Microsome and Inhibitor Type Variability in Log[IC_{50}] and Standard Error

Dependent	Source	DF	MS	Fvalue	ProbF
Log[IC_{50}]	Type[a]	1	0.08165537	19.68	0.0022
	Inhibitor	1	41.60754077	10030.3	<0.0001
	Type*Inhibitor	1	0.00038437	0.09	0.7686
SE_Log[IC_{50}]	Type	1	0.00099434	2.77	0.1344
	Inhibitor	1	0.00148220	4.13	0.0765
	Type*Inhibitor	1	0.00058866	1.64	0.2360

[a]Type refers to microsome type–recombinant vs. placental

Table 15. ANOVA results for Microsome and Inhibitor Type Variability in Hill Slope and Standard Error

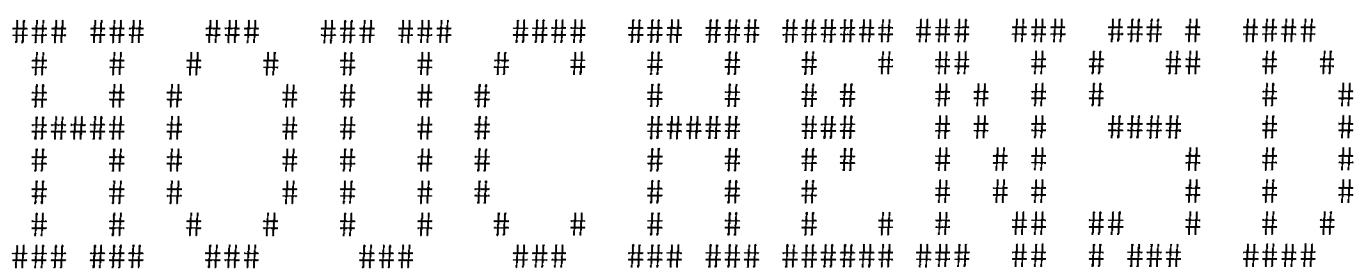
Dependent	Source	DF	MS	FValue	ProbF
HILLSLOPE	Type[a]	1	0.00077638	0.51	0.4952
HILLSLOPE	Inhibitor	1	0.10277628	67.58	<0.0001
HILLSLOPE	Type*Inhibitor	1	0.01030376	6.78	0.0315
SE_Slope	Type	1	0.00391278	6.72	0.0320
SE_Slope	Inhibitor	1	0.00082684	1.42	0.2674
SE_Slope	Type*Inhibitor	1	0.00082201	1.41	0.2687

[a]Type refers to microsome type–recombinant vs. placental

3.2 Compare Estrone and Tritiated Water Measurement Methods

3.2.1 Preliminary Testing of RIA Samples for Interferences and Residual Aromatase Activity

Estrone content of mock assay mixtures containing various combinations of all assay components except NADPH was 25 pg/mL or less as measured by RIA (standard curve range 15-2000 pg/mL). When the assay mixture contained all assay components (including NADPH, as for positive control assays), the estrone content measured was ca. 280 pg/mL, even though this sample was prepared and kept on ice prior to being flash frozen in liquid nitrogen. Therefore it may be possible that some estrone is formed in complete assay mixtures, even when the samples are stored on ice. Complete assay samples to which either inhibitor (ketoconazole or econazole) was added (at the highest concentration tested) contained < 75 pg/mL estrone,



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Final Task Report

Microsomal Aromatase Prevalidation Supplementary Study

WA 4-10 Task 4.0: Establish Inhibition Curves and IC_{50}s for Two Reference Chemicals and Task 5.0: Compare Estrone and Tritiated Water Measurement Methods

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FINAL REPORT

Title: **Microsomal Aromatase Prevalidation Supplementary Study: WA 4-10 Task 4.0: Establish Inhibition Curves and IC_{50}s For Two Reference Chemicals and Task 5.0: Compare Estrone and Tritiated Water Measurement Methods**

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Quality Assurance Statement

Study Title: Microsomal Aromatase Prevalidation Supplementary Study
WA 4-10, Tasks 4 and 5

Sponsor: Battelle Memorial Institute

Study Code: An04-900

Protocol Number: RTI-900-AN

This study was audited by the Sciences and Engineering – Health Sciences Quality Assurance Unit and the results of the inspections and audits were reported to the task leader 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 Task Leader and Management
Protocol Review	March 12, and 15, 2004	March 15, 2004
Data and Report Audit*	July 14-21, 2004	July 22, 2004

*Sections 2.3 and 3.2 of the Report were not audited (they relate to Protocol Section 5.0 which is exempt from QAPP requirements).

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1.0 OBJECTIVES

The objectives of this Work Assignment are to:

- investigate the high variability found during the conduct of Work Assignment (WA) 2-24 for the study of 4-hydroxyandrostenedione (4-OH ASDN) in the recombinant assay,
- generate complete inhibition curves for econazole and ketoconazole, and
- compare two different methods for measuring aromatase activity. One method measures the amount of tritiated water formed by the aromatization of radiolabeled androstendione (ASDN). The second method measures estrone formation by radioimmunoassay (RIA) .

The purpose of this WA is to perform a series of prevalidation studies to address the problems originally identified from WA 2-24 before beginning the interlaboratory validation studies and to compare two different methods for measuring aromatase activity.

This task report includes data from WA 4-10 Task 4.0: Establish Inhibition Curves and IC_{50}s For Two Reference Chemicals and Task 5.0: Compare Estrone and Tritiated Water Measurement Methods. The experiments reported herein are described in the protocol (RTI-900-AN) Sections 4.0 and 5.0.

2.0 MATERIALS AND METHODS

2.1 Establish Inhibition Curves and IC_{50}s For Two Reference Chemicals

2.1.1 Assay Conduct

The aromatase assay was conducted with both human placental and recombinant microsomes using the optimized conditions determined in WA 2-24 and presented below in Table 1 with the addition of varying concentrations of the inhibitors ketoconazole and econazole. Appropriate controls (defined below) were included with each assay set. The following final concentrations (M) of ketoconazole were chosen for assay in consultation with the Sponsor: (log10 units) -3.1, -4, -5, -5.3, -5.6, -6, -7, -8. Similarly, the following concentrations (M) of

econazole were chosen: (log10 units) -7, -8, -8.6, -9, -9.6, -10, -11, -12. Inhibitor and substrate sources, lot numbers and vehicles are described in the WA 4-10 Task 3 report.

Table 1. Optimized Aromatase Assay Conditions

Assay Factor (units)	Assay Type	
	Human Placental	Human Recombinant
Microsomal Protein (mg/mL)[a]	0.0125	0.004
NADPH (mM)[a]	0.3	0.3
[^{3}H]ASDN (nM)[a]	100	100
Incubation Time (min)	15	15

[a] Final concentrations

A Study (Table 2) was defined as a set of assays using a single microsome type with varying concentrations (in triplicate) of the inhibitor (with appropriate controls) conducted by a single technician on a given day. One econazole and one ketoconazole study using a given microsome type was conducted on a single day. The two Studies conducted on a single day employed the same ASDN, NADPH and microsome solutions. All solutions (except buffer) were prepared fresh each day. Stock solutions of ketoconazole were prepared in ethanol and those of econazole were prepared using DMSO. Stock solutions of inhibitors and all dilutions were prepared fresh each day.

Table 2. Study Designations

	Recombinant Assay	Placental Assay
Econazole	Studies 1, 2, 3	Studies 4, 5, 6
Ketoconazole	Studies 7, 8, 9	Studies 10, 11, 12

Positive and negative control samples (as in WA 2-24) were included for each study. Positive controls contained substrate, NADPH, propylene glycol, buffer, vehicle (20 µL EtOH or DMSO) and microsomes. Negative controls contained all positive control assay components except NADPH. Four positive and four negative controls were included with each Study and

treated the same as the other samples. The control's sets were split so that two tubes (of each positive and negative controls) were run at the beginning and two at the end of each study set.

The assays were performed in 13x100 mm test tubes maintained at 37 ± 1°C in a shaking water bath. Propylene glycol (100 µL), [^{3}H]ASDN, NADPH, inhibitor (at varying concentrations) or vehicle and buffer (0.1 M sodium phosphate buffer, pH 7.4) were combined in the test tubes (total volume 1 mL). The volume of solvent (inhibitor solutions or vehicle) added to each tube was 20 µL (1% of final volume). The tubes were placed at 37 ± 1°C in the water bath for 5 min prior to initiation of the assay by the addition of 1 mL of the diluted microsomal suspension. The total assay volume was 2.0 mL, and the tubes were incubated for 15 min. The incubations were stopped by the addition of methylene chloride (2.0 mL); the tubes were vortex-mixed for ca. 5 s and placed on ice. The tubes were then vortex-mixed an additional 20-25 s. The tubes were centrifuged using a Beckman GS-6R centrifuge with GH-3.8 rotor for 10 min at a setting of 1000 rpm. The methylene chloride layer was removed and discarded; the aqueous layers were extracted again with methylene chloride (2 mL). This extraction procedure was performed one additional time, each time discarding the methylene chloride layer. The aqueous layers were transferred to vials and duplicate aliquots (0.5 mL) were transferred to 20-mL liquid scintillation counting vials. Liquid scintillation cocktail (Ultima Gold, Packard, 10 mL) was added to each counting vial and shaken to mix the solution. The radiochemical content of each aliquot was determined by liquid scintillation spectrometry (LSS).

At the end of the incubation time for the recombinant assay tubes, a 200-µL aliquot (for RIA analysis) of the reaction mixture was removed immediately prior to addition of the CH_2Cl_2. The aliquots for RIA were combined with 20 µL of a 11 µM solution of 4-OH ASDN, capped, vortexed to mix and then placed in the -20 °C freezer. The samples were transferred to the -70 °C freezer after they were frozen for storage until analyzed by RIA. RIA analysis was conducted the day after the ^3H_2O aromatase assay.

2.1.2 Data Analysis

Raw LSS data (CPM) were converted to DPM using Process2 (Version 2.1 09251998) software. DPM data and protein assay data were entered into a validated Excel spreadsheet. The spreadsheet calculated DPM/mL for each aliquot of extracted aqueous incubation mixture and average DPM/mL and total DPM for each aqueous portion (after extraction). Multiplication of the volume (mL) of substrate solution added to the incubation by the substrate solution specific

activity (DPM/mL) yielded the total DPM present in the assay tube at initiation. The total DPM remaining in the aqueous portion after extraction divided by the total DPM present in the assay tube at initiation, times 100 yielded the percent of the substrate that was converted to product. The total DPM remaining in the aqueous portion after extraction was corrected for background by subtracting the average DPM present in the aqueous portion of the negative control tubes (for that day/assay). This corrected DPM was then converted to nmol product formed by dividing by the substrate specific activity (DPM/nmol). The activity of the enzyme reaction was expressed in nmol (mg protein)$^{-1}$min^{-1} and was calculated by dividing the amount of estrogen formed (nmol) by the product of mg microsomal protein used times the incubation time, e.g. 15 min. Average activity in the positive control samples for a given Study was calculated. Percent of control activity remaining in the presence of various inhibitor concentrations was calculated by dividing the aromatase activity at a given concentration by the average positive control activity and multiplying by 100.

IC_{50} was calculated using Prism (Version 3.02) software to fit the percent of control activity and log concentration data to a curve using the following equation:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom})/(1 + 10^{((\text{LogIC}_{50} - X)*\text{HillSlope})})$$

where: X is the logarithm of concentration
Y is the percent activity
Bottom is the lower plateau
Top is the upper plateau

2.1.3 Statistical Analysis

2.1.3.1 Quality control comparisons. For purposes of comparison, the control responses were combined across batches and an analysis of variance was carried out on the data. The factors in the analysis were microsome type, batch, portion of batch (beginning or end) and the interaction between batch and portion of batch. The responses were enzymatic activity. The estimated variance between the two replicate runs/batch was based on the pooled value across the quadruplicate replicate runs in a given Study for a given microsome type. In addition, the data was analyzed by multiple comparisons and graphical analyses.

2.1.3.2 Variability assessment. Differences among microsome types and day-to-day variances within microsome types for enzymatic activity at various concentrations of an inhibitor were estimated. The variance components were inspected across the assays to determine their comparability. Analyses were based on mixed effects analysis of variance models using PROC MIXED or PROC GLM in SAS Version 8.2. Microsome types and inhibitor concentrations were treated as fixed effects; day-to-day variation was treated as a random effect. Variation between replicates within a single assay was examined using Levene's test for homogeneity of variance. Graphical analyses were used to examine the changes in the variances across microsome type, inhibitor type, day and inhibitor concentration.

2.1.3.3 Concentration-response curve fits. Analysis of variance tests were carried out on log10(IC_{50}), slope, and associated within day standard errors. Microsome type, inhibitor type and microsome x inhibitor interaction variation was determined for each of the parameters and was tested for significance.

2.2 Compare Estrone and Tritiated Water Measurement Methods

THIS SECTION IS NOT CONDUCTED OR REPORTED UNDER QAPP REQUIREMENTS (see WA 4-10).

Test kits for the determination of estrone content by radioimmunoassay (RIA) were obtained from Diagnostic Systems, Inc. (Webster, TX). The catalog number of the kits was DSL-8700.

2.2.1 General RIA Assay Procedure

The RIA was conducted as described in the kit insert. Each sample and standard for RIA was run in duplicate or triplicate. Each assay set contained tubes for Total Counts, Non-Specific Binding, Standards (range: 15-2000 pg/mL), two Control Concentrations (35 and 300 pg/mL) and Unknowns.

2.2.2 Preliminary Interference and Accuracy Testing

Preliminary experiments were conducted to test for interference of all assay components with the kit. A mixture of buffer, propylene glycol and NADPH (at concentrations used in the ^3H_2O assay) was prepared and tested for interference with the RIA assay. Other ^3H_2O assay components (microsomes, ASDN, and the inhibitors, ketoconazole and econazole, at the highest concentrations used in the ^3H_2O assay) were added in series to the above mixture and these new samples (see Table 3) were also tested for interference. In Mix 4, the NADPH was omitted in order to simulate the negative control assay conditions.

Table 3. Description of Interference Test Samples

Assay Component	Mix 1	Mix 2	Mix 3	Mix 4	Mix 5	Mix 6
Buffer	X	X	X	X	X	X
Propylene glycol	X	X	X	X	X	X
NADPH	X	X	X		X	X
Microsomes		X	X	X	X	X
ASDN			X	X	X	X
Ketoconazole					X	
Econazole						X

Accuracy testing was conducted by fortifying each of the interference testing mixtures with a known amount of estrone and assaying by RIA.

2.2.3 Preliminary Testing to Ensure Aromatase Activity is Abolished

These experiments were conducted to address the concern that viable protein may remain in samples that were either flash frozen or slow frozen and lead to further generation of product when the samples were thawed at room temperature for RIA. Samples that contained microsomes, substrate and all co-factors were assayed by RIA both with and without prior incubation at 37 °C for 15 min and after either slow or flash freezing and thawing (see Table 4). In Mix 4, the NADPH was omitted in order to simulate the negative control assay conditions.

Table 4. Description of Samples for Investigating Residual Aromatase Activity after Freeze/Thaw

Assay Component	Mix 1	Mix 2	Mix 3	Mix 4	Mix 5
Buffer	X	X	X	X	X
Propylene glycol	X	X	X	X	X
NADPH	X	X	X		X
Microsomes	X	X	X	X	X
ASDN	X	X	X	X	X
Econazole					X
Incubated at 37 °C			X		
Slow Freeze	X		X	X	X
Flash Freeze		X			
Assayed immediately on thawing and again after 15 min at room temp	X	X			X

In subsequent experiments, the ability of 4-OH ASDN to abolish the aromatase reaction (and any interference with RIA) was tested (Table 5). A complete aromatase reaction mixture (microsomes, substrate, co-factors) was prepared on ice. One aliquot (200-µL) of this sample was combined with 4-OH ASDN (final concentration -1E-6M [abolished 90% of aromatase activity in previous studies]) and one aliquot (200-µL) was combined with 20 µL EtOH. The aromatase reaction mixture was incubated at 37 °C for 15 min and then two aliquots (200-µL) were combined with 4-OH ASDN and two aliquots (200-µL) were combined with EtOH. One of each of the aliquots combined with 4-OH ASDN and EtOH was further incubated at 37 °C for 15 min. Each aliquot was then slow frozen. The next day each sample was thawed and assayed for estrone content by RIA.

Table 5. Description of Samples for Investigating Residual Aromatase Activity in Presence of 4-OH ASDN

Assay Component	Mix 1	Mix 2	Mix 3	Mix 4	Mix 5	Mix 6
Incubated at 37 °C			X	X	X	X
Aliquot added to 4-OH ASDN	X		X		X	
Aliquot added to EtOH		X		X		X
Second incubation at 37 °C					X	X

3.0 RESULTS

3.1 Inhibition Curves and IC_{50}s for Two Reference Chemicals

3.1.1 Detailed Data Tables

The aromatase assay was conducted using each microsome type (placental and recombinant) and the inhibitors ketoconazole and econazole on three days each as described in Section 2.1.1. The detailed results for each study are presented in Appendix A.

3.1.2 Control Sample Analysis

Negative control tubes (containing all assay components except NADPH) served as blanks for the assay. Generally, the aqueous portion of the negative control tubes contained less than 100 DPM/mL after extraction. In a few instances the negative controls contained in the range of 100-400 DPM/mL (see Appendix A) and in one case (Day 3 Placental assay, ketoconazole set, negative control 3, Appendix A, Table A-9) the negative control contained 706 DPM/mL after extraction. Based on our experience and data obtained during the conduct of WA 2-24, the elevated DPM/mL values in these isolated negative control tubes probably indicate a slight cross-contamination of the assay tube with NADPH. All negative control tubes contained radioactivity equivalent to less than 1% conversion of ASDN to estrone. Overall (3 days x 2 inhibitors x 4 negative control replicates; n=24) the mean aromatase activity in the negative control tubes was 0.0000 ± 0.0007 nmol/mg/min and 0.0000 ± 0.0003 nmol/mg/min for the placental and recombinant assays, respectively.

Means and standard deviations (n=2) for the positive control activities are presented in Table 6. Within an assay day, there was generally little variance (<5%) in positive control activities within an inhibitor, however, this variance was as high as 12% in one instance. Also, variance across inhibitors within an assay day was generally < 10%. The positive control samples for each inhibitor contained a different test article vehicle. Therefore, the absence of any marked variance in the positive control activity across inhibitors within an assay day indicates little variance in the effect of the test article vehicles on aromatase activity. The vehicles were EtOH or DMSO, for ketoconazole or econazole, respectively, at 1% total assay volume.

Table 6. Means and Standard Deviations for Positive Control Activities*

Microsome Type	Vehicle†	Control Type	Portion of Batch (Day)	Day 1 Mean	Day 1 Std Dev	Day 2 Mean	Day 2 Std Dev	Day 3 Mean	Day 3 Std Dev
Placental	DMSO	Positive	Begin	0.0608	0.0117	0.0640	0.0010	0.0597	0.0015
		Positive	End	0.0676	0.0005	0.0626	0.0016	0.0569	0.0007
	Mean ± SD by Vehicle and Day (% CV)			0.0642 ± 0.0078 (12.13%)		0.0633 ± 0.0014 (2.16%)		0.0583 ± 0.0018 (3.12%)	
	EtOH	Positive	Begin	0.0721	0.0010	0.0662	0.0002	0.0655	0.0024
		Positive	End	0.0704	0.0000	0.0640	0.0001	0.0603	0.0013
	Mean ± SD by Vehicle and Day (% CV)			0.0713 ± 0.0011 (1.62%)		0.0651 ± 0.0012 (1.91%)		0.0630 ± 0.0034 (5.42%)	
	Mean ± SD by Day (% CV)			0.0677 ± 0.0064 (9.44%)		0.0642 ± 0.0015 (2.39%)		0.0606 ± 0.0035 (5.84%)	
	Overall Placental Positive Control Mean ± SD			0.0642 ± 0.0051 (7.94%)					
Recombinant	DMSO	Positive	Begin	0.3111	0.0148	0.2381	0.0064	0.2623	0.0172
		Positive	End	0.2914	0.0010	0.2227	0.0082	0.2506	0.0043
	Mean ± SD by Vehicle and Day (% CV)			0.3012 ± 0.0142 (4.72%)		0.2304 ± 0.0107 (4.64%)		0.2564 ± 0.0123 (4.78%)	
	EtOH	Positive	Begin	0.3106	0.0200	0.1609[a]	0.1631	0.2910	0.0156
		Positive	End	0.3011	0.0062	0.2414	0.0072	0.2619	0.0003
	Mean ± SD by Vehicle and Day (% CV)			0.3059 ± 0.0133 (4.34%)		0.2011 ± 0.1051 (52.2%)[b] 0.2530 ± 0.0207 (8.19%)		0.2764 ± 0.0191 (6.90%)	
	Mean ± SD by Day (% CV)			0.3036 ± 0.0130 (4.27%)		0.2158 ± 0.0709 (32.9%)[b] 0.2401 ± 0.0186 (7.75%)		0.2664 ± 0.0183 (6.87%)	
	Overall Recombinant Positive Control Mean ± SD			0.2619 ± 0.0551 (21.0%)[b] 0.2713 ± 0.0309 (11.4%)					

* Control activities from the ^3H_2O release assay (nmol/mg/min). N=2

† Control samples run with the econazole assay sets contained DMSO as vehicle control while those run with the ketoconazole assay sets contained EtOH as vehicle control.

[a] The activity values for the recombinant beginning positive controls with EtOH vehicle (ketoconazole set) were: 0.2762 and 0.0455 nmol/mg/min for tubes 1 and 2, respectively.

[b] Because the Tube 2 activity was an outlier from the mean of all four positive control values for this set, it was excluded from the set and a new mean and SD was calculated. The mean and standard deviation shown in the first row includes all data, the second row contains the mean and standard deviation calculated after removal of the data from Day 2, replicate 2 of the EtOH vehicle (ketoconazole) set.

The analysis of variance results for the control samples are presented in Table 7. As expected, there were statistically significant variances in the positive control activities by microsome type. Statistically significant variances were also detected by day within a microsome type. Where statistically significant variances were detected, the mean activities were further examined for variance by least squares pair-wise comparison (Table 8). No significant day-to-day variance was found in the mean placental positive control values. There were significant day-to-day variances of means for the recombinant positive control activities.

Table 7. ANOVA Results for Positive Control Activities

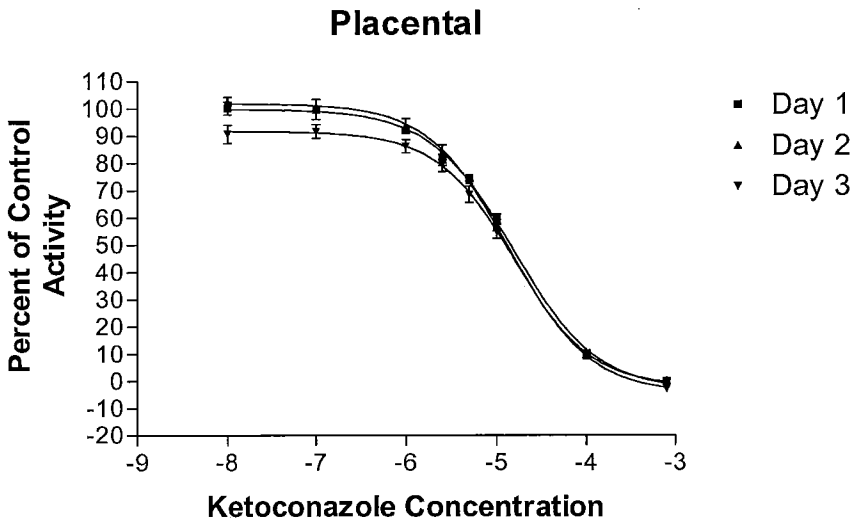
Control Type	Source	DF	Type III SS	Mean Square	F Value	Pr > F
Positive	Type	1	0.4994	0.4994	7338.09	<0.0001
Positive	Inhibitor	1	0.001268	0.001268	18.64	0.0001
Positive	Portion	1	0.001139	0.001139	16.73	0.0002
Positive	Day(Type)	4	0.0144	0.003599	52.88	<0.0001
Positive	Type*Inhibitor	1	0.0004099	0.0004099	6.02	0.019
Positive	Type*Portion	1	0.0009058	0.0009058	13.31	0.0008

Table 8. Least Squares Means Pair-wise Differences and T-Tests

Control	Mean 1 Type	Mean 1 Day	Mean 2 Type	Mean 2 Day	Mean Diff	Tstat	Pvalue
Positive	Placental	1	Placental	2	0.00351	0.8507	0.95562
Positive	Placental	1	Placental	3	0.00712	1.725	0.52473
Positive	Placental	2	Placental	3	0.00361	0.8743	0.95031
Positive	Recombinant	1	Recombinant	2	0.060972	14.231	0.00000
Positive	Recombinant	1	Recombinant	3	0.037139	9.0039	0.00000
Positive	Recombinant	2	Recombinant	3	-0.02383	-5.5627	0.00000

3.1.3 Inhibitor Results

3.1.3.1 Ketoconazole. Aromatase activity was assayed in both microsome types in the presence of varying concentrations of ketoconazole. Each ketoconazole concentration was assayed in triplicate on each of three assay days per microsome type. Graphs of aromatase activity as percent of control activity versus ketoconazole concentration (log(M)) are presented in Figure 1. This data is also presented in Table 9.



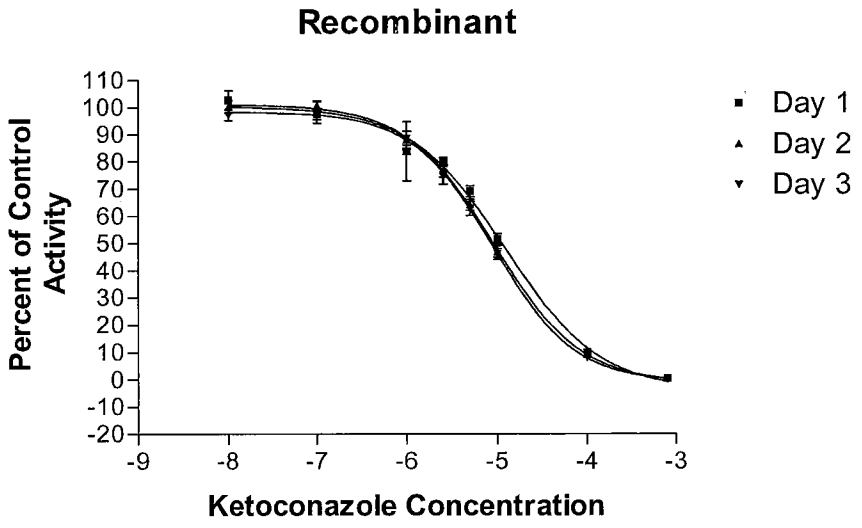
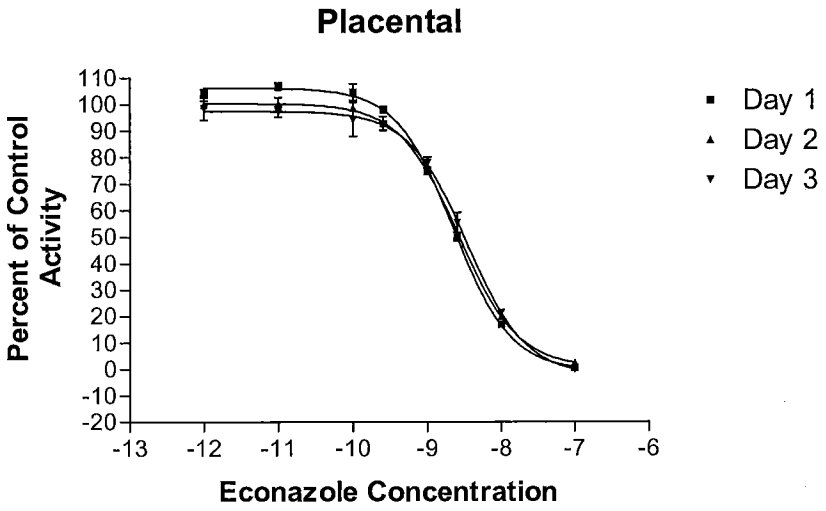


Figure 1. Ketoconazole Inhibition Curves
Concentration units are log (M)

Table 9. Percent of Control Activities for Aromatase Activity in the Presence of Ketoconazole

Concentration Code	Ketoconazole Concentration (Log(M))	Day 1 Replicate 1	Day 1 Replicate 2	Day 1 Replicate 3	Day 2 Replicate 1	Day 2 Replicate 2	Day 2 Replicate 3	Day 3 Replicate 1	Day 3 Replicate 2	Day 3 Replicate 3
		Placental								
1	-3.1	-0.2	-0.1	-0.1	0.2	0.2	0.1	-2.9	-2.8	-2.8
2	-4.0	10.7	10.4	9.4	10.1	8.8	8.8	10.2	10.6	8.3
3	-5.0	60.5	60.6	57.6	58.3	58.5	54.8	57.8	53.0	54.1
4	-5.3	75.0	73.2	75.6	74.2	74.5	72.4	71.8	68.0	65.9
5	-5.6	78.9	82.1	82.6	85.4	85.5	80.7	83.4	77.4	79.2
6	-6.0	91.2	91.8	93.3	95.8	94.5	90.6	88.9	84.5	85.2
7	-7.0	103.7	98.8	96.5	100.9	101.9	99.5	93.4	92.6	88.7
8	-8.0	102.4	99.8	98.0	102.9	103.9	100.9	92.6	92.7	86.8
		Recombinant								
1	-3.1	0.4	0.2	0.3	0.2	0.2	0.3	0.1	0.0	0.1
2	-4.0	10.6	10.5	8.7	9.0	9.7	8.5	8.9	7.8	7.2
3	-5.0	54.0	52.8	47.6	49.9	48.2	42.6	49.7	45.4	42.9
4	-5.3	72.7	68.9	65.7	66.7	65.7	60.0	67.1	63.1	58.0
5	-5.6	81.3	81.6	77.2	81.1	76.0	73.0	80.4	75.4	69.1
6	-6.0	99.6	88.8	63.0	93.1	88.5	82.1	91.5	90.5	83.4
7	-7.0	100.8	97.9	94.0	102.8	101.7	97.3	100.8	103.2	90.2
8	-8.0	107.8	104.0	96.1	102.5	102.4	95.7	97.7	100.2	93.3

3.1.3.2 Econazole. Aromatase activity was assayed in both microsome types in the presence of varying concentrations of econazole. Each econazole concentration was assayed in triplicate on each of three assay days per microsome type. Graphs of aromatase activity as percent of control activity versus econazole concentration (log(M)) are presented in Figure 2. This data is also presented in Table 10.



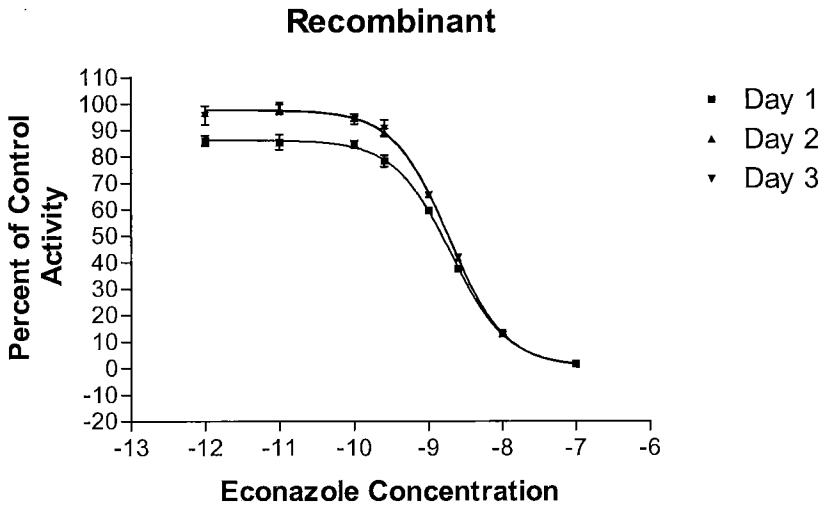


Figure 2. Econazole Inhibition Curves
Concentration units are log(M)

Table 10. Percent of Control Activities for Aromatase Activity in the Presence of Econazole

Concentration Code	Econazole Concentration (Log(M))	Day 1 Replicate 1	Day 1 Replicate 2	Day 1 Replicate 3	Day 2 Replicate 1	Day 2 Replicate 2	Day 2 Replicate 3	Day 3 Replicate 1	Day 3 Replicate 2	Day 3 Replicate 3
		Placental								
1	-7.0	0.7	0.4	0.7	2.3	2.4	2.4	0.1	0.0	-0.2
2	-8.0	17.9	16.8	16.2	19.8	20.1	19.9	21.5	21.2	21.4
3	-8.6	49.4	50.1	49.2	51.6	51.5	51.7	51.1	57.4	57.9
4	-9.0	74.1	75.8	76.0	74.0	75.5	75.2	80.4	77.3	76.5
5	-9.6	97.7	99.2	97.1	94.0	94.5	89.9	93.9	91.1	90.7
6	-10.0	101.3	104.8	107.6	98.6	99.1	98.0	97.4	98.5	86.7
7	-11.0	108.0	106.9	105.4	101.9	100.9	97.2	99.3	97.0	95.3
8	-12.0	105.6	102.4	103.8	101.3	99.0	99.4	103.2	97.8	94.5
		Recombinant								
1	-7.0	1.4	1.5	1.6	1.5	1.6	1.6	1.6	1.5	1.8
2	-8.0	13.1	13.1	13.2	13.6	12.8	12.7	13.7	13.7	13.0
3	-8.6	38.1	38.3	36.3	42.4	41.7	40.6	42.2	41.6	41.7
4	-9.0	61.4	59.5	58.0	67.5	65.8	63.5	64.3	67.2	64.7
5	-9.6	83.4	75.7	76.6	91.4	89.7	86.4	96.4	89.8	85.7
6	-10.0	87.1	84.4	82.6	96.4	92.0	95.7	93.3	96.3	91.5
7	-11.0	90.7	84.6	81.1	101.3	97.5	94.7	101.0	97.4	99.7
8	-12.0	89.9	83.7	84.5	99.0	95.9	95.0	102.2	94.9	89.9

3.1.3.3 Statistical analysis of inhibitor results. Coefficients of variance (CV) for the measured activities of the replicate tubes within a study are presented in Table 11. The samples containing the highest concentration of inhibitor, had very low (or even non-detectable) activities (< 5% of control) which led to much higher CVs than those measured for the other concentrations. When the CVs from those highest inhibitor concentration samples are excluded, the mean CVs across all three assay days for each microsome/inhibitor set were less than 5%, except in the case of the recombinant microsomes/ketoconazole set where the mean CV was 6.9%. The data demonstrate that there is little variance between replicates tubes (at each inhibitor concentration) within a study and across all inhibitor concentrations across three independently conducted assays (days). There was no discernable pattern toward differing variances between microsome types or inhibitors.

Table 11. Coefficients of Variance for Replicates within a Study

Microsome Type	Inhibitor	Concentration Code	Day 1	Day 2	Day 3
Placental	Econazole	1*	28.9	2.1	0.0
		2	5.1	0.9	0.7
		3	0.9	0.1	6.8
		4	1.4	1.1	2.6
		5	1.1	2.7	1.9
		6	3.0	0.6	6.9
		7	1.2	2.5	2.1
		8	1.5	1.2	4.4
	Mean ± SD*		2.3 ± 0.4		
Recombinant	Econazole	1*	5.0	3.3	7.1
		2	0.3	3.5	3.1
		3	2.9	2.1	0.7
		4	2.8	3.1	2.4
		5	5.3	2.8	6.0
		6	2.7	2.5	2.6
		7	5.7	3.4	1.8
		8	3.9	2.2	6.5
	Mean ± SD*		3.2 ± 0.3		
Placental	Ketoconazole	1*	0	55.5	0
		2	7.0	7.8	12.7
		3	2.9	3.6	4.6
		4	1.7	1.6	4.4
		5	2.5	3.3	3.8
		6	1.2	2.8	2.7
		7	3.7	1.2	2.7
		8	2.3	1.5	3.7
	Mean ± SD*		3.7 ± 0.6		

(continued)

Table 11. Coefficients of Variance for Replicates within a Study (continued)

Microsome Type	Inhibitor	Concentration Code	Day 1	Day 2	Day 3
Recombinant	Ketoconazole	1*	40.4	21.3	35.3
		2	10.7	6.7	11.1
		3	6.6	8.2	7.5
		4	5.1	5.6	7.2
		5	3.1	5.3	7.5
		6	22.5	6.3	4.9
		7	3.5	2.9	7.0
		8	5.8	3.9	3.6
	Mean ± SD*		6.9 ± 0.9		

*The activities of all Concentration Code 1 samples were <5% of the positive control activity for their respective set. These very low (in some cases non-detectable) activities typically had much higher variances than the rest of the set. Therefore, all Concentration Code 1 coefficients of variance were excluded from the calculation of Mean and Standard Deviation for each microsome type and inhibitor set.

IC_{50} values for each inhibitor and microsome type are summarized in Table 12. IC_{50} values were similar within an inhibitor and the mean IC_{50} value for the recombinant assay was 69% and 67% of the placental value for econazole and ketoconazole, respectively. Significant day-to-day variance (Table 13) by microsome type was found for both inhibitors. As expected significant variance in activity by microsome type and inhibitor concentration and the interaction between them was detected for both inhibitors (results not shown). Levene's test for homogeneity of variance detected significant variances between replicates within a single assay for inhibitor concentration, day, and all interactions between inhibitor concentration and day for the placental data. Significant differences were also found for all of those parameters in the recombinant assay. The magnitude of these variances appeared to be small compared with the activities, however, and no pattern to the variances was obvious. Therefore, evaluation of weighting schemes for use in the nonlinear curve fitting was not pursued at this time.

Table 12. IC_{50}s by Inhibitor and Microsome Type

Inhibitor	Microsome Type	Study	IC_{50}	IC_{50} Mean ± SD (CV) (by microsome type)
Econazole	Recombinant	1	2.00 nM	
		2	1.89 nM	
		3	1.90 nM	1.93 ± 0.06 nM (3.1%)
	Placental	4	2.27 nM	
		5	2.65 nM	
		6	3.44 nM	2.79 ± 0.60 nM (21.5%)
Ketoconazole	Recombinant	7	12.12 µM	
		8	9.04 µM	
		9	8.98 µM	10.05 ± 1.80 µM (17.0%)
	Placental	10	15.38 µM	
		11	13.16 µM	
		12	16.44 µM	14.99 ± 1.67 µM (11.1%)

Table 13. Tests for Day-to-Day Variability

Inhibitor	Cov Parm	Estimate	Standard Error	Z Value	Pr > |Z|
Ketoconazole	Day(Type[a])	0.0002	0.00012	1.35	0.0892
Ketoconazole	Level[b]*Day(Type[a])	0	0	1.44	0.0747
Ketoconazole	Residual	0.0001	0	6.93	<0.0001
Econazole	Day(Type[a])	0	0.0001	1.35	0.0891
Econazole	Level[b]*Day(Type[a])	0	0	2.61	0.005
Econazole	Residual	0	0	6.93	<0.0001

[a]Type refers to microsome type - recombinant vs. placental
[b]Level refers to inhibitor concentration

Analysis of variance results for log10(IC_{50}), slope, and associated within day standard errors are presented in Tables 14 and 15. Log[IC_{50}] variances were significant for microsome type and inhibitor type. There was a significant variance in slope associated with inhibitor type.

Table 14. ANOVA Results for Microsome and Inhibitor Type Variability in Log[IC_{50}] and Standard Error

Dependent	Source	DF	MS	Fvalue	ProbF
Log[IC_{50}]	Type[a]	1	0.08165537	19.68	0.0022
	Inhibitor	1	41.60754077	10030.3	<0.0001
	Type*Inhibitor	1	0.00038437	0.09	0.7686
SE_Log[IC_{50}]	Type	1	0.00099434	2.77	0.1344
	Inhibitor	1	0.00148220	4.13	0.0765
	Type*Inhibitor	1	0.00058866	1.64	0.2360

[a]Type refers to microsome type–recombinant vs. placental

Table 15. ANOVA results for Microsome and Inhibitor Type Variability in Hill Slope and Standard Error

Dependent	Source	DF	MS	FValue	ProbF
HILLSLOPE	Type[a]	1	0.00077638	0.51	0.4952
HILLSLOPE	Inhibitor	1	0.10277628	67.58	<0.0001
HILLSLOPE	Type*Inhibitor	1	0.01030376	6.78	0.0315
SE_Slope	Type	1	0.00391278	6.72	0.0320
SE_Slope	Inhibitor	1	0.00082684	1.42	0.2674
SE_Slope	Type*Inhibitor	1	0.00082201	1.41	0.2687

[a]Type refers to microsome type–recombinant vs. placental

3.2 Compare Estrone and Tritiated Water Measurement Methods

3.2.1 Preliminary Testing of RIA Samples for Interferences and Residual Aromatase Activity

Estrone content of mock assay mixtures containing various combinations of all assay components except NADPH was 25 pg/mL or less as measured by RIA (standard curve range 15-2000 pg/mL). When the assay mixture contained all assay components (including NADPH, as for positive control assays), the estrone content measured was ca. 280 pg/mL, even though this sample was prepared and kept on ice prior to being flash frozen in liquid nitrogen. Therefore it may be possible that some estrone is formed in complete assay mixtures, even when the samples are stored on ice. Complete assay samples to which either inhibitor (ketoconazole or econazole) was added (at the highest concentration tested) contained < 75 pg/mL estrone,

indicating both low cross reactivity with complete assay components and an apparent inhibition of the formation of estrone in the presence of the inhibitors.

In a second preliminary experiment to determine the effect of slow vs. flash freezing on residual aromatase activity, complete assay mixtures, prepared and stored on ice prior to freezing by either method, contained about 800 pg/mL estrone. When these samples were allowed to sit at room temperature 15 min after thawing, their measured estrone content decreased (650-750 pg/mL) slightly. Once again, assay samples that did not contain NADPH, contained little estrone [below the limit (15 pg/mL) of the standard curve].

The third preliminary experiment investigated the use of 4-OH ASDN as an aromatase inhibitor in RIA samples. Aliquots of complete assay mixtures (prepared on ice) were combined with 4-OH ASDN (1E-6M), slow frozen, and then assayed for estrone content. These samples contained < 30 pg/mL estrone. Aliquots of complete assay mixtures that had been incubated at 37 °C before they were combined with 4-OH ASDN and frozen, contained ca. 1500 pg/mL estrone. There was little change in the estrone content of these samples when they were incubated an additional 15 min at room temperature. Therefore, it appears that 4-OH ASDN successfully inhibits the production of additional estrone in aromatase assay mixtures.

3.2.2 Estrone Content of Recombinant Microsome Aromatase Assay Samples

The estrone content of recombinant aromatase assays with econazole (Studies 1, 2, and 3) and ketoconazole (Studies 7, 8, and 9) as the aromatase inhibitors was measured by RIA. Complete data from each assay is presented in Appendix B (Tables 1-6). The range of the standard curve was 15-2000 pg/mL. Summary data for the assays with comparisons to data from the corresponding ^3H_2O assay are presented in Tables 16 and 17 for econazole and ketoconazole as inhibitor, respectively. Generally, the amount (nmol) of ^3H_2O measured in the tritiated water assay was about 3-fold the amount (nmol) of estrone measured in the RIA assay. At some high concentrations of inhibitor, the ratio was reduced, perhaps due to inhibition of another enzyme that may be involved in the further metabolism of estrone.

Table 16. RIA Results for the Recombinant Assay with Econazole and Comparison with ^3H_2O Assay Data

Control Type/ Inhibitor Concentration Code*	Day 1					Day 2					Day 3				
	RIA[a]		^3H_2O[b]			RIA		^3H_2O			RIA		^3H_2O		
	Mean	SD	Mean	SD	Fold[c]	Mean	SD	Mean	SD	Fold	Mean	SD	Mean	SD	Fold
Positive	0.0100	0.0000	0.0390	0.00	3.8	0.0106	0.0000	0.0360	0.001	3.4	0.0100	0.0000	0.0330	0.00	3.3
1	0.0000	0.0000	0.0000	0.00	2.8	0.0002	0.0000	0.0000	0.000	3.0	0.0000	0.0000	0.0000	0.00	3.0
2	0.0020	0.0000	0.0050	0.00	2.6	0.0020	0.0000	0.0040	0.000	2.4	0.0010	0.0000	0.0040	0.00	2.4
3	0.0050	0.0000	0.0140	0.00	2.6	0.0059	0.0000	0.0150	0.000	2.6	0.0050	0.0000	0.0130	0.00	2.4
4	0.0060	0.0000	0.023	0.00	3.7	0.0066	0.0000	0.0230	0.000	3.6	0.0060	0.0000	0.0210	0.00	3.4
5	0.0080	0.0000	0.0300	0.00	3.5	0.0093	0.0000	0.0320	0.000	3.5	0.0090	0.0000	0.0300	0.00	3.2
6	0.0090	0.0000	0.0330	0.00	3.4	0.0100	0.0000	0.0340	0.000	3.5	0.0100	0.0000	0.0310	0.00	3.0
7	0.0090	0.0000	0.0330	0.00	3.4	0.0102	0.0000	0.0350	0.001	3.5	0.0100	0.0000	0.0330	0.00	3.3
8	0.0100	0.0000	0.0330	0.00	3.4	0.0104	0.0000	0.0350	0.000	3.4	0.0100	0.0000	0.0310	0.00	3.2

* n = 4 for each control; n = 3 for each inhibitor concentration. Positive controls are as described in Section 2.1.1. Negative controls are not included in the summary because all RIA negative controls were < LOQ and ^3H_2O negative controls served as assay blanks.

[a] Values are nmol estrone measured in total assay volume by RIA

[b] Values are nmol ^3H_2O measured in total assay volume by the tritiated water assay, which is a measure of estrone formed

[c] nmol ^3H_2O formed (by tritiated water assay)/ nmol estrone formed (by RIA)

Table 17. RIA Results for the Recombinant Assay with Ketoconazole and Comparison with ^3H_2O Assay Data

Control Type/ Inhibitor Concentration Code*	Day 1					Day 2					Day 3				
	RIA[a]		^3H_2O[b]			RIA		^3H_2O			RIA		^3H_2O		
	Mean	SD	Mean	SD	Fold[c]	Mean	SD	Mean	SD	Fold	Mean	SD	Mean	SD	Fold
Positive	0.0100	0.0000	0.0390	0.001	3.8	0.0080	0.0040	0.0310	0.016	3.9	0.0090	0.0000	0.0350	0.002	3.9
1	0.0000	0.0000	0.0000	0.000	0.2	0.0000	0.0000	0.0000	0.000	0.2	0.0000	0.0000	0.0000	0.000	0.0
2	0.0010	0.0000	0.0040	0.000	2.3	0.0010	0.0000	0.0030	0.000	2.2	0.0010	0.0000	0.0020	0.000	2.2
3	0.0080	0.0000	0.0200	0.001	2.5	0.0070	0.0000	0.0180	0.001	2.7	0.0060	0.0000	0.0160	0.001	2.6
4	0.0060	0.0000	0.0270	0.001	4.2	0.0060	0.0000	0.0250	0.001	4.3	0.0060	0.0000	0.0220	0.001	3.8

(continued)

Table 17. RIA Results for the Recombinant Assay with Ketoconazole and Comparison with ^3H_2O Assay Data (continued)

Control Type/ Inhibitor Concentration Code*	Day 1					Day 2					Day 3				
	RIA[a]		^3H_2O[b]			RIA		^3H_2O			RIA		^3H_2O		
	Mean	SD	Mean	SD	Fold[c]	Mean	SD	Mean	SD	Fold	Mean	SD	Mean	SD	Fold
5	0.0070	0.0000	0.0310	0.001	4.1	0.0070	0.0000	0.0300	0.001	4.1	0.0070	0.0000	0.0260	0.002	3.8
6	0.0090	0.0000	0.0330	0.007	3.4	0.0090	0.0000	0.0350	0.002	3.8	0.0080	0.0000	0.0310	0.001	3.8
7	0.0070	0.0020	0.0380	0.001	5.6	0.0100	0.0000	0.0400	0.001	3.7	0.0090	0.0000	0.0350	0.002	3.8
8	0.0100	0.0010	0.0410	0.002	4.1	0.0100	0.0000	0.0400	0.001	3.7	0.0090	0.0000	0.0340	0.001	3.8

* n =4 for each control; n = for each inhibitor concentration. Positive controls are as described in Section 2.1.1. Negative controls are not included in the summary because all RIA negative controls were < LOQ and ^3H_2O negative controls served as assay blanks.

[a] Values are nmol estrone measured in total assay volume by RIA

[b] Values are nmol ^3H_2O measured in total assay volume by the tritiated water assay, which is equivalent to nmol estrone formed

[c] nmol ^3H_2O formed (by tritiated water assay)/ nmol estrone formed (by RIA)

Aromatase activity was calculated based on the estrone content measure by RIA. Complete data for each assay is presented in Appendix B (Tables 7-12). The activities were expressed as percent of control (see Table 18) and the data were fit to the same model shown in Section 2.1.2 by non linear regression for the calculation of IC_{50} values. The concentration-response curves are presented in Figure 3 and the IC_{50} values are summarized in Table 19. IC_{50} values for econazole calculated based on estrone content determined by RIA (2.73 ± 0.11 nM) are similar to those calculated based on the ^3H_2O release assay (1.93 ± 0.06 nM). The RIA-derived IC_{50} for ketoconazole was 21.1 ± 11.0 μM which was about double the 10.08 ± 1.85 μM measured from the ^3H_2O release assay. Most of the variance in the RIA-derived IC_{50} for ketoconazole appears to arise from a few samples (at 1E-7 M ketoconazole concentration) in the Day 1 assay that contained aberrantly low levels of estrone and have a large effect on the IC_{50} curve. No explanation for the low estrone concentrations measured by RIA in these samples could be found, though it is noted that there was no corresponding finding when these same samples were measured by the tritiated water assay. The mean of the Day 2 and Day 3 IC_{50} for ketoconazole by RIA was 14.9 ± 3.0 μM, which compares more favorably with that measured by the ^3H_2O release assay.

Table 18. Percent of Control Activities Based on RIA Data for the Recombinant Assay

Concentration Code	Inhibitor Concentration (Log(M))	Day 1 Replicate 1	Day 1 Replicate 2	Day 1 Replicate 3	Day 2 Replicate 1	Day 2 Replicate 2	Day 2 Replicate 3	Day 3 Replicate 1	Day 3 Replicate 2	Day 3 Replicate 3
		Econazole								
1	-7.0	1.7	2.3	2.0	1.9	1.8	1.7	1.6	1.8	1.9
2	-8.0	19.0	19.8	18.5	19.5	18.0	17.8	18.3	18.7	17.3
3	-8.6	54.8	56.0	53.8	56.4	55.3	55.8	58.8	57.3	56.0
4	-9.0	57.2	62.9	62.2	64.0	61.7	61.2	61.6	63.5	61.6
5	-9.6	84.6	80.2	86.1	91.0	83.6	88.9	102.4	87.4	87.5
6	-10.0	94.6	91.9	94.9	92.6	91.1	97.5	108.7	100.0	92.2
7	-11.0	98.3	90.7	93.6	99.2	93.0	96.0	107.8	93.7	97.2
8	-12.0	95.3	95.4	95.0	97.0	98.4	97.5	104.8	94.5	93.6
		Ketoconazole								
1	-3.1	4.7	5.5	5.7	5.6	5.3	5.9	4.5	5.6	4.8
2	-4.0	18.0	16.9	15.1	15.0	16.3	14.9	15.4	14.2	12.8
3	-5.0	80.6	82.0	72.1	69.1	67.9	63.2	74.7	66.0	66.8
4	-5.3	68.0	67.1	56.3	59.8	58.0	52.0	71.2	61.0	62.6
5	-5.6	74.6	83.3	66.3	73.3	69.1	69.4	80.1	77.3	70.4
6	-6.0	98.7	88.6	97.7	86.5	88.7	90.7	98.2	94.8	80.7
7	-7.0	41.5	73.5	85.9	100.7	97.8	108.8	101.4	108.5	91.0
8	-8.0	99.1	106.4	85.1	100.2	98.9	105.9	102.2	104.2	91.4

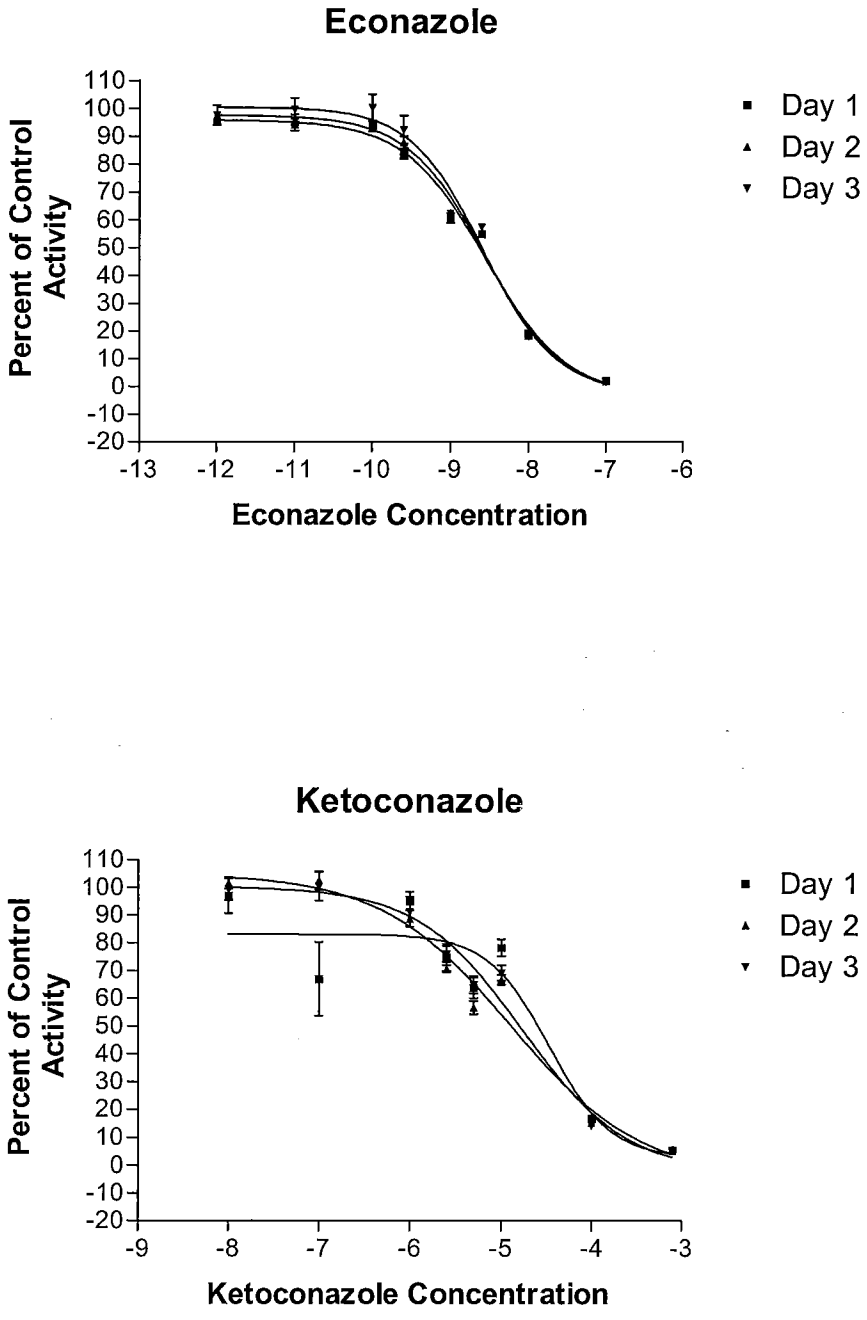


Figure 3. Inhibitor Response Curves for RIA Data
(concentration units are Log(M), data are from recombinant microsomes)

Table 19. IC_{50}s from RIA Assay

Inhibitor	Microsome Type	Study	IC_{50}	IC_{50} Mean ± SD (CV)
Econazole	Recombinant	1	2.81 nM	
		2	2.78 nM	
		3	2.61 nM	2.73 ± 0.11 nM (4.0 %)
Ketoconazole	Recombinant	7	33.6 µM	
		8	12.7 µM	
		9	17.0 µM	21.1 ± 11.0 µM (52.3%)

4.0 DISCUSSION/CONCLUSIONS

Complete inhibition curves for the interaction of the inhibitors ketoconazole and econazole with human placental and recombinant aromatase (CYP19) activity was established. Ketoconazole was tested over the range 8E-4 to 1E-8 M; econazole was assayed over the range 1E-7 to 1E-12 M. The measured IC_{50} for ketoconazole in the recombinant assay was 10.08 ± 1.85 µM, while that for the placental assay was 15.00 ± 1.67 µM. These values are about one-sixth to one-fourth the literature value of > 65 µM reported in the protocol to WA 2-24. However, White et al. (1999) reported an IC_{50} for ketoconazole of 6 µM and noted that literature values vary widely (7-60 µM). Rowlands et al. (1995) reported an IC_{50} for ketoconazole in human placental microsomes of 15 µM while Ayub and Levell (1988) reported an IC_{50} of 7.3 µM for this inhibitor. The measured IC_{50} for econazole in the recombinant assay was 1.93 ± 0.06 nM, while that for the placental assay was 2.79 ± 0.60 nM. These values are an order of magnitude less than the literature values of 30-50 nM reported in the protocol for WA 2-24. There was little variance in the IC_{50} measured for a particular inhibitor using either microsome type.

Estrone content of samples from the recombinant aromatase assay was measured by RIA and compared with the nmol ^3H_2O formed in the tritiated water assay. One mole of ^3H_2O is formed for each mole of estrone formed in the aromatization of androstenedione. Generally, the amount of tritiated water measured in the assays was about 3-fold higher than the amount of estrone measured. At some high concentrations of inhibitor, the ratio was reduced, perhaps due to inhibition of another enzyme that may be involved in the further metabolism of estrone. It is possible that estrone may be further metabolized to another component that may not be

detectable using RIA. Further investigations would be necessary to determine the source of the apparent discrepancy between the amount of estrone and ^3H_2O formed in microsomes.

Aromatase activity in the recombinant microsomes in the presence of ketoconazole or econazole was calculated based on estrone content measured by RIA. IC_{50}s calculated based on these activities were similar to those obtained based on the ^3H_2O release assay. Therefore, it appears that application of either method (RIA or ^3H_2O) gives similar results. Both methods require the use of radiolabeled materials, but the RIA requires the use of the higher energy (but lower half-life) ^{125}I isotope. While the entire ^3H_2O assay can be completed within one day, an additional day may be required to analyze incubation aliquots by RIA. For these reasons, we recommend the continued development of the ^3H_2O assay for screening aromatase activity for the Endocrine Disruptor Screening Program.

5.0 REFERENCES

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APPENDIX A

Detailed Data Tables for Tritiated Water Assay

APPENDIX A

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Table A-1. Placental Assay with Econazole Inhibitor, Day 1

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover					Calculate final assay protein concentration							
Sample Type [a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (uL)	Final volume of diluted microsomes (uL)	[protein] in dilution (mg/mL)	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)
Positive Control	1	2	0.5	1	4373	8746	8583	17166	0.1	186967	9.18	16811	0.0188	0.332	3600	50000	0.024	1	0.012	15	0.0526
			0.5	2	4210	8420			0.1												
	2	2	0.5	1	5632	11264	11218	22436	0.1	186967	12.00	22081	0.0248	0.332	3600	50000	0.024	1	0.012	15	0.0690
			0.5	2	5586	11172			0.1												
	3	2	0.5	1	5581	11162	11035	22070	0.1	186967	11.80	21715	0.0243	0.332	3600	50000	0.024	1	0.012	15	0.0679
			0.5	2	5454	10908			0.1												
	4	2	0.5	1	5477	10954	10932	21864	0.1	186967	11.69	21509	0.0241	0.332	3600	50000	0.024	1	0.012	15	0.0672
			0.5	2	5455	10910			0.1												
Negative Control	1	2	0.5	1	38	76	70	140	0.1	186967	0.07	-215	-0.0002	0.332	3600	50000	0.024	1	0.012	15	— [b]
			0.5	2	32	64			0.1												
	2	2	0.5	1	39	78	85	170	0.1	186967	0.09	-185	-0.0002	0.332	3600	50000	0.024	1	0.012	15	— [b]
			0.5	2	46	92			0.1												
	3	2	0.5	1	174	348	341	682	0.1	186967	0.36	327	0.0004	0.332	3600	50000	0.024	1	0.012	15	— [b]
			0.5	2	167	334			0.1												
	4	2	0.5	1	106	212	214	428	0.1	186967	0.23	73	0.0001	0.332	3600	50000	0.024	1	0.012	15	— [b]
			0.5	2	108	216			0.1												

Table A-1. Placental Assay with Econazole Inhibitor, Day 1 (continued)

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover					Calculate final assay protein concentration							
Sample Type [a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Econazole	1-1	2	0.5	1	115	230	248	496	0.1	186967	0.27	141	0.0002	0.332	3600	50000	0.024	1	0.012	15	0.0004
			0.5	2	133	266			0.1												
	1-2	2	0.5	1	113	226	217	434	0.1	186967	0.23	79	0.0001	0.332	3600	50000	0.024	1	0.012	15	0.0002
			0.5	2	104	208			0.1												
	1-3	2	0.5	1	126	252	245	490	0.1	186967	0.26	135	0.0002	0.332	3600	50000	0.024	1	0.012	15	0.0004
			0.5	2	119	238			0.1												
	2-1	2	0.5	1	996	1992	2019	4038	0.1	186967	2.16	3683	0.0041	0.332	3600	50000	0.024	1	0.012	15	0.0115
			0.5	2	1023	2046			0.1												
	2-2	2	0.5	1	963	1926	1907	3814	0.1	186967	2.04	3459	0.0039	0.332	3600	50000	0.024	1	0.012	15	0.0108
			0.5	2	944	1888			0.1												
	2-3	2	0.5	1	907	1814	1843	3686	0.1	186967	1.97	3331	0.0037	0.332	3600	50000	0.024	1	0.012	15	0.0104
			0.5	2	936	1872			0.1												
	3-1	2	0.5	1	2605	5210	5248	10496	0.1	186967	5.61	10141	0.0114	0.332	3600	50000	0.024	1	0.012	15	0.0317
			0.5	2	2643	5286			0.1												
	3-2	2	0.5	1	2653	5306	5317	10634	0.1	186967	5.69	10279	0.0115	0.332	3600	50000	0.024	1	0.012	15	0.0321
			0.5	2	2664	5328			0.1												

Table A-1. Placental Assay with Econazole Inhibitor, Day 1 (continued)

Sample Info			Calculate DPM in aqueous portion after extraction						Calculate % turnover					Calculate final assay protein concentration							
Sample Type[a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes used in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Econazole	3-3	2	0.5	1	2555	5110	5226	10452	0.1	186967	5.59	10097	0.0113	0.332	3600	50000	0.024	1	0.012	15	0.0316
			0.5	2	2671	5342			0.1												
	4-1	2	0.5	1	3862	7724	7780	15560	0.1	186967	8.32	15205	0.0170	0.332	3600	50000	0.024	1	0.012	15	0.0475
			0.5	2	3918	7836			0.1												
	4-2	2	0.5	1	3964	7928	7960	15920	0.1	186967	8.51	15565	0.0174	0.332	3600	50000	0.024	1	0.012	15	0.0487
			0.5	2	3996	7992			0.1												
	4-3	2	0.5	1	3968	7936	7977	15954	0.1	186967	8.53	15599	0.0175	0.332	3600	50000	0.024	1	0.012	15	0.0488
			0.5	2	4009	8018			0.1												
	5-1	2	0.5	1	5106	10212	10204	20408	0.1	186967	10.92	20053	0.0225	0.332	3600	50000	0.024	1	0.012	15	0.0627
			0.5	2	5098	10196			0.1												
	5-2	2	0.5	1	5231	10462	10365	20730	0.1	186967	11.09	20375	0.0228	0.332	3600	50000	0.024	1	0.012	15	0.0637
			0.5	2	5134	10268			0.1												
	5-3	2	0.5	1	5032	10064	10145	20290	0.1	186967	10.85	19935	0.0223	0.332	3600	50000	0.024	1	0.012	15	0.0623
			0.5	2	5113	10226			0.1												
	6-1	2	0.5	1	5309	10618	10580	21160	0.1	186967	11.32	20805	0.0233	0.332	3600	50000	0.024	1	0.012	15	0.0650
			0.5	2	5271	10542			0.1												

Table A-1. Placental Assay with Econazole Inhibitor, Day 1 (continued)

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover					Calculate final assay protein concentration							
Sample Type [a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Econazole	6-2	2	0.5	1	5444	10888	10932	21864	0.1	186967	11.69	21509	0.0241	0.332	3600	50000	0.024	1	0.012	15	0.0672
			0.5	2	5488	10976			0.1												
	6-3	2	0.5	1	5562	11124	11218	22436	0.1	186967	12.00	22081	0.0248	0.332	3600	50000	0.024	1	0.012	15	0.0690
			0.5	2	5656	11312			0.1												
	7-1	2	0.5	1	5535	11070	11261	22522	0.1	186967	12.05	22167	0.0248	0.332	3600	50000	0.024	1	0.012	15	0.0693
			0.5	2	5726	11452			0.1												
	7-2	2	0.5	1	5595	11190	11155	22310	0.1	186967	11.93	21955	0.0246	0.332	3600	50000	0.024	1	0.012	15	0.0686
			0.5	2	5560	11120			0.1												
	7-3	2	0.5	1	5464	10928	10992	21984	0.1	186967	11.76	21629	0.0242	0.332	3600	50000	0.024	1	0.012	15	0.0676
			0.5	2	5528	11056			0.1												
	8-1	2	0.5	1	5606	11212	11017	22034	0.1	186967	11.78	21679	0.0243	0.332	3600	50000	0.024	1	0.012	15	0.0678
			0.5	2	5411	10822			0.1												
	8-2	2	0.5	1	5323	10646	10688	21376	0.1	186967	11.43	21021	0.0236	0.332	3600	50000	0.024	1	0.012	15	0.0657
			0.5	2	5365	10730			0.1												
	8-3	2	0.5	1	5391	10782	10834	21668	0.1	186967	11.59	21313	0.0239	0.332	3600	50000	0.024	1	0.012	15	0.0666
			0.5	2	5443	10886			0.1												

[a]Positive controls contain all assay components: microsomes, ASDN, NADPH, propylene glycol, buffer and inhibitor vehicle. Negative controls contain all assay components except NADPH. See Section 2.1.1 for further details.
[b]Negative control tubes serve as assay blanks; therefore, no activities are calculated.

Table A-2. Placental Assay with Econazole Inhibitor, Day 2

Sample Info			Calculate DPM in aqueous portion after extraction						Calculate % turnover					Calculate final assay protein concentration							
Sample Type[a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq.	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Positive Control	1	2	0.5	1	5422	10844	10799	21598	0.1	192225	11.24	21493	0.0280	0.410	3600	50000	0.030	1	0.015	15	0.0633
			0.5	2	5377	10754			0.1												
	2	2	0.5	1	5522	11044	11041	22082	0.1	192225	11.49	21977	0.0287	0.410	3600	50000	0.030	1	0.015	15	0.0648
			0.5	2	5519	11038			0.1												
	3	2	0.5	1	5300	10600	10487	20974	0.1	192225	10.91	20869	0.0272	0.410	3600	50000	0.030	1	0.015	15	0.0615
			0.5	2	5187	10374			0.1												
	4	2	0.5	1	5400	10800	10876	21752	0.1	192225	11.32	21647	0.0282	0.410	3600	50000	0.030	1	0.015	15	0.0638
			0.5	2	5476	10952			0.1												
Negative Control	1	2	0.5	1	22	44	46	92	0.1	192225	0.05	-13	0.0000	0.410	3600	50000	0.030	1	0.015	15	—[b]
			0.5	2	24	48			0.1												
	2	2	0.5	1	36	72	54	108	0.1	192225	0.06	3	0.0000	0.410	3600	50000	0.030	1	0.015	15	—[b]
			0.5	2	18	36			0.1												
	3	2	0.5	1	28	56	47	94	0.1	192225	0.05	-11	0.0000	0.410	3600	50000	0.030	1	0.015	15	—[b]
			0.5	2	19	38			0.1												
	4	2	0.5	1	33	66	63	126	0.1	192225	0.07	21	0.0000	0.410	3600	50000	0.030	1	0.015	15	—[b]
			0.5	2	30	60			0.1												

Table A-2. Placental Assay with Econazole Inhibitor, Day 2 (continued)

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover					Calculate final assay protein concentration							
Sample Type[a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Econazole	1-1	2	0.5	1	165	330	301	602	0.1	192225	0.31	497	0.0006	0.410	3600	50000	0.030	1	0.015	15	0.0015
			0.5	2	136	272			0.1												
	1-2	2	0.5	1	152	304	309	618	0.1	192225	0.32	513	0.0007	0.410	3600	50000	0.030	1	0.015	15	0.0015
			0.5	2	157	314			0.1												
	1-3	2	0.5	1	150	300	311	622	0.1	192225	0.32	517	0.0007	0.410	3600	50000	0.030	1	0.015	15	0.0015
			0.5	2	161	322			0.1												
	2-1	2	0.5	1	1076	2152	2179	4358	0.1	192225	2.27	4253	0.0055	0.410	3600	50000	0.030	1	0.015	15	0.0125
			0.5	2	1103	2206			0.1												
	2-2	2	0.5	1	1073	2146	2217	4434	0.1	192225	2.31	4329	0.0056	0.410	3600	50000	0.030	1	0.015	15	0.0128
			0.5	2	1144	2288			0.1												
	2-3	2	0.5	1	1093	2186	2192	4384	0.1	192225	2.28	4279	0.0056	0.410	3600	50000	0.030	1	0.015	15	0.0126
			0.5	2	1099	2198			0.1												
	3-1	2	0.5	1	2808	5616	5598	11196	0.1	192225	5.82	11091	0.0145	0.410	3600	50000	0.030	1	0.015	15	0.0327
			0.5	2	2790	5580			0.1												
	3-2	2	0.5	1	2780	5560	5589	11178	0.1	192225	5.82	11073	0.0144	0.410	3600	50000	0.030	1	0.015	15	0.0326
			0.5	2	2809	5618			0.1												

Table A-2. Placental Assay with Econazole Inhibitor, Day 2 (continued)

Sample Info			Calculate DPM in aqueous portion after extraction						Calculate % turnover					Calculate final assay protein concentration							
Sample Type[a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes used in dilution (uL)	Final volume of diluted microsomes (uL)	[protein] in dilution (mg/mL)	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)
Econazole	3-3	2	0.5	1	2823	5646	5605	11210	0.1	192225	5.83	11105	0.0145	0.410	3600	50000	0.030	1	0.015	15	0.0327
			0.5	2	2782	5564			0.1												
	4-1	2	0.5	1	4014	8028	8002	16004	0.1	192225	8.33	15899	0.0207	0.410	3600	50000	0.030	1	0.015	15	0.0468
			0.5	2	3988	7976			0.1												
	4-2	2	0.5	1	4106	8212	8165	16330	0.1	192225	8.50	16225	0.0212	0.410	3600	50000	0.030	1	0.015	15	0.0478
			0.5	2	4059	8118			0.1												
	4-3	2	0.5	1	4095	8190	8136	16272	0.1	192225	8.47	16167	0.0211	0.410	3600	50000	0.030	1	0.015	15	0.0476
			0.5	2	4041	8082			0.1												
	5-1	2	0.5	1	5100	10200	10157	20314	0.1	192225	10.57	20209	0.0264	0.410	3600	50000	0.030	1	0.015	15	0.0595
			0.5	2	5057	10114			0.1												
	5-2	2	0.5	1	5095	10190	10205	20410	0.1	192225	10.62	20305	0.0265	0.410	3600	50000	0.030	1	0.015	15	0.0598
			0.5	2	5110	10220			0.1												
	5-3	2	0.5	1	4770	9540	9713	19426	0.1	192225	10.11	19321	0.0252	0.410	3600	50000	0.030	1	0.015	15	0.0569
			0.5	2	4943	9886			0.1												
	6-1	2	0.5	1	5302	10604	10645	21290	0.1	192225	11.08	21185	0.0276	0.410	3600	50000	0.030	1	0.015	15	0.0624
			0.5	2	5343	10686			0.1												

Table A-2. Placental Assay with Econazole Inhibitor, Day 2 (continued)

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover					Calculate final assay protein concentration							
Sample Type[a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (Initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes used in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Econazole	6-2	2	0.5	1	5376	10752	10705	21410	0.1	192225	11.14	21305	0.0278	0.410	3600	50000	0.030	1	0.015	15	0.0628
			0.5	2	5329	10658			0.1												
	6-3	2	0.5	1	5205	10410	10586	21172	0.1	192225	11.01	21067	0.0275	0.410	3600	50000	0.030	1	0.015	15	0.0621
			0.5	2	5381	10762			0.1												
	7-1	2	0.5	1	5523	11046	11007	22014	0.1	192225	11.45	21909	0.0286	0.410	3600	50000	0.030	1	0.015	15	0.0646
			0.5	2	5484	10968			0.1												
	7-2	2	0.5	1	5471	10942	10895	21790	0.1	192225	11.34	21685	0.0283	0.410	3600	50000	0.030	1	0.015	15	0.0639
			0.5	2	5424	10848			0.1												
	7-3	2	0.5	1	5305	10610	10497	20994	0.1	192225	10.92	20889	0.0273	0.410	3600	50000	0.030	1	0.015	15	0.0615
			0.5	2	5192	10384			0.1												
	8-1	2	0.5	1	5482	10964	10939	21878	0.1	192225	11.38	21773	0.0284	0.410	3600	50000	0.030	1	0.015	15	0.0641
			0.5	2	5457	10914			0.1												
	8-2	2	0.5	1	5287	10574	10693	21386	0.1	192225	11.13	21281	0.0278	0.410	3600	50000	0.030	1	0.015	15	0.0627
			0.5	2	5406	10812			0.1												
	8-3	2	0.5	1	5408	10816	10740	21480	0.1	192225	11.17	21375	0.0279	0.410	3600	50000	0.030	1	0.015	15	0.0630
			0.5	2	5332	10664			0.1												

[a]Positive controls contain all assay components: microsomes, ASDN, NADPH, propylene glycol, buffer and inhibitor vehicle. Negative controls contain all assay components except NADPH. See Section 2.1.1 for further details.
[b]Negative control tubes serve as assay blanks; therefore, no activities are calculated.

Table A-3. Placental Assay with Econazole Inhibitor, Day 3

Sample Info			Calculate DPM in aqueous portion after extraction						Calculate % turnover					Calculate final assay protein concentration							
Sample Type[a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Positive Control	1	2	0.5	1	5046	10092	10117	20234	0.1	188081	10.76	19629	0.0258	0.408	3600	50000	0.029	1	0.015	15	0.0586
			0.5	2	5071	10142			0.1												
	2	2	0.5	1	5210	10420	10461	20922	0.1	188081	11.12	20317	0.0267	0.408	3600	50000	0.029	1	0.015	15	0.0607
			0.5	2	5251	10502			0.1												
	3	2	0.5	1	4986	9972	9920	19840	0.1	188081	10.55	19235	0.0253	0.408	3600	50000	0.029	1	0.015	15	0.0574
			0.5	2	4934	9868			0.1												
	4	2	0.5	1	4808	9616	9752	19504	0.1	188081	10.37	18899	0.0249	0.408	3600	50000	0.029	1	0.015	15	0.0564
			0.5	2	4944	9888			0.1												
Negative Control	1	2	0.5	1	180	360	354	708	0.1	188081	0.38	103	0.0001	0.408	3600	50000	0.029	1	0.015	15	—[b]
			0.5	2	174	348			0.1												
	2	2	0.5	1	86	172	167	334	0.1	188081	0.18	-272	-0.0004	0.408	3600	50000	0.029	1	0.015	15	—[b]
			0.5	2	81	162			0.1												
	3	2	0.5	1	164	328	342	684	0.1	188081	0.36	79	0.0001	0.408	3600	50000	0.029	1	0.015	15	—[b]
			0.5	2	178	356			0.1												
	4	2	0.5	1	164	328	348	696	0.1	188081	0.37	91	0.0001	0.408	3600	50000	0.029	1	0.015	15	—[b]
			0.5	2	184	368			0.1												

Table A-3. Placental Assay with Econazole Inhibitor, Day 3 (continued)

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover				Calculate final assay protein concentration								
Sample Type[a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Econazole	1-1	2	0.5	1	168	336	315	630	0.1	188081	0.33	25	0.0000	0.408	3600	50000	0.029	1	0.015	15	0.0001
			0.5	2	147	294			0.1												
	1-2	2	0.5	1	152	304	306	612	0.1	188081	0.33	7	0.0000	0.408	3600	50000	0.029	1	0.015	15	0.0000
			0.5	2	154	308			0.1												
	1-3	2	0.5	1	139	278	284	568	0.1	188081	0.30	-38	0.0000	0.408	3600	50000	0.029	1	0.015	15	-0.0001
			0.5	2	145	290			0.1												
	2-1	2	0.5	1	1237	2474	2404	4808	0.1	188081	2.56	4203	0.0055	0.408	3600	50000	0.029	1	0.015	15	0.0126
			0.5	2	1167	2334			0.1												
	2-2	2	0.5	1	1192	2384	2375	4750	0.1	188081	2.53	4145	0.0055	0.408	3600	50000	0.029	1	0.015	15	0.0124
			0.5	2	1183	2366			0.1												
	2-3	2	0.5	1	1188	2376	2391	4782	0.1	188081	2.54	4177	0.0055	0.408	3600	50000	0.029	1	0.015	15	0.0125
			0.5	2	1203	2406			0.1												
	3-1	2	0.5	1	2616	5232	5292	10584	0.1	188081	5.63	9979	0.0131	0.408	3600	50000	0.029	1	0.015	15	0.0298
			0.5	2	2676	5352			0.1												
	3-2	2	0.5	1	2948	5896	5906	11812	0.1	188081	6.28	11207	0.0147	0.408	3600	50000	0.029	1	0.015	15	0.0335
			0.5	2	2958	5916			0.1												

Table A-3. Placental Assay with Econazole Inhibitor, Day 3 (continued)

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover				Calculate final assay protein concentration							
Sample Type [a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Econazole	3-3	2	0.5	1	3003	6006	5957	11914	0.1	188081	6.33	11309	0.0149	0.408	3600	50000	0.029	1	0.015	15	0.0338
			0.5	2	2954	5908			0.1												
	4-1	2	0.5	1	4087	8174	8147	16294	0.1	188081	8.66	15689	0.0206	0.408	3600	50000	0.029	1	0.015	15	0.0469
			0.5	2	4060	8120			0.1												
	4-2	2	0.5	1	3964	7928	7849	15698	0.1	188081	8.35	15093	0.0199	0.408	3600	50000	0.029	1	0.015	15	0.0451
			0.5	2	3885	7770			0.1												
	4-3	2	0.5	1	3943	7886	7769	15538	0.1	188081	8.26	14933	0.0197	0.408	3600	50000	0.029	1	0.015	15	0.0446
			0.5	2	3826	7652			0.1												
	5-1	2	0.5	1	4645	9290	9468	18936	0.1	188081	10.07	18331	0.0241	0.408	3600	50000	0.029	1	0.015	15	0.0547
			0.5	2	4823	9646			0.1												
	5-2	2	0.5	1	4557	9114	9190	18380	0.1	188081	9.77	17775	0.0234	0.408	3600	50000	0.029	1	0.015	15	0.0531
			0.5	2	4633	9266			0.1												
	5-3	2	0.5	1	4508	9016	9154	18308	0.1	188081	9.73	17703	0.0233	0.408	3600	50000	0.029	1	0.015	15	0.0529
			0.5	2	4646	9292			0.1												
	6-1	2	0.5	1	4969	9938	9812	19624	0.1	188081	10.43	19019	0.0250	0.408	3600	50000	0.029	1	0.015	15	0.0568
			0.5	2	4843	9686			0.1												

Table A-3. Placental Assay with Econazole Inhibitor, Day 3 (continued)

Sample Info			Calculate DPM in aqueous portion after extraction						Calculate % turnover					Calculate final assay protein concentration							
Sample Type [a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Econazole	6-2	2	0.5	1	4907	9814	9912	19824	0.1	188081	10.54	19219	0.0253	0.408	3600	50000	0.029	1	0.015	15	0.0574
			0.5	2	5005	10010			0.1												
	6-3	2	0.5	1	4382	8764	8766	17532	0.1	188081	9.32	16927	0.0223	0.408	3600	50000	0.029	1	0.015	15	0.0506
			0.5	2	4384	8768			0.1												
	7-1	2	0.5	1	4927	9854	9997	19994	0.1	188081	10.63	19389	0.0255	0.408	3600	50000	0.029	1	0.015	15	0.0579
			0.5	2	5070	10140			0.1												
	7-2	2	0.5	1	4883	9766	9771	19542	0.1	188081	10.39	18937	0.0249	0.408	3600	50000	0.029	1	0.015	15	0.0566
			0.5	2	4888	9776			0.1												
	7-3	2	0.5	1	4777	9554	9608	19216	0.1	188081	10.22	18611	0.0245	0.408	3600	50000	0.029	1	0.015	15	0.0556
			0.5	2	4831	9662			0.1												
	8-1	2	0.5	1	5201	10402	10376	20752	0.1	188081	11.03	20147	0.0265	0.408	3600	50000	0.029	1	0.015	15	0.0602
			0.5	2	5175	10350			0.1												
	8-2	2	0.5	1	4921	9842	9845	19690	0.1	188081	10.47	19085	0.0251	0.408	3600	50000	0.029	1	0.015	15	0.0570
			0.5	2	4924	9848			0.1												
	8-3	2	0.5	1	4745	9490	9530	19060	0.1	188081	10.13	18455	0.0243	0.408	3600	50000	0.029	1	0.015	15	0.0551
			0.5	2	4785	9570			0.1												

[a]Positive controls contain all assay components: microsomes, ASDN, NADPH, propylene glycol, buffer and inhibitor vehicle. Negative controls contain all assay components except NADPH. See Section 2.1.1 for further details.
[b]Negative control tubes serve as assay blanks; therefore, no activities are calculated.

Table A-4. Recombinant Assay with Econazole Inhibitor, Day 1

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover					Calculate final assay protein concentration								
Sample Type [a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Positive Control	1	2	0.5	1	7894	15788	15785	31570	0.1	162823	19.39	31522	0.0420	0.435	1600	80000	0.009	1	0.004	15	0.3215
			0.5	2	7891	15782			0.1												
	2	2	0.5	1	7449	14898	14758	29516	0.1	162823	18.13	29468	0.0392	0.435	1600	80000	0.009	1	0.004	15	0.3006
			0.5	2	7309	14618			0.1												
	3	2	0.5	1	7069	14138	14275	28550	0.1	162823	17.53	28502	0.0379	0.435	1600	80000	0.009	1	0.004	15	0.2907
			0.5	2	7206	14412			0.1												
	4	2	0.5	1	7135	14270	14344	28688	0.1	162823	17.62	28640	0.0381	0.435	1600	80000	0.009	1	0.004	15	0.2921
			0.5	2	7209	14418			0.1												
Negative Control	1	2	0.5	1	3	6	20	40	0.1	162823	0.02	-8	0.0000	0.435	1600	80000	0.009	1	0.004	15	— [b]
			0.5	2	17	34			0.1												
	2	2	0.5	1	0	0	13	26	0.1	162823	0.02	-22	0.0000	0.435	1600	80000	0.009	1	0.004	15	— [b]
			0.5	2	13	26			0.1												
	3	2	0.5	1	18	36	31	62	0.1	162823	0.04	14	0.0000	0.435	1600	80000	0.009	1	0.004	15	— [b]
			0.5	2	13	26			0.1												
	4	2	0.5	1	14	28	32	64	0.1	162823	0.04	16	0.0000	0.435	1600	80000	0.009	1	0.004	15	— [b]
			0.5	2	18	36			0.1												

Table A-4. Recombinant Assay with Econazole Inhibitor, Day 1 (continued)

Sample Info			Calculate DPM in aqueous portion after extraction						Calculate % turnover					Calculate final assay protein concentration								
Sample Type[a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (Initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Econazole	1-1	2	0.5	1	127	254	234	468	0.1	162823	0.29	420	0.0006	0.435	1600	80000	0.009	1	0.004	15	0.0043
			0.5	2	107	214			0.1												
	1-2	2	0.5	1	107	214	251	502	0.1	162823	0.31	454	0.0006	0.435	1600	80000	0.009	1	0.004	15	0.0046
			0.5	2	144	288			0.1												
	1-3	2	0.5	1	122	244	255	510	0.1	162823	0.31	462	0.0006	0.435	1600	80000	0.009	1	0.004	15	0.0047
			0.5	2	133	266			0.1												
	2-1	2	0.5	1	964	1928	1956	3912	0.1	162823	2.40	3864	0.0051	0.435	1600	80000	0.009	1	0.004	15	0.0394
			0.5	2	992	1984			0.1												
	2-2	2	0.5	1	985	1970	1962	3924	0.1	162823	2.41	3876	0.0052	0.435	1600	80000	0.009	1	0.004	15	0.0395
			0.5	2	977	1954			0.1												
	2-3	2	0.5	1	998	1996	1968	3936	0.1	162823	2.42	3888	0.0052	0.435	1600	80000	0.009	1	0.004	15	0.0397
			0.5	2	970	1940			0.1												
	3-1	2	0.5	1	2787	5574	5652	11304	0.1	162823	6.94	11256	0.0150	0.435	1600	80000	0.009	1	0.004	15	0.1148
			0.5	2	2865	5730			0.1												
	3-2	2	0.5	1	2804	5608	5676	11352	0.1	162823	6.97	11304	0.0150	0.435	1600	80000	0.009	1	0.004	15	0.1153
			0.5	2	2872	5744			0.1												

Table A-4. Recombinant Assay with Econazole Inhibitor, Day 1 (continued)

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover					Calculate final assay protein concentration							
Sample Type [a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (Initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Econazole	3-3	2	0.5	1	2712	5424	5386	10772	0.1	162823	6.62	10724	0.0143	0.435	1600	80000	0.009	1	0.004	15	0.1094
			0.5	2	2674	5348			0.1												
	4-1	2	0.5	1	4460	8920	9088	18176	0.1	162823	11.16	18128	0.0241	0.435	1600	80000	0.009	1	0.004	15	0.1849
			0.5	2	4628	9256			0.1												
	4-2	2	0.5	1	4406	8812	8804	17608	0.1	162823	10.81	17560	0.0234	0.435	1600	80000	0.009	1	0.004	15	0.1791
			0.5	2	4398	8796			0.1												
	4-3	2	0.5	1	4282	8564	8589	17178	0.1	162823	10.55	17130	0.0228	0.435	1600	80000	0.009	1	0.004	15	0.1747
			0.5	2	4307	8614			0.1												
	5-1	2	0.5	1	6199	12398	12335	24670	0.1	162823	15.15	24622	0.0328	0.435	1600	80000	0.009	1	0.004	15	0.2511
			0.5	2	6136	12272			0.1												
	5-2	2	0.5	1	5480	10960	11207	22414	0.1	162823	13.77	22366	0.0298	0.435	1600	80000	0.009	1	0.004	15	0.2281
			0.5	2	5727	11454			0.1												
	5-3	2	0.5	1	5674	11348	11333	22666	0.1	162823	13.92	22618	0.0301	0.435	1600	80000	0.009	1	0.004	15	0.2307
			0.5	2	5659	11318			0.1												
	6-1	2	0.5	1	6507	13014	12888	25776	0.1	162823	15.83	25728	0.0342	0.435	1600	80000	0.009	1	0.004	15	0.2624
			0.5	2	6381	12762			0.1												

Table A-4. Recombinant Assay with Econazole Inhibitor, Day 1 (continued)

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover					Calculate final assay protein concentration							
Sample Type[a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Econazole	6-2	2	0.5	1	6235	12470	12492	24984	0.1	162823	15.34	24936	0.0332	0.435	1600	80000	0.009	1	0.004	15	0.2544
			0.5	2	6257	12514			0.1												
	6-3	2	0.5	1	6049	12098	12216	24432	0.1	162823	15.01	24384	0.0325	0.435	1600	80000	0.009	1	0.004	15	0.2487
			0.5	2	6167	12334			0.1												
	7-1	2	0.5	1	6765	13530	13422	26844	0.1	162823	16.49	26796	0.0357	0.435	1600	80000	0.009	1	0.004	15	0.2733
			0.5	2	6657	13314			0.1												
	7-2	2	0.5	1	6192	12384	12515	25030	0.1	162823	15.37	24982	0.0333	0.435	1600	80000	0.009	1	0.004	15	0.2548
			0.5	2	6323	12646			0.1												
	7-3	2	0.5	1	5975	11950	11994	23988	0.1	162823	14.73	23940	0.0319	0.435	1600	80000	0.009	1	0.004	15	0.2442
			0.5	2	6019	12038			0.1												
	8-1	2	0.5	1	6680	13360	13298	26596	0.1	162823	16.33	26548	0.0353	0.435	1600	80000	0.009	1	0.004	15	0.2708
			0.5	2	6618	13236			0.1												
	8-2	2	0.5	1	6158	12316	12389	24778	0.1	162823	15.22	24730	0.0329	0.435	1600	80000	0.009	1	0.004	15	0.2523
			0.5	2	6231	12462			0.1												
	8-3	2	0.5	1	6227	12454	12499	24998	0.1	162823	15.35	24950	0.0332	0.435	1600	80000	0.009	1	0.004	15	0.2545
			0.5	2	6272	12544			0.1												

[a]Positive controls contain all assay components: microsomes, ASDN, NADPH, propylene glycol, buffer and inhibitor vehicle. Negative controls contain all assay components except NADPH. See Section 2.1.1 for further details.
[b]Negative control tubes serve as assay blanks; therefore, no activities are calculated.

Table A-5. Recombinant Assay with Econazole Inhibitor, Day 2

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover					Calculate final assay protein concentration							
Sample Type[a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (uL)	Final volume of diluted microsomes (uL)	[protein] in dilution (mg/mL)	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)
Positive Control	1	2	0.5	1	8051	16102	16295	32590	0.1	172419	18.90	32526	0.0383	0.527	1600	80000	0.011	1	0.005	15	0.2426
			0.5	2	8244	16488			0.1												
	2	2	0.5	1	7874	15748	15688	31376	0.1	172419	18.20	31312	0.0369	0.527	1600	80000	0.011	1	0.005	15	0.2335
			0.5	2	7814	15628			0.1												
	3	2	0.5	1	7623	15246	15351	30702	0.1	172419	17.81	30638	0.0361	0.527	1600	80000	0.011	1	0.005	15	0.2285
			0.5	2	7728	15456			0.1												
	4	2	0.5	1	7281	14562	14577	29154	0.1	172419	16.91	29090	0.0343	0.527	1600	80000	0.011	1	0.005	15	0.2170
			0.5	2	7296	14592			0.1												
Negative Control	1	2	0.5	1	17	34	27	54	0.1	172419	0.03	-10	0.0000	0.527	1600	80000	0.011	1	0.005	15	—[b]
			0.5	2	10	20			0.1												
	2	2	0.5	1	2	4	14	28	0.1	172419	0.02	-36	0.0000	0.527	1600	80000	0.011	1	0.005	15	—[b]
			0.5	2	12	24			0.1												
	3	2	0.5	1	30	60	50	100	0.1	172419	0.06	36	0.0000	0.527	1600	80000	0.011	1	0.005	15	—[b]
			0.5	2	20	40			0.1												
	4	2	0.5	1	13	26	37	74	0.1	172419	0.04	10	0.0000	0.527	1600	80000	0.011	1	0.005	15	—[b]
			0.5	2	24	48			0.1												

Table A-5. Recombinant Assay with Econazole Inhibitor, Day 2 (continued)

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover					Calculate final assay protein concentration							
Sample Type [a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (Initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Econazole	1-1	2	0.5	1	133	266	269	538	0.1	172419	0.31	474	0.0006	0.527	1600	80000	0.011	1	0.005	15	0.0035
			0.5	2	136	272			0.1												
	1-2	2	0.5	1	145	290	285	570	0.1	172419	0.33	506	0.0006	0.527	1600	80000	0.011	1	0.005	15	0.0038
			0.5	2	140	280			0.1												
	1-3	2	0.5	1	138	276	279	558	0.1	172419	0.32	494	0.0006	0.527	1600	80000	0.011	1	0.005	15	0.0037
			0.5	2	141	282			0.1												
	2-1	2	0.5	1	1080	2160	2125	4250	0.1	172419	2.46	4186	0.0049	0.527	1600	80000	0.011	1	0.005	15	0.0312
			0.5	2	1045	2090			0.1												
	2-2	2	0.5	1	986	1972	2009	4018	0.1	172419	2.33	3954	0.0047	0.527	1600	80000	0.011	1	0.005	15	0.0295
			0.5	2	1023	2046			0.1												
	2-3	2	0.5	1	972	1944	1996	3992	0.1	172419	2.32	3928	0.0046	0.527	1600	80000	0.011	1	0.005	15	0.0293
			0.5	2	1024	2048			0.1												
	3-1	2	0.5	1	3230	6460	6581	13162	0.1	172419	7.63	13098	0.0154	0.527	1600	80000	0.011	1	0.005	15	0.0977
			0.5	2	3351	6702			0.1												
	3-2	2	0.5	1	3212	6424	6467	12934	0.1	172419	7.50	12870	0.0152	0.527	1600	80000	0.011	1	0.005	15	0.0960
			0.5	2	3255	6510			0.1												

Table A-5. Recombinant Assay with Econazole Inhibitor, Day 2 (continued)

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover					Calculate final assay protein concentration							
Sample Type [a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Econazole	3-3	2	0.5	1	3131	6262	6310	12620	0.1	172419	7.32	12556	0.0148	0.527	1600	80000	0.011	1	0.005	15	0.0936
			0.5	2	3179	6358			0.1												
	4-1	2	0.5	1	5225	10450	10463	20926	0.1	172419	12.14	20862	0.0246	0.527	1600	80000	0.011	1	0.005	15	0.1556
			0.5	2	5238	10476			0.1												
	4-2	2	0.5	1	5052	10104	10199	20398	0.1	172419	11.83	20334	0.0240	0.527	1600	80000	0.011	1	0.005	15	0.1517
			0.5	2	5147	10294			0.1												
	4-3	2	0.5	1	4880	9760	9843	19686	0.1	172419	11.42	19622	0.0231	0.527	1600	80000	0.011	1	0.005	15	0.1463
			0.5	2	4963	9926			0.1												
	5-1	2	0.5	1	7033	14066	14155	28310	0.1	172419	16.42	28246	0.0333	0.527	1600	80000	0.011	1	0.005	15	0.2107
			0.5	2	7122	14244			0.1												
	5-2	2	0.5	1	6858	13716	13880	27760	0.1	172419	16.10	27696	0.0326	0.527	1600	80000	0.011	1	0.005	15	0.2066
			0.5	2	7022	14044			0.1												
	5-3	2	0.5	1	6743	13486	13381	26762	0.1	172419	15.52	26698	0.0315	0.527	1600	80000	0.011	1	0.005	15	0.1991
			0.5	2	6638	13276			0.1												
	6-1	2	0.5	1	7360	14720	14929	29858	0.1	172419	17.32	29794	0.0351	0.527	1600	80000	0.011	1	0.005	15	0.2222
			0.5	2	7569	15138			0.1												

Table A-5. Recombinant Assay with Econazole Inhibitor, Day 2 (continued)

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover					Calculate final assay protein concentration							
Sample Type [a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq.	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (Initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Econazole	6-2	2	0.5	1	7137	14274	14244	28488	0.1	172419	16.52	28424	0.0335	0.527	1600	80000	0.011	1	0.005	15	0.2120
			0.5	2	7107	14214			0.1												
	6-3	2	0.5	1	7476	14952	14807	29614	0.1	172419	17.18	29550	0.0348	0.527	1600	80000	0.011	1	0.005	15	0.2204
			0.5	2	7331	14662			0.1												
	7-1	2	0.5	1	7755	15510	15683	31366	0.1	172419	18.19	31302	0.0369	0.527	1600	80000	0.011	1	0.005	15	0.2334
			0.5	2	7928	15856			0.1												
	7-2	2	0.5	1	7546	15092	15094	30188	0.1	172419	17.51	30124	0.0355	0.527	1600	80000	0.011	1	0.005	15	0.2247
			0.5	2	7548	15096			0.1												
	7-3	2	0.5	1	7269	14538	14662	29324	0.1	172419	17.01	29260	0.0345	0.527	1600	80000	0.011	1	0.005	15	0.2182
			0.5	2	7393	14786			0.1												
	8-1	2	0.5	1	7577	15154	15318	30636	0.1	172419	17.77	30572	0.0360	0.527	1600	80000	0.011	1	0.005	15	0.2280
			0.5	2	7741	15482			0.1												
	8-2	2	0.5	1	7394	14788	14842	29684	0.1	172419	17.22	29620	0.0349	0.527	1600	80000	0.011	1	0.005	15	0.2209
			0.5	2	7448	14896			0.1												
	8-3	2	0.5	1	7369	14738	14700	29400	0.1	172419	17.05	29336	0.0346	0.527	1600	80000	0.011	1	0.005	15	0.2188
			0.5	2	7331	14662			0.1												

[a]Positive controls contain all assay components: microsomes, ASDN, NADPH, propylene glycol, buffer and inhibitor vehicle. Negative controls contain all assay components except NADPH. See Section 2.1.1 for further details.
[b]Negative control tubes serve as assay blanks; therefore, no activities are calculated.

Table A-6. Recombinant Assay with Econazole Inhibitor, Day 3

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover				Calculate final assay protein concentration								
Sample Type [a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Positive Control	1	2	0.5	1	7961	15922	15910	31820	0.1	177655	17.91	31783	0.0356	0.433	1600	80000	0.009	1	0.004	15	0.2744
			0.5	2	7949	15898			0.1												
	2	2	0.5	1	7401	14802	14503	29006	0.1	177655	16.33	28969	0.0325	0.433	1600	80000	0.009	1	0.004	15	0.2501
			0.5	2	7102	14204			0.1												
	3	2	0.5	1	7250	14500	14705	29410	0.1	177655	16.55	29373	0.0329	0.433	1600	80000	0.009	1	0.004	15	0.2536
			0.5	2	7455	14910			0.1												
	4	2	0.5	1	7231	14462	14350	28700	0.1	177655	16.15	28663	0.0321	0.433	1600	80000	0.009	1	0.004	15	0.2475
			0.5	2	7119	14238			0.1												
Negative Control	1	2	0.5	1	10	20	21	42	0.1	177655	0.02	5	0.0000	0.433	1600	80000	0.009	1	0.004	15	—[b]
			0.5	2	11	22			0.1												
	2	2	0.5	1	9	18	18	36	0.1	177655	0.02	-2	0.0000	0.433	1600	80000	0.009	1	0.004	15	—[b]
			0.5	2	9	18			0.1												
	3	2	0.5	1	10	20	21	42	0.1	177655	0.02	5	0.0000	0.433	1600	80000	0.009	1	0.004	15	—[b]
			0.5	2	11	22			0.1												
	4	2	0.5	1	8	16	15	30	0.1	177655	0.02	-8	0.0000	0.433	1600	80000	0.009	1	0.004	15	—[b]
			0.5	2	7	14			0.1												

Table A-6. Recombinant Assay with Econazole Inhibitor, Day 3 (continued)

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover					Calculate final assay protein concentration							
Sample Type [a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Econazole	1-1	2	0.5	1	129	258	259	518	0.1	177655	0.29	481	0.0005	0.433	1600	80000	0.009	1	0.004	15	0.0041
			0.5	2	130	260			0.1												
	1-2	2	0.5	1	122	244	245	490	0.1	177655	0.28	453	0.0005	0.433	1600	80000	0.009	1	0.004	15	0.0039
			0.5	2	123	246			0.1												
	1-3	2	0.5	1	143	286	279	558	0.1	177655	0.31	521	0.0006	0.433	1600	80000	0.009	1	0.004	15	0.0045
			0.5	2	136	272			0.1												
	2-1	2	0.5	1	1014	2028	2055	4110	0.1	177655	2.31	4073	0.0046	0.433	1600	80000	0.009	1	0.004	15	0.0352
			0.5	2	1041	2082			0.1												
	2-2	2	0.5	1	1022	2044	2046	4092	0.1	177655	2.30	4055	0.0045	0.433	1600	80000	0.009	1	0.004	15	0.0350
			0.5	2	1024	2048			0.1												
	2-3	2	0.5	1	967	1934	1942	3884	0.1	177655	2.19	3847	0.0043	0.433	1600	80000	0.009	1	0.004	15	0.0332
			0.5	2	975	1950			0.1												
	3-1	2	0.5	1	3176	6352	6282	12564	0.1	177655	7.07	12527	0.0140	0.433	1600	80000	0.009	1	0.004	15	0.1082
			0.5	2	3106	6212			0.1												
	3-2	2	0.5	1	3056	6112	6198	12396	0.1	177655	6.98	12359	0.0139	0.433	1600	80000	0.009	1	0.004	15	0.1067
			0.5	2	3142	6284			0.1												

Table A-6. Recombinant Assay with Econazole Inhibitor, Day 3 (continued)

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover					Calculate final assay protein concentration							
Sample Type [a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Econazole	3-3	2	0.5	1	3083	6166	6216	12432	0.1	177655	7.00	12395	0.0139	0.433	1600	80000	0.009	1	0.004	15	0.1070
			0.5	2	3133	6266			0.1												
	4-1	2	0.5	1	4781	9562	9565	19130	0.1	177655	10.77	19093	0.0214	0.433	1600	80000	0.009	1	0.004	15	0.1649
			0.5	2	4784	9568			0.1												
	4-2	2	0.5	1	4992	9984	9996	19992	0.1	177655	11.25	19955	0.0224	0.433	1600	80000	0.009	1	0.004	15	0.1723
			0.5	2	5004	10008			0.1												
	4-3	2	0.5	1	4806	9612	9623	19246	0.1	177655	10.83	19209	0.0215	0.433	1600	80000	0.009	1	0.004	15	0.1659
			0.5	2	4817	9634			0.1												
	5-1	2	0.5	1	7197	14394	14335	28670	0.1	177655	16.14	28633	0.0321	0.433	1600	80000	0.009	1	0.004	15	0.2472
			0.5	2	7138	14276			0.1												
	5-2	2	0.5	1	6630	13260	13359	26718	0.1	177655	15.04	26681	0.0299	0.433	1600	80000	0.009	1	0.004	15	0.2304
			0.5	2	6729	13458			0.1												
	5-3	2	0.5	1	6311	12622	12746	25492	0.1	177655	14.35	25455	0.0285	0.433	1600	80000	0.009	1	0.004	15	0.2198
			0.5	2	6435	12870			0.1												
	6-1	2	0.5	1	6916	13832	13877	27754	0.1	177655	15.62	27717	0.0311	0.433	1600	80000	0.009	1	0.004	15	0.2393
			0.5	2	6961	13922			0.1												

Table A-6. Recombinant Assay with Econazole Inhibitor, Day 3 (continued)

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover					Calculate final assay protein concentration							
Sample Type[a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Econazole	6-2	2	0.5	1	7143	14286	14322	28644	0.1	177655	16.12	28607	0.0321	0.433	1600	80000	0.009	1	0.004	15	0.2470
			0.5	2	7179	14358			0.1												
	6-3	2	0.5	1	6875	13750	13609	27218	0.1	177655	15.32	27181	0.0305	0.433	1600	80000	0.009	1	0.004	15	0.2347
			0.5	2	6734	13468			0.1												
	7-1	2	0.5	1	7430	14860	15019	30038	0.1	177655	16.91	30001	0.0336	0.433	1600	80000	0.009	1	0.004	15	0.2590
			0.5	2	7589	15178			0.1												
	7-2	2	0.5	1	7251	14502	14486	28972	0.1	177655	16.31	28935	0.0324	0.433	1600	80000	0.009	1	0.004	15	0.2498
			0.5	2	7235	14470			0.1												
	7-3	2	0.5	1	7414	14828	14823	29646	0.1	177655	16.69	29609	0.0332	0.433	1600	80000	0.009	1	0.004	15	0.2557
			0.5	2	7409	14818			0.1												
	8-1	2	0.5	1	7598	15196	15189	30378	0.1	177655	17.10	30341	0.0340	0.433	1600	80000	0.009	1	0.004	15	0.2620
			0.5	2	7591	15182			0.1												
	8-2	2	0.5	1	7175	14350	14105	28210	0.1	177655	15.88	28173	0.0316	0.433	1600	80000	0.009	1	0.004	15	0.2433
			0.5	2	6930	13860			0.1												
	8-3	2	0.5	1	6737	13474	13366	26732	0.1	177655	15.05	26695	0.0299	0.433	1600	80000	0.009	1	0.004	15	0.2305
			0.5	2	6629	13258			0.1												

[a]Positive controls contain all assay components: microsomes, ASDN, NADPH, propylene glycol, buffer and inhibitor vehicle. Negative controls contain all assay components except NADPH. See Section 2.1.1 for further details.
[b]Negative control tubes serve as assay blanks; therefore, no activities are calculated.

Table A-7. Placental Assay with Ketoconazole Inhibitor, Day 1

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover					Calculate final assay protein concentration								
Sample Type [a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Positive Control	1	2	0.5	1	5867	11734	11722	23444	0.1	186967	12.54	23306	0.0261	0.332	3600	50000	0.024	1	0.012	15	0.0729
			0.5	2	5855	11710			0.1												
	2	2	0.5	1	5847	11694	11485	22970	0.1	186967	12.29	22832	0.0256	0.332	3600	50000	0.024	1	0.012	15	0.0714
			0.5	2	5638	11276			0.1												
	3	2	0.5	1	5673	11346	11326	22652	0.1	186967	12.12	22514	0.0252	0.332	3600	50000	0.024	1	0.012	15	0.0704
			0.5	2	5653	11306			0.1												
	4	2	0.5	1	5623	11246	11335	22670	0.1	186967	12.13	22532	0.0253	0.332	3600	50000	0.024	1	0.012	15	0.0704
			0.5	2	5712	11424			0.1												
Negative Control	1	2	0.5	1	0	0	5	10	0.1	186967	0.01	-128	-0.0001	0.332	3600	50000	0.024	1	0.012	15	— [b]
			0.5	2	5	10			0.1												
	2	2	0.5	1	2	4	9	18	0.1	186967	0.01	-120	-0.0001	0.332	3600	50000	0.024	1	0.012	15	— [b]
			0.5	2	7	14			0.1												
	3	2	0.5	1	108	216	202	404	0.1	186967	0.22	266	0.0003	0.332	3600	50000	0.024	1	0.012	15	— [b]
			0.5	2	94	188			0.1												
	4	2	0.5	1	32	64	60	120	0.1	186967	0.06	-18	0.0000	0.332	3600	50000	0.024	1	0.012	15	— [b]
			0.5	2	28	56			0.1												

Table A-7. Placental Assay with Ketoconazole Inhibitor, Day 1 (continued)

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover				Calculate final assay protein concentration							
Sample Type [a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Ketoconazole	1-1	2	0.5	1	20	40	47	94	0.1	186967	0.05	-44	0.0000	0.332	3600	50000	0.024	1	0.012	15	-0.0001
			0.5	2	27	54			0.1												
	1-2	2	0.5	1	22	44	58	116	0.1	186967	0.06	-22	0.0000	0.332	3600	50000	0.024	1	0.012	15	-0.0001
			0.5	2	36	72			0.1												
	1-3	2	0.5	1	30	60	56	112	0.1	186967	0.06	-26	0.0000	0.332	3600	50000	0.024	1	0.012	15	-0.0001
			0.5	2	26	52			0.1												
	2-1	2	0.5	1	649	1298	1290	2580	0.1	186967	1.38	2442	0.0027	0.332	3600	50000	0.024	1	0.012	15	0.0076
			0.5	2	641	1282			0.1												
	2-2	2	0.5	1	620	1240	1259	2518	0.1	186967	1.35	2380	0.0027	0.332	3600	50000	0.024	1	0.012	15	0.0074
			0.5	2	639	1278			0.1												
	2-3	2	0.5	1	573	1146	1137	2274	0.1	186967	1.22	2136	0.0024	0.332	3600	50000	0.024	1	0.012	15	0.0067
			0.5	2	564	1128			0.1												
	3-1	2	0.5	1	3396	6792	6963	13926	0.1	186967	7.45	13788	0.0155	0.332	3600	50000	0.024	1	0.012	15	0.0431
			0.5	2	3567	7134			0.1												
	3-2	2	0.5	1	3475	6950	6978	13956	0.1	186967	7.46	13818	0.0155	0.332	3600	50000	0.024	1	0.012	15	0.0432
			0.5	2	3503	7006			0.1												

Table A-7. Placental Assay with Ketoconazole Inhibitor, Day 1 (continued)

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover					Calculate final assay protein concentration							
Sample Type[a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Ketoconazole	3-3	2	0.5	1	3314	6628	6631	13262	0.1	186967	7.09	13124	0.0147	0.332	3600	50000	0.024	1	0.012	15	0.0410
			0.5	2	3317	6634			0.1												
	4-1	2	0.5	1	4370	8740	8612	17224	0.1	186967	9.21	17086	0.0192	0.332	3600	50000	0.024	1	0.012	15	0.0534
			0.5	2	4242	8484			0.1												
	4-2	2	0.5	1	4197	8394	8414	16828	0.1	186967	9.00	16690	0.0187	0.332	3600	50000	0.024	1	0.012	15	0.0522
			0.5	2	4217	8434			0.1												
	4-3	2	0.5	1	4331	8662	8690	17380	0.1	186967	9.30	17242	0.0193	0.332	3600	50000	0.024	1	0.012	15	0.0539
			0.5	2	4359	8718			0.1												
	5-1	2	0.5	1	4539	9078	9066	18132	0.1	186967	9.70	17994	0.0202	0.332	3600	50000	0.024	1	0.012	15	0.0563
			0.5	2	4527	9054			0.1												
	5-2	2	0.5	1	4638	9276	9427	18854	0.1	186967	10.08	18716	0.0210	0.332	3600	50000	0.024	1	0.012	15	0.0585
			0.5	2	4789	9578			0.1												
	5-3	2	0.5	1	4791	9582	9485	18970	0.1	186967	10.15	18832	0.0211	0.332	3600	50000	0.024	1	0.012	15	0.0589
			0.5	2	4694	9388			0.1												
	6-1	2	0.5	1	5232	10464	10460	20920	0.1	186967	11.19	20782	0.0233	0.332	3600	50000	0.024	1	0.012	15	0.0650
			0.5	2	5228	10456			0.1												

Table A-7. Placental Assay with Ketoconazole Inhibitor, Day 1 (continued)

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover				Calculate final assay protein concentration							
Sample Type[a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Ketoconazole	6-2	2	0.5	1	5333	10666	10532	21064	0.1	186967	11.27	20926	0.0235	0.332	3600	50000	0.024	1	0.012	15	0.0654
			0.5	2	5199	10398			0.1												
	6-3	2	0.5	1	5332	10664	10709	21418	0.1	186967	11.46	21280	0.0239	0.332	3600	50000	0.024	1	0.012	15	0.0665
			0.5	2	5377	10754			0.1												
	7-1	2	0.5	1	5976	11952	11889	23778	0.1	186967	12.72	23640	0.0265	0.332	3600	50000	0.024	1	0.012	15	0.0739
			0.5	2	5913	11826			0.1												
	7-2	2	0.5	1	5701	11402	11329	22658	0.1	186967	12.12	22520	0.0252	0.332	3600	50000	0.024	1	0.012	15	0.0704
			0.5	2	5628	11256			0.1												
	7-3	2	0.5	1	5559	11118	11063	22126	0.1	186967	11.83	21988	0.0246	0.332	3600	50000	0.024	1	0.012	15	0.0687
			0.5	2	5504	11008			0.1												
	8-1	2	0.5	1	5840	11680	11745	23490	0.1	186967	12.56	23352	0.0262	0.332	3600	50000	0.024	1	0.012	15	0.0730
			0.5	2	5905	11810			0.1												
	8-2	2	0.5	1	5722	11444	11443	22886	0.1	186967	12.24	22748	0.0255	0.332	3600	50000	0.024	1	0.012	15	0.0711
			0.5	2	5721	11442			0.1												
	8-3	2	0.5	1	5627	11254	11234	22468	0.1	186967	12.02	22330	0.0250	0.332	3600	50000	0.024	1	0.012	15	0.0698
			0.5	2	5607	11214			0.1												

[a]Positive controls contain all assay components: microsomes, ASDN, NADPH, propylene glycol, buffer and inhibitor vehicle. Negative controls contain all assay components except NADPH. See Section 2.1.1 for further details.
[b]Negative control tubes serve as assay blanks; therefore, no activities are calculated.

Table A-8. Placental Assay with Ketoconazole Inhibitor, Day 2

Sample Info		Calculate DPM in aqueous portion after extraction						Calculate % turnover				Calculate final assay protein concentration								
Sample Type[a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq.	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes used in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Positive Control	1	2	0.5	1	5724	11448	11294	22588	0.1	192225	11.75	22512	0.0294	0.410	3600	50000	0.030	1	0.015	15	0.0663
			0.5	2	5570	11140			0.1												
	2	2	0.5	1	5613	11226	11242	22484	0.1	192225	11.70	22408	0.0292	0.410	3600	50000	0.030	1	0.015	15	0.0660
			0.5	2	5629	11258			0.1												
	3	2	0.5	1	5423	10846	10919	21838	0.1	192225	11.36	21762	0.0284	0.410	3600	50000	0.030	1	0.015	15	0.0641
			0.5	2	5496	10992			0.1												
	4	2	0.5	1	5405	10810	10890	21780	0.1	192225	11.33	21704	0.0283	0.410	3600	50000	0.030	1	0.015	15	0.0639
			0.5	2	5485	10970			0.1												
Negative Control	1	2	0.5	1	24	48	33	66	0.1	192225	0.03	-11	0.0000	0.410	3600	50000	0.030	1	0.015	15	—[b]
			0.5	2	9	18			0.1												
	2	2	0.5	1	24	48	40	80	0.1	192225	0.04	4	0.0000	0.410	3600	50000	0.030	1	0.015	15	—[b]
			0.5	2	16	32			0.1												
	3	2	0.5	1	15	30	37	74	0.1	192225	0.04	-3	0.0000	0.410	3600	50000	0.030	1	0.015	15	—[b]
			0.5	2	22	44			0.1												
	4	2	0.5	1	19	38	43	86	0.1	192225	0.04	10	0.0000	0.410	3600	50000	0.030	1	0.015	15	—[b]
			0.5	2	24	48			0.1												

Table A-8. Placental Assay with Ketoconazole Inhibitor, Day 2 (continued)

Sample Info			Calculate DPM in aqueous portion after extraction						Calculate % turnover				Calculate final assay protein concentration								
Sample Type[a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Ketoconazole	1-1	2	0.5	1	35	70	64	128	0.1	192225	0.07	52	0.0001	0.410	3600	50000	0.030	1	0.015	15	0.0002
			0.5	2	29	58			0.1												
	1-2	2	0.5	1	34	68	61	122	0.1	192225	0.06	46	0.0001	0.410	3600	50000	0.030	1	0.015	15	0.0001
			0.5	2	27	54			0.1												
	1-3	2	0.5	1	20	40	45	90	0.1	192225	0.05	14	0.0000	0.410	3600	50000	0.030	1	0.015	15	0.0000
			0.5	2	25	50			0.1												
	2-1	2	0.5	1	571	1142	1151	2302	0.1	192225	1.20	2226	0.0029	0.410	3600	50000	0.030	1	0.015	15	0.0066
			0.5	2	580	1160			0.1												
	2-2	2	0.5	1	506	1012	1015	2030	0.1	192225	1.06	1954	0.0025	0.410	3600	50000	0.030	1	0.015	15	0.0058
			0.5	2	509	1018			0.1												
	2-3	2	0.5	1	501	1002	1010	2020	0.1	192225	1.05	1944	0.0025	0.410	3600	50000	0.030	1	0.015	15	0.0057
			0.5	2	509	1018			0.1												
	3-1	2	0.5	1	3198	6396	6475	12950	0.1	192225	6.74	12874	0.0168	0.410	3600	50000	0.030	1	0.015	15	0.0379
			0.5	2	3277	6554			0.1												
	3-2	2	0.5	1	3231	6462	6502	13004	0.1	192225	6.76	12928	0.0169	0.410	3600	50000	0.030	1	0.015	15	0.0381
			0.5	2	3271	6542			0.1												

Table A-8. Placental Assay with Ketoconazole Inhibitor, Day 2 (continued)

Sample Info		Calculate DPM in aqueous portion after extraction								Calculate % turnover					Calculate final assay protein concentration								
Sample Type [a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Ketoconazole	3-3	2	0.5	1	3121	6242	6092	12184	0.1	192225	6.34	12108	0.0158	0.410	3600	50000	0.030	1	0.015	15	0.0357
			0.5	2	2971	5942			0.1												
	4-1	2	0.5	1	4097	8194	8236	16472	0.1	192225	8.57	16396	0.0214	0.410	3600	50000	0.030	1	0.015	15	0.0483
			0.5	2	4139	8278			0.1												
	4-2	2	0.5	1	4142	8284	8273	16546	0.1	192225	8.61	16470	0.0215	0.410	3600	50000	0.030	1	0.015	15	0.0485
			0.5	2	4131	8262			0.1												
	4-3	2	0.5	1	4012	8024	8033	16066	0.1	192225	8.36	15990	0.0209	0.410	3600	50000	0.030	1	0.015	15	0.0471
			0.5	2	4021	8042			0.1												
	5-1	2	0.5	1	4703	9406	9473	18946	0.1	192225	9.86	18870	0.0246	0.410	3600	50000	0.030	1	0.015	15	0.0556
			0.5	2	4770	9540			0.1												
	5-2	2	0.5	1	4786	9572	9488	18976	0.1	192225	9.87	18900	0.0247	0.410	3600	50000	0.030	1	0.015	15	0.0557
			0.5	2	4702	9404			0.1												
	5-3	2	0.5	1	4383	8766	8952	17904	0.1	192225	9.31	17828	0.0233	0.410	3600	50000	0.030	1	0.015	15	0.0525
			0.5	2	4569	9138			0.1												
	6-1	2	0.5	1	5201	10402	10618	21236	0.1	192225	11.05	21160	0.0276	0.410	3600	50000	0.030	1	0.015	15	0.0623
			0.5	2	5417	10834			0.1												

Table A-8. Placental Assay with Ketoconazole Inhibitor, Day 2 (continued)

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover					Calculate final assay protein concentration							
Sample Type [a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Ketoconazole	6-2	2	0.5	1	5270	10540	10480	20960	0.1	192225	10.90	20884	0.0272	0.410	3600	50000	0.030	1	0.015	15	0.0615
			0.5	2	5210	10420			0.1												
	6-3	2	0.5	1	5008	10016	10053	20106	0.1	192225	10.46	20030	0.0261	0.410	3600	50000	0.030	1	0.015	15	0.0590
			0.5	2	5045	10090			0.1												
	7-1	2	0.5	1	5609	11218	11183	22366	0.1	192225	11.64	22290	0.0291	0.410	3600	50000	0.030	1	0.015	15	0.0657
			0.5	2	5574	11148			0.1												
	7-2	2	0.5	1	5631	11262	11294	22588	0.1	192225	11.75	22512	0.0294	0.410	3600	50000	0.030	1	0.015	15	0.0663
			0.5	2	5663	11326			0.1												
	7-3	2	0.5	1	5507	11014	11035	22070	0.1	192225	11.48	21994	0.0287	0.410	3600	50000	0.030	1	0.015	15	0.0648
			0.5	2	5528	11056			0.1												
	8-1	2	0.5	1	5703	11406	11411	22822	0.1	192225	11.87	22746	0.0297	0.410	3600	50000	0.030	1	0.015	15	0.0670
			0.5	2	5708	11416			0.1												
	8-2	2	0.5	1	5697	11394	11514	23028	0.1	192225	11.98	22952	0.0299	0.410	3600	50000	0.030	1	0.015	15	0.0676
			0.5	2	5817	11634			0.1												
	8-3	2	0.5	1	5635	11270	11190	22380	0.1	192225	11.64	22304	0.0291	0.410	3600	50000	0.030	1	0.015	15	0.0657
			0.5	2	5555	11110			0.1												

[a]Positive controls contain all assay components: microsomes, ASDN, NADPH, propylene glycol, buffer and inhibitor vehicle. Negative controls contain all assay components except NADPH. See Section 2.1.1 for further details.
[b]Negative control tubes serve as assay blanks; therefore, no activities are calculated.

Table A-9. Placental Assay with Ketoconazole Inhibitor, Day 3

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover					Calculate final assay protein concentration								
Sample Type[a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Positive Control	1	2	0.5	1	5801	11602	11611	23222	0.1	188081	12.35	22519	0.0296	0.408	3600	50000	0.029	1	0.015	15	0.0673
			0.5	2	5810	11620			0.1												
	2	2	0.5	1	5593	11186	11035	22070	0.1	188081	11.73	21367	0.0281	0.408	3600	50000	0.029	1	0.015	15	0.0638
			0.5	2	5442	10884			0.1												
	3	2	0.5	1	5339	10678	10610	21220	0.1	188081	11.28	20517	0.0270	0.408	3600	50000	0.029	1	0.015	15	0.0613
			0.5	2	5271	10542			0.1												
	4	2	0.5	1	5075	10150	10291	20582	0.1	188081	10.94	19879	0.0262	0.408	3600	50000	0.029	1	0.015	15	0.0594
			0.5	2	5216	10432			0.1												
Negative Control	1	2	0.5	1	210	420	429	858	0.1	188081	0.46	155	0.0002	0.408	3600	50000	0.029	1	0.015	15	—[b]
			0.5	2	219	438			0.1												
	2	2	0.5	1	12	24	27	54	0.1	188081	0.03	-650	-0.0009	0.408	3600	50000	0.029	1	0.015	15	—[b]
			0.5	2	15	30			0.1												
	3	2	0.5	1	361	722	706	1412	0.1	188081	0.75	709	0.0009	0.408	3600	50000	0.029	1	0.015	15	—[b]
			0.5	2	345	690			0.1												
	4	2	0.5	1	144	288	245	490	0.1	188081	0.26	-214	-0.0003	0.408	3600	50000	0.029	1	0.015	15	—[b]
			0.5	2	101	202			0.1												

Table A-9. Placental Assay with Ketoconazole Inhibitor, Day 3 (continued)

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover					Calculate final assay protein concentration								
Sample Type[a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (uL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Ketoconazole	1-1	2	0.5	1	29	58	48	96	0.1	188081	0.05	-608	-0.0008	0.408	3600	50000	0.029	1	0.015	15	-0.0018
			0.5	2	19	38			0.1												
	1-2	2	0.5	1	33	66	62	124	0.1	188081	0.07	-580	-0.0008	0.408	3600	50000	0.029	1	0.015	15	-0.0017
			0.5	2	29	58			0.1												
	1-3	2	0.5	1	28	56	61	122	0.1	188081	0.06	-582	-0.0008	0.408	3600	50000	0.029	1	0.015	15	-0.0017
			0.5	2	33	66			0.1												
	2-1	2	0.5	1	723	1446	1423	2846	0.1	188081	1.51	2143	0.0028	0.408	3600	50000	0.029	1	0.015	15	0.0064
			0.5	2	700	1400			0.1												
	2-2	2	0.5	1	738	1476	1473	2946	0.1	188081	1.57	2243	0.0030	0.408	3600	50000	0.029	1	0.015	15	0.0067
			0.5	2	735	1470			0.1												
	2-3	2	0.5	1	599	1198	1227	2454	0.1	188081	1.30	1751	0.0023	0.408	3600	50000	0.029	1	0.015	15	0.0052
			0.5	2	628	1256			0.1												
	3-1	2	0.5	1	3161	6322	6443	12886	0.1	188081	6.85	12183	0.0160	0.408	3600	50000	0.029	1	0.015	15	0.0364
			0.5	2	3282	6564			0.1												
	3-2	2	0.5	1	2957	5914	5940	11880	0.1	188081	6.32	11177	0.0147	0.408	3600	50000	0.029	1	0.015	15	0.0334
			0.5	2	2983	5966			0.1												

Table A-9. Placental Assay with Ketoconazole Inhibitor, Day 3 (continued)

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover				Calculate final assay protein concentration								
Sample Type[a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Ketoconazole	3-3	2	0.5	1	3081	6162	6046	12092	0.1	188081	6.43	11389	0.0150	0.408	3600	50000	0.029	1	0.015	15	0.0340
			0.5	2	2965	5930			0.1												
	4-1	2	0.5	1	3981	7962	7917	15834	0.1	188081	8.42	15131	0.0199	0.408	3600	50000	0.029	1	0.015	15	0.0452
			0.5	2	3936	7872			0.1												
	4-2	2	0.5	1	3700	7400	7518	15036	0.1	188081	7.99	14333	0.0189	0.408	3600	50000	0.029	1	0.015	15	0.0428
			0.5	2	3818	7636			0.1												
	4-3	2	0.5	1	3627	7254	7296	14592	0.1	188081	7.76	13889	0.0183	0.408	3600	50000	0.029	1	0.015	15	0.0415
			0.5	2	3669	7338			0.1												
	5-1	2	0.5	1	4640	9280	9137	18274	0.1	188081	9.72	17571	0.0231	0.408	3600	50000	0.029	1	0.015	15	0.0525
			0.5	2	4497	8994			0.1												
	5-2	2	0.5	1	4285	8570	8508	17016	0.1	188081	9.05	16313	0.0215	0.408	3600	50000	0.029	1	0.015	15	0.0487
			0.5	2	4223	8446			0.1												
	5-3	2	0.5	1	4326	8652	8694	17388	0.1	188081	9.24	16685	0.0220	0.408	3600	50000	0.029	1	0.015	15	0.0498
			0.5	2	4368	8736			0.1												
	6-1	2	0.5	1	4860	9720	9719	19438	0.1	188081	10.33	18735	0.0247	0.408	3600	50000	0.029	1	0.015	15	0.0560
			0.5	2	4859	9718			0.1												

Table A-9. Placental Assay with Ketoconazole Inhibitor, Day 3 (continued)

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover					Calculate final assay protein concentration							
Sample Type[a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (Initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Ketoconazole	6-2	2	0.5	1	4683	9366	9259	18518	0.1	188081	9.85	17815	0.0234	0.408	3600	50000	0.029	1	0.015	15	0.0532
			0.5	2	4576	9152			0.1												
	6-3	2	0.5	1	4606	9212	9332	18664	0.1	188081	9.92	17961	0.0236	0.408	3600	50000	0.029	1	0.015	15	0.0536
			0.5	2	4726	9452			0.1												
	7-1	2	0.5	1	5122	10244	10196	20392	0.1	188081	10.84	19689	0.0259	0.408	3600	50000	0.029	1	0.015	15	0.0588
			0.5	2	5074	10148			0.1												
	7-2	2	0.5	1	5105	10210	10110	20220	0.1	188081	10.75	19517	0.0257	0.408	3600	50000	0.029	1	0.015	15	0.0583
			0.5	2	5005	10010			0.1												
	7-3	2	0.5	1	4917	9834	9701	19402	0.1	188081	10.32	18699	0.0246	0.408	3600	50000	0.029	1	0.015	15	0.0558
			0.5	2	4784	9568			0.1												
	8-1	2	0.5	1	5000	10000	10106	20212	0.1	188081	10.75	19509	0.0257	0.408	3600	50000	0.029	1	0.015	15	0.0583
			0.5	2	5106	10212			0.1												
	8-2	2	0.5	1	5067	10134	10113	20226	0.1	188081	10.75	19523	0.0257	0.408	3600	50000	0.029	1	0.015	15	0.0583
			0.5	2	5046	10092			0.1												
	8-3	2	0.5	1	4830	9660	9498	18996	0.1	188081	10.10	18293	0.0241	0.408	3600	50000	0.029	1	0.015	15	0.0546
			0.5	2	4668	9336			0.1												

[a]Positive controls contain all assay components: microsomes, ASDN, NADPH, propylene glycol, buffer and inhibitor vehicle. Negative controls contain all assay components except NADPH. See Section 2.1.1 for further details.
[b]Negative control tubes serve as assay blanks; therefore, no activities are calculated.

Table A-10. Recombinant Assay with Ketoconazole Inhibitor, Day 1

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover					Calculate final assay protein concentration							
Sample Type[a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Positive Control	1	2	0.5	1	7915	15830	15937	31874	0.1	162823	19.58	31837	0.0424	0.435	1600	80000	0.009	1	0.004	15	0.3247
			0.5	2	8022	16044			0.1												
	2	2	0.5	1	7324	14648	14551	29102	0.1	162823	17.87	29065	0.0387	0.435	1600	80000	0.009	1	0.004	15	0.2965
			0.5	2	7227	14454			0.1												
	3	2	0.5	1	7395	14790	14564	29128	0.1	162823	17.89	29091	0.0387	0.435	1600	80000	0.009	1	0.004	15	0.2967
			0.5	2	7169	14338			0.1												
	4	2	0.5	1	7476	14952	14995	29990	0.1	162823	18.42	29953	0.0399	0.435	1600	80000	0.009	1	0.004	15	0.3055
			0.5	2	7519	15038			0.1												
Negative Control	1	2	0.5	1	5	10	18	36	0.1	162823	0.02	-1	0.0000	0.435	1600	80000	0.009	1	0.004	15	—[b]
			0.5	2	13	26			0.1												
	2	2	0.5	1	1	2	14	28	0.1	162823	0.02	-9	0.0000	0.435	1600	80000	0.009	1	0.004	15	—[b]
			0.5	2	13	26			0.1												
	3	2	0.5	1	14	28	22	44	0.1	162823	0.03	7	0.0000	0.435	1600	80000	0.009	1	0.004	15	—[b]
			0.5	2	8	16			0.1												
	4	2	0.5	1	7	14	20	40	0.1	162823	0.02	3	0.0000	0.435	1600	80000	0.009	1	0.004	15	—[b]
			0.5	2	13	26			0.1												

Table A-10. Recombinant Assay with Ketoconazole Inhibitor, Day 1 (continued)

Sample Info		Calculate DPM in aqueous portion after extraction								Calculate % turnover				Calculate final assay protein concentration								
Sample Type[a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (Initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Ketoconazole	1-1	2	0.5	1	38	76	81	162	0.1	162823	0.10	125	0.0002	0.435	1600	80000	0.009	1	0.004	15	0.0013
			0.5	2	43	86			0.1												
	1-2	2	0.5	1	21	42	45	90	0.1	162823	0.06	53	0.0001	0.435	1600	80000	0.009	1	0.004	15	0.0005
			0.5	2	24	48			0.1												
	1-3	2	0.5	1	25	50	63	126	0.1	162823	0.08	89	0.0001	0.435	1600	80000	0.009	1	0.004	15	0.0009
			0.5	2	38	76			0.1												
	2-1	2	0.5	1	813	1626	1601	3202	0.1	162823	1.97	3165	0.0042	0.435	1600	80000	0.009	1	0.004	15	0.0323
			0.5	2	788	1576			0.1												
	2-2	2	0.5	1	813	1626	1595	3190	0.1	162823	1.96	3153	0.0042	0.435	1600	80000	0.009	1	0.004	15	0.0322
			0.5	2	782	1564			0.1												
	2-3	2	0.5	1	679	1358	1323	2646	0.1	162823	1.63	2609	0.0035	0.435	1600	80000	0.009	1	0.004	15	0.0266
			0.5	2	644	1288			0.1												
	3-1	2	0.5	1	4010	8020	8110	16220	0.1	162823	9.96	16183	0.0215	0.435	1600	80000	0.009	1	0.004	15	0.1651
			0.5	2	4100	8200			0.1												
	3-2	2	0.5	1	3951	7902	7936	15872	0.1	162823	9.75	15835	0.0211	0.435	1600	80000	0.009	1	0.004	15	0.1615
			0.5	2	3985	7970			0.1												

Table A-10. Recombinant Assay with Ketoconazole Inhibitor, Day 1 (continued)

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover				Calculate final assay protein concentration								
Sample Type[a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Ketoconazole	3-3	2	0.5	1	3636	7272	7154	14308	0.1	162823	8.79	14271	0.0190	0.435	1600	80000	0.009	1	0.004	15	0.1456
			0.5	2	3518	7036			0.1												
	4-1	2	0.5	1	5383	10766	10916	21832	0.1	162823	13.41	21795	0.0290	0.435	1600	80000	0.009	1	0.004	15	0.2223
			0.5	2	5533	11066			0.1												
	4-2	2	0.5	1	5092	10184	10346	20692	0.1	162823	12.71	20655	0.0275	0.435	1600	80000	0.009	1	0.004	15	0.2107
			0.5	2	5254	10508			0.1												
	4-3	2	0.5	1	4939	9878	9865	19730	0.1	162823	12.12	19693	0.0262	0.435	1600	80000	0.009	1	0.004	15	0.2009
			0.5	2	4926	9852			0.1												
	5-1	2	0.5	1	6154	12308	12204	24408	0.1	162823	14.99	24371	0.0324	0.435	1600	80000	0.009	1	0.004	15	0.2486
			0.5	2	6050	12100			0.1												
	5-2	2	0.5	1	6193	12386	12256	24512	0.1	162823	15.05	24475	0.0326	0.435	1600	80000	0.009	1	0.004	15	0.2496
			0.5	2	6063	12126			0.1												
	5-3	2	0.5	1	5850	11700	11594	23188	0.1	162823	14.24	23151	0.0308	0.435	1600	80000	0.009	1	0.004	15	0.2361
			0.5	2	5744	11488			0.1												
	6-1	2	0.5	1	7471	14942	14951	29902	0.1	162823	18.36	29865	0.0398	0.435	1600	80000	0.009	1	0.004	15	0.3046
			0.5	2	7480	14960			0.1												

Table A-10. Recombinant Assay with Ketoconazole Inhibitor, Day 1 (continued)

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover					Calculate final assay protein concentration							
Sample Type [a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Ketoconazole	6-2	2	0.5	1	6664	13328	13337	26674	0.1	162823	16.38	26637	0.0355	0.435	1600	80000	0.009	1	0.004	15	0.2717
			0.5	2	6673	13346			0.1												
	6-3	2	0.5	1	4735	9470	9459	18918	0.1	162823	11.62	18881	0.0251	0.435	1600	80000	0.009	1	0.004	15	0.1926
			0.5	2	4724	9448			0.1												
	7-1	2	0.5	1	7473	14946	15137	30274	0.1	162823	18.59	30237	0.0402	0.435	1600	80000	0.009	1	0.004	15	0.3084
			0.5	2	7664	15328			0.1												
	7-2	2	0.5	1	7281	14562	14694	29388	0.1	162823	18.05	29351	0.0391	0.435	1600	80000	0.009	1	0.004	15	0.2994
			0.5	2	7413	14826			0.1												
	7-3	2	0.5	1	7074	14148	14108	28216	0.1	162823	17.33	28179	0.0375	0.435	1600	80000	0.009	1	0.004	15	0.2874
			0.5	2	7034	14068			0.1												
	8-1	2	0.5	1	7987	15974	16179	32358	0.1	162823	19.87	32321	0.0430	0.435	1600	80000	0.009	1	0.004	15	0.3297
			0.5	2	8192	16384			0.1												
	8-2	2	0.5	1	7823	15646	15616	31232	0.1	162823	19.18	31195	0.0415	0.435	1600	80000	0.009	1	0.004	15	0.3182
			0.5	2	7793	15586			0.1												
	8-3	2	0.5	1	7182	14364	14427	28854	0.1	162823	17.72	28817	0.0384	0.435	1600	80000	0.009	1	0.004	15	0.2939
			0.5	2	7245	14490			0.1												

[a]Positive controls contain all assay components: microsomes, ASDN, NADPH, propylene glycol, buffer and inhibitor vehicle. Negative controls contain all assay components except NADPH. See Section 2.1.1 for further details.
[b]Negative control tubes serve as assay blanks; therefore, no activities are calculated.

Table A-11. Recombinant Assay with Ketoconazole Inhibitor, Day 2

Sample Info			Calculate DPM in aqueous portion after extraction						Calculate % turnover					Calculate final assay protein concentration							
Sample Type [a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Positive Control	1	2	0.5	1	9234	18468	18539	37078	0.1	172419	21.50	37037	0.0437	0.527	1600	80000	0.011	1	0.005	15	0.2762
			0.5	2	9305	18610			0.1												
	2	2	0.5	1	1536	3072	3074	6148	0.1	172419	3.57	6107	0.0072	0.527	1600	80000	0.011	1	0.005	15	0.0455
			0.5	2	1538	3076			0.1												
	3	2	0.5	1	7903	15806	15864	31728	0.1	172419	18.40	31687	0.0374	0.527	1600	80000	0.011	1	0.005	15	0.2363
			0.5	2	7961	15922			0.1												
	4	2	0.5	1	8243	16486	16546	33092	0.1	172419	19.19	33051	0.0390	0.527	1600	80000	0.011	1	0.005	15	0.2465
			0.5	2	8303	16606			0.1												
Negative Control	1	2	0.5	1	19	38	31	62	0.1	172419	0.04	21	0.0000	0.527	1600	80000	0.011	1	0.005	15	— [b]
			0.5	2	12	24			0.1												
	2	2	0.5	1	16	32	20	40	0.1	172419	0.02	-2	0.0000	0.527	1600	80000	0.011	1	0.005	15	— [b]
			0.5	2	4	8			0.1												
	3	2	0.5	1	0	0	14	28	0.1	172419	0.02	-14	0.0000	0.527	1600	80000	0.011	1	0.005	15	— [b]
			0.5	2	14	28			0.1												
	4	2	0.5	1	14	28	18	36	0.1	172419	0.02	-6	0.0000	0.527	1600	80000	0.011	1	0.005	15	— [b]
			0.5	2	4	8			0.1												

Table A-11. Recombinant Assay with Ketoconazole Inhibitor, Day 2 (continued)

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover				Calculate final assay protein concentration								
Sample Type[a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (uL)	Final volume of diluted microsomes (uL)	[protein] in dilution (mg/mL)	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)
Ketoconazole	1-1	2	0.5	1	30	60	59	118	0.1	172419	0.07	77	0.0001	0.527	1600	80000	0.011	1	0.005	15	0.0006
			0.5	2	29	58			0.1												
	1-2	2	0.5	1	34	68	62	124	0.1	172419	0.07	83	0.0001	0.527	1600	80000	0.011	1	0.005	15	0.0006
			0.5	2	28	56			0.1												
	1-3	2	0.5	1	32	64	77	154	0.1	172419	0.09	113	0.0001	0.527	1600	80000	0.011	1	0.005	15	0.0008
			0.5	2	45	90			0.1												
	2-1	2	0.5	1	799	1598	1543	3086	0.1	172419	1.79	3045	0.0036	0.527	1600	80000	0.011	1	0.005	15	0.0227
			0.5	2	744	1488			0.1												
	2-2	2	0.5	1	839	1678	1663	3326	0.1	172419	1.93	3285	0.0039	0.527	1600	80000	0.011	1	0.005	15	0.0245
			0.5	2	824	1648			0.1												
	2-3	2	0.5	1	753	1506	1458	2916	0.1	172419	1.69	2875	0.0034	0.527	1600	80000	0.011	1	0.005	15	0.0214
			0.5	2	705	1410			0.1												
	3-1	2	0.5	1	4205	8410	8485	16970	0.1	172419	9.84	16929	0.0200	0.527	1600	80000	0.011	1	0.005	15	0.1263
			0.5	2	4280	8560			0.1												
	3-2	2	0.5	1	4066	8132	8197	16394	0.1	172419	9.51	16353	0.0193	0.527	1600	80000	0.011	1	0.005	15	0.1220
			0.5	2	4131	8262			0.1												

Table A-11. Recombinant Assay with Ketoconazole Inhibitor, Day 2 (continued)

Sample Info			Calculate DPM in aqueous portion after extraction						Calculate % turnover					Calculate final assay protein concentration							
Sample Type[a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Ketoconazole	3-3	2	0.5	1	3616	7232	7246	14492	0.1	172419	8.41	14451	0.0170	0.527	1600	80000	0.011	1	0.005	15	0.1078
			0.5	2	3630	7260			0.1												
	4-1	2	0.5	1	5626	11252	11327	22654	0.1	172419	13.14	22613	0.0267	0.527	1600	80000	0.011	1	0.005	15	0.1686
			0.5	2	5701	11402			0.1												
	4-2	2	0.5	1	5591	11182	11160	22320	0.1	172419	12.95	22279	0.0263	0.527	1600	80000	0.011	1	0.005	15	0.1662
			0.5	2	5569	11138			0.1												
	4-3	2	0.5	1	5067	10134	10195	20390	0.1	172419	11.83	20349	0.0240	0.527	1600	80000	0.011	1	0.005	15	0.1518
			0.5	2	5128	10256			0.1												
	5-1	2	0.5	1	6803	13606	13771	27542	0.1	172419	15.97	27501	0.0324	0.527	1600	80000	0.011	1	0.005	15	0.2051
			0.5	2	6968	13936			0.1												
	5-2	2	0.5	1	6494	12988	12906	25812	0.1	172419	14.97	25771	0.0304	0.527	1600	80000	0.011	1	0.005	15	0.1922
			0.5	2	6412	12824			0.1												
	5-3	2	0.5	1	6258	12516	12407	24814	0.1	172419	14.39	24773	0.0292	0.527	1600	80000	0.011	1	0.005	15	0.1848
			0.5	2	6149	12298			0.1												
	6-1	2	0.5	1	7849	15698	15818	31636	0.1	172419	18.35	31595	0.0372	0.527	1600	80000	0.011	1	0.005	15	0.2356
			0.5	2	7969	15938			0.1												

Table A-11. Recombinant Assay with Ketoconazole Inhibitor, Day 2 (continued)

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover				Calculate final assay protein concentration							
Sample Type[a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (uL)	Final volume of diluted microsomes (uL)	[protein] in dilution (mg/mL)	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)
Ketoconazole	6-2	2	0.5	1	7460	14920	15024	30048	0.1	172419	17.43	30007	0.0354	0.527	1600	80000	0.011	1	0.005	15	0.2238
			0.5	2	7564	15128			0.1												
	6-3	2	0.5	1	6996	13992	13951	27902	0.1	172419	16.18	27861	0.0328	0.527	1600	80000	0.011	1	0.005	15	0.2078
			0.5	2	6955	13910			0.1												
	7-1	2	0.5	1	8670	17340	17462	34924	0.1	172419	20.26	34883	0.0411	0.527	1600	80000	0.011	1	0.005	15	0.2602
			0.5	2	8792	17584			0.1												
	7-2	2	0.5	1	8670	17340	17278	34556	0.1	172419	20.04	34515	0.0407	0.527	1600	80000	0.011	1	0.005	15	0.2574
			0.5	2	8608	17216			0.1												
	7-3	2	0.5	1	8254	16508	16521	33042	0.1	172419	19.16	33001	0.0389	0.527	1600	80000	0.011	1	0.005	15	0.2461
			0.5	2	8267	16534			0.1												
	8-1	2	0.5	1	8662	17324	17402	34804	0.1	172419	20.19	34763	0.0410	0.527	1600	80000	0.011	1	0.005	15	0.2593
			0.5	2	8740	17480			0.1												
	8-2	2	0.5	1	8606	17212	17384	34768	0.1	172419	20.16	34727	0.0409	0.527	1600	80000	0.011	1	0.005	15	0.2590
			0.5	2	8778	17556			0.1												
	8-3	2	0.5	1	8177	16354	16249	32498	0.1	172419	18.85	32457	0.0383	0.527	1600	80000	0.011	1	0.005	15	0.2421
			0.5	2	8072	16144			0.1												

[a]Positive controls contain all assay components: microsomes, ASDN, NADPH, propylene glycol, buffer and inhibitor vehicle. Negative controls contain all assay components except NADPH. See Section 2.1.1 for further details.
[b]Negative control tubes serve as assay blanks; therefore, no activities are calculated.

Table A-12. Recombinant Assay with Ketoconazole Inhibitor, Day 3

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover				Calculate final assay protein concentration								
Sample Type[a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Positive Control	1	2	0.5	1	8826	17652	17534	35068	0.1	177655	19.74	34975	0.0392	0.433	1600	80000	0.009	1	0.004	15	0.3020
			0.5	2	8708	17416			0.1												
	2	2	0.5	1	8136	16272	16257	32514	0.1	177655	18.30	32421	0.0364	0.433	1600	80000	0.009	1	0.004	15	0.2799
			0.5	2	8121	16242			0.1												
	3	2	0.5	1	7653	15306	15222	30444	0.1	177655	17.14	30351	0.0340	0.433	1600	80000	0.009	1	0.004	15	0.2621
			0.5	2	7569	15138			0.1												
	4	2	0.5	1	7543	15086	15197	30394	0.1	177655	17.11	30301	0.0340	0.433	1600	80000	0.009	1	0.004	15	0.2616
			0.5	2	7654	15308			0.1												
Negative Control	1	2	0.5	1	11	22	18	36	0.1	177655	0.02	-57	-0.0001	0.433	1600	80000	0.009	1	0.004	15	—[b]
			0.5	2	7	14			0.1												
	2	2	0.5	1	7	14	23	46	0.1	177655	0.03	-47	-0.0001	0.433	1600	80000	0.009	1	0.004	15	—[b]
			0.5	2	16	32			0.1												
	3	2	0.5	1	53	106	108	216	0.1	177655	0.12	123	0.0001	0.433	1600	80000	0.009	1	0.004	15	—[b]
			0.5	2	55	110			0.1												
	4	2	0.5	1	13	26	37	74	0.1	177655	0.04	-19	0.0000	0.433	1600	80000	0.009	1	0.004	15	—[b]
			0.5	2	24	48			0.1												

Table A-12. Recombinant Assay with Ketoconazole Inhibitor, Day 3 (continued)

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover				Calculate final assay protein concentration								
Sample Type[a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq.	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (Initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Ketoconazole	1-1	2	0.5	1	31	62	58	116	0.1	177655	0.07	23	0.0000	0.433	1600	80000	0.009	1	0.004	15	0.0002
			0.5	2	27	54			0.1												
	1-2	2	0.5	1	25	50	52	104	0.1	177655	0.06	11	0.0000	0.433	1600	80000	0.009	1	0.004	15	0.0001
			0.5	2	27	54			0.1												
	1-3	2	0.5	1	26	52	55	110	0.1	177655	0.06	17	0.0000	0.433	1600	80000	0.009	1	0.004	15	0.0001
			0.5	2	29	58			0.1												
	2-1	2	0.5	1	730	1460	1473	2946	0.1	177655	1.66	2853	0.0032	0.433	1600	80000	0.009	1	0.004	15	0.0246
			0.5	2	743	1486			0.1												
	2-2	2	0.5	1	655	1310	1302	2604	0.1	177655	1.47	2511	0.0028	0.433	1600	80000	0.009	1	0.004	15	0.0217
			0.5	2	647	1294			0.1												
	2-3	2	0.5	1	607	1214	1193	2386	0.1	177655	1.34	2293	0.0026	0.433	1600	80000	0.009	1	0.004	15	0.0198
			0.5	2	586	1172			0.1												
	3-1	2	0.5	1	4036	8072	8001	16002	0.1	177655	9.01	15909	0.0178	0.433	1600	80000	0.009	1	0.004	15	0.1374
			0.5	2	3965	7930			0.1												
	3-2	2	0.5	1	3677	7354	7310	14620	0.1	177655	8.23	14527	0.0163	0.433	1600	80000	0.009	1	0.004	15	0.1254
			0.5	2	3633	7266			0.1												

Table A-12. Recombinant Assay with Ketoconazole Inhibitor, Day 3 (continued)

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover					Calculate final assay protein concentration							
Sample Type [a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (Initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Ketoconazole	3-3	2	0.5	1	3415	6830	6911	13822	0.1	177655	7.78	13729	0.0154	0.433	1600	80000	0.009	1	0.004	15	0.1185
			0.5	2	3496	6992			0.1												
	4-1	2	0.5	1	5407	10814	10779	21558	0.1	177655	12.13	21465	0.0241	0.433	1600	80000	0.009	1	0.004	15	0.1853
			0.5	2	5372	10744			0.1												
	4-2	2	0.5	1	5087	10174	10139	20278	0.1	177655	11.41	20185	0.0226	0.433	1600	80000	0.009	1	0.004	15	0.1743
			0.5	2	5052	10104			0.1												
	4-3	2	0.5	1	4687	9374	9331	18662	0.1	177655	10.50	18569	0.0208	0.433	1600	80000	0.009	1	0.004	15	0.1603
			0.5	2	4644	9288			0.1												
	5-1	2	0.5	1	6387	12774	12910	25820	0.1	177655	14.53	25727	0.0288	0.433	1600	80000	0.009	1	0.004	15	0.2221
			0.5	2	6523	13046			0.1												
	5-2	2	0.5	1	6067	12134	12114	24228	0.1	177655	13.64	24135	0.0271	0.433	1600	80000	0.009	1	0.004	15	0.2084
			0.5	2	6047	12094			0.1												
	5-3	2	0.5	1	5550	11100	11107	22214	0.1	177655	12.50	22121	0.0248	0.433	1600	80000	0.009	1	0.004	15	0.1910
			0.5	2	5557	11114			0.1												
	6-1	2	0.5	1	7375	14750	14688	29376	0.1	177655	16.54	29283	0.0328	0.433	1600	80000	0.009	1	0.004	15	0.2528
			0.5	2	7313	14626			0.1												

Table A-12. Recombinant Assay with Ketoconazole Inhibitor, Day 3 (continued)

Sample Info		Calculate DPM in aqueous portion after extraction							Calculate % turnover					Calculate final assay protein concentration							
Sample Type[a]	Replicate/Concentration Code	Nominal total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube	Total DPM in assay tube (initial)	% conversion to product	Total DPM corrected for background (Blank Tubes)	nmol ^3H_2O formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes usd in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Ketoconazole	6-2	2	0.5	1	7258	14516	14529	29058	0.1	177655	16.36	28965	0.0325	0.433	1600	80000	0.009	1	0.004	15	0.2501
			0.5	2	7271	14542			0.1												
	6-3	2	0.5	1	6743	13486	13403	26806	0.1	177655	15.09	26713	0.0300	0.433	1600	80000	0.009	1	0.004	15	0.2307
			0.5	2	6660	13320			0.1												
	7-1	2	0.5	1	8076	16152	16180	32360	0.1	177655	18.22	32267	0.0362	0.433	1600	80000	0.009	1	0.004	15	0.2786
			0.5	2	8104	16208			0.1												
	7-2	2	0.5	1	8335	16670	16567	33134	0.1	177655	18.65	33041	0.0370	0.433	1600	80000	0.009	1	0.004	15	0.2853
			0.5	2	8232	16464			0.1												
	7-3	2	0.5	1	7228	14456	14490	28980	0.1	177655	16.31	28887	0.0324	0.433	1600	80000	0.009	1	0.004	15	0.2494
			0.5	2	7262	14524			0.1												
	8-1	2	0.5	1	7877	15754	15680	31360	0.1	177655	17.65	31267	0.0351	0.433	1600	80000	0.009	1	0.004	15	0.2700
			0.5	2	7803	15606			0.1												
	8-2	2	0.5	1	8016	16032	16085	32170	0.1	177655	18.11	32077	0.0360	0.433	1600	80000	0.009	1	0.004	15	0.2770
			0.5	2	8069	16138			0.1												
	8-3	2	0.5	1	7410	14820	14983	29966	0.1	177655	16.87	29873	0.0335	0.433	1600	80000	0.009	1	0.004	15	0.2579
			0.5	2	7573	15146			0.1												

[a]Positive controls contain all assay components: microsomes, ASDN, NADPH, propylene glycol, buffer and inhibitor vehicle. Negative controls contain all assay components except NADPH. See Section 2.1.1 for further details.
[b]Negative control tubes serve as assay blanks; therefore, no activities are calculated.

APPENDIX B

Detailed Data Tables for RIA Assay

Appendix B

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Table B-1. Comparisons of RIA and Tritiated Water Assay Results–Ketoconazole–Day 1

Sample type	Replicate/ Level	Nominal total volume (mL)	RIA Ave pg/mL	RIA Total pg estrone	RIA total nmol estrone	^3H_2O assay nmol ^3H_2O formed	Fold difference (^3H_2O / RIA)
Positive control	1	2	1533.8	3067.6	0.0113	0.0424	3.73
	2	2	1400.6	2801.2	0.0104	0.0387	3.73
	3	2	1295.4	2590.8	0.0096	0.0387	4.04
	4	2	1394.9	2789.8	0.0103	0.0399	3.86
Negative control	1	2	< LOQ				
	2	2	< LOQ				
	3	2	< LOQ				
	4	2	< LOQ				
Ketoconazole	1-1	2	65.97	131.94	0.0005	0.0002	0.34
	1-2	2	77.49	154.98	0.0006	0.0001	0.12
	1-3	2	79.58	159.16	0.0006	0.0001	0.20
	2-1	2	253.11	506.22	0.0019	0.0042	2.25
	2-2	2	236.99	473.98	0.0018	0.0042	2.39
	2-3	2	212.83	425.66	0.0016	0.0035	2.21
	3-1	2	1133.3	2266.6	0.0084	0.0215	2.57
	3-2	2	1153.4	2306.8	0.0085	0.0211	2.47
	3-3	2	1014.4	2028.8	0.0075	0.0190	2.53
	4-1	2	956.58	1913.16	0.0071	0.0290	4.10
	4-2	2	943.43	1886.86	0.0070	0.0275	3.94
	4-3	2	791.47	1582.94	0.0059	0.0262	4.48
	5-1	2	1048.5	2097	0.0078	0.0324	4.18
	5-2	2	1171.3	2342.6	0.0087	0.0326	3.76
	5-3	2	931.6	1863.2	0.0069	0.0308	4.47
	6-1	2	1388.2	2776.4	0.0103	0.0398	3.87
	6-2	2	1246.2	2492.4	0.0092	0.0355	3.85
	6-3	2	1373.4	2746.8	0.0102	0.0251	2.47
	7-1	2	583.16	1166.32	0.0043	0.0402	9.33
	7-2	2	1033.4	2066.8	0.0076	0.0391	5.11
	7-3	2	1208.6	2417.2	0.0089	0.0375	4.20
	8-1	2	1394.2	2788.4	0.0103	0.0430	4.17
	8-2	2	1495.7	2991.4	0.0111	0.0415	3.75
	8-3	2	1197.2	2394.4	0.0089	0.0384	4.33

Table B-2. Comparisons of RIA and Tritiated Water Assay Results–Ketoconazole–Day 2

Sample type	Replicate/Level	Nominal total volume (mL)	RIA Ave pg/mL	RIA Total pg estrone	RIA total nmol estrone	^3H_2O assay nmol ^3H_2O formed	Fold difference (^3H_2O / RIA)
Positive control	1	2	1538.7	3077.4	0.0114	0.0437	3.84
	2	2	179.56	359.12	0.0013	0.0072	5.42
	3	2	1326.2	2652.4	0.0098	0.0374	3.81
	4	2	1419.1	2838.2	0.0105	0.0390	3.71
Negative control	1	2	< LOQ				
	2	2	< LOQ				
	3	2	< LOQ				
	4	2	< LOQ				
Ketoconazole	1-1	2	80.51	161.02	0.0006	0.0001	0.15
	1-2	2	75.57	151.14	0.0006	0.0001	0.17
	1-3	2	84.18	168.36	0.0006	0.0001	0.21
	2-1	2	213.95	427.9	0.0016	0.0036	2.27
	2-2	2	233.46	466.92	0.0017	0.0039	2.24
	2-3	2	213.25	426.5	0.0016	0.0034	2.15
	3-1	2	986.81	1973.62	0.0073	0.0200	2.73
	3-2	2	970.23	1940.46	0.0072	0.0193	2.69
	3-3	2	901.91	1803.82	0.0067	0.0170	2.55
	4-1	2	853.72	1707.44	0.0063	0.0267	4.22
	4-2	2	828.55	1657.1	0.0061	0.0263	4.28
	4-3	2	742.73	1485.46	0.0055	0.0240	4.37
	5-1	2	1046.2	2092.4	0.0077	0.0324	4.19
	5-2	2	987.19	1974.38	0.0073	0.0304	4.16
	5-3	2	991.45	1982.9	0.0073	0.0292	3.98
	6-1	2	1235	2470	0.0091	0.0372	4.08
	6-2	2	1266.2	2532.4	0.0094	0.0354	3.78
	6-3	2	1295.3	2590.6	0.0096	0.0328	3.43
	7-1	2	1438.5	2877	0.0106	0.0411	3.86
	7-2	2	1397	2794	0.0103	0.0407	3.94
	7-3	2	1553.2	3106.4	0.0115	0.0389	3.39
	8-1	2	1430.3	2860.6	0.0106	0.0410	3.87
	8-2	2	1411.9	2823.8	0.0104	0.0409	3.92
	8-3	2	1512.4	3024.8	0.0112	0.0383	3.42

Table B-3. Comparisons of RIA and Tritiated Water Assay Results–Ketoconazole–Day 3

Sample type	Replicate/ Level	Nominal total volume (mL)	RIA Ave pg/mL	RIA Total pg estrone	RIA total nmol estrone	^3H_2O assay nmol ^3H_2O formed	Fold difference (^3H_2O / RIA)
Positive control	1	2	1272.4	2544.8	0.0094	0.0392	4.17
	2	2	1145.1	2290.2	0.0085	0.0364	4.29
	3	2	1279.6	2559.2	0.0095	0.0340	3.60
	4	2	1304.2	2608.4	0.0096	0.0340	3.52
Negative control	1	2	< LOQ				
	2	2	< LOQ				
	3	2	< LOQ				
	4	2	< LOQ				
Ketoconazole	1-1	2	56.44	112.88	0.0004	0.0000	0.06
	1-2	2	69.82	139.64	0.0005	0.0000	0.02
	1-3	2	59.83	119.66	0.0004	0.0000	0.04
	2-1	2	193.17	386.34	0.0014	0.0032	2.24
	2-2	2	177.48	354.96	0.0013	0.0028	2.14
	2-3	2	160.58	321.16	0.0012	0.0026	2.16
	3-1	2	934.02	1868.04	0.0069	0.0178	2.58
	3-2	2	824.78	1649.56	0.0061	0.0163	2.67
	3-3	2	835	1670	0.0062	0.0154	2.49
	4-1	2	890.08	1780.16	0.0066	0.0241	3.66
	4-2	2	762.51	1525.02	0.0056	0.0226	4.01
	4-3	2	782.19	1564.38	0.0058	0.0208	3.60
	5-1	2	1001.5	2003	0.0074	0.0288	3.89
	5-2	2	966.34	1932.68	0.0071	0.0271	3.79
	5-3	2	880.26	1760.52	0.0065	0.0248	3.81
	6-1	2	1227.8	2455.6	0.0091	0.0328	3.62
	6-2	2	1185.1	2370.2	0.0088	0.0325	3.70
	6-3	2	1008.4	2016.8	0.0075	0.0300	4.02
	7-1	2	1267.9	2535.8	0.0094	0.0362	3.86
	7-2	2	1356.2	2712.4	0.0100	0.0370	3.69
	7-3	2	1137.9	2275.8	0.0084	0.0324	3.85
	8-1	2	1277.3	2554.6	0.0094	0.0351	3.71
	8-2	2	1303.4	2606.8	0.0096	0.0360	3.73
	8-3	2	1142.6	2285.2	0.0085	0.0335	3.96

Table B-4. Comparisons of RIA and Tritiated Water Assay Results–Econazole–Day 1

Sample type	Replicate /Level	Nominal total volume (mL)	RIA Ave pg/mL	RIA Total pg estrone	RIA total nmol estrone	^3H_2O assay nmol ^3H_2O formed	Fold difference (^3H_2O / RIA)
Positive control	1	2	1467.9	2935.8	0.0109	0.0420	3.86
	2	2	1376.9	2753.8	0.0102	0.0392	3.85
	3	2	1370.1	2740.2	0.0101	0.0379	3.74
	4	2	1443.4	2886.8	0.0107	0.0381	3.57
Negative control	1	2	< LOQ				
	2	2	< LOQ				
	3	2	< LOQ				
	4	2	< LOQ				
Econazole	1-1	2	24.23	48.46	0.0002	0.0006	3.12
	1-2	2	32.89	65.78	0.0002	0.0006	2.48
	1-3	2	27.9	55.8	0.0002	0.0006	2.98
	2-1	2	268.91	537.82	0.0020	0.0051	2.59
	2-2	2	280.64	561.28	0.0021	0.0052	2.49
	2-3	2	261.42	522.84	0.0019	0.0052	2.68
	3-1	2	775.55	1551.1	0.0057	0.0150	2.61
	3-2	2	791.79	1583.58	0.0059	0.0150	2.57
	3-3	2	760.92	1521.84	0.0056	0.0143	2.54
	4-1	2	809.25	1618.5	0.0060	0.0241	4.03
	4-2	2	889.37	1778.74	0.0066	0.0234	3.55
	4-3	2	879.84	1759.68	0.0065	0.0228	3.50
	5-1	2	1197.1	2394.2	0.0089	0.0328	3.70
	5-2	2	1134.6	2269.2	0.0084	0.0298	3.55
	5-3	2	1217.3	2434.6	0.0090	0.0301	3.34
	6-1	2	1338.7	2677.4	0.0099	0.0342	3.46
	6-2	2	1299.9	2599.8	0.0096	0.0332	3.45
	6-3	2	1341.9	2683.8	0.0099	0.0325	3.27
	7-1	2	1390.5	2781	0.0103	0.0357	3.47
	7-2	2	1283.4	2566.8	0.0095	0.0333	3.50
	7-3	2	1323.9	2647.8	0.0098	0.0319	3.25
	8-1	2	1347.5	2695	0.0100	0.0353	3.55
	8-2	2	1349.2	2698.4	0.0100	0.0329	3.30
	8-3	2	1343.9	2687.8	0.0099	0.0332	3.34

Table B-5. Comparisons of RIA and Tritiated Water Assay Results–Econazole–Day 2

Sample type	Replicate /Level	Nominal total volume (mL)	RIA Ave pg/mL	RIA Total pg estrone	RIA total nmol estrone	^3H_2O assay nmol ^3H_2O formed	Fold difference (^3H_2O / RIA)
Positive control	1	2	1528.9	3057.8	0.0113	0.0383	3.39
	2	2	1468.2	2936.4	0.0109	0.0369	3.40
	3	2	1436.1	2872.2	0.0106	0.0361	3.40
	4	2	1313.3	2626.6	0.0097	0.0343	3.53
Negative control	1	2	< LOQ				
	2	2	< LOQ				
	3	2	< LOQ				
	4	2	< LOQ				
Econazole	1-1	2	26.94	53.88	0.0002	0.0006	2.80
	1-2	2	26.11	52.22	0.0002	0.0006	3.09
	1-3	2	24.08	48.16	0.0002	0.0006	3.27
	2-1	2	280.23	560.46	0.0021	0.0049	2.38
	2-2	2	259.05	518.1	0.0019	0.0047	2.43
	2-3	2	255.56	511.12	0.0019	0.0046	2.45
	3-1	2	809.63	1619.26	0.0060	0.0154	2.58
	3-2	2	793.97	1587.94	0.0059	0.0152	2.58
	3-3	2	801.51	1603.02	0.0059	0.0148	2.50
	4-1	2	919.49	1838.98	0.0068	0.0246	3.62
	4-2	2	887.11	1774.22	0.0066	0.0240	3.65
	4-3	2	879.9	1759.8	0.0065	0.0231	3.55
	5-1	2	1307.5	2615	0.0097	0.0333	3.44
	5-2	2	1201.7	2403.4	0.0089	0.0326	3.67
	5-3	2	1277.7	2555.4	0.0095	0.0315	3.33
	6-1	2	1330.6	2661.2	0.0098	0.0351	3.57
	6-2	2	1309	2618	0.0097	0.0335	3.46
	6-3	2	1400.6	2801.2	0.0104	0.0348	3.36
	7-1	2	1425.2	2850.4	0.0105	0.0369	3.50
	7-2	2	1335.9	2671.8	0.0099	0.0355	3.59
	7-3	2	1379.7	2759.4	0.0102	0.0345	3.38
	8-1	2	1393	2786	0.0103	0.0360	3.50
	8-2	2	1414.1	2828.2	0.0105	0.0349	3.34
	8-3	2	1401	2802	0.0104	0.0346	3.34

Table B-6. Comparisons of RIA and Tritiated Water Assay Results–Econazole–Day 3

Sample type	Replicate/ Level	Nominal total volume (mL)	RIA Ave pg/mL	RIA Total pg estrone	RIA total nmol estrone	^3H_2O assay nmol ^3H_2O formed	Fold difference (^3H_2O / RIA)
Positive control	1	2	1387.5	2775	0.0103	0.0356	3.47
	2	2	1384.9	2769.8	0.0102	0.0325	3.17
	3	2	1468.6	2937.2	0.0109	0.0329	3.03
	4	2	1277.4	2554.8	0.0094	0.0321	3.40
Negative control	1	2	< LOQ				
	2	2	< LOQ				
	3	2	< LOQ				
	4	2	< LOQ				
Econazole	1-1	2	22.11	44.22	0.0002	0.0005	3.29
	1-2	2	25.29	50.58	0.0002	0.0005	2.71
	1-3	2	25.67	51.34	0.0002	0.0006	3.07
	2-1	2	251.84	503.68	0.0019	0.0046	2.45
	2-2	2	257.71	515.42	0.0019	0.0045	2.38
	2-3	2	238.92	477.84	0.0018	0.0043	2.44
	3-1	2	810.8	1621.6	0.0060	0.0140	2.34
	3-2	2	790.02	1580.04	0.0058	0.0139	2.37
	3-3	2	772.52	1545.04	0.0057	0.0139	2.43
	4-1	2	849.3	1698.6	0.0063	0.0214	3.41
	4-2	2	876.36	1752.72	0.0065	0.0224	3.45
	4-3	2	849.57	1699.14	0.0063	0.0215	3.43
	5-1	2	1412.7	2825.4	0.0105	0.0321	3.07
	5-2	2	1205.3	2410.6	0.0089	0.0299	3.36
	5-3	2	1207.7	2415.4	0.0089	0.0285	3.19
	6-1	2	1499.1	2998.2	0.0111	0.0311	2.80
	6-2	2	1379	2758	0.0102	0.0321	3.14
	6-3	2	1272.4	2544.8	0.0094	0.0305	3.24
	7-1	2	1487.2	2974.4	0.0110	0.0336	3.06
	7-2	2	1292.7	2585.4	0.0096	0.0324	3.39
	7-3	2	1340.6	2681.2	0.0099	0.0332	3.35
	8-1	2	1445.4	2890.8	0.0107	0.0340	3.18
	8-2	2	1303.6	2607.2	0.0096	0.0316	3.28
	8-3	2	1291.5	2583	0.0096	0.0299	3.13

Table B-7. RIA-Based Aromatase Activity–Econazole Inhibitor–Day 1

Sample Info			Calculate final assay protein concentration							
Sample Type	Replicate/Level	nmol estrone formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes used in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Positive Control	1	0.0109	0.435	1600	80000	0.009	1	0.004	15	0.0832
	2	0.0102	0.435	1600	80000	0.009	1	0.004	15	0.0781
	3	0.0101	0.435	1600	80000	0.009	1	0.004	15	0.0777
	4	0.0107	0.435	1600	80000	0.009	1	0.004	15	0.0818
Negative Control	1	< LOQ	0.435	1600	80000	0.009	1	0.004	15	
	2	< LOQ	0.435	1600	80000	0.009	1	0.004	15	
	3	< LOQ	0.435	1600	80000	0.009	1	0.004	15	
	4	< LOQ	0.435	1600	80000	0.009	1	0.004	15	
Econazole	1-1	0.0002	0.435	1600	80000	0.009	1	0.004	15	0.0014
	1-2	0.0002	0.435	1600	80000	0.009	1	0.004	15	0.0019
	1-3	0.0002	0.435	1600	80000	0.009	1	0.004	15	0.0016
	2-1	0.0020	0.435	1600	80000	0.009	1	0.004	15	0.0152
	2-2	0.0021	0.435	1600	80000	0.009	1	0.004	15	0.0159
	2-3	0.0019	0.435	1600	80000	0.009	1	0.004	15	0.0148
	3-1	0.0057	0.435	1600	80000	0.009	1	0.004	15	0.0440
	3-2	0.0059	0.435	1600	80000	0.009	1	0.004	15	0.0449
	3-3	0.0056	0.435	1600	80000	0.009	1	0.004	15	0.0431
	4-1	0.0060	0.435	1600	80000	0.009	1	0.004	15	0.0459
	4-2	0.0066	0.435	1600	80000	0.009	1	0.004	15	0.0504
	4-3	0.0065	0.435	1600	80000	0.009	1	0.004	15	0.0499
	5-1	0.0089	0.435	1600	80000	0.009	1	0.004	15	0.0679
	5-2	0.0084	0.435	1600	80000	0.009	1	0.004	15	0.0643

Table B-7. RIA-Based Aromatase Activity–Econazole Inhibitor–Day 1 (continued)

Sample Info			Calculate final assay protein concentration							
Sample Type	Replicate/Level	nmol estrone formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes used in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Econazole	5-3	0.0090	0.435	1600	80000	0.009	1	0.004	15	0.0690
	6-1	0.0099	0.435	1600	80000	0.009	1	0.004	15	0.0759
	6-2	0.0096	0.435	1600	80000	0.009	1	0.004	15	0.0737
	6-3	0.0099	0.435	1600	80000	0.009	1	0.004	15	0.0761
	7-1	0.0103	0.435	1600	80000	0.009	1	0.004	15	0.0788
	7-2	0.0095	0.435	1600	80000	0.009	1	0.004	15	0.0728
	7-3	0.0098	0.435	1600	80000	0.009	1	0.004	15	0.0750
	8-1	0.0100	0.435	1600	80000	0.009	1	0.004	15	0.0764
	8-2	0.0100	0.435	1600	80000	0.009	1	0.004	15	0.0765
	8-3	0.0099	0.435	1600	80000	0.009	1	0.004	15	0.0762

Table B-8. RIA-Based Aromatase Activity–Econazole Inhibitor–Day 2

Sample Info			Calculate final assay protein concentration							
Sample Type	Replicate/Level	nmol estrone formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes used in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	Volume diluted microsomes used in assay tube (mL)	Final [protein] in assay (mg/mL)	Incubation time (min)	
Positive Control	1	0.0113	0.527	1600	80000	0.011	1	0.005	15	0.0716
	2	0.0109	0.527	1600	80000	0.011	1	0.005	15	0.0687
	3	0.0106	0.527	1600	80000	0.011	1	0.005	15	0.0672
	4	0.0097	0.527	1600	80000	0.011	1	0.005	15	0.0615
Negative Control	1	< LOQ	0.527	1600	80000	0.011	1	0.005	15	
	2	< LOQ	0.527	1600	80000	0.011	1	0.005	15	
	3	< LOQ	0.527	1600	80000	0.011	1	0.005	15	
	4	< LOQ	0.527	1600	80000	0.011	1	0.005	15	
Econazole	1-1	0.0002	0.527	1600	80000	0.011	1	0.005	15	0.0013
	1-2	0.0002	0.527	1600	80000	0.011	1	0.005	15	0.0012
	1-3	0.0002	0.527	1600	80000	0.011	1	0.005	15	0.0011
	2-1	0.0021	0.527	1600	80000	0.011	1	0.005	15	0.0131
	2-2	0.0019	0.527	1600	80000	0.011	1	0.005	15	0.0121
	2-3	0.0019	0.527	1600	80000	0.011	1	0.005	15	0.0120
	3-1	0.0060	0.527	1600	80000	0.011	1	0.005	15	0.0379
	3-2	0.0059	0.527	1600	80000	0.011	1	0.005	15	0.0372
	3-3	0.0059	0.527	1600	80000	0.011	1	0.005	15	0.0375
	4-1	0.0068	0.527	1600	80000	0.011	1	0.005	15	0.0430
	4-2	0.0066	0.527	1600	80000	0.011	1	0.005	15	0.0415
	4-3	0.0065	0.527	1600	80000	0.011	1	0.005	15	0.0412
	5-1	0.0097	0.527	1600	80000	0.011	1	0.005	15	0.0612

Table B-8. RIA-Based Aromatase Activity–Econazole Inhibitor–Day 2 (continued)

Sample Info			Calculate final assay protein concentration							
Sample Type	Replicate/Level	nmol estrone formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes used in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Econazole	5-2	0.0089	0.527	1600	80000	0.011	1	0.005	15	0.0562
	5-3	0.0095	0.527	1600	80000	0.011	1	0.005	15	0.0598
	6-1	0.0098	0.527	1600	80000	0.011	1	0.005	15	0.0623
	6-2	0.0097	0.527	1600	80000	0.011	1	0.005	15	0.0613
	6-3	0.0104	0.527	1600	80000	0.011	1	0.005	15	0.0656
	7-1	0.0105	0.527	1600	80000	0.011	1	0.005	15	0.0667
	7-2	0.0099	0.527	1600	80000	0.011	1	0.005	15	0.0625
	7-3	0.0102	0.527	1600	80000	0.011	1	0.005	15	0.0646
	8-1	0.0103	0.527	1600	80000	0.011	1	0.005	15	0.0652
	8-2	0.0105	0.527	1600	80000	0.011	1	0.005	15	0.0662
	8-3	0.0104	0.527	1600	80000	0.011	1	0.005	15	0.0656

Table B-9. RIA-Based Aromatase Activity - Econazole Inhibitor–Day 3

Sample Info			Calculate final assay protein concentration							
Sample Type	Replicate/Level	nmol estrone formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes used in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Positive Control	1	0.0103	0.433	1600	80000	0.009	1	0.004	15	0.0790
	2	0.0102	0.433	1600	80000	0.009	1	0.004	15	0.0789
	3	0.0109	0.433	1600	80000	0.009	1	0.004	15	0.0837
	4	0.0094	0.433	1600	80000	0.009	1	0.004	15	0.0728
Negative Control	1	< LOQ	0.433	1600	80000	0.009	1	0.004	15	
	2	< LOQ	0.433	1600	80000	0.009	1	0.004	15	
	3	< LOQ	0.433	1600	80000	0.009	1	0.004	15	
	4	< LOQ	0.433	1600	80000	0.009	1	0.004	15	
Econazole	1-1	0.0002	0.433	1600	80000	0.009	1	0.004	15	0.0013
	1-2	0.0002	0.433	1600	80000	0.009	1	0.004	15	0.0014
	1-3	0.0002	0.433	1600	80000	0.009	1	0.004	15	0.0015
	2-1	0.0019	0.433	1600	80000	0.009	1	0.004	15	0.0143
	2-2	0.0019	0.433	1600	80000	0.009	1	0.004	15	0.0147
	2-3	0.0018	0.433	1600	80000	0.009	1	0.004	15	0.0136
	3-1	0.0060	0.433	1600	80000	0.009	1	0.004	15	0.0462
	3-2	0.0058	0.433	1600	80000	0.009	1	0.004	15	0.0450
	3-3	0.0057	0.433	1600	80000	0.009	1	0.004	15	0.0440
	4-1	0.0063	0.433	1600	80000	0.009	1	0.004	15	0.0484
	4-2	0.0065	0.433	1600	80000	0.009	1	0.004	15	0.0499
	4-3	0.0063	0.433	1600	80000	0.009	1	0.004	15	0.0484
	5-1	0.0105	0.433	1600	80000	0.009	1	0.004	15	0.0805

Table B-9. RIA-Based Aromatase Activity–Econazole Inhibitor–Day 3 (continued)

Sample Info			Calculate final assay protein concentration							
Sample Type	Replicate/Level	nmol estrone formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes used in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Econazole	5-2	0.0089	0.433	1600	80000	0.009	1	0.004	15	0.0687
	5-3	0.0089	0.433	1600	80000	0.009	1	0.004	15	0.0688
	6-1	0.0111	0.433	1600	80000	0.009	1	0.004	15	0.0854
	6-2	0.0102	0.433	1600	80000	0.009	1	0.004	15	0.0786
	6-3	0.0094	0.433	1600	80000	0.009	1	0.004	15	0.0725
	7-1	0.0110	0.433	1600	80000	0.009	1	0.004	15	0.0847
	7-2	0.0096	0.433	1600	80000	0.009	1	0.004	15	0.0736
	7-3	0.0099	0.433	1600	80000	0.009	1	0.004	15	0.0764
	8-1	0.0107	0.433	1600	80000	0.009	1	0.004	15	0.0823
	8-2	0.0096	0.433	1600	80000	0.009	1	0.004	15	0.0743
	8-3	0.0096	0.433	1600	80000	0.009	1	0.004	15	0.0736

Table B-10. RIA-Based Aromatase Activity–Ketoconazole Inhibitor–Day 1

Sample Info			Calculate final assay protein concentration								
Sample Type	Replicate/Level	nmol estrone formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes used in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Positive Control	1	0.0113	0.435	1600	80000	0.009	1	0.004	15	0.0869
	2	0.0104	0.435	1600	80000	0.009	1	0.004	15	0.0794
	3	0.0096	0.435	1600	80000	0.009	1	0.004	15	0.0734
	4	0.0103	0.435	1600	80000	0.009	1	0.004	15	0.0791
Negative Control	1	< LOQ	0.435	1600	80000	0.009	1	0.004	15	
	2	< LOQ	0.435	1600	80000	0.009	1	0.004	15	
	3	< LOQ	0.435	1600	80000	0.009	1	0.004	15	
	4	< LOQ	0.435	1600	80000	0.009	1	0.004	15	
Ketoconazole	1-1	0.0005	0.435	1600	80000	0.009	1	0.004	15	0.0037
	1-2	0.0006	0.435	1600	80000	0.009	1	0.004	15	0.0044
	1-3	0.0006	0.435	1600	80000	0.009	1	0.004	15	0.0045
	2-1	0.0019	0.435	1600	80000	0.009	1	0.004	15	0.0143
	2-2	0.0018	0.435	1600	80000	0.009	1	0.004	15	0.0134
	2-3	0.0016	0.435	1600	80000	0.009	1	0.004	15	0.0121
	3-1	0.0084	0.435	1600	80000	0.009	1	0.004	15	0.0642
	3-2	0.0085	0.435	1600	80000	0.009	1	0.004	15	0.0654
	3-3	0.0075	0.435	1600	80000	0.009	1	0.004	15	0.0575
	4-1	0.0071	0.435	1600	80000	0.009	1	0.004	15	0.0542
	4-2	0.0070	0.435	1600	80000	0.009	1	0.004	15	0.0535
	4-3	0.0059	0.435	1600	80000	0.009	1	0.004	15	0.0449

Table B-10. RIA-Based Aromatase Activity–Ketoconazole Inhibitor–Day 1 (continued)

Sample Info		Calculate final assay protein concentration								
Sample Type	Replicate/Level	nmol estrone formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes used in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Ketoconazole	5-1	0.0078	0.435	1600	80000	0.009	1	0.004	15	0.0594
	5-2	0.0087	0.435	1600	80000	0.009	1	0.004	15	0.0664
	5-3	0.0069	0.435	1600	80000	0.009	1	0.004	15	0.0528
	6-1	0.0103	0.435	1600	80000	0.009	1	0.004	15	0.0787
	6-2	0.0092	0.435	1600	80000	0.009	1	0.004	15	0.0706
	6-3	0.0102	0.435	1600	80000	0.009	1	0.004	15	0.0779
	7-1	0.0043	0.435	1600	80000	0.009	1	0.004	15	0.0331
	7-2	0.0076	0.435	1600	80000	0.009	1	0.004	15	0.0586
	7-3	0.0089	0.435	1600	80000	0.009	1	0.004	15	0.0685
	8-1	0.0103	0.435	1600	80000	0.009	1	0.004	15	0.0790
	8-2	0.0111	0.435	1600	80000	0.009	1	0.004	15	0.0848
	8-3	0.0089	0.435	1600	80000	0.009	1	0.004	15	0.0679

Table B-11. RIA-Based Aromatase Activity–Ketoconazole Inhibitor– Day 2

Sample Info			Calculate final assay protein concentration							
Sample Type	Replicate/Level	nmol estrone formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes used in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Positive Control	1	0.0114	0.527	1600	80000	0.011	1	0.005	15	0.0720
	2	0.0013	0.527	1600	80000	0.011	1	0.005	15	0.0084
	3	0.0098	0.527	1600	80000	0.011	1	0.005	15	0.0621
	4	0.0105	0.527	1600	80000	0.011	1	0.005	15	0.0664
Negative Control	1	< LOQ	0.527	1600	80000	0.011	1	0.005	15	
	2	< LOQ	0.527	1600	80000	0.011	1	0.005	15	
	3	< LOQ	0.527	1600	80000	0.011	1	0.005	15	
	4	< LOQ	0.527	1600	80000	0.011	1	0.005	15	
Ketoconazole	1-1	0.0006	0.527	1600	80000	0.011	1	0.005	15	0.0038
	1-2	0.0006	0.527	1600	80000	0.011	1	0.005	15	0.0035
	1-3	0.0006	0.527	1600	80000	0.011	1	0.005	15	0.0039
	2-1	0.0016	0.527	1600	80000	0.011	1	0.005	15	0.0100
	2-2	0.0017	0.527	1600	80000	0.011	1	0.005	15	0.0109
	2-3	0.0016	0.527	1600	80000	0.011	1	0.005	15	0.0100
	3-1	0.0073	0.527	1600	80000	0.011	1	0.005	15	0.0462
	3-2	0.0072	0.527	1600	80000	0.011	1	0.005	15	0.0454
	3-3	0.0067	0.527	1600	80000	0.011	1	0.005	15	0.0422
	4-1	0.0063	0.527	1600	80000	0.011	1	0.005	15	0.0400
	4-2	0.0061	0.527	1600	80000	0.011	1	0.005	15	0.0388
	4-3	0.0055	0.527	1600	80000	0.011	1	0.005	15	0.0348

Table B-11. RIA-Based Aromatase Activity–Ketoconazole Inhibitor–Day 2 (continued)

Sample Info			Calculate final assay protein concentration							
Sample Type	Replicate/Level	nmol estrone formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes used in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Ketoconazole	5-1	0.0077	0.527	1600	80000	0.011	1	0.005	15	0.0490
	5-2	0.0073	0.527	1600	80000	0.011	1	0.005	15	0.0462
	5-3	0.0073	0.527	1600	80000	0.011	1	0.005	15	0.0464
	6-1	0.0091	0.527	1600	80000	0.011	1	0.005	15	0.0578
	6-2	0.0094	0.527	1600	80000	0.011	1	0.005	15	0.0593
	6-3	0.0096	0.527	1600	80000	0.011	1	0.005	15	0.0606
	7-1	0.0106	0.527	1600	80000	0.011	1	0.005	15	0.0673
	7-2	0.0103	0.527	1600	80000	0.011	1	0.005	15	0.0654
	7-3	0.0115	0.527	1600	80000	0.011	1	0.005	15	0.0727
	8-1	0.0106	0.527	1600	80000	0.011	1	0.005	15	0.0669
	8-2	0.0104	0.527	1600	80000	0.011	1	0.005	15	0.0661
	8-3	0.0112	0.527	1600	80000	0.011	1	0.005	15	0.0708

Table B-12. RIA-Based Aromatase Activity–Ketoconazole Inhibitor–Day 3

Sample Info		Calculate final assay protein concentration								
Sample Type	Replicate/Level	nmol estrone formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes used in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Positive Control	1	0.0094	0.433	1600	80000	0.009	1	0.004	15	0.0725
	2	0.0085	0.433	1600	80000	0.009	1	0.004	15	0.0652
	3	0.0095	0.433	1600	80000	0.009	1	0.004	15	0.0729
	4	0.0096	0.433	1600	80000	0.009	1	0.004	15	0.0743
Negative Control	1	< LOQ	0.433	1600	80000	0.009	1	0.004	15	
	2	< LOQ	0.433	1600	80000	0.009	1	0.004	15	
	3	< LOQ	0.433	1600	80000	0.009	1	0.004	15	
	4	< LOQ	0.433	1600	80000	0.009	1	0.004	15	
Ketoconazole	1-1	0.0004	0.433	1600	80000	0.009	1	0.004	15	0.0032
	1-2	0.0005	0.433	1600	80000	0.009	1	0.004	15	0.0040
	1-3	0.0004	0.433	1600	80000	0.009	1	0.004	15	0.0034
	2-1	0.0014	0.433	1600	80000	0.009	1	0.004	15	0.0110
	2-2	0.0013	0.433	1600	80000	0.009	1	0.004	15	0.0101
	2-3	0.0012	0.433	1600	80000	0.009	1	0.004	15	0.0091
	3-1	0.0069	0.433	1600	80000	0.009	1	0.004	15	0.0532
	3-2	0.0061	0.433	1600	80000	0.009	1	0.004	15	0.0470
	3-3	0.0062	0.433	1600	80000	0.009	1	0.004	15	0.0476
	4-1	0.0066	0.433	1600	80000	0.009	1	0.004	15	0.0507
	4-2	0.0056	0.433	1600	80000	0.009	1	0.004	15	0.0434
	4-3	0.0058	0.433	1600	80000	0.009	1	0.004	15	0.0446

Table B-12. RIA-Based Aromatase Activity–Ketoconazole Inhibitor–Day 3 (continued)

Sample Info			Calculate final assay protein concentration							
Sample Type	Replicate/Level	nmol estrone formed	[protein] stock microsomes (mg/mL)	Volume of stock microsomes used in dilution (µL)	Final volume of diluted microsomes (µL)	[protein] in dilution (mg/mL)	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)
Ketoconazole	5-1	0.0074	0.433	1600	80000	0.009	1	0.004	15	0.0571
	5-2	0.0071	0.433	1600	80000	0.009	1	0.004	15	0.0550
	5-3	0.0065	0.433	1600	80000	0.009	1	0.004	15	0.0501
	6-1	0.0091	0.433	1600	80000	0.009	1	0.004	15	0.0699
	6-2	0.0088	0.433	1600	80000	0.009	1	0.004	15	0.0675
	6-3	0.0075	0.433	1600	80000	0.009	1	0.004	15	0.0574
	7-1	0.0094	0.433	1600	80000	0.009	1	0.004	15	0.0722
	7-2	0.0100	0.433	1600	80000	0.009	1	0.004	15	0.0773
	7-3	0.0084	0.433	1600	80000	0.009	1	0.004	15	0.0648
	8-1	0.0094	0.433	1600	80000	0.009	1	0.004	15	0.0728
	8-2	0.0096	0.433	1600	80000	0.009	1	0.004	15	0.0742
	8-3	0.0085	0.433	1600	80000	0.009	1	0.004	15	0.0651