The following report presents results of a study conducted by a contract laboratory for the National Toxicology Program (NTP). The report may not have been peer reviewed. The findings and conclusions for this study should not be construed to represent the view of NTP or the U.S. Government.

CeeTox
In vitro models to predict toxicity

Human Recombinant Aromatase Assay:

Final Report

DATA REQUIREMENT(S): OPPTS 890.1200

AUTHOR(S): [redacted]

STUDY COMPLETION DATE: 10 February 2012

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LABORATORY PROJECT ID:
Report Number: 9070-100107AROM
Study Number: 9070-100107AROM
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SPONSOR(S):
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STATEMENT OF DATA CONFIDENTIALITY CLAIMS

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Study Number: 9070-100107AROM

Study Title: Human Recombinant Aromatase Assay

I, the undersigned, hereby declare that this study was conducted in compliance EPA GLP regulations (Title 40 Part 160) with the exception of section 160.113. Dose concentrations of test substance and control substances were not verified using analytical methods.

The study was conducted according to the procedures herein described and this report represents a true and accurate record of the results obtained. There were no deviations that impacted the quality or integrity of the study data. Any deviations that occurred during the course of the study will be noted in this report, with the full write-ups included in the study binder.

[redacted]

10 FEBRUARY 2012
Date

Study Director
CeeTox, Inc.

[redacted]

Date

Study Toxicologist
Investigative Toxicology Division
Integrated Laboratory Systems, Inc

FLAGGING STATEMENT

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QUALITY ASSURANCE STATEMENT

Study Title: Human Recombinant Aromatase Assay

Study Number: 9070-100107AROM

In accordance with CeeTox, Inc.'s policies and Quality Assurance standard operating procedures for Good Laboratory Practice (GLP), the conduct of this study has been audited as follows:

Date(s) of Inspection/Audit	Inspection/Audit	Date(s) reported to Study Director	Date(s) reported to Management
27 June 2011	Study Protocol	27 June 2011	27 June 2011
29 June 2011	In-Process	18 July 2011	18 July 2011
09 December 2011	Study Databook	09 December 2011	09 December 2011
07 February 2012	Draft Report	07 February 2012	07 February 2012

The signature below indicates the summary table is an accurate representation of Quality Assurance's involvement with this study.

[redacted]

10 Feb 2012
Date

Quality Assurance Auditor
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GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Name	Title
[redacted]	Study Director
	Director of Project Management
	Director of Laboratory Operations
	Senior Scientist/Endocrine Group Leader
	Scientist
	Scientist

Study Dates

Study initiation date: 24 June 2011
Experimental start date: 28 June 2011
Experimental termination date: 27 July 2011
Study completion date: 10 February 2012

Deviations from the Protocol

See Appendix 2. There were three deviations however they did not impact the integrity of the data in this report.

Retention of Samples

Test substances will be either returned to the Sponsor or destroyed following finalization of the study report.

Test Substance Reference Number

- 2-ethylhexyl p-methoxycinnamate, lot A0293319 (Referred to as Methoxycinnamate)
- 2-ethylhexyl 2-cyano-3,3-diphenylacrylate, lot 01697MJ (Referred to as Octocrylene)
- Octyl salicylate, lot 44698PJ (Referred to as Octylsalicylate)
- 2-hydroxy-4-methoxybenzophenone, lot 20100801 (Referred to as Oxybenzone)

Data Retention and Archiving

At the study closure, all study records including all original raw data and original final report, will be shipped to the sponsor at the following address:

NTP Archives
[redacted]
615 Davis Drive, Suite 300
Durham, NC 27713

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1.0 EXECUTIVE SUMMARY

1.1 Study Design

The objective of this study was to evaluate the ability of four test substances to act as inhibitors of aromatase activity using human CYP19 (aromatase) and P450 reductase Supersomes ™ purchased from Gentest ™ as the test system. The substrate for the assay is androstenedione (ASDN), which is converted by aromatase to estrone.

Final concentrations of each test substance tested in the aromatase assay were 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, $10^{-4.5}$, 10^{-4}, and 10^{-3} M. Test substances were:

- 2-ethylhexyl p-methoxycinnamate, lot A0293319 (Referred to as Methoxycinnamate)
- 2-ethylhexyl 2-cyano-3,3-diphenylacrylate, lot 01697MJ (Referred to as Octocrylene)
- Octyl salicylate, lot 44698PJ (Referred to as Octylsalicylate)
- 2-hydroxy-4-methoxybenzophenone, lot 20100801 (Referred to as Oxybenzone)

Three independent runs of the aromatase assay were conducted. In each independent run, each concentration of test substance was tested in triplicate. In addition, the positive control inhibitor 4-hydroxyandrostenedione (4OH-ASDN) was included each time the aromatase assay was performed. Increasing concentrations of 4OH-ASDN decrease the aromatase activity in a concentration dependent manner. The OPPTS 890.1200 guideline outlines the preferred performance criteria for each run.

1.2 Results

In three independent runs of the assay, increasing concentrations of methoxycinnamate, octocrylene, and octylsalicylate showed negligible decreases in aromatase activity (all $\geq$90% of control values). Oxybenzone was 51% of control value at 10^{-4} M. All test substances were soluble in the assay buffer at concentrations of $\leq 10^{-4}$ M. Thus, the suitable top concentration for all test substances for use in the aromatase assay was established at 10^{-4} M.

1.3 Conclusion

The guidelines require that the mean aromatase enzyme activity level at the highest test concentration be used to determine whether the test substance is an inhibitor, non-inhibitor, or equivocal for activity at the aromatase enzyme. According to the data interpretation procedure outlined by the EPA for aromatase inhibition (Table 10, Section 3.10.5 Data Interpretation Criteria; OPPTS 890.1200), Methoxycinnamate, Octocrylene, and Octylsalicylate, were classified as non-inhibitors, with mean aromatase activities of 100% ($\pm$ 6% SD), 94% ($\pm$ 1% SD), and 90% ($\pm$ 2% SD), respectively, at the highest soluble test concentration of 10^{-4} M. Oxybenzone was classified as equivocal, as it produced a mean aromatase activity level of 51% control activity $\pm$ 13% SD at the highest soluble test concentration of 10^{-4} M.

2.0 INTRODUCTION

2.1 Purpose

The objective of this study was to evaluate the ability of four test substances to inhibit the catalytic activity of aromatase. This assay is a Tier 1 screening tool intended to identify test substances that may affect the endocrine system (e.g., steroidogenesis) by inhibiting catalytic activity of aromatase, the enzyme responsible for the conversion of androgens to estrogens.

The results of this study are intended to be used in conjunction with results from other Tier 1 screening studies (OPPTS 890 test guideline series) that constitute the full screening battery under the Endocrine Disruptor Screening Program (EDSP). Together, the results from the screening battery will be used by the US EPA to identify substances that have the potential to interact with the estrogen, androgen, or thyroid system. Results of the Tier 1 screening battery, along with other scientifically relevant information, are to be used in a weight-of-evidence determination of a substance's potential to interact with these systems. The fact that a substance may interact with a hormone system does not mean that when the substance is used, it will cause adverse effects in humans or ecological systems. The Tier 1 battery is intended for screening purposes only and should not be used for endocrine classification or risk assessment.

2.2 Regulatory Citations

OPPTS 890.1200: Endocrine Disruptor Screening Program, *in vitro* Aromatase (Human Recombinant).

3.0 MATERIALS AND METHODS

3.1 Test Substances

Table 1 (A-D) contains identity and property information provided by the Sponsor for four test substances:

- 2-ethylhexyl p-methoxycinnamate, lot A0293319 (Referred to as Methoxycinnamate)
- 2-ethylhexyl 2-cyano-3,3-diphenylacrylate, lot 01697MJ (Referred to as Octocrylene)
- Octyl salicylate, lot 44698PJ (Referred to as Octylsalicylate)
- 2-hydroxy-4-methoxybenzophenone, lot 20100801 (Referred to as Oxybenzone)

Table 1A. Test Substance 2-ethylhexyl p-methoxycinnamate, lot A0293319 (Referred to as Methoxycinnamate)

Test Substance Name:	**Methoxycinnamate** (octyl 4-methoxycinnamate; or 2-ethylhexyl p-methoxycinnamate)
Manufacturer:	Acros Organics (cat # 29116)
CAS Number:	5466-77-3
Description:	Clear colourless to yellow liquid
Solvent Used:	DMSO
Lot Number:	A0293319
Expiry Date:	Not provided
Purity:	99.8%
Molecular Formula:	$C_{18}H_{26}O_3$
Molecular Weight:	290.39
Storage Conditions:	Room temp (e.g., ambient)

Table 1B. Test Substance 2-ethylhexyl 2-cyano-3,3-diphenylacrylate, lot 01697MJ (Referred to as Octocrylene)

Test Substance Name:	**Octocrylene** (2-ethylhexyl 2-cyano-3,3-diphenylacrylate)
Manufacturer:	Sigma-Aldrich (cat # 415820)
CAS Number:	6197-30-4
Description:	Viscous yellow liquid
Solvent Used:	DMSO
Lot Number:	01697MJ
Expiry Date:	Not provided
Purity:	99.2%
Molecular Formula:	$C_{24}H_{27}NO_2$
Molecular Weight:	361.48
Storage Conditions:	Room temp (e.g., ambient)

Table 1C. Test Substance octyl salicylate, lot 44698PJ (Referred to as Octylsalicylate)

Test Substance Name:	**Octyl salicylate** (2-Ethylhexyl salicylate; or Octylsalicylate)
Manufacturer:	Sigma-Aldrich (cat # W514500)
CAS Number:	118-60-5
Description:	Colourless liquid
Solvent Used:	DMSO
Lot Number:	44698PJ
Expiry Date:	Not provided
Purity:	99.6%
Molecular Formula:	$C_{15}H_{22}O_3$
Molecular Weight:	250.33
Storage Conditions:	Room temp (e.g., ambient)

Table 1D. Test Substance 2-hydroxy-4-methoxybenzophenone, lot 20100801 (Referred to as Oxybenzone)

Test Substance Name:	**Oxybenzone** (2-hydroxy-4-methoxybenzophenone)
Manufacturer:	Ivy Fine Chemicals Corp. (cat # HH13-026)
CAS Number:	131-57-7
Description:	Light yellow powder
Solvent Used:	DMSO
Lot Number:	20100801
Expiry Date:	1 August 2012
Purity:	99.9%
Molecular Formula:	$C_{14}H_{12}O_3$
Molecular Weight:	228.25
Storage Conditions:	Room temp (e.g., ambient)

Note: A certificate of analysis was provided by the Sponsor, stored in the study data and appended to the study report (Appendix 3). Confirmation of the identity of the test chemical, characterization and stability were verified by the Sponsor. Test chemical will be either returned to the Sponsor or destroyed following finalization of the study report.

3.2 Positive Control

The known aromatase inhibitor, 4-hydroxyandrostendione (4OH-ASDN), was used as the positive control for aromatase inhibition. Table 2 contains identity and property information for 4OH-ASDN (Formestane).

Table 2. Positive Control Substance

Positive Control Name:	4OH-ASDN (Formestane)
Positive Control Manufacturer:	Sigma-Aldrich (cat # F2552)
CAS Number:	566-48-3
Description:	White powder, slightly crystalline
Solvent Used:	DMSO
Batch Number:	081K2133
Expiry Date:	March 2015
Purity:	99.6%
Molecular Formula	$C_{19}H_{26}O_3$
Molecular Weight:	302.41
Storage Conditions:	-4^{o}C

A certificate of analysis for 4OH-ASDN is stored in the study data binder and appended to the study report, (Appendix 3).

The 4OH-ASDN was formulated in 100% dimethylsulfoxide (DMSO; lot RNBB7617, expires 2/2013). Fresh dilutions of the stock solution were prepared on the day of use. Dilutions were prepared such that the target concentrations of control substance (Table 2) could be achieved by

the addition of 20 µL of the dilution to a 2 mL total assay volume with final DMSO concentrations ≤ 1%.

3.3 Aromatase Substrate

The substrate for the aromatase assay was androstenedione (4-Androstene-3,17-dione or ASDN). Non-radiolabeled and radiolabeled androstenedione ([1β-^{3}H]-androstenedione, [^{3}H] ASDN) were used. The non-radiolabeled ASDN was 99.8% pure. The radiolabeled [^{3}H] ASDN stock was >97% radiochemically pure and was supplied at a specific activity of 26.3 Ci/mmol.

Table 3. Non-radiolabeled Substrate

Substrate Name (Non-radiolabeled):	Androstenedione (4-Androstene-3,17-dione, or ASDN)
Substrate Manufacturer:	Steraloids, Inc. (cat # A6030-100)
CAS Number:	63-05-8
Description:	White powder, slightly crystalline
Solvent Used:	Ethanol
Batch Number:	L1712
Expiry Date:	April 2016
Purity:	99.8%
Molecular Formula	$C_{19}H_{26}O_2$
Molecular Weight:	286.41
Storage Conditions:	Room temp (e.g., ambient)

A certificate of analysis for ASDN is stored in the study data binder and appended to the study report, (Appendix 3).

Table 4. Radiolabeled Substrate

Substrate Name (Radiolabeled):	[1β-^{3}H]-Androstenedione, or [^{3}H] ASDN
Substrate Manufacturer:	Perkin Elmer (cat # NET-926)
CAS Number:	63-05-8
Description:	White powder, slightly crystalline
Solvent Used:	Ethanol
Batch Number:	619344
Expiry Date:	10 Jan 2012
Radiochemical Purity:	>97%
Molecular Formula	$C_{19}H_{26}O_2$
Molecular Weight:	286.41
Storage Conditions:	-80^{o}C
Specific Activity (Lot):	26.3 Ci/mmol
Specific Activity (Stock):	15-30 Ci/mmol

A certificate of analysis for [^{3}H]ASDN is stored in the study data binder and appended to the study report, (Appendix 3).

3.3.1 Radiochemical Purity and Preparation of Substrate Solution for use in Aromatase Assay

The radiochemical purity of the [^{3}H] ASDN was >97% percent. The specific activity of the stock, [^{3}H]ASDN, was too high for direct use in the assay. Therefore, a solution containing a mixture of the nonradiolabeled and radiolabeled ASDN was prepared. The 1 mCi/ml [^{3}H] ASDN stock was diluted to 0.3 to 0.5 Ci/mmol by the addition of buffer (0.1 M sodium phosphate, pH 7.4) and radioinert ASDN. This substrate solution had a concentration of 2 µM ASDN and a radiochemical content of about 1 µCi/ml. The final concentration of the ASDN in the assay was 100 nM and the amount of tritium added to each incubation tube was approximately 0.1 µCi.

3.3.1.1 Calculations

Calculations for Specific Activity Adjustment for [^{3}H]ASDN:

[^{3}H]ASDN, NET-926 (Lot# 619344; MW 286.41; Specific Activity 26.3 Ci/mmol)

- 1 mCi/mL
- 0.974 TBq/mmol
- 37 MBq/mL EtOH

$$= \frac{37\ \text{MBq/mL}}{0.974\ \text{TBq/mmol}} = 37.99\ \mu\text{M in Ethanol}$$

Adjustment of specific activity to be between 0.3 and 0.5 Ci/mmol:

Prepared 1:100 dilution of the [^{3}H] ASDN so that aliquots contained 10 µCi/mL at 380 nM, or 0.00872 µg ASDN. Aliquots prepared and stored frozen.

Aliquots thawed and combined with 1 µg/mL radioinert ASDN and assay buffer to prepare the ASDN Substrate Solution (8 mL):

= 0.8 mL [^{3}H] ASDN (10 µCi/mL at 0.38 µM)
= 4.6 mL Unlabeled ASDN (1 µg/mL, or 3.5 µM)
= 2.6 mL Assay Buffer

This resulted in a 2 µM ASDN (2 nmol/mL) solution with approximately 1 µCi/mL (a specific activity between 0.3 mCi/mmol and 0.5 mCi/mmol).

The non-decayed nominal tritium activity in a 20 µL sample (read in Packard TriCarb LSC) should be 44,400 DPM, and thus 1 mL = 1 µCi = 2,220,000 DPM (e.g., 50 x 44,400 dpm).

Thus, the above ASDN stock of 2 nmol/mL should be 0.5 mCi/mmol.

Accuracy of the activity of the solution was checked by determining the DPM in the LSC and comparing it to the decayed nominal activity (e.g., it should be off by no more than 6%).

EXAMPLE:

- Average of 20 µL reads = 42,390 DPM with nominal decayed activity calculated as 43,180 DPM/20 µL
- This was determined to be 98.2% of nominal activity, so no adjustment needed.
- 42,390 DPM x 50 (to get from 20 µL to 1 mL) = 2,119,500 DPM
- 2,119,500 DPM / 2,220,000 DPM = 0.955
- 1 µCi = 2,220,000 DPM so the stock is 0.955 µCi, with 2 nmol/mL ASDN
- Specific activity of stock is thus 0.477 µCi/nmol, or 0.477 Ci/mmol

3.3.2 Preparation of Test Substances

Test substances were formulated in dimethylsulfoxide (DMSO). The total volume of DMSO used in each assay was 1% of the total assay volume (20 µL in 2 mL total assay volume) in order to minimize the potential of this vehicle to inhibit the aromatase enzyme (CYP19). Fresh dilutions of the stock solution of test substances were prepared on the day of use such that the target concentration (10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, $10^{-4.5}$, 10^{-4}, and 10^{-3} M) was achieved by the addition of 20 µL of the dilution to a 2 mL total assay volume. Dose concentrations of test and control substances were not verified using analytical methods as outlined in the protocol and GLP compliance statement of this report.

DMSO was chosen over ethanol as the solvent of choice for the following reasons: 1) DMSO was listed as one of the vehicles acceptable for use in OPPTS 890.1200 guideline; 2) DMSO was not as volatile as ethanol and so evaporation was less of a concern in the assay, and 3) DMSO was more accurate to pipette because of density and viscosity.

3.4 Microsomes

3.4.1 Human Recombinant Microsomes

Human recombinant microsomes were purchased from Gentest™ (Woburn, MA: www.gentest.com). The product name was Human CYP19 (Aromatase) and P450 reductase Supersomes ™ (Runs 1-2: catalog number 456260, lot 03897; Run 3: catalog number 456260, lot 19701). The vendor package inserts (batch data sheets) provided values for protein concentration, cytochrome c reductase activity, and aromatase activity and is included in the report (Appendix 3). Microsomes were stored at -80 ± 10°C.

3.4.2 Protein Assay

Protein content of the microsomes was supplied by the vendor (BD Gentest; 7.4 mg/mL for lot 03897 and 3.7 mg/mL for lot 19701; Appendix 3).

3.4.3 Cytochrome P450 (CYP19) Aromatase Activity

Aromatase activity of the microsome preparation was provided by the vendor (BD Gentest; 6.0 pmol product/(min x pmol P450) for lot 03897 and 5.7 pmol product/(min x pmol P450) for lot 09701; Appendix 3).

3.4.4 Human Recombinant Microsome Preparation

Initial preparation of the human recombinant microsomes involved thawing the microsomes rapidly in a 37 ± 2°C water bath and performing a two-step dilution. Following thawing, microsomes were placed in an ice bath and diluted to 0.8 mg/mL with buffer (0.1 M sodium phosphate, pH 7.4). Microsomes were further diluted to 0.008 mg/mL and aliquoted into individual vials. After aliquoting the microsomes into individual vials, the vials were returned to the -80 ± 10°C freezer for storage (information regarding stability to freeze thaw cycles was provided on the batch data sheet). This minimized freeze-thaw cycles to no more than one.

The assay used vials containing 0.008 mg/mL protein and final concentration was approximately 0.004 mg/mL of microsomal protein per assay tube. Rate of conversion of androstanedione to $^{3}H_2O$ was checked in each run to ensure suitability of microsomes. All runs met the acceptance criteria of 0.100 nmol/mg-protein/min minimum activity as forth in OPPTS 890.1200 guideline.

3.5 Other Assay Components

3.5.1 Buffer

The assay buffer was 0.1 M sodium phosphate buffer, pH 7.4. Sodium phosphate monobasic (Sigma S5011, lots 019K01021 and 70M001962V) and sodium phosphate dibasic (Sigma S5136, lots 077K01281 and 050M02174V) were used to prepare this buffer. 0.1 M solutions of each reagent were prepared in purified water and then combined to achieve a final pH of 7.4.

3.5.2 Propylene Glycol

Propylene glycol (Spectrum P1456, lot YE1040) was added to the assay directly as described below.

3.5.3 NADPH

NADPH (β-nicotinamide adenine dinucleotide phosphate, reduced form, tetrasodium salt) was the required co-factor for CYP19. A 6 mM stock solution was prepared in assay buffer (0.1 M sodium phosphate, pH 7.4) and the final concentration in the assay was 0.3 mM NADPH (Calbiochem 481973, lot D00102947). NADPH was prepared fresh each day the assay was performed and was kept on ice prior to use in the assay.

3.6 Test System

As per guideline (OPPTS 890.1200) recombinant microsomes (Human CYP19 + P450 Reductase Supersomes™) were used as the test system for this study.

3.7 Aromatase Assay Method

The assays were performed in 13 x 100 mm test tubes maintained at 37 ± 2°C in a shaking water bath. Propylene glycol, [^{3}H] ASDN, NADPH, and assay buffer were combined in the test tubes, with or without test substances or the positive control chemical for a total volume of 1 mL. The final concentrations for the major components of the assay are presented in Table 5 below. The test tubes and microsomal suspensions were placed at 37 ± 2°C in the water bath for approximately 5 minutes prior to the initiation of the assay by the addition of 1 mL of the diluted microsomal suspension. The total assay volume was 2 mL. The tubes were then incubated for approximately 15 minutes at 37 ± 2°C. The reactions were then terminated by the addition of 2 mL of ice-cold methylene chloride and vortex-mixed for approximately 5 seconds and placed on ice. The tubes were then re-vortex-mixed for an additional 20 to 25 seconds to extract the unreacted ASDN. The methylene chloride layer was removed (bottom layer) and discarded and the aqueous layer was extracted two more times, as outlined above. Two 0.5 mL aliquots of the top aqueous layers were then transferred to duplicate liquid scintillation vials containing 10 mL of liquid scintillation cocktail and then mixed.

Table 5. Optimized Aromatase Assay Conditions

Assay Factor (units)	Human Recombinant
Microsomal Protein (mg/mL)	0.004
NADPH (mM)	0.3
[^{3}H]ASDN (nM)	100
Propylene glycol	5%
Incubation Time (min)	15

Analysis of the samples was performed using a Packard TriCarb LSC (model 2910TR, serial DG03117657). Radioactivity found in the aqueous fractions is from the $^3H_20$ formed upon hydrolysis of [^{3}H] ASDN. One H_20 molecule is released per molecule of ASDN converted to estrone in a stereospecific reaction. Therefore, the amount of estrone product formed was determined by dividing the total amount of $^3H_20$ formed by the specific activity of the [^{3}H] ASDN substrate (expressed in dpm/mL). Results are presented as the activity of the enzyme reaction and expressed in nmol (mg protein)$^{-1}$ min $^{-1}$.

Three types of control samples were included for each run. These included:

- Full enzyme (aromatase) activity controls (substrate, NADPH, propylene glycol, buffer, vehicle (used for preparation of test substance solutions) and microsomes).
- Background activity controls (all components that are in the full aromatase activity controls except NADPH).
- Positive controls (4OH-ASDN run at 8 concentrations in same manner as test substance).

Prior to conducting this assay using test substances, a full-scale assay consisting of three independent runs were conducted using the positive control (4OH-ASDN) and the four proficiency chemicals outlined in the OPPTS 890.1200 guideline. The results of this proficiency demonstration are maintained at CeeTox. Proficiency was demonstrated when the positive

control met the performance criteria outlined in Section 3.8 below and by the correct classification of the proficiency chemicals.

3.8 Positive Control Assays and Determination of the Response of Aromatase Activity to Test Substances

Positive control 4-OH ASDN and test substances were tested in three independent runs, and for each run, eight concentrations were tested in triplicate (N=3). Four full activity controls and four background activity controls were included with each run of the assay. All controls were split in half so that two tubes (for full and background activity) were run at the beginning of the assay and two of each (full and background activity) were run at the end of each assay.

Table 6. Tubes Needed for Determination of CYP19 Aromatase Assay

Sample Type	Repetitions (tubes)	Description
Full Activity Control	4	All test components[(a)] plus solvent vehicle
Background Activity Control	4	Same as full activity control, but no NADPH

(a) The complete assay ("all test components") contains buffer, propylene glycol, microsomal protein, [3H]ASDN, and NADPH.

As set forth in OPPTS 890.1200 guideline, the mean aromatase activity in the full activity control samples must be ≥0.100 nmol/mg-protein/min for the assay run to be considered acceptable. In addition, the mean background control activity must be ≤ 15% (Tables 24-27) of the full activity control and the concentration response curve data generated for 4OH-ASDN must meet the performance criteria conditions listed in Table 7 below (see Table 23 for 4OH-ASDN proficiency results).

Table 7. Performance Criteria for the Positive Control

	Parameter	Lower Limit	Upper Limit
Positive Control	Slope	-1.2	-0.8
	Top (%)	90	110
	Bottom (%)	-5	+6
	Log IC_{50}	-7.3	-7.0

3.8.1 4-OH ASDN Positive Control Analysis

The positive control 4-OH ASDN (Formestane) was used to demonstrate that the assay was being conducted properly for each run. The positive control was tested in the aromatase assay according to the methods described in Section 3.7 and 3.8 above using the study design shown in Table 8 below.

Table 8. Positive Control Study Design

Sample Type	Repetition (tubes)	Description	4OH-ASDN Conc. (M)
Full Activity Control	4	All test components. No inhibitor	N/A
Background Activity Control	4	Same as full activity control, but no NADPH	N/A
4OH-ASDN Conc 1	3	Complete assay with 4-OH ASDN (positive control) added	$1X10^{-5}$
4OH-ASDN Conc 2	3	same	$1X10^{-6}$
4OH-ASDN Conc 3	3	same	$1X10^{-6.5}$
4OH-ASDN Conc 4	3	same	$1X10^{-7}$
4OH-ASDN Conc 5	3	same	$1X10^{-7.5}$
4OH-ASDN Conc 6	3	same	$1X10^{-8}$
4OH-ASDN Conc 7	3	same	$1X10^{-9}$
4OH-ASDN Conc 8	3	same	$1X10^{-10}$

3.8.2 Test Substance Analysis

Test substances were tested in three independent runs and each run was conducted independently of the other runs using the aromatase assay methods described in Section 3.7 and 3.8 above with the study design shown in Table 9 below.

After completion of the first run, the data were reviewed and solubility assessed by visual inspection to determine if test concentrations of test substances should be adjusted for subsequent runs of the assay (See Section 3.9 Solubility Assessment below).

Table 9. Test Substance Study Design

Sample Type	Repetition	Description	Reference or Substance Conc (M)
Full Activity Control	4	All test components plus solvent vehicle*	N/A
Background Activity Control	4	Same as full activity control, but no NADPH	N/A
Positive Control Conc1	2	Complete assay with 4OH-ASDN added	$1X10^{-5}$
Positive Control Conc2	2	same	$1X10^{-6}$
Positive Control Conc3	2	same	$1X10^{-6.5}$
Positive Control Conc4	2	same	$1X10^{-7}$
Positive Control Conc5	2	same	$1X10^{-7.5}$
Positive Control Conc6	2	same	$1X10^{-8}$
Positive Control Conc7	2	same	$1X10^{-9}$
Positive Control Conc8	2	same	$1X10^{-10}$
Test substance Conc1	3	Compete assay with test substance added	$1X10^{-3}$
Test substance Conc2	3	same	$1X10^{-4}$
Test substance Conc3	3	same	$1X10^{-5}$
Test substance Conc4	3	same	$1X10^{-6}$
Test substance Conc5	3	same	$1X10^{-7}$
Test substance Conc6	3	same	$1X10^{-8}$
Test substance Conc7	3	same	$1X10^{-9}$
Test substance Conc8	3	same	$1X10^{-10}$

N/A = not applicable
Conc = concentration
*The complete assay ("all test components")

3.9 Solubility Assessment of Test Substances

Solubility of the test substance was assessed in the first run of the assay by visual observation using the precipitation code shown below:

0 = Negative
+ = Small Amount
++ = Moderate Amount
+++ = Substantial Amount

3.9.1 Solubility Assessment and Concentration Ranges

- If insolubility (cloudiness or a precipitate) was visually observed at the highest concentration (10^{-3} M), then the highest concentration would be adjusted for the second and third runs at the highest concentration that appeared soluble using log or half-log concentrations; i.e., $10^{-4.5}$ M, 10^{-4} M, etc. Concentrations lower than 10^{-5} M for the highest concentration were not tested.

The lowest concentration to be tested was 10^{-10} M. Low concentrations were required to obtain the "top of the curve". That is, the full enzymatic activity was obtained at the two lowest concentrations of the test substance in order to define the top of the concentration-response curve.

3.10 Data Evaluation

3.10.1 Aromatase Activity and Percent of Control Calculations

Relevant data was entered into the aromatase assay spreadsheet for calculations of aromatase activity and percent control (see Tables 11-22 and Appendix 1: Raw and Normalized DPM Data). The spreadsheet was created in Excel and calculated the DPM/mL for each aliquot of the extracted aqueous incubation mixture, average DPM/mL and total DPM for each aqueous portion (after extraction). The volume (mL) of substrate solution added to the incubation multiplied by the substrates 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 background activity control tubes (Appendix 1: Raw and Normalized DPM Data). 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 product)$^{-1}$min^{-1} and was calculated by dividing the amount of ^3H_2O formed (nmol) by the product of mg microsome protein used times the incubation time (15 minutes). Average activity in the full activity control samples was calculated. Percent of control activity remaining in the presence of the various test chemical concentrations, including the positive control, was calculated by dividing the aromatase activity at a given concentration by the average full activity control and multiplying by 100.

Nominally one might expect the percent of control activity values for an inhibitor to vary between approximately 0 percent near the high inhibition concentrations and approximately 100 percent near the low inhibition concentrations. However due to experimental variation, individual observed percent of control values sometimes extended slightly below 0 percent or above 100 percent.

3.10.2 Model Fitting

The response curves were fitted by weighted least squares nonlinear regression analysis with weights equal to 1/Y. Model fits were carried out using a 4-parameter regression model (XLfit; IDBS; Version 5.2.0.0; Fit Model 208) and Tukey's Bi-Weight statistical analysis for outlier analysis.

Concentration response trend curves were fitted to the percent of control activity values within each of the replicate tubes at each test chemical concentration. Concentration was expressed on the log or half-log scale.

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

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

The above equation is equivalent to the XLfit Model 208 (IDBS; Version 5.2.0.0), or the 4 Parameter Logistic Model.

Concentration response models were fitted for each test run for each test substance and control(s).

Y = percent of control activity in the inhibitor tube.

X = Logarithm (base 10) of the concentration.

T = average DPMs across the repeat tubes with the same test substance concentration that define the Top of the curve.

B = average DPMs across the repeat tubes with the same test substance concentration that define the Bottom of the curve.

β = slope of the concentrations response curve (β will be negative).

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

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

For each run for each test substance the individual percent of control values were plotted versus logarithm of the test chemical concentration. The fitted concentration response curves were superimposed on the plot. Individual plots were prepared for each run for each test substance (Figures 1-4) along with plotted means (Figures 5, 8, 11, and 14).

Additional plots for each test substance were prepared to compare the percent of control activity values across runs. For each run the average percent of control values versus logarithm of test chemical concentration were plotted on the same plot. Plotting symbols distinguished among runs for a given test substance. The fitted concentration response curves for each run were superimposed on the plots (Figures 6, 9, 12, and 15). On separate plots the average percent of control values for each run were plotted versus logarithm of test substance concentration. The average concentration response curve across runs was superimposed on the same plot for each test substance (Figures 7, 10, 13, and 16).

3.10.4 Quality Control-Analysis of Variance Comparisons of Full Enzyme Activity Control and Background Activity Control as Percent of Control

Within each run of each test substance quadruplicate repetitions were made of the control tubes (Full Activity Control and Background Activity Control). Half the repetitions were carried out at the beginning of the run and half at the end. Control responses were adjusted for background DPMs, divided by the average of the (background adjusted) full activity (TA) control values, and expressed as percent of control. The average of the four background activity controls (NSB) within a run had to be approximately 0 % (with an acceptable range of -5 to +6%) and the average of the four full activity controls (TA) within a run had to be approximately 100% (with an acceptable range of 90 – 110%).

The mean background activity control also had to be ≤ 15% of the full activity control, the limit established in the guidelines (Tables 24-27).

3.10.5 Data Interpretation

Data from this assay were used to classify the test substances according to their ability to inhibit aromatase. To be classified as an inhibitor, the data must fit the 4-parameter regression model to yield an inhibition curve and result in greater than 50% inhibition at the highest concentration. The value of the inhibition curve at each of three runs at the highest concentration were averaged and compared with the following criteria. If the data did fit the model, the average activity of the data points at the highest concentration was used.

Table 10. Data Interpretation Criteria

Criteria		Classification
Data fit 4-parameter nonlinear regression model	Curve crosses 50%	Inhibitor
	Average lowest portion of curves across runs is between 50% and 75% Activity	Equivocal
	Average lowest portion of curves across runs is greater than 75%	Non-inhibitor
Data do not fit the model	----	

3.11 Statistical Software and Analysis

Concentration curves were fitted to the data using non-linear regression analysis features in a commercial software package (e.g., IDBS XLfit v5.2.0.0). For data generated at CeeTox, basic statistical analysis was performed on the data, which included means of replicates, standard deviation of the mean, standard error of the mean, and coefficient of variation.

4.0 RESULTS AND DISCUSSION

4.1 Concentration Range for the Test Substance

In the first run of the aromatase activity assay, test substances were tested at following concentrations: 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3} M. In general, test substances were found to be soluble at concentrations of $\leq 10^{-4}$ M (see Table 28). Consequently, runs 2 and 3 of the assay were conducted test concentrations of 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, $10^{-4.5}$ and 10^{-4} M.

4.2 Aromatase Assay Acceptance Criteria

In three independent runs of the positive control assay (4OH-ASDN) (see Table 23), the mean Hill slope, IC_{50}, bottom curve (%), and top curve (%) were calculated. The range of values achieved for these parameters in three independent runs of the assay are shown below, along with the performance criteria ranges established in the OPPTS 890.1200 guideline. All values were within the acceptable ranges specified in Section 3.8 (see Table 7), with the following minor exception below:

- In Run 2 of the assay, the IC50 was marginally higher than the specified range (log IC50 = -7.46) compared to the specified guideline range of -7.3 to -7.0, and the Hill Slope was marginally lower than specified range (slope = -0.75) compared to the specified guideline range of -1.2 to -0.8.

The above differences were minor and not considered to reflect a true deviation from the specified ranges. Therefore, all independent runs of the assay were considered to have met the assay acceptance criteria and were considered to be definitive.

Top of Curve = 97.51% to 103.96%	(Guideline Range = 90% – 110%)
Bottom Curve = -1.85% to 0.78%	(Guideline Range = -5% to 6%)
Hill Slope = -0.98 to -0.75	(Guideline Range = -1.2 to -0.8)
Log IC_{50} = -7.46 to -7.26	(Guideline Range = -7.3 to -7)

4.3 Quality Control Analysis Acceptance Criteria

In three independent runs of the assay, the average of the four background activity controls (NSB) within a run had to be approximately 0 % (with an acceptable range of -5 to +6%) and the average of the four full activity controls (TA) within a run had to be approximately 100% (with an acceptable range of 90 – 110%).

All runs were within specifications. In addition, the mean background activity controls were ≤ 15% of the full activity controls, the limit established in the guidelines (Tables 24-27).

The mean aromatase activity values in the full activity control samples were at least 0.241 nmol/mg-protein/min in the runs, well above the 0.100 nmol/mg-protein/min minimum acceptable activity limit set forth in OPPTS 890.1200 guideline.

4.4 Aromatase Assay Results

The four test substances were evaluated in three independent runs of the assay conducted on 28 June 2011, 29 June 2011, and 27 July 2011. Solubility/precipitation of test substances in the assay buffer was assessed visually in the first run of the assay. The results of these analyses are presented in Tables 11-22. Based on these results, the suitable top concentration of test substances for use in the aromatase assays was determined to be 10^{-4} M and concentrations of the test substance used in the latter runs were adjusted accordingly. The positive control inhibitor 4OH-ASDN was included with each run each time the aromatase assay was performed to ensure results passed the performance criteria as set forth in OPPTS 890.1200 guidelines. In three independent runs of the aromatase assay, mean aromatase activity was determined to be:

Methoxycinnamate: 100% (± 6% SD) of control activity = Non-inhibitor

Octocrylene: 94% (± 1% SD) of control activity = Non-inhibitor

Octylsalicylate: 90% (± 2% SD) of control activity = Non-inhibitor

Oxybenzone: 51% (± 13% SD) of control activity = Equivocal

4.5 Discussion

In three independent runs of the assay, test substances were tested at final concentrations of 10^{-10} to 10^{-4} M. Methoxycinnamate, octocrylene, and octylsalicylate were shown to be non-inhibitors. Oxybenzone was within the 50-75% range of the EDSP guideline (Table 10, Section 3.10.5 Data Interpretation). As such, oxybenzone was classified as equivocal in its response.

5.0 CONCLUSIONS

Methoxycinnamate, octocrylene, and octylsalicylate were determined to be non-inhibitors, and Oxybenzone was determined to be equivocal in its response as defined by EDSP guideline OPPTS 890.1200 (Table 10, Section 3.10.5 Data Interpretation).

6.0 REFERENCES

- Endocrine Disruptor Screening Program Test Guidelines OPPTS 890.1200: Aromatase (Human Recombinant); US EPA 740-C-09-004 (October 2009).

- Integrated Summary Report on Aromatase; Battelle and US EPA (December 11, 2007).

TABLES SECTION (RESULTS)

TABLE 11: Results of Run 1 Aromatase Activity Assay: 4OH-ASDN and Methoxycinnamate (28 June 2011)

Concentration of 4OH-ASDN (M)	Aromatase Activity (% of VC)		Individual Aromatase Activity (% of VC)		
	Mean	SD	Value 1	Value 2	Value 3
TA	103.52	0.432	103.83	103.21	ND
NSB	-0.01	0.000	-0.01	-0.01	ND
10^{-5}	0.77	0.033	0.79	0.74	ND
10^{-6}	5.97	0.119	6.05	5.88	ND
$10^{-6.5}$	16.53	0.062	16.57	16.48	ND
10^{-7}	35.79	0.261	35.60	35.97	ND
$10^{-7.5}$	65.95	0.422	65.65	66.25	ND
10^{-8}	85.77	0.332	86.01	85.54	ND
10^{-9}	100.51	1.219	101.38	99.65	ND
10^{-10}	103.02	3.648	100.44	105.60	ND
Concentration of Methoxycinnamate (M)	**Aromatase Activity (% of VC)**		**Individual Aromatase Activity (% of VC)**		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	96.48	2.030	95.04	97.92	ND
NSB	0.01	0.057	-0.03	0.05	ND
10^{-3}	97.68	0.802	98.16	96.75	98.12
10^{-4}	101.59	4.127	101.30	97.61	105.85
10^{-5}	102.66	0.981	102.36	103.75	101.86
10^{-6}	98.67	3.083	100.40	100.50	95.11
10^{-7}	92.94	7.517	94.51	99.55	84.76
10^{-8}	98.64	7.715	96.37	92.31	107.23
10^{-9}	98.85	4.193	95.61	97.35	103.58
10^{-10}	95.52	4.213	90.66	98.11	97.79

VC = Vehicle Control
TA = Full Activity Control
NSB = Background Activity Control
SD = Standard Deviation
ND = Not Determined

TABLE 12: Results of Run 2 Aromatase Activity Assay: 4OH-ASDN and Methoxycinnamate (29 June 2011)

Concentration of 4OH-ASDN (M)	Aromatase Activity (% of VC)		Individual Aromatase Activity (% of VC)		
	Mean	SD	Value 1	Value 2	Value 3
TA	104.45	4.576	101.22	107.69	ND
NSB	-0.02	0.037	0.01	-0.04	ND
10^{-5}	0.67	0.064	0.62	0.71	ND
10^{-6}	5.19	0.172	5.31	5.07	ND
$10^{-6.5}$	13.58	0.347	13.83	13.34	ND
10^{-7}	30.99	0.219	30.84	31.15	ND
$10^{-7.5}$	57.15	3.254	59.45	54.85	ND
10^{-8}	68.54	23.821	85.39	51.70	ND
10^{-9}	101.09	0.495	101.44	100.74	ND
10^{-10}	100.79	4.589	97.55	104.04	ND
Concentration of Methoxycinnamate (M)	**Aromatase Activity (% of VC)**		**Individual Aromatase Activity (% of VC)**		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	95.55	5.198	91.87	99.22	ND
NSB	0.02	0.027	0.03	0.00	ND
10^{-4}	105.03	1.873	106.70	105.38	103.01
$10^{-4.5}$	98.51	9.561	103.91	104.16	87.48
10^{-5}	104.32	2.705	105.45	106.27	101.23
10^{-6}	95.69	10.187	96.67	105.35	85.05
10^{-7}	100.98	1.079	101.67	101.53	99.73
10^{-8}	80.49	20.984	101.08	59.14	81.24
10^{-9}	91.24	16.736	72.20	97.93	103.60
10^{-10}	98.71	1.101	97.85	99.95	98.32

VC = Vehicle Control
TA = Full Activity Control
NSB = Background Activity Control
SD = Standard Deviation
ND = Not Determined

TABLE 13: Results of Run 3 Aromatase Activity Assay: 4OH-ASDN and Methoxycinnamate (27 July 2011)

Concentration of 4OH-ASDN (M)	Aromatase Activity (% of VC)		Individual Aromatase Activity (% of VC)		
	Mean	SD	Value 1	Value 2	Value 3
TA	102.63	0.904	103.27	101.99	ND
NSB	-0.01	0.015	-0.02	0.00	ND
10^{-5}	1.41	0.032	1.38	1.43	ND
10^{-6}	5.79	0.096	5.73	5.86	ND
$10^{-6.5}$	15.66	0.132	15.75	15.57	ND
10^{-7}	34.42	0.747	34.95	33.89	ND
$10^{-7.5}$	63.78	0.164	63.66	63.90	ND
10^{-8}	85.09	2.195	86.64	83.54	ND
10^{-9}	99.58	1.353	100.54	98.62	ND
10^{-10}	103.52	1.370	102.55	104.49	ND
Concentration of Methoxycinnamate (M)	**Aromatase Activity (% of VC)**		**Individual Aromatase Activity (% of VC)**		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	97.37	5.820	93.25	101.48	ND
NSB	0.01	0.002	0.01	0.00	ND
10^{-4}	93.95	2.156	91.46	95.09	95.29
$10^{-4.5}$	98.39	0.923	99.41	98.13	97.62
10^{-5}	98.14	1.324	99.67	97.31	97.44
10^{-6}	90.47	17.341	98.68	102.17	70.54
10^{-7}	101.32	1.232	100.07	102.53	101.37
10^{-8}	103.87	1.042	103.35	105.07	103.19
10^{-9}	99.57	1.803	98.09	99.04	101.58
10^{-10}	100.16	1.269	100.85	98.70	100.94

VC = Vehicle Control
TA = Full Activity Control
NSB = Background Activity Control
SD = Standard Deviation
ND = Not Determined

TABLE 14: Results of Run 1 Aromatase Activity Assay: 4OH-ASDN and Octocrylene (28 June 2011)

Concentration of 4OH-ASDN (M)	Aromatase Activity (% of VC)		Individual Aromatase Activity (% of VC)		
	Mean	SD	Value 1	Value 2	Value 3
TA	102.19	0.426	102.49	101.89	ND
NSB	-0.01	0.000	-0.01	-0.01	ND
10^{-5}	0.75	0.033	0.78	0.73	ND
10^{-6}	5.89	0.117	5.97	5.80	ND
$10^{-6.5}$	16.31	0.061	16.35	16.27	ND
10^{-7}	35.32	0.258	35.14	35.50	ND
$10^{-7.5}$	65.10	0.417	64.81	65.40	ND
10^{-8}	84.67	0.328	84.90	84.44	ND
10^{-9}	99.22	1.203	100.07	98.37	ND
10^{-10}	101.69	3.601	99.15	104.24	ND
Concentration of Octocrylene (M)	**Aromatase Activity (% of VC)**		**Individual Aromatase Activity (% of VC)**		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	97.81	1.297	98.73	96.89	ND
NSB	0.01	0.005	0.01	0.01	ND
10^{-3}	83.84	5.278	89.01	78.46	84.07
10^{-4}	93.39	3.647	95.11	89.20	95.85
10^{-5}	98.75	3.082	98.62	95.74	101.90
10^{-6}	99.75	0.312	99.60	100.11	99.55
10^{-7}	97.24	0.944	96.22	98.08	97.42
10^{-8}	98.47	4.894	94.50	103.94	96.97
10^{-9}	95.51	6.819	100.48	98.32	87.74
10^{-10}	96.77	0.988	95.91	97.85	96.55

VC = Vehicle Control
TA = Full Activity Control
NSB = Background Activity Control
SD = Standard Deviation
ND = Not Determined

TABLE 15: Results of Run 2 Aromatase Activity Assay: 4OH-ASDN and Octocrylene (29 June 2011)

Concentration of 4OH-ASDN (M)	Aromatase Activity (% of VC)		Individual Aromatase Activity (% of VC)		
	Mean	SD	Value 1	Value 2	Value 3
TA	97.97	4.292	94.94	101.01	ND
NSB	-0.02	0.035	0.00	-0.05	ND
10^{-5}	0.62	0.060	0.58	0.66	ND
10^{-6}	4.86	0.161	4.97	4.74	ND
$10^{-6.5}$	12.73	0.325	12.96	12.50	ND
10^{-7}	29.06	0.205	28.92	29.21	ND
$10^{-7.5}$	53.60	3.052	55.76	51.44	ND
10^{-8}	64.29	22.345	80.09	48.49	ND
10^{-9}	94.81	0.464	95.14	94.48	ND
10^{-10}	94.54	4.305	91.49	97.58	ND
Concentration of Octocrylene (M)	**Aromatase Activity (% of VC)**		**Individual Aromatase Activity (% of VC)**		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	102.03	12.608	93.11	110.95	ND
NSB	0.02	0.009	0.02	0.03	ND
10^{-4}	94.55	1.491	93.53	93.86	96.27
$10^{-4.5}$	95.14	1.632	96.81	93.54	95.07
10^{-5}	94.91	1.807	95.14	93.00	96.60
10^{-6}	93.18	1.853	91.30	95.01	93.24
10^{-7}	97.07	3.863	98.37	100.11	92.72
10^{-8}	105.68	5.635	107.79	99.30	109.95
10^{-9}	93.72	0.179	93.74	93.53	93.89
10^{-10}	106.76	7.512	98.33	112.74	109.20

VC = Vehicle Control
TA = Full Activity Control
NSB = Background Activity Control
SD = Standard Deviation
ND = Not Determined

TABLE 16: Results of Run 3 Aromatase Activity Assay: 4OH-ASDN and Octocrylene (27 July 2011)

Concentration of 4OH-ASDN (M)	Aromatase Activity (% of VC)		Individual Aromatase Activity (% of VC)		
	Mean	SD	Value 1	Value 2	Value 3
TA	102.05	0.899	102.69	101.42	ND
NSB	-0.01	0.015	-0.02	0.00	ND
10^{-5}	1.39	0.032	1.37	1.41	ND
10^{-6}	5.75	0.096	5.69	5.82	ND
$10^{-6.5}$	15.56	0.131	15.66	15.47	ND
10^{-7}	34.22	0.743	34.75	33.70	ND
$10^{-7.5}$	63.42	0.163	63.30	63.53	ND
10^{-8}	84.61	2.182	86.15	83.07	ND
10^{-9}	99.02	1.346	99.97	98.07	ND
10^{-10}	102.94	1.362	101.98	103.90	ND
Concentration of Octocrylene (M)	**Aromatase Activity (% of VC)**		**Individual Aromatase Activity (% of VC)**		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	97.95	6.424	102.49	93.40	ND
NSB	0.01	0.039	-0.01	0.04	ND
10^{-4}	94.08	0.595	93.79	94.76	93.68
$10^{-4.5}$	92.67	0.865	92.74	93.50	91.77
10^{-5}	87.05	9.674	91.88	93.36	75.92
10^{-6}	93.90	0.338	93.58	93.85	94.25
10^{-7}	103.61	1.051	103.91	104.48	102.44
10^{-8}	94.35	18.234	103.72	105.99	73.33
10^{-9}	101.17	0.558	100.71	101.79	101.02
10^{-10}	97.10	7.261	101.69	88.73	100.89

VC = Vehicle Control
TA = Full Activity Control
NSB = Background Activity Control
SD = Standard Deviation
ND = Not Determined

TABLE 17: Results of Run 1 Aromatase Activity Assay: 4OH-ASDN and Octylsalicylate (28 June 2011)

Concentration of 4OH-ASDN (M)	Aromatase Activity (% of VC)		Individual Aromatase Activity (% of VC)		
	Mean	SD	Value 1	Value 2	Value 3
TA	98.36	0.410	98.65	98.07	ND
NSB	-0.03	0.000	-0.03	-0.03	ND
10^{-5}	0.70	0.032	0.73	0.68	ND
10^{-6}	5.65	0.113	5.73	5.57	ND
$10^{-6.5}$	15.68	0.059	15.72	15.64	ND
10^{-7}	33.98	0.248	33.81	34.16	ND
$10^{-7.5}$	62.65	0.401	62.37	62.94	ND
10^{-8}	81.49	0.316	81.71	81.27	ND
10^{-9}	95.50	1.159	96.32	94.68	ND
10^{-10}	97.88	3.467	95.43	100.33	ND
Concentration of Octylsalicylate (M)	**Aromatase Activity (% of VC)**		**Individual Aromatase Activity (% of VC)**		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	101.64	1.853	100.33	102.95	ND
NSB	0.03	0.000	0.03	0.03	ND
10^{-3}	84.47	0.734	83.90	85.30	84.21
10^{-4}	91.94	3.781	95.81	88.26	91.73
10^{-5}	103.19	1.008	102.23	104.24	103.08
10^{-6}	101.40	2.500	98.56	102.37	103.26
10^{-7}	101.83	0.744	102.64	101.68	101.17
10^{-8}	97.79	3.405	94.09	100.79	98.49
10^{-9}	97.11	1.268	95.76	98.28	97.28
10^{-10}	98.05	1.349	99.37	98.11	96.68

VC = Vehicle Control
TA = Full Activity Control
NSB = Background Activity Control
SD = Standard Deviation
ND = Not Determined

TABLE 18: Results of Run 2 Aromatase Activity Assay: 4OH-ASDN and Octylsalicylate (29 June 2011)

Concentration of 4OH-ASDN (M)	Aromatase Activity (% of VC)		Individual Aromatase Activity (% of VC)		
	Mean	SD	Value 1	Value 2	Value 3
TA	100.54	4.405	97.43	103.65	ND
NSB	-0.02	0.036	0.01	-0.04	ND
10^{-5}	0.64	0.062	0.60	0.68	ND
10^{-6}	4.99	0.165	5.11	4.88	ND
$10^{-6.5}$	13.07	0.334	13.31	12.84	ND
10^{-7}	29.83	0.211	29.68	29.98	ND
$10^{-7.5}$	55.01	3.132	57.23	52.80	ND
10^{-8}	65.97	22.930	82.19	49.76	ND
10^{-9}	97.30	0.476	97.64	96.96	ND
10^{-10}	97.02	4.417	93.89	100.14	ND
Concentration of Octylsalicylate (M)	**Aromatase Activity (% of VC)**		**Individual Aromatase Activity (% of VC)**		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	99.46	1.768	100.71	98.21	ND
NSB	0.02	0.010	0.03	0.01	ND
10^{-4}	88.88	3.223	90.43	91.04	85.17
$10^{-4.5}$	96.86	6.812	93.39	104.71	92.49
10^{-5}	103.35	2.927	100.43	106.28	103.34
10^{-6}	104.14	1.942	106.07	102.19	104.15
10^{-7}	93.47	12.918	95.33	105.36	79.72
10^{-8}	102.50	1.308	103.78	102.54	101.17
10^{-9}	93.49	12.921	100.64	101.26	78.58
10^{-10}	98.83	3.494	94.80	100.96	100.73

VC = Vehicle Control
TA = Full Activity Control
NSB = Background Activity Control
SD = Standard Deviation
ND = Not Determined

TABLE 19: Results of Run 3 Aromatase Activity Assay: 4OH-ASDN and Octylsalicylate (27 July 2011)

Concentration of 4OH-ASDN (M)	Aromatase Activity (% of VC)		Individual Aromatase Activity (% of VC)		
	Mean	SD	Value 1	Value 2	Value 3
TA	102.42	0.902	103.06	101.78	ND
NSB	-0.01	0.015	-0.03	0.00	ND
10^{-5}	1.40	0.032	1.37	1.42	ND
10^{-6}	5.77	0.096	5.71	5.84	ND
$10^{-6.5}$	15.62	0.132	15.71	15.53	ND
10^{-7}	34.34	0.746	34.87	33.82	ND
$10^{-7.5}$	63.65	0.164	63.53	63.76	ND
10^{-8}	84.91	2.190	86.46	83.37	ND
10^{-9}	99.38	1.350	100.33	98.42	ND
10^{-10}	103.31	1.367	102.34	104.28	ND
Concentration of Octylsalicylate (M)	**Aromatase Activity (% of VC)**		**Individual Aromatase Activity (% of VC)**		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	97.58	0.642	97.12	98.03	ND
NSB	0.01	0.015	0.00	0.03	ND
10^{-4}	88.3	0.564	88.45	88.77	87.67
$10^{-4.5}$	88.72	1.777	88.08	87.35	90.73
10^{-5}	94.68	0.782	95.33	94.91	93.81
10^{-6}	104.22	1.648	105.97	102.70	104.00
10^{-7}	103.04	2.441	105.36	100.49	103.26
10^{-8}	104.50	1.665	102.58	105.57	105.34
10^{-9}	102.11	2.539	103.49	99.18	103.65
10^{-10}	103.26	1.853	101.45	103.17	105.15

VC = Vehicle Control
TA = Full Activity Control
NSB = Background Activity Control
SD = Standard Deviation
ND = Not Determined

TABLE 20: Results of Run 1 Aromatase Activity Assay: 4OH-ASDN and Oxybenzone (28 June 2011)

Concentration of 4OH-ASDN (M)	Aromatase Activity (% of VC)		Individual Aromatase Activity (% of VC)		
	Mean	SD	Value 1	Value 2	Value 3
TA	100.05	0.417	100.35	99.76	ND
NSB	0.01	0.000	0.01	0.01	ND
10^{-5}	0.76	0.032	0.78	0.73	ND
10^{-6}	5.78	0.115	5.86	5.70	ND
$10^{-6.5}$	15.98	0.060	16.03	15.94	ND
10^{-7}	34.60	0.252	34.42	34.77	ND
$10^{-7.5}$	63.75	0.408	63.46	64.04	ND
10^{-8}	82.90	0.321	83.13	82.68	ND
10^{-9}	97.15	1.178	97.98	96.32	ND
10^{-10}	99.57	3.525	97.07	102.06	ND
Concentration of Oxybenzone (M)	**Aromatase Activity (% of VC)**		**Individual Aromatase Activity (% of VC)**		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	99.95	1.586	101.07	98.82	ND
NSB	-0.01	0.028	-0.03	0.01	ND
10^{-3}	32.90	16.260	46.44	14.87	37.38
10^{-4}	52.22	10.414	62.11	53.21	41.35
10^{-5}	87.98	2.323	86.82	86.47	90.66
10^{-6}	98.87	2.597	96.64	98.25	101.72
10^{-7}	100.00	3.819	96.08	103.71	100.20
10^{-8}	99.31	2.652	96.25	100.70	100.98
10^{-9}	99.00	1.347	98.00	98.46	100.53
10^{-10}	100.70	1.840	102.38	100.97	98.73

VC = Vehicle Control
TA = Full Activity Control
NSB = Background Activity Control
SD = Standard Deviation
ND = Not Determined

TABLE 21: Results of Run 2 Aromatase Activity Assay: 4OH-ASDN and Oxybenzone (29 June 2011)

Concentration of 4OH-ASDN (M)	Aromatase Activity (% of VC)		Individual Aromatase Activity (% of VC)		
	Mean	SD	Value 1	Value 2	Value 3
TA	99.88	4.376	96.78	102.97	ND
NSB	-0.03	0.035	-0.01	-0.06	ND
10^{-5}	0.62	0.061	0.58	0.67	ND
10^{-6}	4.95	0.164	5.06	4.83	ND
$10^{-6.5}$	12.97	0.331	13.21	12.74	ND
10^{-7}	29.63	0.209	29.48	29.77	ND
$10^{-7.5}$	54.64	3.112	56.84	52.44	ND
10^{-8}	65.54	22.781	81.64	49.43	ND
10^{-9}	96.66	0.473	96.99	96.32	ND
10^{-10}	96.38	4.389	93.28	99.48	ND
Concentration of Oxybenzone (M)	**Aromatase Activity (% of VC)**		**Individual Aromatase Activity (% of VC)**		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	100.12	3.465	97.67	102.57	ND
NSB	0.03	0.058	-0.01	0.07	ND
10^{-4}	62.81	6.047	69.13	62.22	57.09
$10^{-4.5}$	71.26	2.134	73.60	70.75	69.42
10^{-5}	87.23	0.615	87.59	86.52	87.57
10^{-6}	96.72	0.964	97.55	96.96	95.66
10^{-7}	99.79	1.892	101.72	99.72	97.94
10^{-8}	97.04	0.749	97.69	96.22	97.21
10^{-9}	102.46	5.827	99.88	98.36	109.13
10^{-10}	96.33	6.512	103.56	94.52	90.91

VC = Vehicle Control
TA = Full Activity Control
NSB = Background Activity Control
SD = Standard Deviation
ND = Not Determined

TABLE 22: Results of Run 3 Aromatase Activity Assay: 4OH-ASDN and Oxybenzone (27 July 2011)

Concentration of 4OH-ASDN (M)	Aromatase Activity (% of VC)		Individual Aromatase Activity (% of VC)		
	Mean	SD	Value 1	Value 2	Value 3
TA	100.01	0.881	100.64	99.39	ND
NSB	-0.01	0.015	-0.02	0.00	ND
10^{-5}	1.37	0.031	1.35	1.39	ND
10^{-6}	5.65	0.094	5.58	5.71	ND
$10^{-6.5}$	15.26	0.129	15.35	15.17	ND
10^{-7}	33.54	0.728	34.06	33.03	ND
$10^{-7.5}$	62.15	0.160	62.04	62.27	ND
10^{-8}	82.92	2.139	84.43	81.41	ND
10^{-9}	97.04	1.319	97.97	96.11	ND
10^{-10}	100.88	1.335	99.94	101.82	ND
Concentration of Oxybenzone (M)	**Aromatase Activity (% of VC)**		**Individual Aromatase Activity (% of VC)**		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	99.99	0.068	100.04	99.94	ND
NSB	0.01	0.007	0.00	0.01	ND
10^{-4}	37.52	3.837	36.21	34.51	41.84
$10^{-4.5}$	63.88	1.061	63.19	65.10	63.36
10^{-5}	92.29	0.691	92.51	91.52	92.84
10^{-6}	99.83	4.835	102.07	94.29	103.14
10^{-7}	100.81	1.773	101.23	98.87	102.34
10^{-8}	88.73	20.804	98.86	102.53	64.80
10^{-9}	101.72	0.683	102.23	100.95	101.98
10^{-10}	100.61	1.696	98.67	101.82	101.34

VC = Vehicle Control
TA = Full Activity Control
NSB = Background Activity Control
SD = Standard Deviation
ND = Not Determined

TABLE 23: Hill Slope, LogIC_{50}, Top of Curve (%), and Bottom of Curve (%) Values for the Reference Chemical 4OH- ASDN

Name	Hill Slope			Log IC50		
	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3
4OH-ASDN	-0.97	-0.75	-0.98	-7.26	-7.46	-7.30
4OH-ASDN	-0.97	-0.75	-0.98	-7.26	-7.46	-7.30
4OH-ASDN	-0.97	-0.75	-0.98	-7.26	-7.46	-7.30
4OH-ASDN	-0.97	-0.75	-0.98	-7.26	-7.46	-7.30

Name	Top of Curve (%)			Bottom of Curve (%)		
	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3
4OH-ASDN	102.87	103.96	102.81	0.23	-1.85	0.78
4OH-ASDN	101.54	97.51	102.22	0.23	-1.75	0.76
4OH-ASDN	97.73	100.07	102.59	0.20	-1.79	0.77
4OH-ASDN	99.42	99.41	100.18	0.24	-1.79	0.76

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	Parameter	Lower	Upper
4OH-ASDN	Slope	-1.2	-0.8
	Top (%)	90	110
	Bottom (%)	-5	6
	Log IC50	-7.3	-7.0

TABLE 24: Individual and Mean Full Activity Control and Background Activity Control Values for the Assay Runs (Methoxycinnamate Runs)

Tube Position	Full Activity Control (TA; Full Activity %)			Background Activity Control (NSB; Non-Specific Binding; No Activity %)		
	Run 1	**Run 2**	**Run 3**	**Run 1**	**Run 2**	**Run 3**
Beginning	103.83	101.22	103.27	-0.01	0.01	-0.02
	103.21	107.69	101.99	-0.01	-0.04	0.00
End	95.04	91.87	93.25	-0.03	0.03	0.01
	97.92	99.22	101.48	0.05	0.00	0.00
Means	**100.0**	**100.0**	**100.0**	**0.0**	**0.0**	**0.0**
% of Full Activity	**NA**	**NA**	**NA**	**0.0**	**0.0**	**0.0**

NOTE: NA = not applicable.

ACCEPTANCE CRITERIA

Full Activity Control (TA) Average = Range of 90 to 110%
Background Activity Control (NSB) Average = Range of -5 to +6%

Mean background control activity ≤ 15% of the full activity control

TABLE 25: Individual and Mean Full Activity Control and Background Activity Control Values for the Assay Runs (Octocrylene Runs)

Tube Position	Full Activity Control (TA; Full Activity %)			Background Activity Control (NSB; Non-Specific Binding; No Activity %)		
	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3
Beginning	102.49	94.94	102.69	-0.01	0.00	-0.02
	101.89	101.01	101.42	-0.01	-0.05	0.00
End	98.73	93.11	102.49	0.01	0.02	-0.01
	96.89	110.95	93.40	0.01	0.03	0.04
Means	**100.0**	**100.0**	**100.0**	**0.0**	**0.0**	**0.0**
% of Full Activity	**NA**	**NA**	**NA**	**0.0**	**0.0**	**0.0**

NOTE: NA = not applicable.

ACCEPTANCE CRITERIA

Full Activity Control (TA) Average = Range of 90 to 110%
Background Activity Control (NSB) Average = Range of -5 to +6%

Mean background control activity ≤ 15% of the full activity control

TABLE 26: Individual and Mean Full Activity Control and Background Activity Control Values for the Assay Runs (Octylsalicylate Runs)

Tube Position	Full Activity Control (TA; Full Activity %)			Background Activity Control (NSB; Non-Specific Binding; No Activity %)		
	Run 1	**Run 2**	**Run 3**	**Run 1**	**Run 2**	**Run 3**
Beginning	98.65	97.43	103.06	-0.03	0.01	-0.03
	98.07	103.65	101.78	-0.03	-0.04	0.00
End	100.33	100.71	97.12	0.03	0.03	0.00
	102.95	98.21	98.03	0.03	0.01	0.03
Means	**100.0**	**100.0**	**100.0**	**0.0**	**0.0**	**0.0**
% of Full Activity	**NA**	**NA**	**NA**	**0.0**	**0.0**	**0.0**

NOTE: NA = not applicable.

ACCEPTANCE CRITERIA

Full Activity Control (TA) Average = Range of 90 to 110%
Background Activity Control (NSB) Average = Range of -5 to +6%

Mean background control activity ≤ 15% of the full activity control

TABLE 27: Individual and Mean Full Activity Control and Background Activity Control Values for the Assay Runs (Oxybenzone Runs)

Tube Position	Full Activity Control (TA; Full Activity %)			Background Activity Control (NSB; Non-Specific Binding; No Activity %)		
	Run 1	**Run 2**	**Run 3**	**Run 1**	**Run 2**	**Run 3**
Beginning	100.35	96.78	100.64	0.01	-0.01	-0.02
	99.76	102.97	99.39	0.01	-0.06	0.00
End	101.07	97.67	100.04	-0.03	-0.01	0.00
	98.82	102.57	99.94	0.01	0.07	0.01
Means	**100.0**	**100.0**	**100.0**	**0.0**	**0.0**	**0.0**
% of Full Activity	**NA**	**NA**	**NA**	**0.0**	**0.0**	**0.0**

NOTE: NA = not applicable.

ACCEPTANCE CRITERIA

Full Activity Control (TA) Average = Range of 90 to 110%
Background Activity Control (NSB) Average = Range of -5 to +6%

Mean background control activity ≤ 15% of the full activity control

TABLE 28: Solubility Results

Test Substance	Precipitation Code			Comments
	Run 1	Run 2	Run 3	Rx tubes 37°C after addition of Supersomes™
Methoxycinnamate, 10^{-3} M	+++	ND	ND	Cloudy
Methoxycinnamate, 10^{-4} M	+	0	0	
Methoxycinnamate, $10^{-4.5}$ M	ND	0	0	
Methoxycinnamate, 10^{-5} M	0	0	0	
Octocrylene, 10^{-3} M	+++	ND	ND	Cloudy
Octocrylene, 10^{-4} M	+ [(a)]	0	+ [(b)]	[(a)] Very slightly cloudy; [(b)] Cloudy
Octocrylene, $10^{-4.5}$ M	ND	0	0	
Octocrylene, 10^{-5} M	0	0	0	
Oxylsalicylate, 10^{-3} M	+++	ND	ND	Oily substance at top
Oxylsalicylate, 10^{-4} M	++	0	0	
Oxylsalicylate, $10^{-4.5}$ M	ND	0	0	
Oxylsalicylate, 10^{-5} M	0	0	0	
Oxybenzone, 10^{-3} M	++	ND	ND	Cloudy and precipitated
Oxybenzone, 10^{-4} M	0	+	0	
Oxybenzone, $10^{-4.5}$ M	ND	0	0	
Oxybenzone, 10^{-5} M	0	0	0	

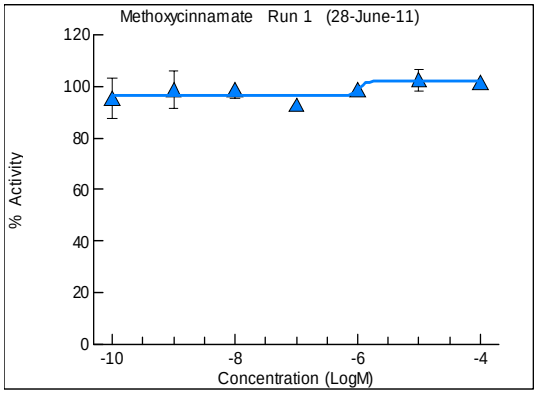
Precipitation Code (Visual):

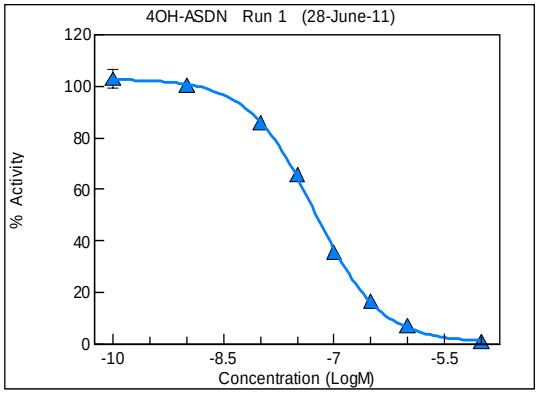
0 = Negative
+ = Small Amount
++ = Moderate Amount
+++ = Substantial Amount
ND = Not determined

FIGURES SECTION

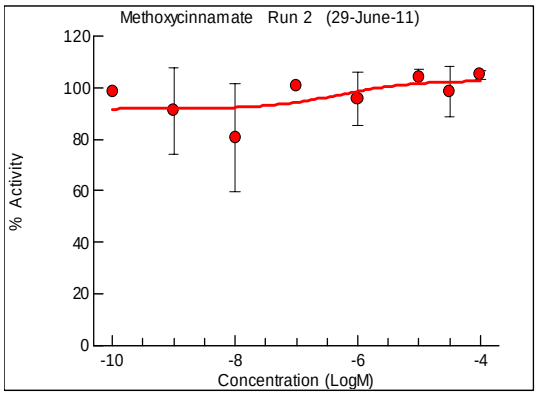
FIGURE 1: Runs 1-3: Methoxycinnamate and 4OH-ASDN

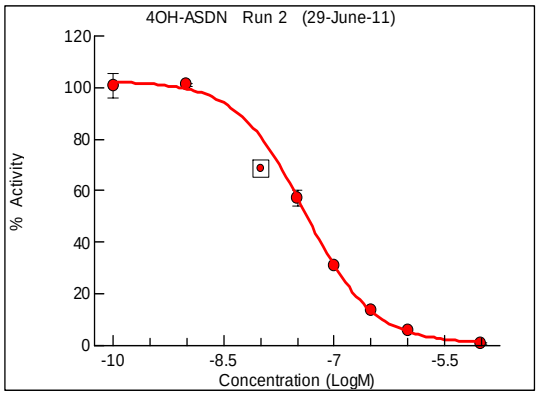
Run 1: 28 June 2011



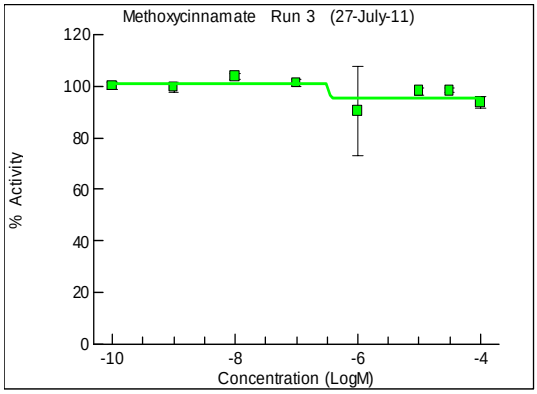


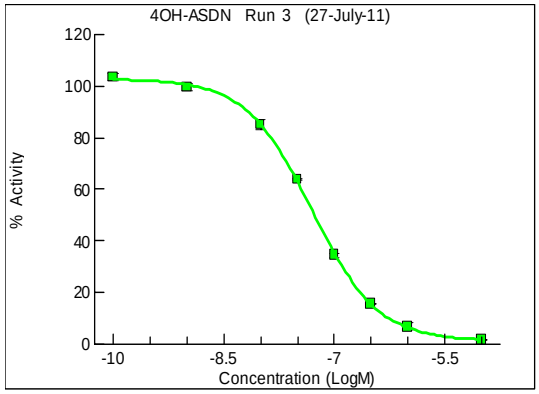
Run 2: 29 June 2011





Run 3: 27 July 2011



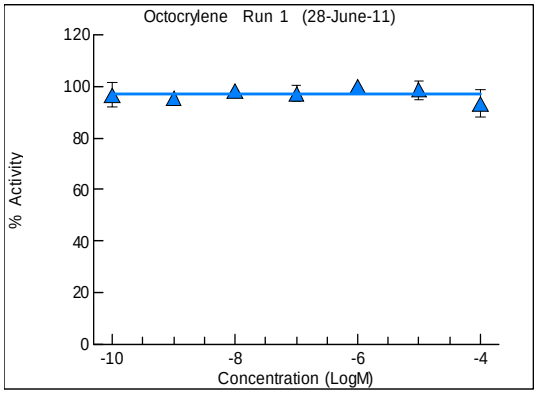


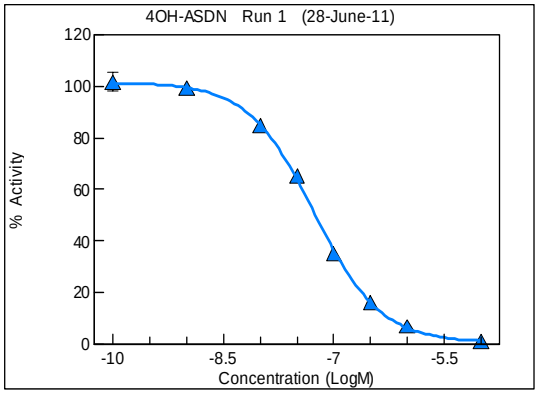
The graphs above represent the data (Means ± Standard Error of the Mean) from three independent runs of the assay (n =3/concentration for test substance; n=2/concentration for 4OH-ASDN).

NOTE: Only soluble concentrations shown (e.g., excluding 10^{-3} M for test substance). Also, 4OH-ASDN value (10^{-8} M) enclosed in the symbol removed from run 2 because of high CV% (34.8%) and thus variability with other runs.

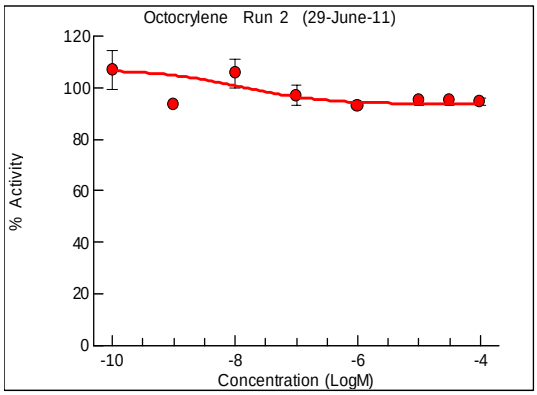
FIGURE 2: Runs 1-3: Octocrylene and 4OH-ASDN

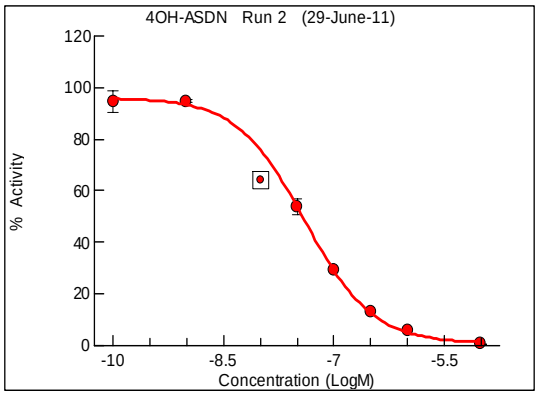
Run 1: 28 June 2011



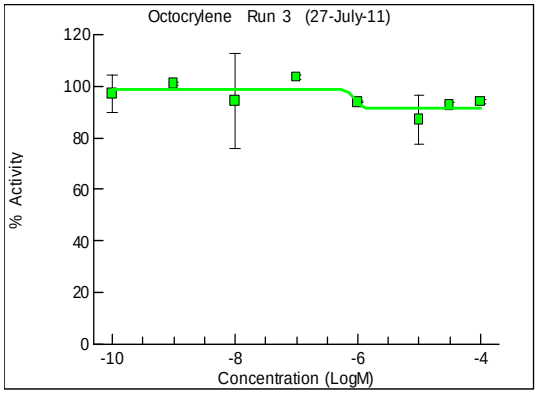


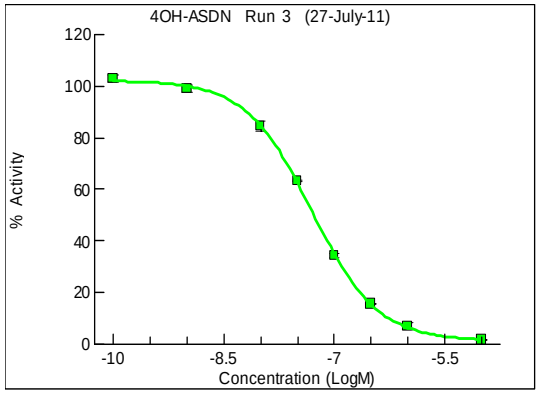
Run 2: 29 June 2011





Run 3: 27 July 2011



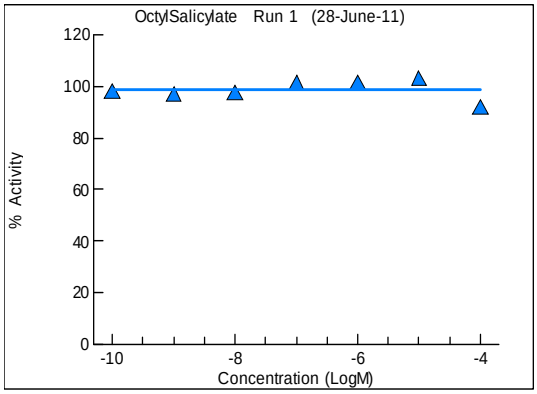


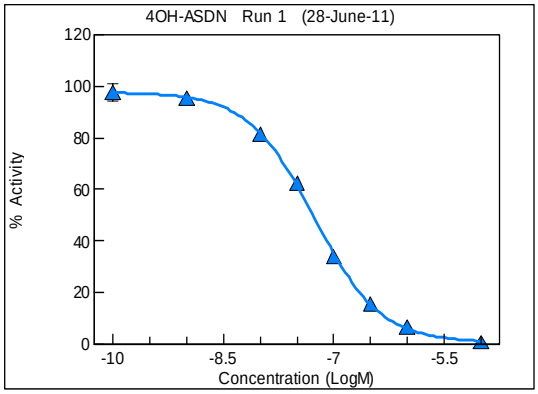
The graphs above represent the data (Means ± Standard Error of the Mean) from three independent runs of the assay (n =3/concentration for test substance; n=2/concentration for 4OH-ASDN).

NOTE: Only soluble concentrations shown (e.g., excluding 10^{-3} M for test substance). Also, 4OH-ASDN value (10^{-8} M) enclosed in the symbol removed from run 2 because of high CV% (34.8%) and thus variability with other runs.

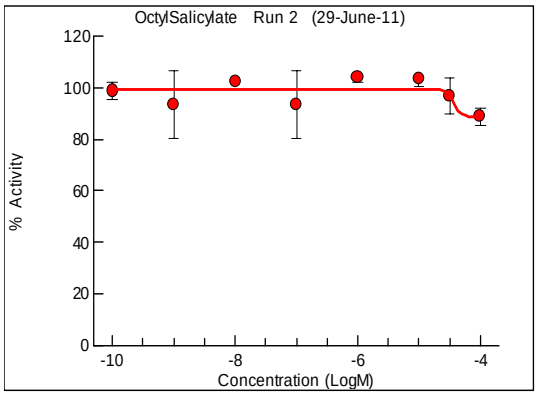
FIGURE 3: Runs 1-3: OctylSalicylate and 4OH-ASDN

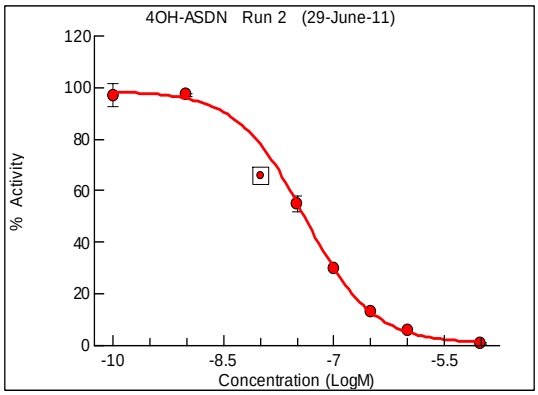
Run 1: 28 June 2011



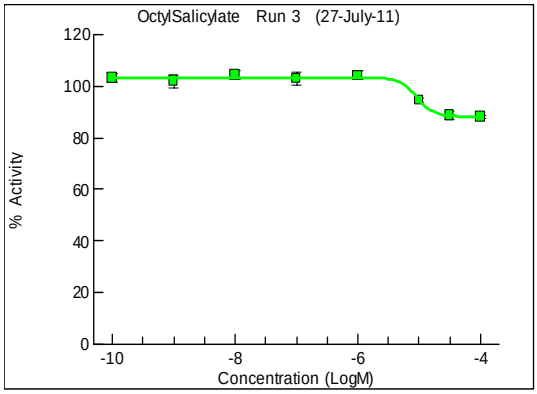


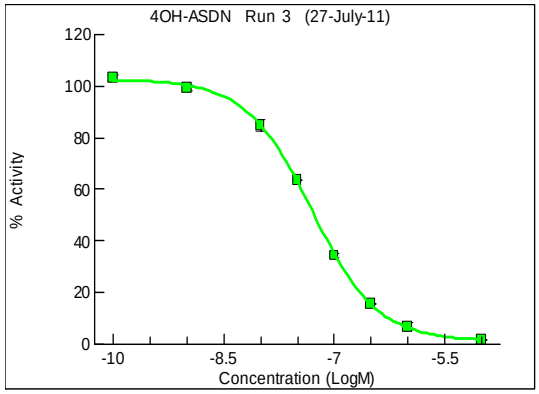
Run 2: 29 June 2011





Run 3: 27 July 2011



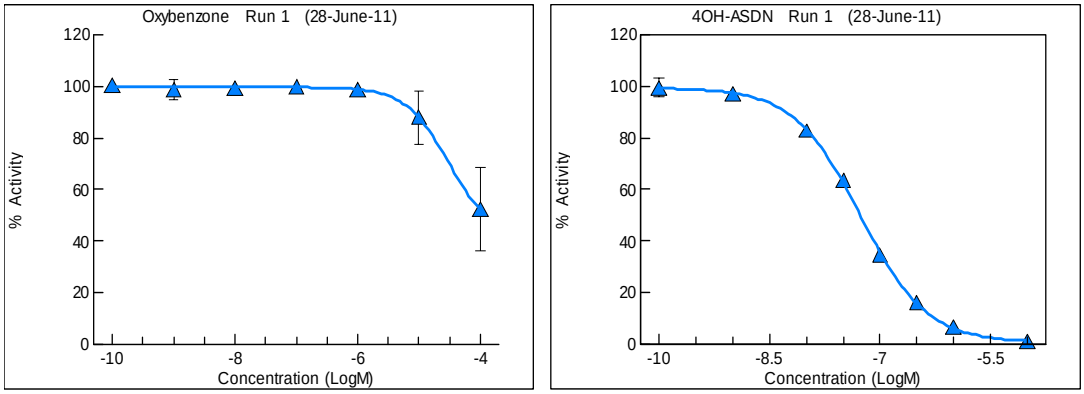


The graphs above represent the data (Means ± Standard Error of the Mean) from three independent runs of the assay (n =3/concentration for test substance; n=2/concentration for 4OH-ASDN).

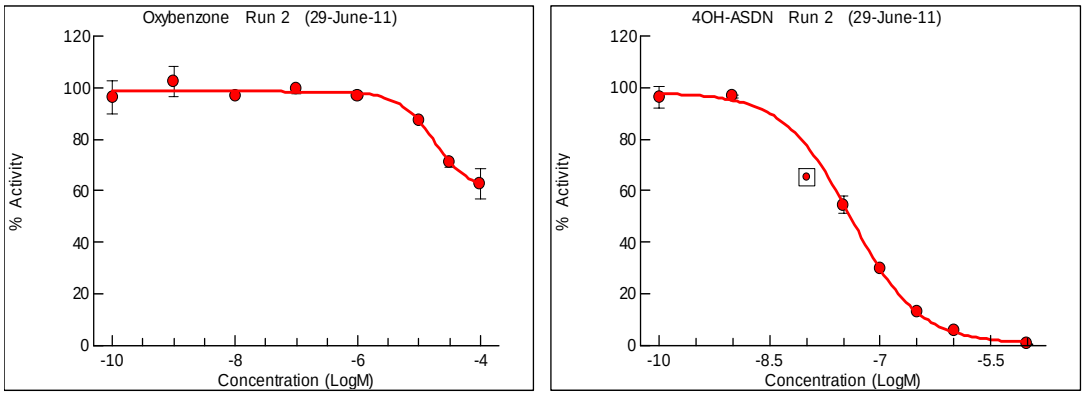
NOTE: Only soluble concentrations shown (e.g., excluding 10^{-3} M for test substance). Also, 4OH-ASDN value (10^{-8} M) enclosed in the symbol removed from run 2 because of high CV% (34.8%) and thus variability with other runs.

FIGURE 4: Runs 1-3: Oxybenzone and 4OH-ASDN

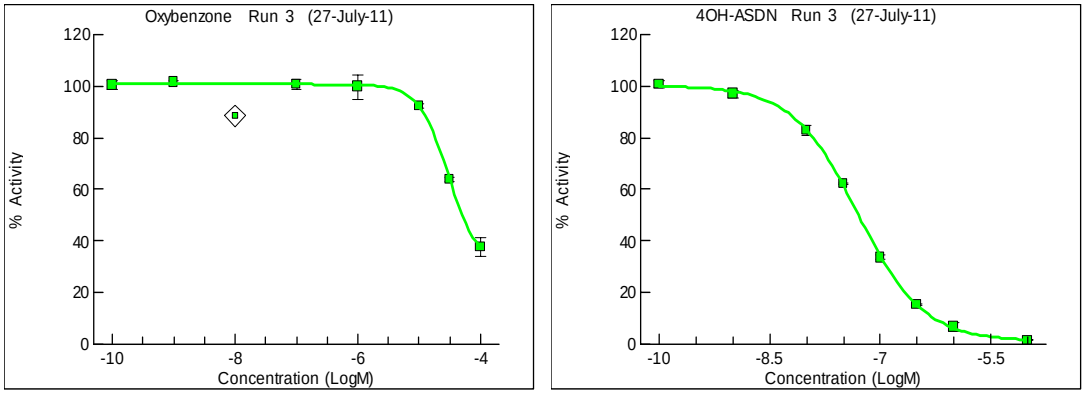
Run 1: 28 June 2011



Run 2: 29 June 2011



Run 3: 27 July 2011



The graphs above represent the data (Means ± Standard Error of the Mean) from three independent runs of the assay (n =3/concentration for test substance; n=2/concentration for 4OH-ASDN).

NOTE: Only soluble concentrations shown (e.g., excluding 10^{-3} M for test substance). Also, oxybenzone value enclosed in the symbol represents outlier removed during the regression analysis using Tukey's Bi-Weight statistical analysis. 4OH-ASDN value (10^{-8} M) enclosed in the symbol removed from run 2 because of high CV% (34.8%) and thus variability with other runs.

FIGURE 5: Mean Response of Runs 1-3: Methoxycinnamate and 4OH-ASDN

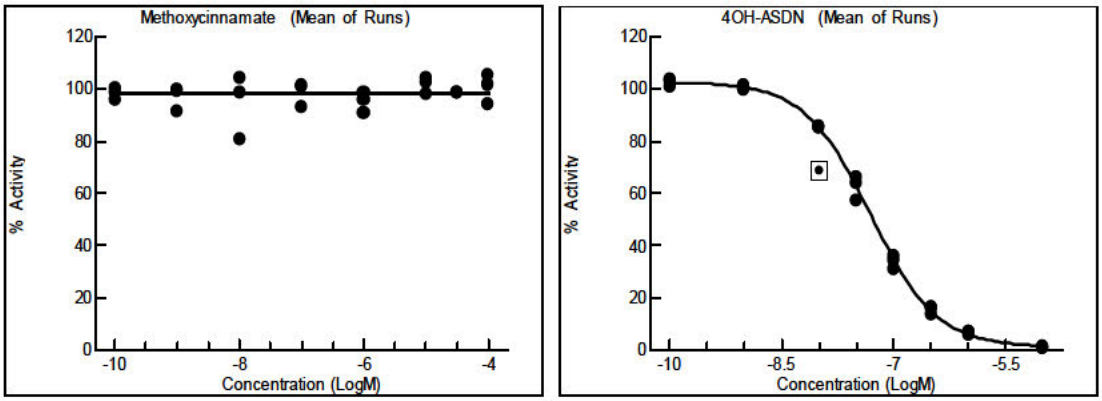


FIGURE 6: Combined Response of Runs 1-3: Methoxycinnamate and 4OH-ASDN

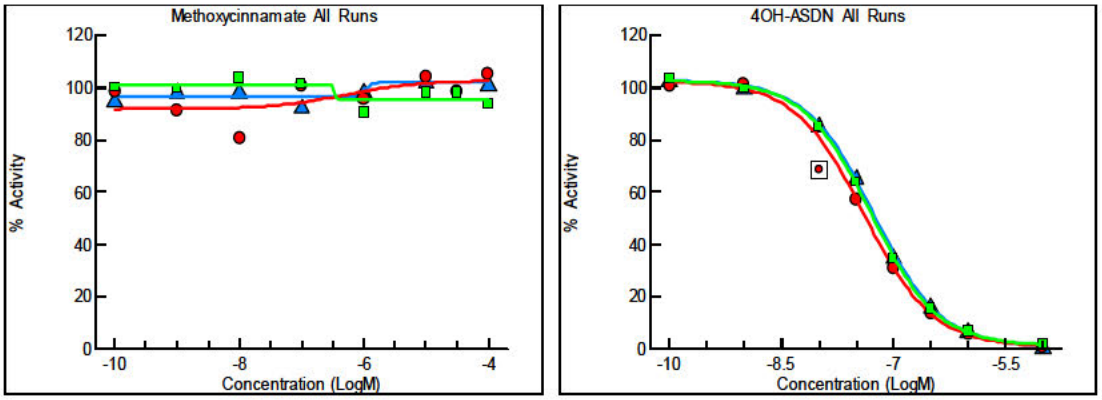
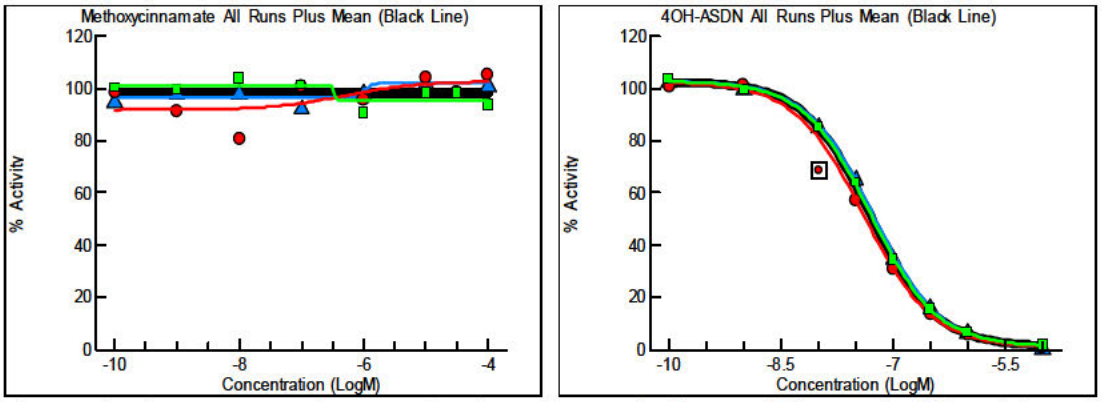


FIGURE 7: Combined Response of Mean and Runs 1-3: Methoxycinnamate and 4OH-ASDN



The graphs above represent the mean data (Means ± Standard Error of the Mean) of three independent runs of the assay (n =3/concentration for test substance; n=2/concentration for 4OH-ASDN).

NOTE: Mean of three runs is the bold, black line. 4OH-ASDN value (10^{-8} M) enclosed in the symbol was removed from run 2 because of high CV% (34.8%) and thus variability with other runs.

FIGURE 8: Mean Response of Runs 1-3: Octocrylene and 4OH-ASDN

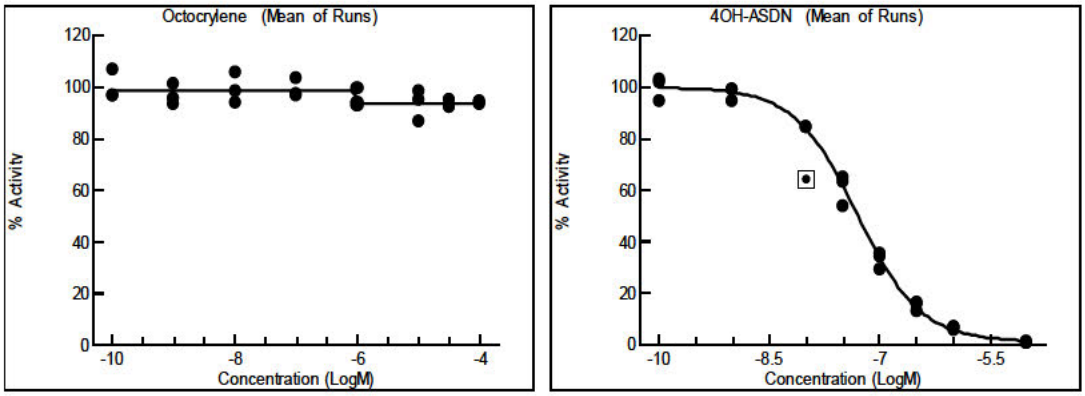


FIGURE 9: Combined Response of Runs 1-3: Octocrylene and 4OH-ASDN

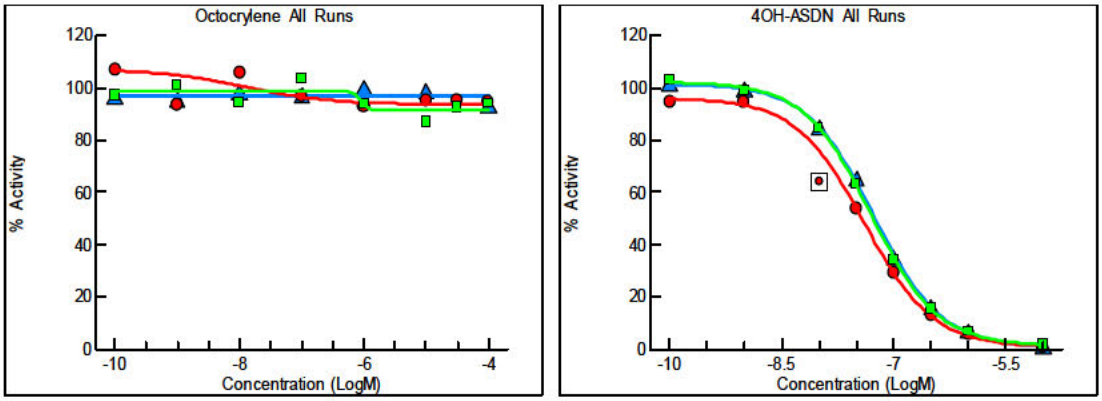
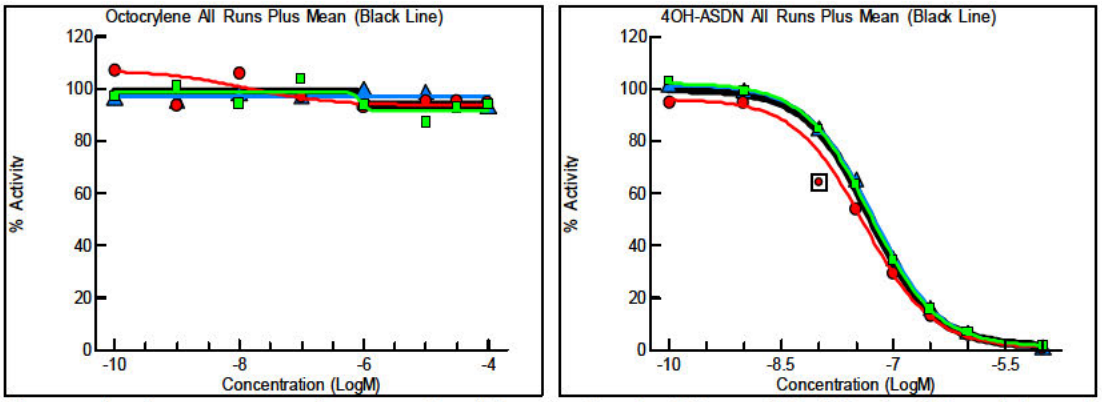


FIGURE 10: Combined Response of Mean and Runs 1-3: Octocrylene and 4OH-ASDN



The graphs above represent the mean data (Means ± Standard Error of the Mean) of three independent runs of the assay (n =3/concentration for test substance; n=2/concentration for 4OH-ASDN).

NOTE: Mean of three runs is the bold, black line. 4OH-ASDN value (10^{-8} M) enclosed in the symbol was removed from run 2 because of high CV% (34.8%) and thus variability with other runs.

FIGURE 11: Mean Response of Runs 1-3: OctylSalicylate and 4OH-ASDN

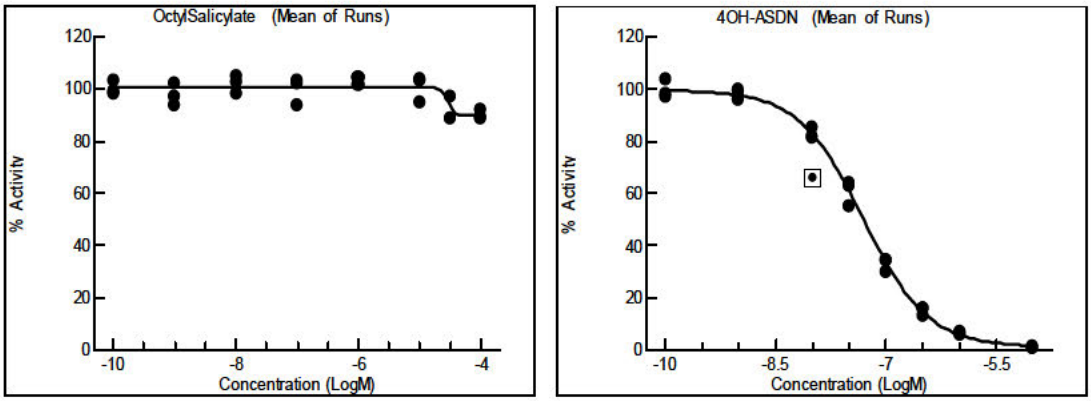


FIGURE 12: Combined Response of Runs 1-3: OctylSalicylate and 4OH-ASDN

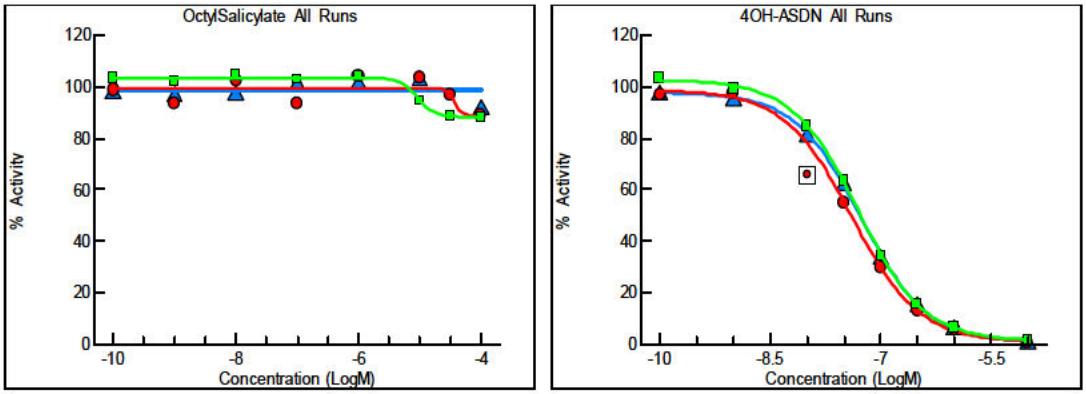
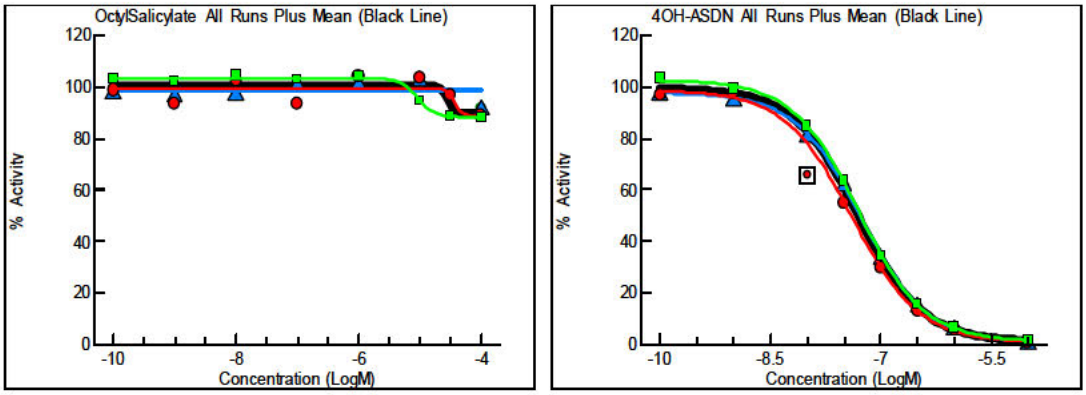


FIGURE 13: Combined Response of Mean and Runs 1-3: OctylSalicylate and 4OH-ASDN



The graphs above represent the mean data (Means ± Standard Error of the Mean) of three independent runs of the assay (n =3/concentration for test substance; n=2/concentration for 4OH-ASDN).

NOTE: Mean of three runs is the bold, black line. 4OH-ASDN value (10^{-8} M) enclosed in the symbol was removed from run 2 because of high CV% (34.8%) and thus variability with other runs.

FIGURE 14: Mean Response of Runs 1-3: Oxybenzone and 4OH-ASDN

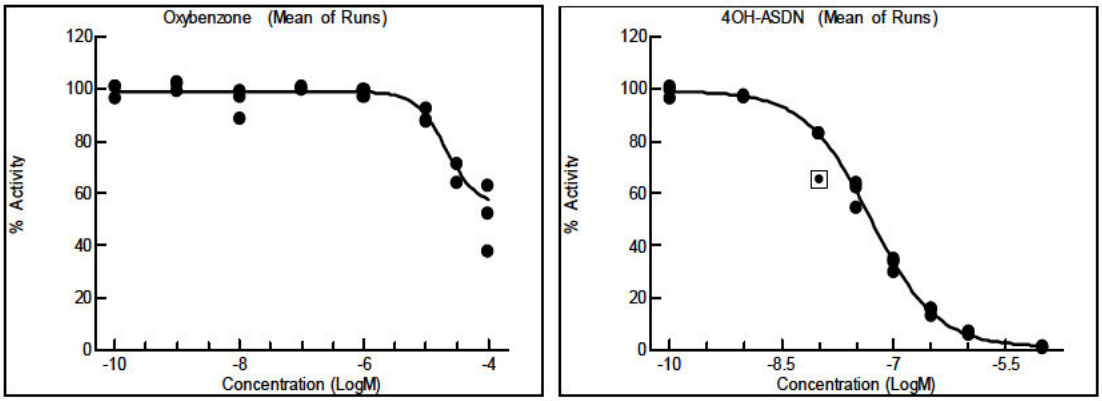


FIGURE 15: Combined Response of Runs 1-3: Oxybenzone and 4OH-ASDN

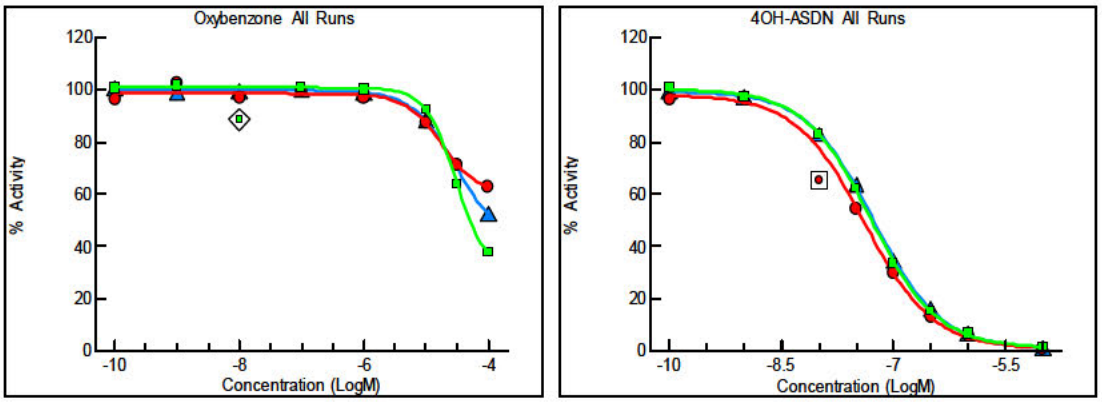
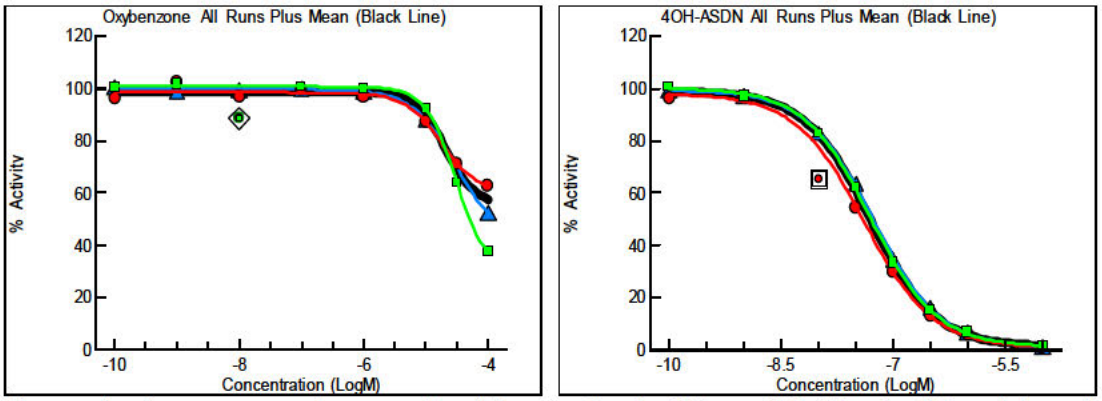


FIGURE 16: Combined Response of Mean and Runs 1-3: Oxybenzone and 4OH-ASDN



The graphs above represent the mean data (Means ± Standard Error of the Mean) of three independent runs of the assay (n =3/concentration for test substance; n=2/concentration for 4OH-ASDN).

NOTE: Mean of three runs is the bold, black line. Also, oxybenzone value enclosed in the symbol represents outlier removed during the regression analysis using Tukey's Bi-Weight statistical analysis. 4OH-ASDN value (10^{-8} M) enclosed in the symbol removed from run 2 because of high CV% (34.8%) and thus variability with other runs.

APPENDICES SECTION

APPENDIX 1: Run 1: Assay Information (Methoxycinnamate)

Experiment Date:	28-Jun-11	Study Number:	9070-100107AROM		
Test substance:	Methoxycinnamate				
2/3/2012 11:52					
	specific activity based on decay for 4/20/10		42240.0	DPM	
	20 uL count of 3H-ASDN (mean)		41270.7	DPM	
	0.5 mL count for total activity		7494.9	DPM	
	microsomal protein/assay		0.008	mg	
	Reaction time		15	min	
	20 uL count of 3H-ASDN (DPM)		42157	40831	40824

Assays Conducted by:	[redacted]				
Each assay contained 100 uL 3H-ASDN		206353.3	DPM	0.200	(nmoles)
Total product 3H-H20 per assay		29979.5	DPM	0.029	(nmoles)
Percent conversion to product (3H-H20) (percent)		14.5			
Rate of conversion to 3H-H20 in total activity assay		0.242	nmol/(mg protein-min)		
Average activity of control Tubes		0.241	nmol/(mg protein-min)		
	Average full enzyme activity controls (percent +/-SD)	100.0	4.2		
	Average background activity controls (percent +/- SD)	0.0	0.0		

Spreadsheet locked on: 06/30/2011
Green shaded areas: unlocked cells for data entry

APPENDIX 1: Run 1: Raw and Normalized DPM Data (4OH-ASDN and Methoxycinnamate): Part 1 of 4

Sample Type	Concentration	DPM1/aliquot (aliquot 1)	DPM1/aliquot (aliquot 2)	DPM1/mL (aliquot 1)	DPM2/mL (aliquot 2)	Average DPM/mL	Stdev DPM/mL	CV DPM/mL (%)	Total DPM	Total DPM present in assay	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
TA		7816.0	7744.0	15632.0	15488.0	15560.0	101.82	0.65	31120.0	206353.3	15.1	30954.0	0.030	0.250
TA		7763.0	7706.0	15526.0	15412.0	15469.0	80.61	0.52	30938.0	206353.3	15.0	30772.0	0.030	0.249
NSB		42.0	40.0	84.0	80.0	82.0	2.83	3.45	164.0	206353.3	0.1	-2.0	0.000	0.000
NSB		43.0	39.0	86.0	78.0	82.0	5.66	6.90	164.0	206353.3	0.1	-2.0	0.000	0.000
4OH-ASDN	-5	101.0	100.0	202.0	200.0	201.0	1.41	0.70	402.0	206353.3	0.2	236.0	0.000	0.002
4OH-ASDN		99.0	95.0	198.0	190.0	194.0	5.66	2.92	388.0	206353.3	0.2	222.0	0.000	0.002
4OH-ASDN	-6	479.0	506.0	958.0	1012.0	985.0	38.18	3.88	1970.0	206353.3	1.0	1804.0	0.002	0.015
4OH-ASDN		472.0	488.0	944.0	976.0	960.0	22.63	2.36	1920.0	206353.3	0.9	1754.0	0.002	0.014
4OH-ASDN	-6.5	1274.0	1279.0	2548.0	2558.0	2553.0	7.07	0.28	5106.0	206353.3	2.5	4940.0	0.005	0.040
4OH-ASDN		1264.0	1276.0	2528.0	2552.0	2540.0	16.97	0.67	5080.0	206353.3	2.5	4914.0	0.005	0.040
4OH-ASDN	-7	2693.0	2697.0	5386.0	5394.0	5390.0	5.66	0.10	10780.0	206353.3	5.2	10614.0	0.010	0.086
4OH-ASDN		2754.0	2691.0	5508.0	5382.0	5445.0	89.10	1.64	10890.0	206353.3	5.3	10724.0	0.010	0.087
4OH-ASDN	-7.5	4942.0	4928.0	9884.0	9856.0	9870.0	19.80	0.20	19740.0	206353.3	9.6	19574.0	0.019	0.158
4OH-ASDN		5052.0	4907.0	10104.0	9814.0	9959.0	205.06	2.06	19918.0	206353.3	9.7	19752.0	0.019	0.160
4OH-ASDN	-8	6457.0	6447.0	12914.0	12894.0	12904.0	14.14	0.11	25808.0	206353.3	12.5	25642.0	0.025	0.207
4OH-ASDN		6558.0	6276.0	13116.0	12552.0	12834.0	398.81	3.11	25668.0	206353.3	12.4	25502.0	0.025	0.206
4OH-ASDN	-9	7577.0	7618.0	15154.0	15236.0	15195.0	57.98	0.38	30390.0	206353.3	14.7	30224.0	0.029	0.244
4OH-ASDN		7579.0	7359.0	15158.0	14718.0	14938.0	311.13	2.08	29876.0	206353.3	14.5	29710.0	0.029	0.240
4OH-ASDN	-10	7536.0	7519.0	15072.0	15038.0	15055.0	24.04	0.16	30110.0	206353.3	14.6	29944.0	0.029	0.242
4OH-ASDN		7945.0	7879.0	15890.0	15758.0	15824.0	93.34	0.59	31648.0	206353.3	15.3	31482.0	0.031	0.254

TA = Full Activity Control (Total Activity); NSB = Background Activity Control (Non-Specific Binding)

APPENDIX 1: Run 1: Raw and Normalized DPM Data (4OH-ASDN and Methoxycinnamate): Part 2 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
103.83	103.52	0.305	0.432	0.417
103.21				
-0.01	-0.01	0.000	0.000	0.000
-0.01				
0.79	0.77	0.023	0.033	4.323
0.74				
6.05	5.97	0.084	0.119	1.987
5.88				
16.57	16.53	0.044	0.062	0.373
16.48				
35.60	35.79	0.184	0.261	0.729
35.97				
65.65	65.95	0.299	0.422	0.640
66.25				
86.01	85.77	0.235	0.332	0.387
85.54				
101.38	100.51	0.862	1.219	1.213
99.65				
100.44	103.02	2.579	3.648	3.541
105.60				

APPENDIX 1: Run 1: Raw and Normalized DPM Data (4OH-ASDN and Methoxycinnamate): Part 3 of 4

Sample Type	Concentration	DPM1/aliquot (aliquot 1)	DPM1/aliquot (aliquot 2)	DPM1/mL (aliquot 1)	DPM2/mL (aliquot 2)	Average DPM/mL	Stdev DPM/mL	CV DPM/mL (%)	Total DPM	Total DPM present in assay	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
Methoxycinnamate	-3	7226.0	7490.0	14452.0	14980.0	14716.0	373.35	2.54	29432.0	206353.3	14.3	29266.0	0.028	0.236
Methoxycinnamate	-3	7201.0	7305.0	14402.0	14610.0	14506.0	147.08	1.01	29012.0	206353.3	14.1	28846.0	0.028	0.233
Methoxycinnamate	-3	7351.0	7359.0	14702.0	14718.0	14710.0	11.31	0.08	29420.0	206353.3	14.3	29254.0	0.028	0.236
Methoxycinnamate	-4	7627.0	7556.0	15254.0	15112.0	15183.0	100.41	0.66	30366.0	206353.3	14.7	30200.0	0.029	0.244
Methoxycinnamate	-4	7383.0	7251.0	14766.0	14502.0	14634.0	186.68	1.28	29268.0	206353.3	14.2	29102.0	0.028	0.235
Methoxycinnamate	-4	8079.0	7783.0	16158.0	15566.0	15862.0	418.61	2.64	31724.0	206353.3	15.4	31558.0	0.031	0.255
Methoxycinnamate	-5	7677.0	7664.0	15354.0	15328.0	15341.0	18.38	0.12	30682.0	206353.3	14.9	30516.0	0.030	0.246
Methoxycinnamate	-5	7771.0	7778.0	15542.0	15556.0	15549.0	9.90	0.06	31098.0	206353.3	15.1	30932.0	0.030	0.250
Methoxycinnamate	-5	7703.0	7564.0	15406.0	15128.0	15267.0	196.58	1.29	30534.0	206353.3	14.8	30368.0	0.029	0.245
Methoxycinnamate	-6	7499.0	7551.0	14998.0	15102.0	15050.0	73.54	0.49	30100.0	206353.3	14.6	29934.0	0.029	0.242
Methoxycinnamate	-6	7517.0	7547.0	15034.0	15094.0	15064.0	42.43	0.28	30128.0	206353.3	14.6	29962.0	0.029	0.242
Methoxycinnamate	-6	7225.0	7036.0	14450.0	14072.0	14261.0	267.29	1.87	28522.0	206353.3	13.8	28356.0	0.027	0.229
Methoxycinnamate	-7	7052.0	7119.0	14104.0	14238.0	14171.0	94.75	0.67	28342.0	206353.3	13.7	28176.0	0.027	0.228
Methoxycinnamate	-7	7402.0	7520.0	14804.0	15040.0	14922.0	166.88	1.12	29844.0	206353.3	14.5	29678.0	0.029	0.240
Methoxycinnamate	-7	6347.0	6371.0	12694.0	12742.0	12718.0	33.94	0.27	25436.0	206353.3	12.3	25270.0	0.024	0.204
Methoxycinnamate	-8	7075.0	7373.0	14150.0	14746.0	14448.0	421.44	2.92	28896.0	206353.3	14.0	28730.0	0.028	0.232
Methoxycinnamate	-8	6813.0	7031.0	13626.0	14062.0	13844.0	308.30	2.23	27688.0	206353.3	13.4	27522.0	0.027	0.222
Methoxycinnamate	-8	7937.0	8131.0	15874.0	16262.0	16068.0	274.36	1.71	32136.0	206353.3	15.6	31970.0	0.031	0.258
Methoxycinnamate	-9	7087.0	7248.0	14174.0	14496.0	14335.0	227.69	1.59	28670.0	206353.3	13.9	28504.0	0.028	0.230
Methoxycinnamate	-9	7346.0	7249.0	14692.0	14498.0	14595.0	137.18	0.94	29190.0	206353.3	14.1	29024.0	0.028	0.234
Methoxycinnamate	-9	7768.0	7756.0	15536.0	15512.0	15524.0	16.97	0.11	31048.0	206353.3	15.0	30882.0	0.030	0.249
Methoxycinnamate	-10	6841.0	6756.0	13682.0	13512.0	13597.0	120.21	0.88	27194.0	206353.3	13.2	27028.0	0.026	0.218
Methoxycinnamate	-10	7387.0	7321.0	14774.0	14642.0	14708.0	93.34	0.63	29416.0	206353.3	14.3	29250.0	0.028	0.236
Methoxycinnamate	-10	7302.0	7358.0	14604.0	14716.0	14660.0	79.20	0.54	29320.0	206353.3	14.2	29154.0	0.028	0.235
TA		7187.0	7064.0	14374.0	14128.0	14251.0	173.95	1.22	28502.0	206353.3	13.8	28336.0	0.027	0.229
TA		7367.0	7312.0	14734.0	14624.0	14679.0	77.78	0.53	29358.0	206353.3	14.2	29192.0	0.028	0.236
NSB		34.0	44.0	68.0	88.0	78.0	14.14	18.13	156.0	206353.3	0.1	-10.0	0.000	0.000
NSB		48.0	42.0	96.0	84.0	90.0	8.49	9.43	180.0	206353.3	0.1	14.0	0.000	0.000

TA = Full Activity Control (Total Activity); NSB = Background Activity Control (Non-Specific Binding)

APPENDIX 1: Run 1: Raw and Normalized DPM Data (4OH-ASDN and Methoxycinnamate): Part 4 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
98.16	97.68	0.463	0.802	0.821
96.75				
98.12				
101.30	101.59	2.383	4.127	4.062
97.61				
105.85				
102.36	102.66	0.566	0.981	0.955
103.75				
101.86				
100.40	98.67	1.780	3.083	3.125
100.50				
95.11				
94.51	92.94	4.340	7.517	8.088
99.55				
84.76				
96.37	98.64	4.454	7.715	7.821
92.31				
107.23				
95.61	98.85	2.421	4.193	4.242
97.35				
103.58				
90.66	95.52	2.432	4.213	4.411
98.11				
97.79				
95.04	96.48	1.436	2.030	2.104
97.92				
-0.03	0.01	0.040	0.057	848.528
0.05				

APPENDIX 1: Run 1: Assay Information (Octylcrylene)

Experiment Date:	28-Jun-11	Study Number:	9070-100107AROM		
Test substance:	Octocrylene				
2/3/2012 13:38					
	specific activity based on decay for 4/20/10	42240.0	DPM		
	20 uL count of 3H-ASDN (mean)	41270.7	DPM		
	0.5 mL count for total activity	7592.0	DPM		
	microsomal protein/assay	0.008	mg		
	Reaction time	15	min		
	20 uL count of 3H-ASDN (DPM)	42157	40831	40824	

Assays Conducted by:	[redacted]			Spreadsheet locked on: 06/30/2011	
				Green shaded areas: unlocked cells for data entry	
Each assay contained 100 uL 3H-ASDN	206353.3	DPM	0.200	(nmoles)	
Total product 3H-H20 per assay	30368.0	DPM	0.029	(nmoles)	
Percent conversion to product (3H-H20) (percent)	14.7				
Rate of conversion to 3H-H20 in total activity assay	0.245	nmol/(mg protein-min)			
Average activity of control Tubes	0.244	nmol/(mg protein-min)			
Average full enzyme activity controls (percent +/-SD)	100.0	2.6			
Average background activity controls (percent +/- SD)	0.0	0.0			

APPENDIX 1: Run 1: Raw and Normalized DPM Data (4OH-ASDN and Octylcrylene): Part 1 of 4

Sample Type	Concentration	DPM1/aliquot (aliquot 1)	DPM1/aliquot (aliquot 2)	DPM1/mL (aliquot 1)	DPM2/mL (aliquot 2)	Average DPM/mL	Stdev DPM/mL	CV DPM/mL (%)	Total DPM	Total DPM present in assay	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
TA		7816.0	7744.0	15632.0	15488.0	15560.0	101.82	0.65	31120.0	206353.3	15.1	30952.5	0.030	0.250
TA		7763.0	7706.0	15526.0	15412.0	15469.0	80.61	0.52	30938.0	206353.3	15.0	30770.5	0.030	0.249
NSB		42.0	40.0	84.0	80.0	82.0	2.83	3.45	164.0	206353.3	0.1	-3.5	0.000	0.000
NSB		43.0	39.0	86.0	78.0	82.0	5.66	6.90	164.0	206353.3	0.1	-3.5	0.000	0.000
4OH-ASDN	-5	101.0	100.0	202.0	200.0	201.0	1.41	0.70	402.0	206353.3	0.2	234.5	0.000	0.002
4OH-ASDN		99.0	95.0	198.0	190.0	194.0	5.66	2.92	388.0	206353.3	0.2	220.5	0.000	0.002
4OH-ASDN	-6	479.0	506.0	958.0	1012.0	985.0	38.18	3.88	1970.0	206353.3	1.0	1802.5	0.002	0.015
4OH-ASDN		472.0	488.0	944.0	976.0	960.0	22.63	2.36	1920.0	206353.3	0.9	1752.5	0.002	0.014
4OH-ASDN	-6.5	1274.0	1279.0	2548.0	2558.0	2553.0	7.07	0.28	5106.0	206353.3	2.5	4938.5	0.005	0.040
4OH-ASDN		1264.0	1276.0	2528.0	2552.0	2540.0	16.97	0.67	5080.0	206353.3	2.5	4912.5	0.005	0.040
4OH-ASDN	-7	2693.0	2697.0	5386.0	5394.0	5390.0	5.66	0.10	10780.0	206353.3	5.2	10612.5	0.010	0.086
4OH-ASDN		2754.0	2691.0	5508.0	5382.0	5445.0	89.10	1.64	10890.0	206353.3	5.3	10722.5	0.010	0.087
4OH-ASDN	-7.5	4942.0	4928.0	9884.0	9856.0	9870.0	19.80	0.20	19740.0	206353.3	9.6	19572.5	0.019	0.158
4OH-ASDN		5052.0	4907.0	10104.0	9814.0	9959.0	205.06	2.06	19918.0	206353.3	9.7	19750.5	0.019	0.160
4OH-ASDN	-8	6457.0	6447.0	12914.0	12894.0	12904.0	14.14	0.11	25808.0	206353.3	12.5	25640.5	0.025	0.207
4OH-ASDN		6558.0	6276.0	13116.0	12552.0	12834.0	398.81	3.11	25668.0	206353.3	12.4	25500.5	0.025	0.206
4OH-ASDN	-9	7577.0	7618.0	15154.0	15236.0	15195.0	57.98	0.38	30390.0	206353.3	14.7	30222.5	0.029	0.244
4OH-ASDN		7579.0	7359.0	15158.0	14718.0	14938.0	311.13	2.08	29876.0	206353.3	14.5	29708.5	0.029	0.240
4OH-ASDN	-10	7536.0	7519.0	15072.0	15038.0	15055.0	24.04	0.16	30110.0	206353.3	14.6	29942.5	0.029	0.242
4OH-ASDN		7945.0	7879.0	15890.0	15758.0	15824.0	93.34	0.59	31648.0	206353.3	15.3	31480.5	0.031	0.254

TA = Full Activity Control (Total Activity); NSB = Background Activity Control (Non-Specific Binding)

APPENDIX 1: Run 1: Raw and Normalized DPM Data (4OH-ASDN and Octylcrylene): Part 2 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
102.49	102.19	0.301	0.426	0.417
101.89				
-0.01	-0.01	0.000	0.000	0.000
-0.01				
0.78	0.75	0.023	0.033	4.351
0.73				
5.97	5.89	0.083	0.117	1.989
5.80				
16.35	16.31	0.043	0.061	0.373
16.27				
35.14	35.32	0.182	0.258	0.729
35.50				
64.81	65.10	0.295	0.417	0.640
65.40				
84.90	84.67	0.232	0.328	0.387
84.44				
100.07	99.22	0.851	1.203	1.213
98.37				
99.15	101.69	2.546	3.601	3.541
104.24				

APPENDIX 1: Run 1: Raw and Normalized DPM Data (4OH-ASDN and Octylcrylene): Part 3 of 4

Sample Type	Concentration	DPM1/aliquot (aliquot 1)	DPM1/aliquot (aliquot 2)	DPM1/mL (aliquot 1)	DPM2/mL (aliquot 2)	Average DPM/mL	Stdev DPM/mL	CV DPM/mL (%)	Total DPM	Total DPM present in assay	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
Octocrylene	-3	6759.0	6765.0	13518.0	13530.0	13524.0	8.49	0.06	27048.0	206353.3	13.1	26880.5	0.026	0.217
Octocrylene	-3	5943.0	5988.0	11886.0	11976.0	11931.0	63.64	0.53	23862.0	206353.3	11.6	23694.5	0.023	0.191
Octocrylene	-3	6416.0	6362.0	12832.0	12724.0	12778.0	76.37	0.60	25556.0	206353.3	12.4	25388.5	0.025	0.205
Octocrylene	-4	7217.0	7229.0	14434.0	14458.0	14446.0	16.97	0.12	28892.0	206353.3	14.0	28724.5	0.028	0.232
Octocrylene	-4	7350.0	6203.0	14700.0	12406.0	13553.0	1622.10	11.97	27106.0	206353.3	13.1	26938.5	0.026	0.218
Octocrylene	-4	7267.0	7291.0	14534.0	14582.0	14558.0	33.94	0.23	29116.0	206353.3	14.1	28948.5	0.028	0.234
Octocrylene	-5	7448.0	7527.0	14896.0	15054.0	14975.0	111.72	0.75	29950.0	206353.3	14.5	29782.5	0.029	0.241
Octocrylene	-5	7318.0	7223.0	14636.0	14446.0	14541.0	134.35	0.92	29082.0	206353.3	14.1	28914.5	0.028	0.234
Octocrylene	-5	7676.0	7795.0	15352.0	15590.0	15471.0	168.29	1.09	30942.0	206353.3	15.0	30774.5	0.030	0.249
Octocrylene	-6	7568.0	7555.0	15136.0	15110.0	15123.0	18.38	0.12	30246.0	206353.3	14.7	30078.5	0.029	0.243
Octocrylene	-6	7638.0	7563.0	15276.0	15126.0	15201.0	106.07	0.70	30402.0	206353.3	14.7	30234.5	0.029	0.244
Octocrylene	-6	7599.0	7517.0	15198.0	15034.0	15116.0	115.97	0.77	30232.0	206353.3	14.7	30064.5	0.029	0.243
Octocrylene	-7	7251.0	7362.0	14502.0	14724.0	14613.0	156.98	1.07	29226.0	206353.3	14.2	29058.5	0.028	0.235
Octocrylene	-7	7506.0	7388.0	15012.0	14776.0	14894.0	166.88	1.12	29788.0	206353.3	14.4	29620.5	0.029	0.239
Octocrylene	-7	7393.0	7402.0	14786.0	14804.0	14795.0	12.73	0.09	29590.0	206353.3	14.3	29422.5	0.029	0.238
Octocrylene	-8	7271.0	7083.0	14542.0	14166.0	14354.0	265.87	1.85	28708.0	206353.3	13.9	28540.5	0.028	0.231
Octocrylene	-8	7972.0	7807.0	15944.0	15614.0	15779.0	233.35	1.48	31558.0	206353.3	15.3	31390.5	0.030	0.254
Octocrylene	-8	7413.0	7314.0	14826.0	14628.0	14727.0	140.01	0.95	29454.0	206353.3	14.3	29286.5	0.028	0.237
Octocrylene	-9	7668.0	7588.0	15336.0	15176.0	15256.0	113.14	0.74	30512.0	206353.3	14.8	30344.5	0.029	0.245
Octocrylene	-9	7405.0	7525.0	14810.0	15050.0	14930.0	169.71	1.14	29860.0	206353.3	14.5	29692.5	0.029	0.240
Octocrylene	-9	6647.0	6685.0	13294.0	13370.0	13332.0	53.74	0.40	26664.0	206353.3	12.9	26496.5	0.026	0.214
Octocrylene	-10	7117.0	7449.0	14234.0	14898.0	14566.0	469.52	3.22	29132.0	206353.3	14.1	28964.5	0.028	0.234
Octocrylene	-10	7448.0	7411.0	14896.0	14822.0	14859.0	52.33	0.35	29718.0	206353.3	14.4	29550.5	0.029	0.239
Octocrylene	-10	7127.0	7536.0	14254.0	15072.0	14663.0	578.41	3.94	29326.0	206353.3	14.2	29158.5	0.028	0.236
TA		7552.0	7440.0	15104.0	14880.0	14992.0	158.39	1.06	29984.0	206353.3	14.5	29816.5	0.029	0.241
TA		7252.0	7463.0	14504.0	14926.0	14715.0	298.40	2.03	29430.0	206353.3	14.3	29262.5	0.028	0.236
NSB		40.0	45.0	80.0	90.0	85.0	7.07	8.32	170.0	206353.3	0.1	2.5	0.000	0.000
NSB		40.0	46.0	80.0	92.0	86.0	8.49	9.87	172.0	206353.3	0.1	4.5	0.000	0.000

TA = Full Activity Control (Total Activity); NSB = Background Activity Control (Non-Specific Binding)

APPENDIX 1: Run 1: Raw and Normalized DPM Data (4OH-ASDN and Octylcrylene): Part 4 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
89.01	83.84	3.047	5.278	6.295
78.46				
84.07				
95.11	93.39	2.106	3.647	3.906
89.20				
95.85				
98.62	98.75	1.779	3.082	3.121
95.74				
101.90				
99.60	99.75	0.180	0.312	0.313
100.11				
99.55				
96.22	97.24	0.545	0.944	0.971
98.08				
97.42				
94.50	98.47	2.825	4.894	4.970
103.94				
96.97				
100.48	95.51	3.937	6.819	7.140
98.32				
87.74				
95.91	96.77	0.571	0.988	1.021
97.85				
96.55				
98.73	97.81	0.917	1.297	1.326
96.89				
0.01	0.01	0.003	0.005	40.406
0.01				

APPENDIX 1: Run 1: Assay Information (Octylsalicylate)

Experiment Date:	28-Jun-11	Study Number:	9070-100107AROM		
Test substance:	OctylSalicylate				
2/3/2012 14:22					
	specific activity based on decay for 4/20/10		42240.0	DPM	
	20 uL count of 3H-ASDN (mean)		41270.7	DPM	
	0.5 mL count for total activity		7886.3	DPM	
	microsomal protein/assay		0.008	mg	
	Reaction time		15	min	
	20 uL count of 3H-ASDN (DPM)		42157	40831	40824

Assays Conducted by:	[redacted]			Spreadsheet locked on: 06/30/2011	
				Green shaded areas: unlocked cells for data entry	
Each assay contained 100 uL 3H-ASDN		206353.3	DPM	0.200	(nmoles)
Total product 3H-H20 per assay		31545.0	DPM	0.031	(nmoles)
Percent conversion to product (3H-H2O) (percent)		15.3			
Rate of conversion to 3H-H2O in total activity assay		0.255	nmol/(mg protein-min)		
Average activity of control Tubes		0.253	nmol/(mg protein-min)		
	Average full enzyme activity controls (percent +/-SD)	100.0	2.2		
	Average background activity controls (percent +/- SD)	0.0	0.0		

APPENDIX 1: Run 1: Raw and Normalized DPM Data (4OH-ASDN and (Octylsalicylate): Part 1 of 4

Sample Type	Concentration	DPM1/aliquot (aliquot 1)	DPM1/aliquot (aliquot 2)	DPM1/mL (aliquot 1)	DPM2/mL (aliquot 2)	Average DPM/mL	Stdev DPM/mL	CV DPM/mL (%)	Total DPM	Total DPM present in assay	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
TA		7816.0	7744.0	15632.0	15488.0	15560.0	101.82	0.65	31120.0	206353.3	15.1	30946.0	0.030	0.250
TA		7763.0	7706.0	15526.0	15412.0	15469.0	80.61	0.52	30938.0	206353.3	15.0	30764.0	0.030	0.248
NSB		42.0	40.0	84.0	80.0	82.0	2.83	3.45	164.0	206353.3	0.1	-10.0	0.000	0.000
NSB		43.0	39.0	86.0	78.0	82.0	5.66	6.90	164.0	206353.3	0.1	-10.0	0.000	0.000
4OH-ASDN	-5	101.0	100.0	202.0	200.0	201.0	1.41	0.70	402.0	206353.3	0.2	228.0	0.000	0.002
4OH-ASDN		99.0	95.0	198.0	190.0	194.0	5.66	2.92	388.0	206353.3	0.2	214.0	0.000	0.002
4OH-ASDN	-6	479.0	506.0	958.0	1012.0	985.0	38.18	3.88	1970.0	206353.3	1.0	1796.0	0.002	0.015
4OH-ASDN		472.0	488.0	944.0	976.0	960.0	22.63	2.36	1920.0	206353.3	0.9	1746.0	0.002	0.014
4OH-ASDN	-6.5	1274.0	1279.0	2548.0	2558.0	2553.0	7.07	0.28	5106.0	206353.3	2.5	4932.0	0.005	0.040
4OH-ASDN		1264.0	1276.0	2528.0	2552.0	2540.0	16.97	0.67	5080.0	206353.3	2.5	4906.0	0.005	0.040
4OH-ASDN	-7	2693.0	2697.0	5386.0	5394.0	5390.0	5.66	0.10	10780.0	206353.3	5.2	10606.0	0.010	0.086
4OH-ASDN		2754.0	2691.0	5508.0	5382.0	5445.0	89.10	1.64	10890.0	206353.3	5.3	10716.0	0.010	0.087
4OH-ASDN	-7.5	4942.0	4928.0	9884.0	9856.0	9870.0	19.80	0.20	19740.0	206353.3	9.6	19566.0	0.019	0.158
4OH-ASDN		5052.0	4907.0	10104.0	9814.0	9959.0	205.06	2.06	19918.0	206353.3	9.7	19744.0	0.019	0.159
4OH-ASDN	-8	6457.0	6447.0	12914.0	12894.0	12904.0	14.14	0.11	25808.0	206353.3	12.5	25634.0	0.025	0.207
4OH-ASDN		6558.0	6276.0	13116.0	12552.0	12834.0	398.81	3.11	25668.0	206353.3	12.4	25494.0	0.025	0.206
4OH-ASDN	-9	7577.0	7618.0	15154.0	15236.0	15195.0	57.98	0.38	30390.0	206353.3	14.7	30216.0	0.029	0.244
4OH-ASDN		7579.0	7359.0	15158.0	14718.0	14938.0	311.13	2.08	29876.0	206353.3	14.5	29702.0	0.029	0.240
4OH-ASDN	-10	7536.0	7519.0	15072.0	15038.0	15055.0	24.04	0.16	30110.0	206353.3	14.6	29936.0	0.029	0.242
4OH-ASDN		7945.0	7879.0	15890.0	15758.0	15824.0	93.34	0.59	31648.0	206353.3	15.3	31474.0	0.031	0.254

TA = Full Activity Control (Total Activity); NSB = Background Activity Control (Non-Specific Binding)

APPENDIX 1: Run 1: Raw and Normalized DPM Data (4OH-ASDN and (Octylsalicylate): Part 1 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
98.65	98.36	0.290	0.410	0.417
98.07				
-0.03	-0.03	0.000	0.000	0.000
-0.03				
0.73	0.70	0.022	0.032	4.479
0.68				
5.73	5.65	0.080	0.113	1.996
5.57				
15.72	15.68	0.041	0.059	0.374
15.64				
33.81	33.98	0.175	0.248	0.730
34.16				
62.37	62.65	0.284	0.401	0.640
62.94				
81.71	81.49	0.223	0.316	0.387
81.27				
96.32	95.50	0.819	1.159	1.213
94.68				
95.43	97.88	2.451	3.467	3.542
100.33				

APPENDIX 1: Run 1: Raw and Normalized DPM Data (4OH-ASDN and (Octylsalicylate): Part 3 of 4

Sample Type	Concentration	DPM1/aliquot (aliquot 1)	DPM1/aliquot (aliquot 2)	DPM1/mL (aliquot 1)	DPM2/mL (aliquot 2)	Average DPM/mL	Stdev DPM/mL	CV DPM/mL (%)	Total DPM	Total DPM present in assay	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
OctylSalicylate	-3	6604.0	6643.0	13208.0	13286.0	13247.0	55.15	0.42	26494.0	206353.3	12.8	26320.0	0.026	0.213
OctylSalicylate	-3	6716.0	6750.0	13432.0	13500.0	13466.0	48.08	0.36	26932.0	206353.3	13.1	26758.0	0.026	0.216
OctylSalicylate	-3	6574.0	6721.0	13148.0	13442.0	13295.0	207.89	1.56	26590.0	206353.3	12.9	26416.0	0.026	0.213
OctylSalicylate	-4	7786.0	7330.0	15572.0	14660.0	15116.0	644.88	4.27	30232.0	206353.3	14.7	30058.0	0.029	0.243
OctylSalicylate	-4	6945.0	6986.0	13890.0	13972.0	13931.0	57.98	0.42	27862.0	206353.3	13.5	27688.0	0.027	0.224
OctylSalicylate	-4	7231.0	7245.0	14462.0	14490.0	14476.0	19.80	0.14	28952.0	206353.3	14.0	28778.0	0.028	0.232
OctylSalicylate	-5	8140.0	7983.0	16280.0	15966.0	16123.0	222.03	1.38	32246.0	206353.3	15.6	32072.0	0.031	0.259
OctylSalicylate	-5	8166.0	8272.0	16332.0	16544.0	16438.0	149.91	0.91	32876.0	206353.3	15.9	32702.0	0.032	0.264
OctylSalicylate	-5	8152.0	8104.0	16304.0	16208.0	16256.0	67.88	0.42	32512.0	206353.3	15.8	32338.0	0.031	0.261
OctylSalicylate	-6	7722.0	7824.0	15444.0	15648.0	15546.0	144.25	0.93	31092.0	206353.3	15.1	30918.0	0.030	0.250
OctylSalicylate	-6	7974.0	8171.0	15948.0	16342.0	16145.0	278.60	1.73	32290.0	206353.3	15.6	32116.0	0.031	0.259
OctylSalicylate	-6	8116.0	8168.0	16232.0	16336.0	16284.0	73.54	0.45	32568.0	206353.3	15.8	32394.0	0.031	0.262
OctylSalicylate	-7	8127.0	8059.0	16254.0	16118.0	16186.0	96.17	0.59	32372.0	206353.3	15.7	32198.0	0.031	0.260
OctylSalicylate	-7	8062.0	7974.0	16124.0	15948.0	16036.0	124.45	0.78	32072.0	206353.3	15.5	31898.0	0.031	0.258
OctylSalicylate	-7	7926.0	8030.0	15852.0	16060.0	15956.0	147.08	0.92	31912.0	206353.3	15.5	31738.0	0.031	0.256
OctylSalicylate	-8	7921.0	6924.0	15842.0	13848.0	14845.0	1409.97	9.50	29690.0	206353.3	14.4	29516.0	0.029	0.238
OctylSalicylate	-8	8040.0	7856.0	16080.0	15712.0	15896.0	260.22	1.64	31792.0	206353.3	15.4	31618.0	0.031	0.255
OctylSalicylate	-8	7626.0	7909.0	15252.0	15818.0	15535.0	400.22	2.58	31070.0	206353.3	15.1	30896.0	0.030	0.250
OctylSalicylate	-9	7472.0	7636.0	14944.0	15272.0	15108.0	231.93	1.54	30216.0	206353.3	14.6	30042.0	0.029	0.243
OctylSalicylate	-9	7769.0	7734.0	15538.0	15468.0	15503.0	49.50	0.32	31006.0	206353.3	15.0	30832.0	0.030	0.249
OctylSalicylate	-9	7729.0	7617.0	15458.0	15234.0	15346.0	158.39	1.03	30692.0	206353.3	14.9	30518.0	0.030	0.246
OctylSalicylate	-10	7796.0	7878.0	15592.0	15756.0	15674.0	115.97	0.74	31348.0	206353.3	15.2	31174.0	0.030	0.252
OctylSalicylate	-10	7742.0	7734.0	15484.0	15468.0	15476.0	11.31	0.07	30952.0	206353.3	15.0	30778.0	0.030	0.249
OctylSalicylate	-10	7596.0	7655.0	15192.0	15310.0	15251.0	83.44	0.55	30502.0	206353.3	14.8	30328.0	0.029	0.245
TA		7807.0	8018.0	15614.0	16036.0	15825.0	298.40	1.89	31650.0	206353.3	15.3	31476.0	0.031	0.254
TA		8179.0	8057.0	16358.0	16114.0	16236.0	172.53	1.06	32472.0	206353.3	15.7	32298.0	0.031	0.261
NSB		48.0	44.0	96.0	88.0	92.0	5.66	6.15	184.0	206353.3	0.1	10.0	0.000	0.000
NSB		50.0	42.0	100.0	84.0	92.0	11.31	12.30	184.0	206353.3	0.1	10.0	0.000	0.000

TA = Full Activity Control (Total Activity); NSB = Background Activity Control (Non-Specific Binding)

APPENDIX 1: Run 1: Raw and Normalized DPM Data (4OH-ASDN and (Octylsalicylate): Part 4 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
83.90	84.47	0.424	0.734	0.869
85.30				
84.21				
95.81	91.94	2.183	3.781	4.113
88.26				
91.73				
102.23	103.19	0.582	1.008	0.977
104.24				
103.08				
98.56	101.40	1.443	2.500	2.466
102.37				
103.26				
102.64	101.83	0.430	0.744	0.731
101.68				
101.17				
94.09	97.79	1.966	3.405	3.482
100.79				
98.49				
95.76	97.11	0.732	1.268	1.306
98.28				
97.28				
99.37	98.05	0.779	1.349	1.376
98.11				
96.68				
100.33	101.64	1.310	1.853	1.823
102.95				
0.03	0.03	0.000	0.000	0.000
0.03				

APPENDIX 1: Run 1: Assay Information (Oxybenzone)

Experiment Date:	28-Jun-11	Study Number:	9070-100107AROM
Test substance:	Oxybenzone		
2/3/2012 14:29			

specific activity based on decay for 4/20/10	42240.0	DPM
20 uL count of 3H-ASDN (mean)	41270.7	DPM
0.5 mL count for total activity	7753.1	DPM
microsomal protein/assay	0.008	mg
Reaction time	15	min

20 uL count of 3H-ASDN (DPM)	42157	40831	40824

Assays Conducted by: [redacted]

Spreadsheet locked on: 06/30/2011

Green shaded areas: unlocked cells for data entry

Each assay contained 100 uL 3H-ASDN	206353.3	DPM	0.200	(nmoles)
Total product 3H-H20 per assay	31012.5	DPM	0.030	(nmoles)
Percent conversion to product (3H-H2O) (percent)	15.0			
Rate of conversion to 3H-H2O in total activity assay	0.250	nmol/(mg protein-min)		
Average activity of control Tubes	0.249	nmol/(mg protein-min)		

Average full enzyme activity controls (percent +/-SD)	100.0	0.9
Average background activity controls (percent +/- SD)	0.0	0.0

APPENDIX 1: Run 1: Raw and Normalized DPM Data (4OH-ASDN and Oxybenzone): Part 1 of 4

Sample Type	Concentration	DPM1/aliquot (aliquot 1)	DPM1/aliquot (aliquot 2)	DPM1/mL (aliquot 1)	DPM2/mL (aliquot 2)	Average DPM/mL	Stdev DPM/mL	CV DPM/mL (%)	Total DPM	Total DPM present in assay	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
TA		7816.0	7744.0	15632.0	15488.0	15560.0	101.82	0.65	31120.0	206353.3	15.1	30958.0	0.030	0.250
TA		7763.0	7706.0	15526.0	15412.0	15469.0	80.61	0.52	30938.0	206353.3	15.0	30776.0	0.030	0.249
NSB		42.0	40.0	84.0	80.0	82.0	2.83	3.45	164.0	206353.3	0.1	2.0	0.000	0.000
NSB		43.0	39.0	86.0	78.0	82.0	5.66	6.90	164.0	206353.3	0.1	2.0	0.000	0.000
4OH-ASDN	-5	101.0	100.0	202.0	200.0	201.0	1.41	0.70	402.0	206353.3	0.2	240.0	0.000	0.002
4OH-ASDN		99.0	95.0	198.0	190.0	194.0	5.66	2.92	388.0	206353.3	0.2	226.0	0.000	0.002
4OH-ASDN	-6	479.0	506.0	958.0	1012.0	985.0	38.18	3.88	1970.0	206353.3	1.0	1808.0	0.002	0.015
4OH-ASDN		472.0	488.0	944.0	976.0	960.0	22.63	2.36	1920.0	206353.3	0.9	1758.0	0.002	0.014
4OH-ASDN	-6.5	1274.0	1279.0	2548.0	2558.0	2553.0	7.07	0.28	5106.0	206353.3	2.5	4944.0	0.005	0.040
4OH-ASDN		1264.0	1276.0	2528.0	2552.0	2540.0	16.97	0.67	5080.0	206353.3	2.5	4918.0	0.005	0.040
4OH-ASDN	-7	2693.0	2697.0	5386.0	5394.0	5390.0	5.66	0.10	10780.0	206353.3	5.2	10618.0	0.010	0.086
4OH-ASDN		2754.0	2691.0	5508.0	5382.0	5445.0	89.10	1.64	10890.0	206353.3	5.3	10728.0	0.010	0.087
4OH-ASDN	-7.5	4942.0	4928.0	9884.0	9856.0	9870.0	19.80	0.20	19740.0	206353.3	9.6	19578.0	0.019	0.158
4OH-ASDN		5052.0	4907.0	10104.0	9814.0	9959.0	205.06	2.06	19918.0	206353.3	9.7	19756.0	0.019	0.160
4OH-ASDN	-8	6457.0	6447.0	12914.0	12894.0	12904.0	14.14	0.11	25808.0	206353.3	12.5	25646.0	0.025	0.207
4OH-ASDN		6558.0	6276.0	13116.0	12552.0	12834.0	398.81	3.11	25668.0	206353.3	12.4	25506.0	0.025	0.206
4OH-ASDN	-9	7577.0	7618.0	15154.0	15236.0	15195.0	57.98	0.38	30390.0	206353.3	14.7	30228.0	0.029	0.244
4OH-ASDN		7579.0	7359.0	15158.0	14718.0	14938.0	311.13	2.08	29876.0	206353.3	14.5	29714.0	0.029	0.240
4OH-ASDN	-10	7536.0	7519.0	15072.0	15038.0	15055.0	24.04	0.16	30110.0	206353.3	14.6	29948.0	0.029	0.242
4OH-ASDN		7945.0	7879.0	15890.0	15758.0	15824.0	93.34	0.59	31648.0	206353.3	15.3	31486.0	0.031	0.254

TA = Full Activity Control (Total Activity); NSB = Background Activity Control (Non-Specific Binding)

APPENDIX 1: Run 1: Raw and Normalized DPM Data (4OH-ASDN and Oxybenzone): Part 2 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
100.35	100.05	0.295	0.417	0.417
99.76				
0.01	0.01	0.000	0.000	0.000
0.01				
0.78	0.76	0.023	0.032	4.249
0.73				
5.86	5.78	0.081	0.115	1.983
5.70				
16.03	15.98	0.042	0.060	0.373
15.94				
34.42	34.60	0.178	0.252	0.729
34.77				
63.46	63.75	0.288	0.408	0.640
64.04				
83.13	82.90	0.227	0.321	0.387
82.68				
97.98	97.15	0.833	1.178	1.213
96.32				
97.07	99.57	2.493	3.525	3.540
102.06				

APPENDIX 1: Run 1: Raw and Normalized DPM Data (4OH-ASDN and Oxybenzone): Part 3 of 4

Sample Type	Concentration	DPM1/aliquot (aliquot 1)	DPM1/aliquot (aliquot 2)	DPM1/mL (aliquot 1)	DPM2/mL (aliquot 2)	Average DPM/mL	Stdev DPM/mL	CV DPM/mL (%)	Total DPM	Total DPM present in assay	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
Oxybenzone	-3	3657.0	3588.0	7314.0	7176.0	7245.0	97.58	1.35	14490.0	206353.3	7.0	14328.0	0.014	0.116
Oxybenzone	-3	1193.0	1181.0	2386.0	2362.0	2374.0	16.97	0.71	4748.0	206353.3	2.3	4586.0	0.004	0.037
Oxybenzone	-3	2901.0	2946.0	5802.0	5892.0	5847.0	63.64	1.09	11694.0	206353.3	5.7	11532.0	0.011	0.093
Oxybenzone	-4	4834.0	4827.0	9668.0	9654.0	9661.0	9.90	0.10	19322.0	206353.3	9.4	19160.0	0.019	0.155
Oxybenzone	-4	4148.0	4141.0	8296.0	8282.0	8289.0	9.90	0.12	16578.0	206353.3	8.0	16416.0	0.016	0.133
Oxybenzone	-4	3241.0	3218.0	6482.0	6436.0	6459.0	32.53	0.50	12918.0	206353.3	6.3	12756.0	0.012	0.103
Oxybenzone	-5	6633.0	6840.0	13266.0	13680.0	13473.0	292.74	2.17	26946.0	206353.3	13.1	26784.0	0.026	0.216
Oxybenzone	-5	6826.0	6593.0	13652.0	13186.0	13419.0	329.51	2.46	26838.0	206353.3	13.0	26676.0	0.026	0.215
Oxybenzone	-5	7062.0	7003.0	14124.0	14006.0	14065.0	83.44	0.59	28130.0	206353.3	13.6	27968.0	0.027	0.226
Oxybenzone	-6	7581.0	7407.0	15162.0	14814.0	14988.0	246.07	1.64	29976.0	206353.3	14.5	29814.0	0.029	0.241
Oxybenzone	-6	7508.0	7729.0	15016.0	15458.0	15237.0	312.54	2.05	30474.0	206353.3	14.8	30312.0	0.029	0.245
Oxybenzone	-6	7843.0	7929.0	15686.0	15858.0	15772.0	121.62	0.77	31544.0	206353.3	15.3	31382.0	0.030	0.253
Oxybenzone	-7	7459.0	7443.0	14918.0	14886.0	14902.0	22.63	0.15	29804.0	206353.3	14.4	29642.0	0.029	0.239
Oxybenzone	-7	8051.0	8028.0	16102.0	16056.0	16079.0	32.53	0.20	32158.0	206353.3	15.6	31996.0	0.031	0.258
Oxybenzone	-7	7912.0	7625.0	15824.0	15250.0	15537.0	405.88	2.61	31074.0	206353.3	15.1	30912.0	0.030	0.250
Oxybenzone	-8	7453.0	7475.0	14906.0	14950.0	14928.0	31.11	0.21	29856.0	206353.3	14.5	29694.0	0.029	0.240
Oxybenzone	-8	7821.0	7793.0	15642.0	15586.0	15614.0	39.60	0.25	31228.0	206353.3	15.1	31066.0	0.030	0.251
Oxybenzone	-8	7792.0	7865.0	15584.0	15730.0	15657.0	103.24	0.66	31314.0	206353.3	15.2	31152.0	0.030	0.252
Oxybenzone	-9	7617.0	7581.0	15234.0	15162.0	15198.0	50.91	0.33	30396.0	206353.3	14.7	30234.0	0.029	0.244
Oxybenzone	-9	7728.0	7541.0	15456.0	15082.0	15269.0	264.46	1.73	30538.0	206353.3	14.8	30376.0	0.029	0.245
Oxybenzone	-9	7813.0	7775.0	15626.0	15550.0	15588.0	53.74	0.34	31176.0	206353.3	15.1	31014.0	0.030	0.250
Oxybenzone	-10	7813.0	8061.0	15626.0	16122.0	15874.0	350.72	2.21	31748.0	206353.3	15.4	31586.0	0.031	0.255
Oxybenzone	-10	7618.0	8038.0	15236.0	16076.0	15656.0	593.97	3.79	31312.0	206353.3	15.2	31150.0	0.030	0.252
Oxybenzone	-10	7493.0	7818.0	14986.0	15636.0	15311.0	459.62	3.00	30622.0	206353.3	14.8	30460.0	0.030	0.246
TA		7821.0	7850.0	15642.0	15700.0	15671.0	41.01	0.26	31342.0	206353.3	15.2	31180.0	0.030	0.252
TA		7609.0	7716.0	15218.0	15432.0	15325.0	151.32	0.99	30650.0	206353.3	14.9	30488.0	0.030	0.246
NSB		38.0	39.0	76.0	78.0	77.0	1.41	1.84	154.0	206353.3	0.1	-8.0	0.000	0.000
NSB		45.0	38.0	90.0	76.0	83.0	9.90	11.93	166.0	206353.3	0.1	4.0	0.000	0.000

TA = Full Activity Control (Total Activity); NSB = Background Activity Control (Non-Specific Binding)

APPENDIX 1: Run 1: Raw and Normalized DPM Data (4OH-ASDN and Oxybenzone): Part 4 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
46.44	32.90	9.387	16.260	49.427
14.87				
37.38				
62.11	52.22	6.013	10.414	19.943
53.21				
41.35				
86.82	87.98	1.341	2.323	2.641
86.47				
90.66				
96.64	98.87	1.499	2.597	2.627
98.25				
101.72				
96.08	100.00	2.205	3.819	3.819
103.71				
100.20				
96.25	99.31	1.531	2.652	2.670
100.70				
100.98				
98.00	99.00	0.777	1.347	1.360
98.46				
100.53				
102.38	100.70	1.063	1.840	1.828
100.97				
98.73				
101.07	99.95	1.122	1.586	1.587
98.82				
-0.03	-0.01	0.019	0.028	424.264
0.01				

APPENDIX 1: Run 2: Assay Information (Methoxycinnamate)

Experiment Date:	29-Jun-11	Study Number:	9070-100107AROM	
Test substance:	Methoxycinnamate			
2/3/2012 14:41				
	specific activity based on decay for 4/20/10	42227.0	DPM	
	20 uL count of 3H-ASDN (mean)	40447.0	DPM	
	0.5 mL count for total activity	10548.6	DPM	
	microsomal protein/assay	0.008	mg	
	Reaction time	15	min	
	20 uL count of 3H-ASDN (DPM)	41993	39792	39556

Assays Conducted by:	[redacted]			Spreadsheet locked on: 06/30/2011	
				Green shaded areas: unlocked cells for data entry	
Each assay contained 100 uL 3H-ASDN		202235.0	DPM	0.200	(nmoles)
Total product 3H-H20 per assay		42194.5	DPM	0.042	(nmoles)
Percent conversion to product (3H-H2O) (percent)		20.9			
Rate of conversion to 3H-H2O in total activity assay		0.348	nmol/(mg protein-min)		
Average activity of control Tubes		0.346	nmol/(mg protein-min)		
	Average full enzyme activity controls (percent +/-SD)	100.0	6.5		
	Average background activity controls (percent +/- SD)	0.0	0.0		

APPENDIX 1: Run 2: Raw and Normalized DPM Data (4OH-ASDN and Methoxycinnamate): Part 1 of 4

Sample Type	Concentration	DPM1/aliquot (aliquot 1)	DPM1/aliquot (aliquot 2)	DPM1/mL (aliquot 1)	DPM2/mL (aliquot 2)	Average DPM/mL	Stdev DPM/mL	CV DPM/mL (%)	Total DPM	Total DPM present in assay	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
TA		10672.0	10681.0	21344.0	21362.0	21353.0	12.73	0.06	42706.0	202235.0	21.1	42544.5	0.042	0.351
TA		11398.0	11315.0	22796.0	22630.0	22713.0	117.38	0.52	45426.0	202235.0	22.5	45264.5	0.045	0.373
NSB		42.0	41.0	84.0	82.0	83.0	1.41	1.70	166.0	202235.0	0.1	4.5	0.000	0.000
NSB		38.0	34.0	76.0	68.0	72.0	5.66	7.86	144.0	202235.0	0.1	-17.5	0.000	0.000
4OH-ASDN	-5	108.0	104.0	216.0	208.0	212.0	5.66	2.67	424.0	202235.0	0.2	262.5	0.000	0.002
4OH-ASDN		115.0	116.0	230.0	232.0	231.0	1.41	0.61	462.0	202235.0	0.2	300.5	0.000	0.002
4OH-ASDN	-6	599.0	598.0	1198.0	1196.0	1197.0	1.41	0.12	2394.0	202235.0	1.2	2232.5	0.002	0.018
4OH-ASDN		563.0	583.0	1126.0	1166.0	1146.0	28.28	2.47	2292.0	202235.0	1.1	2130.5	0.002	0.018
4OH-ASDN	-6.5	1497.0	1490.0	2994.0	2980.0	2987.0	9.90	0.33	5974.0	202235.0	3.0	5812.5	0.006	0.048
4OH-ASDN		1420.0	1464.0	2840.0	2928.0	2884.0	62.23	2.16	5768.0	202235.0	2.9	5606.5	0.006	0.046
4OH-ASDN	-7	3298.0	3264.0	6596.0	6528.0	6562.0	48.08	0.73	13124.0	202235.0	6.5	12962.5	0.013	0.107
4OH-ASDN		3308.0	3319.0	6616.0	6638.0	6627.0	15.56	0.23	13254.0	202235.0	6.6	13092.5	0.013	0.108
4OH-ASDN	-7.5	6390.0	6186.0	12780.0	12372.0	12576.0	288.50	2.29	25152.0	202235.0	12.4	24990.5	0.025	0.206
4OH-ASDN		5849.0	5760.0	11698.0	11520.0	11609.0	125.87	1.08	23218.0	202235.0	11.5	23056.5	0.023	0.190
4OH-ASDN	-8	9106.0	8920.0	18212.0	17840.0	18026.0	263.04	1.46	36052.0	202235.0	17.8	35890.5	0.035	0.296
4OH-ASDN		5453.0	5493.0	10906.0	10986.0	10946.0	56.57	0.52	21892.0	202235.0	10.8	21730.5	0.021	0.179
4OH-ASDN	-9	10687.0	10712.0	21374.0	21424.0	21399.0	35.36	0.17	42798.0	202235.0	21.2	42636.5	0.042	0.351
4OH-ASDN		10481.0	10771.0	20962.0	21542.0	21252.0	410.12	1.93	42504.0	202235.0	21.0	42342.5	0.042	0.349
4OH-ASDN	-10	10116.0	10466.0	20232.0	20932.0	20582.0	494.97	2.40	41164.0	202235.0	20.4	41002.5	0.041	0.338
4OH-ASDN		11053.0	10893.0	22106.0	21786.0	21946.0	226.27	1.03	43892.0	202235.0	21.7	43730.5	0.043	0.360

TA = Full Activity Control (Total Activity); NSB = Background Activity Control (Non-Specific Binding)

APPENDIX 1: Run 2: Raw and Normalized DPM Data (4OH-ASDN and Methoxycinnamate): Part 2 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
101.22	104.45	3.236	4.576	4.381
107.69				
0.01	-0.02	0.026	0.037	239.328
-0.04				
0.62	0.67	0.045	0.064	9.545
0.71				
5.31	5.19	0.121	0.172	3.306
5.07				
13.83	13.58	0.245	0.347	2.551
13.34				
30.84	30.99	0.155	0.219	0.706
31.15				
59.45	57.15	2.301	3.254	5.693
54.85				
85.39	68.54	16.844	23.821	34.753
51.70				
101.44	101.09	0.350	0.495	0.489
100.74				
97.55	100.79	3.245	4.589	4.553
104.04				

APPENDIX 1: Run 2: Raw and Normalized DPM Data (4OH-ASDN and Methoxycinnamate): Part 3 of 4

Sample Type	Concentration	DPM1/aliquot (aliquot 1)	DPM1/aliquot (aliquot 2)	DPM1/mL (aliquot 1)	DPM2/mL (aliquot 2)	Average DPM/mL	Stdev DPM/mL	CV DPM/mL (%)	Total DPM	Total DPM present in assay	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
Methoxycinnamate	-4	11194.0	11312.0	22388.0	22624.0	22506.0	166.88	0.74	45012.0	202235.0	22.3	44850.5	0.044	0.370
Methoxycinnamate	-4	11277.0	10950.0	22554.0	21900.0	22227.0	462.45	2.08	44454.0	202235.0	22.0	44292.5	0.044	0.365
Methoxycinnamate	-4	11016.0	10713.0	22032.0	21426.0	21729.0	428.51	1.97	43458.0	202235.0	21.5	43296.5	0.043	0.357
Methoxycinnamate	-4.5	10938.0	10980.0	21876.0	21960.0	21918.0	59.40	0.27	43836.0	202235.0	21.7	43674.5	0.043	0.360
Methoxycinnamate	-4.5	11049.0	10923.0	22098.0	21846.0	21972.0	178.19	0.81	43944.0	202235.0	21.7	43782.5	0.043	0.361
Methoxycinnamate	-4.5	9094.0	9371.0	18188.0	18742.0	18465.0	391.74	2.12	36930.0	202235.0	18.3	36768.5	0.036	0.303
Methoxycinnamate	-5	11251.0	10991.0	22502.0	21982.0	22242.0	367.70	1.65	44484.0	202235.0	22.0	44322.5	0.044	0.365
Methoxycinnamate	-5	11212.0	11204.0	22424.0	22408.0	22416.0	11.31	0.05	44832.0	202235.0	22.2	44670.5	0.044	0.368
Methoxycinnamate	-5	10662.0	10694.0	21324.0	21388.0	21356.0	45.25	0.21	42712.0	202235.0	21.1	42550.5	0.042	0.351
Methoxycinnamate	-6	10326.0	10071.0	20652.0	20142.0	20397.0	360.62	1.77	40794.0	202235.0	20.2	40632.5	0.040	0.335
Methoxycinnamate	-6	10976.0	11246.0	21952.0	22492.0	22222.0	381.84	1.72	44444.0	202235.0	22.0	44282.5	0.044	0.365
Methoxycinnamate	-6	8982.0	8973.0	17964.0	17946.0	17955.0	12.73	0.07	35910.0	202235.0	17.8	35748.5	0.035	0.295
Methoxycinnamate	-7	10783.0	10665.0	21566.0	21330.0	21448.0	166.88	0.78	42896.0	202235.0	21.2	42734.5	0.042	0.352
Methoxycinnamate	-7	10587.0	10831.0	21174.0	21662.0	21418.0	345.07	1.61	42836.0	202235.0	21.2	42674.5	0.042	0.352
Methoxycinnamate	-7	10500.0	10541.0	21000.0	21082.0	21041.0	57.98	0.28	42082.0	202235.0	20.8	41920.5	0.041	0.345
Methoxycinnamate	-8	10627.0	10698.0	21254.0	21396.0	21325.0	100.41	0.47	42650.0	202235.0	21.1	42488.5	0.042	0.350
Methoxycinnamate	-8	6220.0	6289.0	12440.0	12578.0	12509.0	97.58	0.78	25018.0	202235.0	12.4	24856.5	0.025	0.205
Methoxycinnamate	-8	8583.0	8571.0	17166.0	17142.0	17154.0	16.97	0.10	34308.0	202235.0	17.0	34146.5	0.034	0.281
Methoxycinnamate	-9	7697.0	7557.0	15394.0	15114.0	15254.0	197.99	1.30	30508.0	202235.0	15.1	30346.5	0.030	0.250
Methoxycinnamate	-9	10338.0	10324.0	20676.0	20648.0	20662.0	19.80	0.10	41324.0	202235.0	20.4	41162.5	0.041	0.339
Methoxycinnamate	-9	10930.0	10924.0	21860.0	21848.0	21854.0	8.49	0.04	43708.0	202235.0	21.6	43546.5	0.043	0.359
Methoxycinnamate	-10	10225.0	10421.0	20450.0	20842.0	20646.0	277.19	1.34	41292.0	202235.0	20.4	41130.5	0.041	0.339
Methoxycinnamate	-10	10474.0	10613.0	20948.0	21226.0	21087.0	196.58	0.93	42174.0	202235.0	20.9	42012.5	0.042	0.346
Methoxycinnamate	-10	10275.0	10470.0	20550.0	20940.0	20745.0	275.77	1.33	41490.0	202235.0	20.5	41328.5	0.041	0.341
TA		9610.0	9779.0	19220.0	19558.0	19389.0	239.00	1.23	38778.0	202235.0	19.2	38616.5	0.038	0.318
TA		10374.0	10560.0	20748.0	21120.0	20934.0	263.04	1.26	41868.0	202235.0	20.7	41706.5	0.041	0.344
NSB		44.0	44.0	88.0	88.0	88.0	0.00	0.00	176.0	202235.0	0.1	14.5	0.000	0.000
NSB		38.0	42.0	76.0	84.0	80.0	5.66	7.07	160.0	202235.0	0.1	-1.5	0.000	0.000

TA = Full Activity Control (Total Activity); NSB = Background Activity Control (Non-Specific Binding)

APPENDIX 1: Run 2: Raw and Normalized DPM Data (4OH-ASDN and Methoxycinnamate): Part 4 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
106.70	105.03	1.081	1.873	1.783
105.38				
103.01				
103.91	98.51	5.520	9.561	9.705
104.16				
87.48				
105.45	104.32	1.562	2.705	2.593
106.27				
101.23				
96.67	95.69	5.881	10.187	10.646
105.35				
85.05				
101.67	100.98	0.623	1.079	1.069
101.53				
99.73				
101.08	80.49	12.115	20.984	26.072
59.14				
81.24				
72.20	91.24	9.662	16.736	18.342
97.93				
103.60				
97.85	98.71	0.636	1.101	1.115
99.95				
98.32				
91.87	95.55	3.676	5.198	5.440
99.22				
0.03	0.02	0.019	0.027	174.057
0.00				

APPENDIX 1: Run 2: Assay Information (Octocrylene)

Experiment Date:	29-Jun-11	Study Number:	9070-100107AROM		
Test substance:	Octocrylene				
2/3/2012 14:53					
	specific activity based on decay for 4/20/10		42227.0	DPM	
	20 uL count of 3H-ASDN (mean)		40447.0	DPM	
	0.5 mL count for total activity		11243.9	DPM	
	microsomal protein/assay		0.008	mg	
	Reaction time		15	min	
	20 uL count of 3H-ASDN (DPM)		41993	39792	39556

Assays Conducted by:	[redacted]			Spreadsheet locked on: 06/30/2011	
				Green shaded areas: unlocked cells for data entry	
Each assay contained 100 uL 3H-ASDN		202236.0	DPM	0.200	(nmoles)
Total product 3H-H20 per assay		44975.5	DPM	0.044	(nmoles)
Percent conversion to product (3H-H2O) (percent)		22.2			
Rate of conversion to 3H-H2O in total activity assay		0.371	nmol/(mg protein-min)		
Average activity of control Tubes		0.369	nmol/(mg protein-min)		
	Average full enzyme activity controls (percent +/-SD)	100.0	8.0		
	Average background activity controls (percent +/- SD)	0.0	0.0		

APPENDIX 1: Run 2: Raw and Normalized DPM Data (4OH-ASDN and Octocrylene): Part 1 of 4

Sample Type	Concentration	DPM1/aliquot (aliquot 1)	DPM1/aliquot (aliquot 2)	DPM1/mL (aliquot 1)	DPM2/mL (aliquot 2)	Average DPM/mL	Stdev DPM/mL	CV DPM/mL (%)	Total DPM	Total DPM present in assay	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
TA		10672.0	10681.0	21344.0	21362.0	21353.0	12.73	0.06	42706.0	202235.0	21.1	42540.0	0.042	0.351
TA		11398.0	11315.0	22796.0	22630.0	22713.0	117.38	0.52	45426.0	202235.0	22.5	45260.0	0.045	0.373
NSB		42.0	41.0	84.0	82.0	83.0	1.41	1.70	166.0	202235.0	0.1	0.0	0.000	0.000
NSB		38.0	34.0	76.0	68.0	72.0	5.66	7.86	144.0	202235.0	0.1	-22.0	0.000	0.000
4OH-ASDN	-5	108.0	104.0	216.0	208.0	212.0	5.66	2.67	424.0	202235.0	0.2	258.0	0.000	0.002
4OH-ASDN		115.0	116.0	230.0	232.0	231.0	1.41	0.61	462.0	202235.0	0.2	296.0	0.000	0.002
4OH-ASDN	-6	599.0	598.0	1198.0	1196.0	1197.0	1.41	0.12	2394.0	202235.0	1.2	2228.0	0.002	0.018
4OH-ASDN		563.0	583.0	1126.0	1166.0	1146.0	28.28	2.47	2292.0	202235.0	1.1	2126.0	0.002	0.018
4OH-ASDN	-6.5	1497.0	1490.0	2994.0	2980.0	2987.0	9.90	0.33	5974.0	202235.0	3.0	5808.0	0.006	0.048
4OH-ASDN		1420.0	1464.0	2840.0	2928.0	2884.0	62.23	2.16	5768.0	202235.0	2.9	5602.0	0.006	0.046
4OH-ASDN	-7	3298.0	3264.0	6596.0	6528.0	6562.0	48.08	0.73	13124.0	202235.0	6.5	12958.0	0.013	0.107
4OH-ASDN		3308.0	3319.0	6616.0	6638.0	6627.0	15.56	0.23	13254.0	202235.0	6.6	13088.0	0.013	0.108
4OH-ASDN	-7.5	6390.0	6186.0	12780.0	12372.0	12576.0	288.50	2.29	25152.0	202235.0	12.4	24986.0	0.025	0.206
4OH-ASDN		5849.0	5760.0	11698.0	11520.0	11609.0	125.87	1.08	23218.0	202235.0	11.5	23052.0	0.023	0.190
4OH-ASDN	-8	9106.0	8920.0	18212.0	17840.0	18026.0	263.04	1.46	36052.0	202235.0	17.8	35886.0	0.035	0.296
4OH-ASDN		5453.0	5493.0	10906.0	10986.0	10946.0	56.57	0.52	21892.0	202235.0	10.8	21726.0	0.021	0.179
4OH-ASDN	-9	10687.0	10712.0	21374.0	21424.0	21399.0	35.36	0.17	42798.0	202235.0	21.2	42632.0	0.042	0.351
4OH-ASDN		10481.0	10771.0	20962.0	21542.0	21252.0	410.12	1.93	42504.0	202235.0	21.0	42338.0	0.042	0.349
4OH-ASDN	-10	10116.0	10466.0	20232.0	20932.0	20582.0	494.97	2.40	41164.0	202235.0	20.4	40998.0	0.041	0.338
4OH-ASDN		11053.0	10893.0	22106.0	21786.0	21946.0	226.27	1.03	43892.0	202235.0	21.7	43726.0	0.043	0.360

TA = Full Activity Control (Total Activity); NSB = Background Activity Control (Non-Specific Binding)

APPENDIX 1: Run 2: Raw and Normalized DPM Data (4OH-ASDN and Octocrylene): Part 2 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
94.94	97.97	3.035	4.292	4.381
101.01				
0.00	-0.02	0.025	0.035	141.421
-0.05				
0.58	0.62	0.042	0.060	9.700
0.66				
4.97	4.86	0.114	0.161	3.313
4.74				
12.96	12.73	0.230	0.325	2.553
12.50				
28.92	29.06	0.145	0.205	0.706
29.21				
55.76	53.60	2.158	3.052	5.694
51.44				
80.09	64.29	15.800	22.345	34.759
48.49				
95.14	94.81	0.328	0.464	0.489
94.48				
91.49	94.54	3.044	4.305	4.554
97.58				

APPENDIX 1: Run 2: Raw and Normalized DPM Data (4OH-ASDN and Octocrylene): Part 3 of 4

Sample Type	Concentration	DPM1/aliquot (aliquot 1)	DPM1/aliquot (aliquot 2)	DPM1/mL (aliquot 1)	DPM2/mL (aliquot 2)	Average DPM/mL	Stdev DPM/mL	CV DPM/mL (%)	Total DPM	Total DPM present in assay	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
Octocrylene	-4	10475.0	10564.0	20950.0	21128.0	21039.0	125.87	0.60	42078.0	202235.0	20.8	41912.0	0.041	0.345
Octocrylene	-4	10613.0	10500.0	21226.0	21000.0	21113.0	159.81	0.76	42226.0	202235.0	20.9	42060.0	0.042	0.347
Octocrylene	-4	10571.0	11080.0	21142.0	22160.0	21651.0	719.83	3.32	43302.0	202235.0	21.4	43136.0	0.043	0.355
Octocrylene	-4.5	10885.0	10887.0	21770.0	21774.0	21772.0	2.83	0.01	43544.0	202235.0	21.5	43378.0	0.043	0.357
Octocrylene	-4.5	10446.0	10595.0	20892.0	21190.0	21041.0	210.72	1.00	42082.0	202235.0	20.8	41916.0	0.041	0.345
Octocrylene	-4.5	10661.0	10722.0	21322.0	21444.0	21383.0	86.27	0.40	42766.0	202235.0	21.1	42600.0	0.042	0.351
Octocrylene	-5	10684.0	10715.0	21368.0	21430.0	21399.0	43.84	0.20	42798.0	202235.0	21.2	42632.0	0.042	0.351
Octocrylene	-5	10455.0	10465.0	20910.0	20930.0	20920.0	14.14	0.07	41840.0	202235.0	20.7	41674.0	0.041	0.343
Octocrylene	-5	10832.0	10893.0	21664.0	21786.0	21725.0	86.27	0.40	43450.0	202235.0	21.5	43284.0	0.043	0.357
Octocrylene	-6	10383.0	10156.0	20766.0	20312.0	20539.0	321.03	1.56	41078.0	202235.0	20.3	40912.0	0.040	0.337
Octocrylene	-6	10545.0	10824.0	21090.0	21648.0	21369.0	394.57	1.85	42738.0	202235.0	21.1	42572.0	0.042	0.351
Octocrylene	-6	10502.0	10472.0	21004.0	20944.0	20974.0	42.43	0.20	41948.0	202235.0	20.7	41782.0	0.041	0.344
Octocrylene	-7	11174.0	10948.0	22348.0	21896.0	22122.0	319.61	1.44	44244.0	202235.0	21.9	44078.0	0.044	0.363
Octocrylene	-7	11033.0	11480.0	22066.0	22960.0	22513.0	632.15	2.81	45026.0	202235.0	22.3	44860.0	0.044	0.370
Octocrylene	-7	10431.0	10426.0	20862.0	20852.0	20857.0	7.07	0.03	41714.0	202235.0	20.6	41548.0	0.041	0.342
Octocrylene	-8	12268.0	11966.0	24536.0	23932.0	24234.0	427.09	1.76	48468.0	202235.0	24.0	48302.0	0.048	0.398
Octocrylene	-8	11268.0	11062.0	22536.0	22124.0	22330.0	291.33	1.30	44660.0	202235.0	22.1	44494.0	0.044	0.367
Octocrylene	-8	12358.0	12360.0	24716.0	24720.0	24718.0	2.83	0.01	49436.0	202235.0	24.4	49270.0	0.049	0.406
Octocrylene	-9	10563.0	10522.0	21126.0	21044.0	21085.0	57.98	0.27	42170.0	202235.0	20.9	42004.0	0.042	0.346
Octocrylene	-9	10351.0	10687.0	20702.0	21374.0	21038.0	475.18	2.26	42076.0	202235.0	20.8	41910.0	0.041	0.345
Octocrylene	-9	10553.0	10565.0	21106.0	21130.0	21118.0	16.97	0.08	42236.0	202235.0	20.9	42070.0	0.042	0.347
Octocrylene	-10	10905.0	11208.0	21810.0	22416.0	22113.0	428.51	1.94	44226.0	202235.0	21.9	44060.0	0.044	0.363
Octocrylene	-10	12723.0	12620.0	25446.0	25240.0	25343.0	145.66	0.57	50686.0	202235.0	25.1	50520.0	0.050	0.416
Octocrylene	-10	12235.0	12314.0	24470.0	24628.0	24549.0	111.72	0.46	49098.0	202235.0	24.3	48932.0	0.048	0.403
TA		10502.0	10443.0	21004.0	20886.0	20945.0	83.44	0.40	41890.0	202235.0	20.7	41724.0	0.041	0.344
TA		12516.0	12424.0	25032.0	24848.0	24940.0	130.11	0.52	49880.0	202235.0	24.7	49714.0	0.049	0.410
NSB		44.0	43.0	88.0	86.0	87.0	1.41	1.63	174.0	202235.0	0.1	8.0	0.000	0.000
NSB		45.0	45.0	90.0	90.0	90.0	0.00	0.00	180.0	202235.0	0.1	14.0	0.000	0.000

TA = Full Activity Control (Total Activity); NSB = Background Activity Control (Non-Specific Binding)

APPENDIX 1: Run 2: Raw and Normalized DPM Data (4OH-ASDN and Octocrylene): Part 4 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
93.53	94.55	0.861	1.491	1.577
93.86				
96.27				
96.81	95.14	0.943	1.632	1.716
93.54				
95.07				
95.14	94.91	1.043	1.807	1.904
93.00				
96.60				
91.30	93.18	1.070	1.853	1.989
95.01				
93.24				
98.37	97.07	2.231	3.863	3.980
100.11				
92.72				
107.79	105.68	3.253	5.635	5.332
99.30				
109.95				
93.74	93.72	0.104	0.179	0.191
93.53				
93.89				
98.33	106.76	4.337	7.512	7.037
112.74				
109.20				
93.11	102.03	8.916	12.608	12.358
110.95				
0.02	0.02	0.007	0.009	38.569
0.03				

APPENDIX 1: Run 2: Assay Information (Octylsalicylate)

Experiment Date:	29-Jun-11	Study Number:	9070-100107AROM		
Test substance:	OctylSalicylate				
2/3/2012 14:58					
	specific activity based on decay for 4/20/10		42227.0	DPM	
	20 uL count of 3H-ASDN (mean)		40447.0	DPM	
	0.5 mL count for total activity		10957.5	DPM	
	microsomal protein/assay		0.008	mg	
	Reaction time		15	min	
	20 uL count of 3H-ASDN (DPM)		41993	39792	39556

Assays Conducted by:	[redacted]			Spreadsheet locked on: 06/30/2011	
				Green shaded areas: unlocked cells for data entry	
Each assay contained 100 uL 3H-ASDN		202235.0	DPM	0.200	(nmoles)
Total product 3H-H20 per assay		43830.0	DPM	0.043	(nmoles)
Percent conversion to product (3H-H2O) (percent)		21.7			
Rate of conversion to 3H-H2O in total activity assay		0.361	nmol/(mg protein-min)		
Average activity of control Tubes		0.360	nmol/(mg protein-min)		
	Average full enzyme activity controls (percent +/-SD)	100.0	2.8		
	Average background activity controls (percent +/- SD)	0.0	0.0		

APPENDIX 1: Run 2: Raw and Normalized DPM Data (4OH-ASDN and Octylsalicylate): Part 1 of 4

Sample Type	Concentration	DPM1/aliquot (aliquot 1)	DPM1/aliquot (aliquot 2)	DPM1/mL (aliquot 1)	DPM2/mL (aliquot 2)	Average DPM/mL	Stdev DPM/mL	CV DPM/mL (%)	Total DPM	Total DPM present in assay	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
TA		10672.0	10681.0	21344.0	21362.0	21353.0	12.73	0.06	42706.0	202235.0	21.1	42543.0	0.042	0.351
TA		11398.0	11315.0	22796.0	22630.0	22713.0	117.38	0.52	45426.0	202235.0	22.5	45263.0	0.045	0.373
NSB		42.0	41.0	84.0	82.0	83.0	1.41	1.70	166.0	202235.0	0.1	3.0	0.000	0.000
NSB		38.0	34.0	76.0	68.0	72.0	5.66	7.86	144.0	202235.0	0.1	-19.0	0.000	0.000
4OH-ASDN	-5	108.0	104.0	216.0	208.0	212.0	5.66	2.67	424.0	202235.0	0.2	261.0	0.000	0.002
4OH-ASDN		115.0	116.0	230.0	232.0	231.0	1.41	0.61	462.0	202235.0	0.2	299.0	0.000	0.002
4OH-ASDN	-6	599.0	598.0	1198.0	1196.0	1197.0	1.41	0.12	2394.0	202235.0	1.2	2231.0	0.002	0.018
4OH-ASDN		563.0	583.0	1126.0	1166.0	1146.0	28.28	2.47	2292.0	202235.0	1.1	2129.0	0.002	0.018
4OH-ASDN	-6.5	1497.0	1490.0	2994.0	2980.0	2987.0	9.90	0.33	5974.0	202235.0	3.0	5811.0	0.006	0.048
4OH-ASDN		1420.0	1464.0	2840.0	2928.0	2884.0	62.23	2.16	5768.0	202235.0	2.9	5605.0	0.006	0.046
4OH-ASDN	-7	3298.0	3264.0	6596.0	6528.0	6562.0	48.08	0.73	13124.0	202235.0	6.5	12961.0	0.013	0.107
4OH-ASDN		3308.0	3319.0	6616.0	6638.0	6627.0	15.56	0.23	13254.0	202235.0	6.6	13091.0	0.013	0.108
4OH-ASDN	-7.5	6390.0	6186.0	12780.0	12372.0	12576.0	288.50	2.29	25152.0	202235.0	12.4	24989.0	0.025	0.206
4OH-ASDN		5849.0	5760.0	11698.0	11520.0	11609.0	125.87	1.08	23218.0	202235.0	11.5	23055.0	0.023	0.190
4OH-ASDN	-8	9106.0	8920.0	18212.0	17840.0	18026.0	263.04	1.46	36052.0	202235.0	17.8	35889.0	0.035	0.296
4OH-ASDN		5453.0	5493.0	10906.0	10986.0	10946.0	56.57	0.52	21892.0	202235.0	10.8	21729.0	0.021	0.179
4OH-ASDN	-9	10687.0	10712.0	21374.0	21424.0	21399.0	35.36	0.17	42798.0	202235.0	21.2	42635.0	0.042	0.351
4OH-ASDN		10481.0	10771.0	20962.0	21542.0	21252.0	410.12	1.93	42504.0	202235.0	21.0	42341.0	0.042	0.349
4OH-ASDN	-10	10116.0	10466.0	20232.0	20932.0	20582.0	494.97	2.40	41164.0	202235.0	20.4	41001.0	0.041	0.338
4OH-ASDN		11053.0	10893.0	22106.0	21786.0	21946.0	226.27	1.03	43892.0	202235.0	21.7	43729.0	0.043	0.360

TA = Full Activity Control (Total Activity); NSB = Background Activity Control (Non-Specific Binding)

APPENDIX 1: Run 2: Raw and Normalized DPM Data (4OH-ASDN and Octylsalicylate): Part 2 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
97.43	100.54	3.114	4.405	4.381
103.65				
0.01	-0.02	0.025	0.036	194.454
-0.04				
0.60	0.64	0.044	0.062	9.596
0.68				
5.11	4.99	0.117	0.165	3.308
4.88				
13.31	13.07	0.236	0.334	2.552
12.84				
29.68	29.83	0.149	0.211	0.706
29.98				
57.23	55.01	2.214	3.132	5.693
52.80				
82.19	65.97	16.214	22.930	34.755
49.76				
97.64	97.30	0.337	0.476	0.489
96.96				
93.89	97.02	3.124	4.417	4.553
100.14				

APPENDIX 1: Run 2: Raw and Normalized DPM Data (4OH-ASDN and Octylsalicylate): Part 3 of 4

Sample Type	Concentration	DPM1/aliquot (aliquot 1)	DPM1/aliquot (aliquot 2)	DPM1/mL (aliquot 1)	DPM2/mL (aliquot 2)	Average DPM/mL	Stdev DPM/mL	CV DPM/mL (%)	Total DPM	Total DPM present in assay	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
OctylSalicylate	-4	9914.0	9911.0	19828.0	19822.0	19825.0	4.24	0.02	39650.0	202235.0	19.6	39487.0	0.039	0.325
OctylSalicylate	-4	9829.0	10129.0	19658.0	20258.0	19958.0	424.26	2.13	39916.0	202235.0	19.7	39753.0	0.039	0.328
OctylSalicylate	-4	9443.0	9235.0	18886.0	18470.0	18678.0	294.16	1.57	37356.0	202235.0	18.5	37193.0	0.037	0.307
OctylSalicylate	-4.5	10332.0	10140.0	20664.0	20280.0	20472.0	271.53	1.33	40944.0	202235.0	20.2	40781.0	0.040	0.336
OctylSalicylate	-4.5	11362.0	11582.0	22724.0	23164.0	22944.0	311.13	1.36	45888.0	202235.0	22.7	45725.0	0.045	0.377
OctylSalicylate	-4.5	10335.0	9940.0	20670.0	19880.0	20275.0	558.61	2.76	40550.0	202235.0	20.1	40387.0	0.040	0.333
OctylSalicylate	-5	11097.0	10911.0	22194.0	21822.0	22008.0	263.04	1.20	44016.0	202235.0	21.8	43853.0	0.043	0.361
OctylSalicylate	-5	11693.0	11593.0	23386.0	23186.0	23286.0	141.42	0.61	46572.0	202235.0	23.0	46409.0	0.046	0.382
OctylSalicylate	-5	11327.0	11317.0	22654.0	22634.0	22644.0	14.14	0.06	45288.0	202235.0	22.4	45125.0	0.045	0.372
OctylSalicylate	-6	11697.0	11544.0	23394.0	23088.0	23241.0	216.37	0.93	46482.0	202235.0	23.0	46319.0	0.046	0.382
OctylSalicylate	-6	11320.0	11073.0	22640.0	22146.0	22393.0	349.31	1.56	44786.0	202235.0	22.1	44623.0	0.044	0.368
OctylSalicylate	-6	11561.0	11261.0	23122.0	22522.0	22822.0	424.26	1.86	45644.0	202235.0	22.6	45481.0	0.045	0.375
OctylSalicylate	-7	10409.0	10487.0	20818.0	20974.0	20896.0	110.31	0.53	41792.0	202235.0	20.7	41629.0	0.041	0.343
OctylSalicylate	-7	11448.0	11637.0	22896.0	23274.0	23085.0	267.29	1.16	46170.0	202235.0	22.8	46007.0	0.045	0.379
OctylSalicylate	-7	8742.0	8746.0	17484.0	17492.0	17488.0	5.66	0.03	34976.0	202235.0	17.3	34813.0	0.034	0.287
OctylSalicylate	-8	11308.0	11433.0	22616.0	22866.0	22741.0	176.78	0.78	45482.0	202235.0	22.5	45319.0	0.045	0.373
OctylSalicylate	-8	11130.0	11339.0	22260.0	22678.0	22469.0	295.57	1.32	44938.0	202235.0	22.2	44775.0	0.044	0.369
OctylSalicylate	-8	11020.0	11150.0	22040.0	22300.0	22170.0	183.85	0.83	44340.0	202235.0	21.9	44177.0	0.044	0.364
OctylSalicylate	-9	10963.0	11092.0	21926.0	22184.0	22055.0	182.43	0.83	44110.0	202235.0	21.8	43947.0	0.043	0.362
OctylSalicylate	-9	11083.0	11108.0	22166.0	22216.0	22191.0	35.36	0.16	44382.0	202235.0	21.9	44219.0	0.044	0.364
OctylSalicylate	-9	8636.0	8602.0	17272.0	17204.0	17238.0	48.08	0.28	34476.0	202235.0	17.0	34313.0	0.034	0.283
OctylSalicylate	-10	10493.0	10286.0	20986.0	20572.0	20779.0	292.74	1.41	41558.0	202235.0	20.5	41395.0	0.041	0.341
OctylSalicylate	-10	11203.0	10921.0	22406.0	21842.0	22124.0	398.81	1.80	44248.0	202235.0	21.9	44085.0	0.044	0.363
OctylSalicylate	-10	11122.0	10953.0	22244.0	21906.0	22075.0	239.00	1.08	44150.0	202235.0	21.8	43987.0	0.044	0.363
TA		11181.0	10889.0	22362.0	21778.0	22070.0	412.95	1.87	44140.0	202235.0	21.8	43977.0	0.043	0.362
TA		10725.0	10799.0	21450.0	21598.0	21524.0	104.65	0.49	43048.0	202235.0	21.3	42885.0	0.042	0.353
NSB		41.0	46.0	82.0	92.0	87.0	7.07	8.13	174.0	202235.0	0.1	11.0	0.000	0.000
NSB		42.0	42.0	84.0	84.0	84.0	0.00	0.00	168.0	202235.0	0.1	5.0	0.000	0.000

TA = Full Activity Control (Total Activity); NSB = Background Activity Control (Non-Specific Binding)

APPENDIX 1: Run 2: Raw and Normalized DPM Data (4OH-ASDN and Octylsalicylate): Part 4 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
90.43	88.88	1.861	3.223	3.627
91.04				
85.17				
93.39	96.86	3.933	6.812	7.033
104.71				
92.49				
100.43	103.35	1.690	2.927	2.832
106.28				
103.34				
106.07	104.14	1.121	1.942	1.865
102.19				
104.15				
95.33	93.47	7.458	12.918	13.821
105.36				
79.72				
103.78	102.50	0.755	1.308	1.276
102.54				
101.17				
100.64	93.49	7.460	12.921	13.820
101.26				
78.58				
94.80	98.83	2.017	3.494	3.535
100.96				
100.73				
100.71	99.46	1.250	1.768	1.778
98.21				
0.03	0.02	0.007	0.010	53.033
0.01				

APPENDIX 1: Run 2: Assay Information (Oxybenzone)

Experiment Date:	29-Jun-11	Study Number:	9070-100107AROM		
Test substance:	Oxybenzone				
2/3/2012 15:05					
	specific activity based on decay for 4/20/10		42227.0	DPM	
	20 uL count of 3H-ASDN (mean)		40447.0	DPM	
	0.5 mL count for total activity		11029.9	DPM	
	microsomal protein/assay		0.008	mg	
	Reaction time		15	min	
	20 uL count of 3H-ASDN (DPM)		41993	39792	39556

Assays Conducted by:	[redacted]			Spreadsheet locked on: 06/30/2011	
				Green shaded areas: unlocked cells for data entry	
Each assay contained 100 uL 3H-ASDN		202235.0	DPM	0.200	(nmoles)
Total product 3H-H20 per assay		44119.5	DPM	0.044	(nmoles)
Percent conversion to product (3H-H2O) (percent)		21.8			
Rate of conversion to 3H-H2O in total activity assay		0.364	nmol/(mg protein-min)		
Average activity of control Tubes		0.362	nmol/(mg protein-min)		
	Average full enzyme activity controls (percent +/-SD)	100.0	3.2		
	Average background activity controls (percent +/- SD)	0.0	0.1		

APPENDIX 1: Run 2: Raw and Normalized DPM Data (4OH-ASDN and Oxybenzone): Part 1 of 4

Sample Type	Concentration	DPM1/aliquot (aliquot 1)	DPM1/aliquot (aliquot 2)	DPM1/mL (aliquot 1)	DPM2/mL (aliquot 2)	Average DPM/mL	Stdev DPM/mL	CV DPM/mL (%)	Total DPM	Total DPM present in assay	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
TA		10672.0	10681.0	21344.0	21362.0	21353.0	12.73	0.06	42706.0	202235.0	21.1	42537.5	0.042	0.351
TA		11398.0	11315.0	22796.0	22630.0	22713.0	117.38	0.52	45426.0	202235.0	22.5	45257.5	0.045	0.373
NSB		42.0	41.0	84.0	82.0	83.0	1.41	1.70	166.0	202235.0	0.1	-2.5	0.000	0.000
NSB		38.0	34.0	76.0	68.0	72.0	5.66	7.86	144.0	202235.0	0.1	-24.5	0.000	0.000
4OH-ASDN	-5	108.0	104.0	216.0	208.0	212.0	5.66	2.67	424.0	202235.0	0.2	255.5	0.000	0.002
4OH-ASDN		115.0	116.0	230.0	232.0	231.0	1.41	0.61	462.0	202235.0	0.2	293.5	0.000	0.002
4OH-ASDN	-6	599.0	598.0	1198.0	1196.0	1197.0	1.41	0.12	2394.0	202235.0	1.2	2225.5	0.002	0.018
4OH-ASDN		563.0	583.0	1126.0	1166.0	1146.0	28.28	2.47	2292.0	202235.0	1.1	2123.5	0.002	0.018
4OH-ASDN	-6.5	1497.0	1490.0	2994.0	2980.0	2987.0	9.90	0.33	5974.0	202235.0	3.0	5805.5	0.006	0.048
4OH-ASDN		1420.0	1464.0	2840.0	2928.0	2884.0	62.23	2.16	5768.0	202235.0	2.9	5599.5	0.006	0.046
4OH-ASDN	-7	3298.0	3264.0	6596.0	6528.0	6562.0	48.08	0.73	13124.0	202235.0	6.5	12955.5	0.013	0.107
4OH-ASDN		3308.0	3319.0	6616.0	6638.0	6627.0	15.56	0.23	13254.0	202235.0	6.6	13085.5	0.013	0.108
4OH-ASDN	-7.5	6390.0	6186.0	12780.0	12372.0	12576.0	288.50	2.29	25152.0	202235.0	12.4	24983.5	0.025	0.206
4OH-ASDN		5849.0	5760.0	11698.0	11520.0	11609.0	125.87	1.08	23218.0	202235.0	11.5	23049.5	0.023	0.190
4OH-ASDN	-8	9106.0	8920.0	18212.0	17840.0	18026.0	263.04	1.46	36052.0	202235.0	17.8	35883.5	0.035	0.296
4OH-ASDN		5453.0	5493.0	10906.0	10986.0	10946.0	56.57	0.52	21892.0	202235.0	10.8	21723.5	0.021	0.179
4OH-ASDN	-9	10687.0	10712.0	21374.0	21424.0	21399.0	35.36	0.17	42798.0	202235.0	21.2	42629.5	0.042	0.351
4OH-ASDN		10481.0	10771.0	20962.0	21542.0	21252.0	410.12	1.93	42504.0	202235.0	21.0	42335.5	0.042	0.349
4OH-ASDN	-10	10116.0	10466.0	20232.0	20932.0	20582.0	494.97	2.40	41164.0	202235.0	20.4	40995.5	0.041	0.338
4OH-ASDN		11053.0	10893.0	22106.0	21786.0	21946.0	226.27	1.03	43892.0	202235.0	21.7	43723.5	0.043	0.360

TA = Full Activity Control (Total Activity); NSB = Background Activity Control (Non-Specific Binding)

APPENDIX 1: Run 2: Raw and Normalized DPM Data (4OH-ASDN and Oxybenzone): Part 2 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
96.78	99.88	3.094	4.376	4.381
102.97				
-0.01	-0.03	0.025	0.035	115.232
-0.06				
0.58	0.62	0.043	0.061	9.789
0.67				
5.06	4.95	0.116	0.164	3.317
4.83				
13.21	12.97	0.234	0.331	2.554
12.74				
29.48	29.63	0.148	0.209	0.706
29.77				
56.84	54.64	2.200	3.112	5.694
52.44				
81.64	65.54	16.109	22.781	34.762
49.43				
96.99	96.66	0.334	0.473	0.489
96.32				
93.28	96.38	3.103	4.389	4.554
99.48				

APPENDIX 1: Run 2: Raw and Normalized DPM Data (4OH-ASDN and Oxybenzone): Part 3 of 4

Sample Type	Concentration	DPM1/aliquot (aliquot 1)	DPM1/aliquot (aliquot 2)	DPM1/mL (aliquot 1)	DPM2/mL (aliquot 2)	Average DPM/mL	Stdev DPM/mL	CV DPM/mL (%)	Total DPM	Total DPM present in assay	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
Oxybenzone	-4	7755.0	7522.0	15510.0	15044.0	15277.0	329.51	2.16	30554.0	202235.0	15.1	30385.5	0.030	0.250
Oxybenzone	-4	6836.0	6922.0	13672.0	13844.0	13758.0	121.62	0.88	27516.0	202235.0	13.6	27347.5	0.027	0.225
Oxybenzone	-4	6288.0	6341.0	12576.0	12682.0	12629.0	74.95	0.59	25258.0	202235.0	12.5	25089.5	0.025	0.207
Oxybenzone	-4.5	8111.0	8147.0	16222.0	16294.0	16258.0	50.91	0.31	32516.0	202235.0	16.1	32347.5	0.032	0.267
Oxybenzone	-4.5	7802.0	7830.0	15604.0	15660.0	15632.0	39.60	0.25	31264.0	202235.0	15.5	31095.5	0.031	0.256
Oxybenzone	-4.5	7591.0	7749.0	15182.0	15498.0	15340.0	223.45	1.46	30680.0	202235.0	15.2	30511.5	0.030	0.251
Oxybenzone	-5	9687.0	9646.0	19374.0	19292.0	19333.0	57.98	0.30	38666.0	202235.0	19.1	38497.5	0.038	0.317
Oxybenzone	-5	9307.0	9790.0	18614.0	19580.0	19097.0	683.07	3.58	38194.0	202235.0	18.9	38025.5	0.038	0.313
Oxybenzone	-5	9783.0	9546.0	19566.0	19092.0	19329.0	335.17	1.73	38658.0	202235.0	19.1	38489.5	0.038	0.317
Oxybenzone	-6	10750.0	10771.0	21500.0	21542.0	21521.0	29.70	0.14	43042.0	202235.0	21.3	42873.5	0.042	0.353
Oxybenzone	-6	10489.0	10903.0	20978.0	21806.0	21392.0	585.48	2.74	42784.0	202235.0	21.2	42615.5	0.042	0.351
Oxybenzone	-6	10592.0	10515.0	21184.0	21030.0	21107.0	108.89	0.52	42214.0	202235.0	20.9	42045.5	0.042	0.347
Oxybenzone	-7	11194.0	11243.0	22388.0	22486.0	22437.0	69.30	0.31	44874.0	202235.0	22.2	44705.5	0.044	0.368
Oxybenzone	-7	10899.0	11100.0	21798.0	22200.0	21999.0	284.26	1.29	43998.0	202235.0	21.8	43829.5	0.043	0.361
Oxybenzone	-7	10738.0	10868.0	21476.0	21736.0	21606.0	183.85	0.85	43212.0	202235.0	21.4	43043.5	0.043	0.355
Oxybenzone	-8	10909.0	10644.0	21818.0	21288.0	21553.0	374.77	1.74	43106.0	202235.0	21.3	42937.5	0.042	0.354
Oxybenzone	-8	10414.0	10816.0	20828.0	21632.0	21230.0	568.51	2.68	42460.0	202235.0	21.0	42291.5	0.042	0.349
Oxybenzone	-8	10913.0	10533.0	21826.0	21066.0	21446.0	537.40	2.51	42892.0	202235.0	21.2	42723.5	0.042	0.352
Oxybenzone	-9	11029.0	11005.0	22058.0	22010.0	22034.0	33.94	0.15	44068.0	202235.0	21.8	43899.5	0.043	0.362
Oxybenzone	-9	10790.0	10910.0	21580.0	21820.0	21700.0	169.71	0.78	43400.0	202235.0	21.5	43231.5	0.043	0.356
Oxybenzone	-9	12062.0	12004.0	24124.0	24008.0	24066.0	82.02	0.34	48132.0	202235.0	23.8	47963.5	0.047	0.395
Oxybenzone	-10	11457.0	11384.0	22914.0	22768.0	22841.0	103.24	0.45	45682.0	202235.0	22.6	45513.5	0.045	0.375
Oxybenzone	-10	10500.0	10355.0	21000.0	20710.0	20855.0	205.06	0.98	41710.0	202235.0	20.6	41541.5	0.041	0.342
Oxybenzone	-10	10608.0	9455.0	21216.0	18910.0	20063.0	1630.59	8.13	40126.0	202235.0	19.8	39957.5	0.040	0.329
TA		10995.0	10553.0	21990.0	21106.0	21548.0	625.08	2.90	43096.0	202235.0	21.3	42927.5	0.042	0.354
TA		11319.0	11306.0	22638.0	22612.0	22625.0	18.38	0.08	45250.0	202235.0	22.4	45081.5	0.045	0.372
NSB		40.0	42.0	80.0	84.0	82.0	2.83	3.45	164.0	202235.0	0.1	-4.5	0.000	0.000
NSB		50.0	50.0	100.0	100.0	100.0	0.00	0.00	200.0	202235.0	0.1	31.5	0.000	0.000

TA = Full Activity Control (Total Activity); NSB = Background Activity Control (Non-Specific Binding)

APPENDIX 1: Run 2: Raw and Normalized DPM Data (4OH-ASDN and Oxybenzone): Part 4 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
69.13	62.81	3.491	6.047	9.626
62.22				
57.09				
73.60	71.26	1.232	2.134	2.995
70.75				
69.42				
87.59	87.23	0.355	0.615	0.705
86.52				
87.57				
97.55	96.72	0.557	0.964	0.997
96.96				
95.66				
101.72	99.79	1.092	1.892	1.896
99.72				
97.94				
97.69	97.04	0.432	0.749	0.772
96.22				
97.21				
99.88	102.46	3.364	5.827	5.687
98.36				
109.13				
103.56	96.33	3.760	6.512	6.761
94.52				
90.91				
97.67	100.12	2.450	3.465	3.461
102.57				
-0.01	0.03	0.041	0.058	188.562
0.07				

APPENDIX 1: Run 3: Assay Information (Methoxycinnamate)

Experiment Date:	27-Jul-11	Study Number:	9070-100107AROM	
Test substance:	Methoxycinnamate			
2/3/2012 15:10				
	specific activity based on decay for 4/20/10	42026.0	DPM	
	20 uL count of 3H-ASDN (mean)	41068.7	DPM	
	0.5 mL count for total activity	18781.1	DPM	
	microsomal protein/assay	0.008	mg	
	Reaction time	15	min	
	20 uL count of 3H-ASDN (DPM)	41269	40762	41175

Assays Conducted by:	[redacted]			Spreadsheet locked on: 06/30/2011	
				Green shaded areas: unlocked cells for data entry	
Each assay contained 100 uL 3H-ASDN		205343.3	DPM	0.200	(nmoles)
Total product 3H-H20 per assay		75124.5	DPM	0.073	(nmoles)
Percent conversion to product (3H-H2O) (percent)		36.6			
Rate of conversion to 3H-H2O in total activity assay		0.610	nmol/(mg protein-min)		
Average activity of control Tubes		0.608	nmol/(mg protein-min)		
	Average full enzyme activity controls (percent +/-SD)	100.0	4.6		
	Average background activity controls (percent +/- SD)	0.0	0.0		

APPENDIX 1: Run 3: Raw and Normalized DPM Data (4OH-ASDN and Methoxycinnamate): Part 1 of 4

Sample Type	Concentration	DPM1/aliquot (aliquot 1)	DPM1/aliquot (aliquot 2)	DPM1/mL (aliquot 1)	DPM2/mL (aliquot 2)	Average DPM/mL	Stdev DPM/mL	CV DPM/mL (%)	Total DPM	Total DPM present in assay	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
TA		19548.0	19240.0	39096.0	38480.0	38788.0	435.58	1.12	77576.0	205343.3	37.8	77393.5	0.075	0.628
TA		19191.0	19118.0	38382.0	38236.0	38309.0	103.24	0.27	76618.0	205343.3	37.3	76435.5	0.074	0.620
NSB		43.0	42.0	86.0	84.0	85.0	1.41	1.66	170.0	205343.3	0.1	-12.5	0.000	0.000
NSB		46.0	47.0	92.0	94.0	93.0	1.41	1.52	186.0	205343.3	0.1	3.5	0.000	0.000
4OH-ASDN	-5	308.0	302.0	616.0	604.0	610.0	8.49	1.39	1220.0	205343.3	0.6	1037.5	0.001	0.008
4OH-ASDN		322.0	305.0	644.0	610.0	627.0	24.04	3.83	1254.0	205343.3	0.6	1071.5	0.001	0.009
4OH-ASDN	-6	1125.0	1112.0	2250.0	2224.0	2237.0	18.38	0.82	4474.0	205343.3	2.2	4291.5	0.004	0.035
4OH-ASDN		1152.0	1136.0	2304.0	2272.0	2288.0	22.63	0.99	4576.0	205343.3	2.2	4393.5	0.004	0.036
4OH-ASDN	-6.5	3011.0	2983.0	6022.0	5966.0	5994.0	39.60	0.66	11988.0	205343.3	5.8	11805.5	0.011	0.096
4OH-ASDN		2956.0	2968.0	5912.0	5936.0	5924.0	16.97	0.29	11848.0	205343.3	5.8	11665.5	0.011	0.095
4OH-ASDN	-7	6562.0	6625.0	13124.0	13250.0	13187.0	89.10	0.68	26374.0	205343.3	12.8	26191.5	0.026	0.213
4OH-ASDN		6383.0	6408.0	12766.0	12816.0	12791.0	35.36	0.28	25582.0	205343.3	12.5	25399.5	0.025	0.206
4OH-ASDN	-7.5	11969.0	11978.0	23938.0	23956.0	23947.0	12.73	0.05	47894.0	205343.3	23.3	47711.5	0.046	0.387
4OH-ASDN		12108.0	11926.0	24216.0	23852.0	24034.0	257.39	1.07	48068.0	205343.3	23.4	47885.5	0.047	0.389
4OH-ASDN	-8	16277.0	16280.0	32554.0	32560.0	32557.0	4.24	0.01	65114.0	205343.3	31.7	64931.5	0.063	0.527
4OH-ASDN		15752.0	15642.0	31504.0	31284.0	31394.0	155.56	0.50	62788.0	205343.3	30.6	62605.5	0.061	0.508
4OH-ASDN	-9	19037.0	18727.0	38074.0	37454.0	37764.0	438.41	1.16	75528.0	205343.3	36.8	75345.5	0.073	0.612
4OH-ASDN		18408.0	18639.0	36816.0	37278.0	37047.0	326.68	0.88	74094.0	205343.3	36.1	73911.5	0.072	0.600
4OH-ASDN	-10	19320.0	19199.0	38640.0	38398.0	38519.0	171.12	0.44	77038.0	205343.3	37.5	76855.5	0.075	0.624
4OH-ASDN		19312.0	19933.0	38624.0	39866.0	39245.0	878.23	2.24	78490.0	205343.3	38.2	78307.5	0.076	0.636

TA = Full Activity Control (Total Activity); NSB = Background Activity Control (Non-Specific Binding)

APPENDIX 1: Run 3: Raw and Normalized DPM Data (4OH-ASDN and Methoxycinnamate): Part 2 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
103.27	102.63	0.639	0.904	0.881
101.99				
-0.02	-0.01	0.011	0.015	251.416
0.00				
1.38	1.41	0.023	0.032	2.280
1.43				
5.73	5.79	0.068	0.096	1.661
5.86				
15.75	15.66	0.093	0.132	0.844
15.57				
34.95	34.42	0.528	0.747	2.171
33.89				
63.66	63.78	0.116	0.164	0.257
63.90				
86.64	85.09	1.552	2.195	2.579
83.54				
100.54	99.58	0.957	1.353	1.359
98.62				
102.55	103.52	0.969	1.370	1.323
104.49				

APPENDIX 1: Run 3: Raw and Normalized DPM Data (4OH-ASDN and Methoxycinnamate): Part 3 of 4

Sample Type	Concentration	DPM1/aliquot (aliquot 1)	DPM1/aliquot (aliquot 2)	DPM1/mL (aliquot 1)	DPM2/mL (aliquot 2)	Average DPM/mL	Stdev DPM/mL	CV DPM/mL (%)	Total DPM	Total DPM present in assay	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
Methoxycinnamate	-4	17609.0	16753.0	35218.0	33506.0	34362.0	1210.57	3.52	68724.0	205343.3	33.5	68541.5	0.067	0.556
Methoxycinnamate	-4	17787.0	17934.0	35574.0	35868.0	35721.0	207.89	0.58	71442.0	205343.3	34.8	71259.5	0.069	0.578
Methoxycinnamate	-4	18098.0	17700.0	36196.0	35400.0	35798.0	562.86	1.57	71596.0	205343.3	34.9	71413.5	0.070	0.580
Methoxycinnamate	-4.5	18626.0	18716.0	37252.0	37432.0	37342.0	127.28	0.34	74684.0	205343.3	36.4	74501.5	0.073	0.605
Methoxycinnamate	-4.5	18554.0	18308.0	37108.0	36616.0	36862.0	347.90	0.94	73724.0	205343.3	35.9	73541.5	0.072	0.597
Methoxycinnamate	-4.5	18257.0	18414.0	36514.0	36828.0	36671.0	222.03	0.61	73342.0	205343.3	35.7	73159.5	0.071	0.594
Methoxycinnamate	-5	18670.0	18767.0	37340.0	37534.0	37437.0	137.18	0.37	74874.0	205343.3	36.5	74691.5	0.073	0.606
Methoxycinnamate	-5	18351.0	18203.0	36702.0	36406.0	36554.0	209.30	0.57	73108.0	205343.3	35.6	72925.5	0.071	0.592
Methoxycinnamate	-5	18382.0	18222.0	36764.0	36444.0	36604.0	226.27	0.62	73208.0	205343.3	35.7	73025.5	0.071	0.593
Methoxycinnamate	-6	18450.0	18618.0	36900.0	37236.0	37068.0	237.59	0.64	74136.0	205343.3	36.1	73953.5	0.072	0.600
Methoxycinnamate	-6	19283.0	19094.0	38566.0	38188.0	38377.0	267.29	0.70	76754.0	205343.3	37.4	76571.5	0.075	0.621
Methoxycinnamate	-6	13418.0	13107.0	26836.0	26214.0	26525.0	439.82	1.66	53050.0	205343.3	25.8	52867.5	0.051	0.429
Methoxycinnamate	-7	19056.0	18532.0	38112.0	37064.0	37588.0	741.05	1.97	75176.0	205343.3	36.6	74993.5	0.073	0.609
Methoxycinnamate	-7	19211.0	19300.0	38422.0	38600.0	38511.0	125.87	0.33	77022.0	205343.3	37.5	76839.5	0.075	0.624
Methoxycinnamate	-7	18919.0	19157.0	37838.0	38314.0	38076.0	336.58	0.88	76152.0	205343.3	37.1	75969.5	0.074	0.617
Methoxycinnamate	-8	19280.0	19537.0	38560.0	39074.0	38817.0	363.45	0.94	77634.0	205343.3	37.8	77451.5	0.075	0.629
Methoxycinnamate	-8	19268.0	20193.0	38536.0	40386.0	39461.0	1308.15	3.32	78922.0	205343.3	38.4	78739.5	0.077	0.639
Methoxycinnamate	-8	19326.0	19431.0	38652.0	38862.0	38757.0	148.49	0.38	77514.0	205343.3	37.7	77331.5	0.075	0.628
Methoxycinnamate	-9	18471.0	18376.0	36942.0	36752.0	36847.0	134.35	0.36	73694.0	205343.3	35.9	73511.5	0.072	0.597
Methoxycinnamate	-9	18690.0	18514.0	37380.0	37028.0	37204.0	248.90	0.67	74408.0	205343.3	36.2	74225.5	0.072	0.602
Methoxycinnamate	-9	19223.0	18931.0	38446.0	37862.0	38154.0	412.95	1.08	76308.0	205343.3	37.2	76125.5	0.074	0.618
Methoxycinnamate	-10	18741.0	19138.0	37482.0	38276.0	37879.0	561.44	1.48	75758.0	205343.3	36.9	75575.5	0.074	0.613
Methoxycinnamate	-10	18328.0	18746.0	36656.0	37492.0	37074.0	591.14	1.59	74148.0	205343.3	36.1	73965.5	0.072	0.600
Methoxycinnamate	-10	18853.0	19062.0	37706.0	38124.0	37915.0	295.57	0.78	75830.0	205343.3	36.9	75647.5	0.074	0.614
TA		17643.0	17391.0	35286.0	34782.0	35034.0	356.38	1.02	70068.0	205343.3	34.1	69885.5	0.068	0.567
TA		18852.0	19266.0	37704.0	38532.0	38118.0	585.48	1.54	76236.0	205343.3	37.1	76053.5	0.074	0.617
NSB		44.0	50.0	88.0	100.0	94.0	8.49	9.03	188.0	205343.3	0.1	5.5	0.000	0.000
NSB		41.0	52.0	82.0	104.0	93.0	15.56	16.73	186.0	205343.3	0.1	3.5	0.000	0.000

TA = Full Activity Control (Total Activity); NSB = Background Activity Control (Non-Specific Binding)

APPENDIX 1: Run 3: Raw and Normalized DPM Data (4OH-ASDN and Methoxycinnamate): Part 4 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
91.46	93.95	1.245	2.156	2.295
95.09				
95.29				
99.41	98.39	0.533	0.923	0.938
98.13				
97.62				
99.67	98.14	0.764	1.324	1.349
97.31				
97.44				
98.68	90.47	10.012	17.341	19.169
102.17				
70.54				
100.07	101.32	0.711	1.232	1.216
102.53				
101.37				
103.35	103.87	0.601	1.042	1.003
105.07				
103.19				
98.09	99.57	1.041	1.803	1.811
99.04				
101.58				
100.85	100.16	0.733	1.269	1.267
98.70				
100.94				
93.25	97.37	4.115	5.820	5.977
101.48				
0.01	0.01	0.001	0.002	31.427
0.00				

TA = Full Activity Control (Total Activity); NSB = Background Activity Control (Non-Specific Binding)

APPENDIX 1: Run 3: Assay Information (Octocrylene)

Experiment Date:	27-Jul-11	Study Number:	9070-100107AROM
Test substance:	Octocrylene		
2/3/2012 15:16			

specific activity based on decay for 4/20/10	42026.0	DPM
20 uL count of 3H-ASDN (mean)	41068.7	DPM
0.5 mL count for total activity	18887.3	DPM
microsomal protein/assay	0.008	mg
Reaction time	15	min

20 uL count of 3H-ASDN (DPM)	41269	40762	41175

Assays Conducted by: [redacted]

Spreadsheet locked on: 06/30/2011

Green shaded areas: unlocked cells for data entry

Each assay contained 100 uL 3H-ASDN	205343.3	DPM	0.200	(nmoles)
Total product 3H-H20 per assay	75549.0	DPM	0.074	(nmoles)
Percent conversion to product (3H-H2O) (percent)	36.8			
Rate of conversion to 3H-H2O in total activity assay	0.613	nmol/(mg protein-min)		
Average activity of control Tubes	0.612	nmol/(mg protein-min)		

Average full enzyme activity controls (percent +/-SD)	100.0	4.4
Average background activity controls (percent +/- SD)	0.0	0.0

APPENDIX 1: Run 3: Raw and Normalized DPM Data (4OH-ASDN and Octocrylene): Part 1 of 4

Sample Type	Concentration	DPM1/aliquot (aliquot 1)	DPM1/aliquot (aliquot 2)	DPM1/mL (aliquot 1)	DPM2/mL (aliquot 2)	Average DPM/mL	Stdev DPM/mL	CV DPM/mL (%)	Total DPM	Total DPM present in assay	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
TA		19548.0	19240.0	39096.0	38480.0	38788.0	435.58	1.12	77576.0	205343.3	37.8	77387.5	0.075	0.628
TA		19191.0	19118.0	38382.0	38236.0	38309.0	103.24	0.27	76618.0	205343.3	37.3	76429.5	0.074	0.620
NSB		43.0	42.0	86.0	84.0	85.0	1.41	1.66	170.0	205343.3	0.1	-18.5	0.000	0.000
NSB		46.0	47.0	92.0	94.0	93.0	1.41	1.52	186.0	205343.3	0.1	-2.5	0.000	0.000
4OH-ASDN	-5	308.0	302.0	616.0	604.0	610.0	8.49	1.39	1220.0	205343.3	0.6	1031.5	0.001	0.008
4OH-ASDN		322.0	305.0	644.0	610.0	627.0	24.04	3.83	1254.0	205343.3	0.6	1065.5	0.001	0.009
4OH-ASDN	-6	1125.0	1112.0	2250.0	2224.0	2237.0	18.38	0.82	4474.0	205343.3	2.2	4285.5	0.004	0.035
4OH-ASDN		1152.0	1136.0	2304.0	2272.0	2288.0	22.63	0.99	4576.0	205343.3	2.2	4387.5	0.004	0.036
4OH-ASDN	-6.5	3011.0	2983.0	6022.0	5966.0	5994.0	39.60	0.66	11988.0	205343.3	5.8	11799.5	0.011	0.096
4OH-ASDN		2956.0	2968.0	5912.0	5936.0	5924.0	16.97	0.29	11848.0	205343.3	5.8	11659.5	0.011	0.095
4OH-ASDN	-7	6562.0	6625.0	13124.0	13250.0	13187.0	89.10	0.68	26374.0	205343.3	12.8	26185.5	0.026	0.213
4OH-ASDN		6383.0	6408.0	12766.0	12816.0	12791.0	35.36	0.28	25582.0	205343.3	12.5	25393.5	0.025	0.206
4OH-ASDN	-7.5	11969.0	11978.0	23938.0	23956.0	23947.0	12.73	0.05	47894.0	205343.3	23.3	47705.5	0.046	0.387
4OH-ASDN		12108.0	11926.0	24216.0	23852.0	24034.0	257.39	1.07	48068.0	205343.3	23.4	47879.5	0.047	0.389
4OH-ASDN	-8	16277.0	16280.0	32554.0	32560.0	32557.0	4.24	0.01	65114.0	205343.3	31.7	64925.5	0.063	0.527
4OH-ASDN		15752.0	15642.0	31504.0	31284.0	31394.0	155.56	0.50	62788.0	205343.3	30.6	62599.5	0.061	0.508
4OH-ASDN	-9	19037.0	18727.0	38074.0	37454.0	37764.0	438.41	1.16	75528.0	205343.3	36.8	75339.5	0.073	0.611
4OH-ASDN		18408.0	18639.0	36816.0	37278.0	37047.0	326.68	0.88	74094.0	205343.3	36.1	73905.5	0.072	0.600
4OH-ASDN	-10	19320.0	19199.0	38640.0	38398.0	38519.0	171.12	0.44	77038.0	205343.3	37.5	76849.5	0.075	0.624
4OH-ASDN		19312.0	19933.0	38624.0	39866.0	39245.0	878.23	2.24	78490.0	205343.3	38.2	78301.5	0.076	0.636

TA = Full Activity Control (Total Activity); NSB = Background Activity Control (Non-Specific Binding)

APPENDIX 1: Run 3: Raw and Normalized DPM Data (4OH-ASDN and Octocrylene): Part 2 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
102.69	102.05	0.636	0.899	0.881
101.42				
-0.02	-0.01	0.011	0.015	107.750
0.00				
1.37	1.39	0.023	0.032	2.293
1.41				
5.69	5.75	0.068	0.096	1.663
5.82				
15.66	15.56	0.093	0.131	0.844
15.47				
34.75	34.22	0.525	0.743	2.172
33.70				
63.30	63.42	0.115	0.163	0.257
63.53				
86.15	84.61	1.543	2.182	2.579
83.07				
99.97	99.02	0.951	1.346	1.359
98.07				
101.98	102.94	0.963	1.362	1.324
103.90				

APPENDIX 1: Run 3: Raw and Normalized DPM Data (4OH-ASDN and Octocrylene): Part 3 of 4

Sample Type	Concentration	DPM1/aliquot (aliquot 1)	DPM1/aliquot (aliquot 2)	DPM1/mL (aliquot 1)	DPM2/mL (aliquot 2)	Average DPM/mL	Stdev DPM/mL	CV DPM/mL (%)	Total DPM	Total DPM present in assay	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
Octocrylene	-4	17678.0	17756.0	35356.0	35512.0	35434.0	110.31	0.31	70868.0	205343.3	34.5	70679.5	0.069	0.574
Octocrylene	-4	17732.0	18068.0	35464.0	36136.0	35800.0	475.18	1.33	71600.0	205343.3	34.9	71411.5	0.070	0.580
Octocrylene	-4	17761.0	17632.0	35522.0	35264.0	35393.0	182.43	0.52	70786.0	205343.3	34.5	70597.5	0.069	0.573
Octocrylene	-4.5	17621.0	17418.0	35242.0	34836.0	35039.0	287.09	0.82	70078.0	205343.3	34.1	69889.5	0.068	0.567
Octocrylene	-4.5	17717.0	17607.0	35434.0	35214.0	35324.0	155.56	0.44	70648.0	205343.3	34.4	70459.5	0.069	0.572
Octocrylene	-4.5	17393.0	17281.0	34786.0	34562.0	34674.0	158.39	0.46	69348.0	205343.3	33.8	69159.5	0.067	0.561
Octocrylene	-5	17332.0	17384.0	34664.0	34768.0	34716.0	73.54	0.21	69432.0	205343.3	33.8	69243.5	0.067	0.562
Octocrylene	-5	17417.0	17857.0	34834.0	35714.0	35274.0	622.25	1.76	70548.0	205343.3	34.4	70359.5	0.069	0.571
Octocrylene	-5	14369.0	14331.0	28738.0	28662.0	28700.0	53.74	0.19	57400.0	205343.3	28.0	57211.5	0.056	0.464
Octocrylene	-6	17693.0	17663.0	35386.0	35326.0	35356.0	42.43	0.12	70712.0	205343.3	34.4	70523.5	0.069	0.572
Octocrylene	-6	17826.0	17633.0	35652.0	35266.0	35459.0	272.94	0.77	70918.0	205343.3	34.5	70729.5	0.069	0.574
Octocrylene	-6	17792.0	17817.0	35584.0	35634.0	35609.0	35.36	0.10	71218.0	205343.3	34.7	71029.5	0.069	0.577
Octocrylene	-7	19473.0	19774.0	38946.0	39548.0	39247.0	425.68	1.08	78494.0	205343.3	38.2	78305.5	0.076	0.636
Octocrylene	-7	19755.0	19708.0	39510.0	39416.0	39463.0	66.47	0.17	78926.0	205343.3	38.4	78737.5	0.077	0.639
Octocrylene	-7	19323.0	19372.0	38646.0	38744.0	38695.0	69.30	0.18	77390.0	205343.3	37.7	77201.5	0.075	0.627
Octocrylene	-8	19788.0	19388.0	39576.0	38776.0	39176.0	565.69	1.44	78352.0	205343.3	38.2	78163.5	0.076	0.634
Octocrylene	-8	19914.0	20116.0	39828.0	40232.0	40030.0	285.67	0.71	80060.0	205343.3	39.0	79871.5	0.078	0.648
Octocrylene	-8	13761.0	13965.0	27522.0	27930.0	27726.0	288.50	1.04	55452.0	205343.3	27.0	55263.5	0.054	0.449
Octocrylene	-9	19115.0	18927.0	38230.0	37854.0	38042.0	265.87	0.70	76084.0	205343.3	37.1	75895.5	0.074	0.616
Octocrylene	-9	19137.0	19313.0	38274.0	38626.0	38450.0	248.90	0.65	76900.0	205343.3	37.4	76711.5	0.075	0.623
Octocrylene	-9	19182.0	18977.0	38364.0	37954.0	38159.0	289.91	0.76	76318.0	205343.3	37.2	76129.5	0.074	0.618
Octocrylene	-10	19435.0	18977.0	38870.0	37954.0	38412.0	647.71	1.69	76824.0	205343.3	37.4	76635.5	0.075	0.622
Octocrylene	-10	19563.0	13966.0	39126.0	27932.0	33529.0	7915.35	23.61	67058.0	205343.3	32.7	66869.5	0.065	0.543
Octocrylene	-10	18936.0	19173.0	37872.0	38346.0	38109.0	335.17	0.88	76218.0	205343.3	37.1	76029.5	0.074	0.617
TA		19537.0	19175.0	39074.0	38350.0	38712.0	511.95	1.32	77424.0	205343.3	37.7	77235.5	0.075	0.627
TA		18857.0	16432.0	37714.0	32864.0	35289.0	3429.47	9.72	70578.0	205343.3	34.4	70389.5	0.069	0.571
NSB		42.0	47.0	84.0	94.0	89.0	7.07	7.95	178.0	205343.3	0.1	-10.5	0.000	0.000
NSB		59.0	51.0	118.0	102.0	110.0	11.31	10.29	220.0	205343.3	0.1	31.5	0.000	0.000

TA = Full Activity Control (Total Activity); NSB = Background Activity Control (Non-Specific Binding)

APPENDIX 1: Run 3: Raw and Normalized DPM Data (4OH-ASDN and Octocrylene): Part 4 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
93.79	94.08	0.343	0.595	0.632
94.76				
93.68				
92.74	92.67	0.499	0.865	0.933
93.50				
91.77				
91.88	87.05	5.585	9.674	11.112
93.36				
75.92				
93.58	93.90	0.195	0.338	0.360
93.85				
94.25				
103.91	103.61	0.607	1.051	1.014
104.48				
102.44				
103.72	94.35	10.527	18.234	19.326
105.99				
73.33				
100.71	101.17	0.322	0.558	0.551
101.79				
101.02				
101.69	97.10	4.192	7.261	7.477
88.73				
100.89				
102.49	97.95	4.542	6.424	6.558
93.40				
-0.01	0.01	0.028	0.039	282.843
0.04				

APPENDIX 1: Run 3: Assay Information (Octylsalicylate)

Experiment Date:	27-Jul-11	Study Number:	9070-100107AROM	
Test substance:	OctylSalicylate			
2/3/2012 15:21				
	specific activity based on decay for 4/20/10	42026.0	DPM	
	20 uL count of 3H-ASDN (mean)	41068.7	DPM	
	0.5 mL count for total activity	18819.8	DPM	
	microsomal protein/assay	0.008	mg	
	Reaction time	15	min	
	20 uL count of 3H-ASDN (DPM)	41269	40762	41175

Assays Conducted by:	[redacted]			Spreadsheet locked on: 06/30/2011	
				Green shaded areas: unlocked cells for data entry	
Each assay contained 100 uL 3H-ASDN		205343.3	DPM	0.200	(nmoles)
Total product 3H-H20 per assay		75279.0	DPM	0.073	(nmoles)
Percent conversion to product (3H-H2O) (percent)		36.7			
Rate of conversion to 3H-H2O in total activity assay		0.611	nmol/(mg protein-min)		
Average activity of control Tubes		0.609	nmol/(mg protein-min)		
	Average full enzyme activity controls (percent +/-SD)	100.0	2.9		
	Average background activity controls (percent +/- SD)	0.0	0.0		

APPENDIX 1: Run 3: Raw and Normalized DPM Data (4OH-ASDN and Octylsalicylate): Part 1 of 4

Sample Type	Concentration	DPM1/aliquot (aliquot 1)	DPM1/aliquot (aliquot 2)	DPM1/mL (aliquot 1)	DPM2/mL (aliquot 2)	Average DPM/mL	Stdev DPM/mL	CV DPM/mL (%)	Total DPM	Total DPM present in assay	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
TA		19548.0	19240.0	39096.0	38480.0	38788.0	435.58	1.12	77576.0	205343.3	37.8	77387.0	0.075	0.628
TA		19191.0	19118.0	38382.0	38236.0	38309.0	103.24	0.27	76618.0	205343.3	37.3	76429.0	0.074	0.620
NSB		43.0	42.0	86.0	84.0	85.0	1.41	1.66	170.0	205343.3	0.1	-19.0	0.000	0.000
NSB		46.0	47.0	92.0	94.0	93.0	1.41	1.52	186.0	205343.3	0.1	-3.0	0.000	0.000
4OH-ASDN	-5	308.0	302.0	616.0	604.0	610.0	8.49	1.39	1220.0	205343.3	0.6	1031.0	0.001	0.008
4OH-ASDN		322.0	305.0	644.0	610.0	627.0	24.04	3.83	1254.0	205343.3	0.6	1065.0	0.001	0.009
4OH-ASDN	-6	1125.0	1112.0	2250.0	2224.0	2237.0	18.38	0.82	4474.0	205343.3	2.2	4285.0	0.004	0.035
4OH-ASDN		1152.0	1136.0	2304.0	2272.0	2288.0	22.63	0.99	4576.0	205343.3	2.2	4387.0	0.004	0.036
4OH-ASDN	-6.5	3011.0	2983.0	6022.0	5966.0	5994.0	39.60	0.66	11988.0	205343.3	5.8	11799.0	0.011	0.096
4OH-ASDN		2956.0	2968.0	5912.0	5936.0	5924.0	16.97	0.29	11848.0	205343.3	5.8	11659.0	0.011	0.095
4OH-ASDN	-7	6562.0	6625.0	13124.0	13250.0	13187.0	89.10	0.68	26374.0	205343.3	12.8	26185.0	0.026	0.213
4OH-ASDN		6383.0	6408.0	12766.0	12816.0	12791.0	35.36	0.28	25582.0	205343.3	12.5	25393.0	0.025	0.206
4OH-ASDN	-7.5	11969.0	11978.0	23938.0	23956.0	23947.0	12.73	0.05	47894.0	205343.3	23.3	47705.0	0.046	0.387
4OH-ASDN		12108.0	11926.0	24216.0	23852.0	24034.0	257.39	1.07	48068.0	205343.3	23.4	47879.0	0.047	0.389
4OH-ASDN	-8	16277.0	16280.0	32554.0	32560.0	32557.0	4.24	0.01	65114.0	205343.3	31.7	64925.0	0.063	0.527
4OH-ASDN		15752.0	15642.0	31504.0	31284.0	31394.0	155.56	0.50	62788.0	205343.3	30.6	62599.0	0.061	0.508
4OH-ASDN	-9	19037.0	18727.0	38074.0	37454.0	37764.0	438.41	1.16	75528.0	205343.3	36.8	75339.0	0.073	0.611
4OH-ASDN		18408.0	18639.0	36816.0	37278.0	37047.0	326.68	0.88	74094.0	205343.3	36.1	73905.0	0.072	0.600
4OH-ASDN	-10	19320.0	19199.0	38640.0	38398.0	38519.0	171.12	0.44	77038.0	205343.3	37.5	76849.0	0.075	0.624
4OH-ASDN		19312.0	19933.0	38624.0	39866.0	39245.0	878.23	2.24	78490.0	205343.3	38.2	78301.0	0.076	0.636

TA = Full Activity Control (Total Activity); NSB = Background Activity Control (Non-Specific Binding)

APPENDIX 1: Run 3: Raw and Normalized DPM Data (4OH-ASDN and Octylsalicylate): Part 2 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
103.06	102.42	0.638	0.902	0.881
101.78				
-0.03	-0.01	0.011	0.015	102.852
0.00				
1.37	1.40	0.023	0.032	2.294
1.42				
5.71	5.77	0.068	0.096	1.663
5.84				
15.71	15.62	0.093	0.132	0.844
15.53				
34.87	34.34	0.527	0.746	2.172
33.82				
63.53	63.65	0.116	0.164	0.257
63.76				
86.46	84.91	1.549	2.190	2.579
83.37				
100.33	99.38	0.955	1.350	1.359
98.42				
102.34	103.31	0.967	1.367	1.324
104.28				

APPENDIX 1: Run 3: Raw and Normalized DPM Data (4OH-ASDN and Octylsalicylate): Part 3 of 4

Sample Type	Concentration	DPM1/aliquot (aliquot 1)	DPM1/aliquot (aliquot 2)	DPM1/mL (aliquot 1)	DPM2/mL (aliquot 2)	Average DPM/mL	Stdev DPM/mL	CV DPM/mL (%)	Total DPM	Total DPM present in assay	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
OctylSalicylate	-4	17010.0	16292.0	34020.0	32584.0	33302.0	1015.41	3.05	66604.0	205343.3	32.4	66415.0	0.065	0.539
OctylSalicylate	-4	17171.0	16253.0	34342.0	32506.0	33424.0	1298.25	3.88	66848.0	205343.3	32.6	66659.0	0.065	0.541
OctylSalicylate	-4	16373.0	16639.0	32746.0	33278.0	33012.0	376.18	1.14	66024.0	205343.3	32.2	65835.0	0.064	0.534
OctylSalicylate	-4.5	16847.0	16318.0	33694.0	32636.0	33165.0	748.12	2.26	66330.0	205343.3	32.3	66141.0	0.064	0.537
OctylSalicylate	-4.5	16326.0	16565.0	32652.0	33130.0	32891.0	338.00	1.03	65782.0	205343.3	32.0	65593.0	0.064	0.532
OctylSalicylate	-4.5	17165.0	16994.0	34330.0	33988.0	34159.0	241.83	0.71	68318.0	205343.3	33.3	68129.0	0.066	0.553
OctylSalicylate	-5	18030.0	17855.0	36060.0	35710.0	35885.0	247.49	0.69	71770.0	205343.3	35.0	71581.0	0.070	0.581
OctylSalicylate	-5	18146.0	17583.0	36292.0	35166.0	35729.0	796.20	2.23	71458.0	205343.3	34.8	71269.0	0.069	0.578
OctylSalicylate	-5	17589.0	17728.0	35178.0	35456.0	35317.0	196.58	0.56	70634.0	205343.3	34.4	70445.0	0.069	0.572
OctylSalicylate	-6	19930.0	19952.0	39860.0	39904.0	39882.0	31.11	0.08	79764.0	205343.3	38.8	79575.0	0.078	0.646
OctylSalicylate	-6	19416.0	19237.0	38832.0	38474.0	38653.0	253.14	0.65	77306.0	205343.3	37.6	77117.0	0.075	0.626
OctylSalicylate	-6	19529.0	19611.0	39058.0	39222.0	39140.0	115.97	0.30	78280.0	205343.3	38.1	78091.0	0.076	0.634
OctylSalicylate	-7	19763.0	19888.0	39526.0	39776.0	39651.0	176.78	0.45	79302.0	205343.3	38.6	79113.0	0.077	0.642
OctylSalicylate	-7	18926.0	18898.0	37852.0	37796.0	37824.0	39.60	0.10	75648.0	205343.3	36.8	75459.0	0.073	0.612
OctylSalicylate	-7	19527.0	19336.0	39054.0	38672.0	38863.0	270.11	0.70	77726.0	205343.3	37.9	77537.0	0.076	0.629
OctylSalicylate	-8	19268.0	19341.0	38536.0	38682.0	38609.0	103.24	0.27	77218.0	205343.3	37.6	77029.0	0.075	0.625
OctylSalicylate	-8	19931.0	19801.0	39862.0	39602.0	39732.0	183.85	0.46	79464.0	205343.3	38.7	79275.0	0.077	0.643
OctylSalicylate	-8	20024.0	19622.0	40048.0	39244.0	39646.0	568.51	1.43	79292.0	205343.3	38.6	79103.0	0.077	0.642
OctylSalicylate	-9	19563.0	19387.0	39126.0	38774.0	38950.0	248.90	0.64	77900.0	205343.3	37.9	77711.0	0.076	0.631
OctylSalicylate	-9	19165.0	18165.0	38330.0	36330.0	37330.0	1414.21	3.79	74660.0	205343.3	36.4	74471.0	0.073	0.604
OctylSalicylate	-9	19728.0	19282.0	39456.0	38564.0	39010.0	630.74	1.62	78020.0	205343.3	38.0	77831.0	0.076	0.632
OctylSalicylate	-10	19242.0	18942.0	38484.0	37884.0	38184.0	424.26	1.11	76368.0	205343.3	37.2	76179.0	0.074	0.618
OctylSalicylate	-10	19413.0	19415.0	38826.0	38830.0	38828.0	2.83	0.01	77656.0	205343.3	37.8	77467.0	0.075	0.629
OctylSalicylate	-10	19639.0	19935.0	39278.0	39870.0	39574.0	418.61	1.06	79148.0	205343.3	38.5	78959.0	0.077	0.641
TA		18460.0	18100.0	36920.0	36200.0	36560.0	509.12	1.39	73120.0	205343.3	35.6	72931.0	0.071	0.592
TA		18548.0	18353.0	37096.0	36706.0	36901.0	275.77	0.75	73802.0	205343.3	35.9	73613.0	0.072	0.597
NSB		46.0	50.0	92.0	100.0	96.0	5.66	5.89	192.0	205343.3	0.1	3.0	0.000	0.000
NSB		48.0	56.0	96.0	112.0	104.0	11.31	10.88	208.0	205343.3	0.1	19.0	0.000	0.000

TA = Full Activity Control (Total Activity); NSB = Background Activity Control (Non-Specific Binding)

APPENDIX 1: Run 3: Raw and Normalized DPM Data (4OH-ASDN and Octylsalicylate): Part 4 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
88.45	88.30	0.325	0.564	0.638
88.77				
87.67				
88.08	88.72	1.026	1.777	2.003
87.35				
90.73				
95.33	94.68	0.451	0.782	0.825
94.91				
93.81				
105.97	104.22	0.952	1.648	1.582
102.70				
104.00				
105.36	103.04	1.409	2.441	2.369
100.49				
103.26				
102.58	104.50	0.961	1.665	1.593
105.57				
105.34				
103.49	102.11	1.466	2.539	2.486
99.18				
103.65				
101.45	103.26	1.070	1.853	1.794
103.17				
105.15				
97.12	97.58	0.454	0.642	0.658
98.03				
0.00	0.01	0.011	0.015	102.852
0.03				

APPENDIX 1: Run 3: Assay Information (Oxybenzone)

Experiment Date:	27-Jul-11	Study Number:	9070-100107AROM	
Test substance:	Oxybenzone			
2/3/2012 15:25				
	specific activity based on decay for 4/20/10	42026.0	DPM	
	20 uL count of 3H-ASDN (mean)	41068.7	DPM	
	0.5 mL count for total activity	19271.8	DPM	
	microsomal protein/assay	0.008	mg	
	Reaction time	15	min	
	20 uL count of 3H-ASDN (DPM)	41269	40762	41175

Assays Conducted by:	[redacted]			Spreadsheet locked on: 06/30/2011	
				Green shaded areas: unlocked cells for data entry	
Each assay contained 100 uL 3H-ASDN		205343.3	DPM	0.200	(nmoles)
Total product 3H-H20 per assay		77087.0	DPM	0.075	(nmoles)
Percent conversion to product (3H-H2O) (percent)		37.5			
Rate of conversion to 3H-H2O in total activity assay		0.626	nmol/(mg protein-min)		
Average activity of control Tubes		0.624	nmol/(mg protein-min)		
	Average full enzyme activity controls (percent +/-SD)	100.0	0.5		
	Average background activity controls (percent +/- SD)	0.0	0.0		

APPENDIX 1: Run 3: Raw and Normalized DPM Data (4OH-ASDN and Oxybenzone): Part 1 of 4

Sample Type	Concentration	DPM1/aliquot (aliquot 1)	DPM1/aliquot (aliquot 2)	DPM1/mL (aliquot 1)	DPM2/mL (aliquot 2)	Average DPM/mL	Stdev DPM/mL	CV DPM/mL (%)	Total DPM	Total DPM present in assay	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
TA		19548.0	19240.0	39096.0	38480.0	38788.0	435.58	1.12	77576.0	205343.3	37.8	77393.0	0.075	0.628
TA		19191.0	19118.0	38382.0	38236.0	38309.0	103.24	0.27	76618.0	205343.3	37.3	76435.0	0.074	0.620
NSB		43.0	42.0	86.0	84.0	85.0	1.41	1.66	170.0	205343.3	0.1	-13.0	0.000	0.000
NSB		46.0	47.0	92.0	94.0	93.0	1.41	1.52	186.0	205343.3	0.1	3.0	0.000	0.000
4OH-ASDN	-5	308.0	302.0	616.0	604.0	610.0	8.49	1.39	1220.0	205343.3	0.6	1037.0	0.001	0.008
4OH-ASDN		322.0	305.0	644.0	610.0	627.0	24.04	3.83	1254.0	205343.3	0.6	1071.0	0.001	0.009
4OH-ASDN	-6	1125.0	1112.0	2250.0	2224.0	2237.0	18.38	0.82	4474.0	205343.3	2.2	4291.0	0.004	0.035
4OH-ASDN		1152.0	1136.0	2304.0	2272.0	2288.0	22.63	0.99	4576.0	205343.3	2.2	4393.0	0.004	0.036
4OH-ASDN	-6.5	3011.0	2983.0	6022.0	5966.0	5994.0	39.60	0.66	11988.0	205343.3	5.8	11805.0	0.011	0.096
4OH-ASDN		2956.0	2968.0	5912.0	5936.0	5924.0	16.97	0.29	11848.0	205343.3	5.8	11665.0	0.011	0.095
4OH-ASDN	-7	6562.0	6625.0	13124.0	13250.0	13187.0	89.10	0.68	26374.0	205343.3	12.8	26191.0	0.026	0.213
4OH-ASDN		6383.0	6408.0	12766.0	12816.0	12791.0	35.36	0.28	25582.0	205343.3	12.5	25399.0	0.025	0.206
4OH-ASDN	-7.5	11969.0	11978.0	23938.0	23956.0	23947.0	12.73	0.05	47894.0	205343.3	23.3	47711.0	0.046	0.387
4OH-ASDN		12108.0	11926.0	24216.0	23852.0	24034.0	257.39	1.07	48068.0	205343.3	23.4	47885.0	0.047	0.389
4OH-ASDN	-8	16277.0	16280.0	32554.0	32560.0	32557.0	4.24	0.01	65114.0	205343.3	31.7	64931.0	0.063	0.527
4OH-ASDN		15752.0	15642.0	31504.0	31284.0	31394.0	155.56	0.50	62788.0	205343.3	30.6	62605.0	0.061	0.508
4OH-ASDN	-9	19037.0	18727.0	38074.0	37454.0	37764.0	438.41	1.16	75528.0	205343.3	36.8	75345.0	0.073	0.612
4OH-ASDN		18408.0	18639.0	36816.0	37278.0	37047.0	326.68	0.88	74094.0	205343.3	36.1	73911.0	0.072	0.600
4OH-ASDN	-10	19320.0	19199.0	38640.0	38398.0	38519.0	171.12	0.44	77038.0	205343.3	37.5	76855.0	0.075	0.624
4OH-ASDN		19312.0	19933.0	38624.0	39866.0	39245.0	878.23	2.24	78490.0	205343.3	38.2	78307.0	0.076	0.636

TA = Full Activity Control (Total Activity); NSB = Background Activity Control (Non-Specific Binding)

APPENDIX 1: Run 3: Raw and Normalized DPM Data (4OH-ASDN and Oxybenzone): Part 2 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
100.64	100.01	0.623	0.881	0.881
99.39				
-0.02	-0.01	0.010	0.015	226.274
0.00				
1.35	1.37	0.022	0.031	2.281
1.39				
5.58	5.65	0.066	0.094	1.661
5.71				
15.35	15.26	0.091	0.129	0.844
15.17				
34.06	33.54	0.515	0.728	2.171
33.03				
62.04	62.15	0.113	0.160	0.257
62.27				
84.43	82.92	1.512	2.139	2.579
81.41				
97.97	97.04	0.932	1.319	1.359
96.11				
99.94	100.88	0.944	1.335	1.323
101.82				

APPENDIX 1: Run 3: Raw and Normalized DPM Data (4OH-ASDN and Oxybenzone): Part 3 of 4

Sample Type	Concentration	DPM1/aliquot (aliquot 1)	DPM1/aliquot (aliquot 2)	DPM1/mL (aliquot 1)	DPM2/mL (aliquot 2)	Average DPM/mL	Stdev DPM/mL	CV DPM/mL (%)	Total DPM	Total DPM present in assay	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
Oxybenzone	-4	6900.0	7114.0	13800.0	14228.0	14014.0	302.64	2.16	28028.0	205343.3	13.6	27845.0	0.027	0.226
Oxybenzone	-4	6701.0	6662.0	13402.0	13324.0	13363.0	55.15	0.41	26726.0	205343.3	13.0	26543.0	0.026	0.215
Oxybenzone	-4	8188.0	7993.0	16376.0	15986.0	16181.0	275.77	1.70	32362.0	205343.3	15.8	32179.0	0.031	0.261
Oxybenzone	-4.5	12242.0	12146.0	24484.0	24292.0	24388.0	135.76	0.56	48776.0	205343.3	23.8	48593.0	0.047	0.394
Oxybenzone	-4.5	12730.0	12395.0	25460.0	24790.0	25125.0	473.76	1.89	50250.0	205343.3	24.5	50067.0	0.049	0.406
Oxybenzone	-4.5	12111.0	12343.0	24222.0	24686.0	24454.0	328.10	1.34	48908.0	205343.3	23.8	48725.0	0.047	0.395
Oxybenzone	-5	17971.0	17691.0	35942.0	35382.0	35662.0	395.98	1.11	71324.0	205343.3	34.7	71141.0	0.069	0.577
Oxybenzone	-5	17723.0	17558.0	35446.0	35116.0	35281.0	233.35	0.66	70562.0	205343.3	34.4	70379.0	0.069	0.571
Oxybenzone	-5	17910.0	17882.0	35820.0	35764.0	35792.0	39.60	0.11	71584.0	205343.3	34.9	71401.0	0.070	0.580
Oxybenzone	-6	19603.0	19737.0	39206.0	39474.0	39340.0	189.50	0.48	78680.0	205343.3	38.3	78497.0	0.076	0.637
Oxybenzone	-6	18114.0	18232.0	36228.0	36464.0	36346.0	166.88	0.46	72692.0	205343.3	35.4	72509.0	0.071	0.589
Oxybenzone	-6	19984.0	19768.0	39968.0	39536.0	39752.0	305.47	0.77	79504.0	205343.3	38.7	79321.0	0.077	0.644
Oxybenzone	-7	19511.0	19504.0	39022.0	39008.0	39015.0	9.90	0.03	78030.0	205343.3	38.0	77847.0	0.076	0.632
Oxybenzone	-7	18910.0	19198.0	37820.0	38396.0	38108.0	407.29	1.07	76216.0	205343.3	37.1	76033.0	0.074	0.617
Oxybenzone	-7	19618.0	19825.0	39236.0	39650.0	39443.0	292.74	0.74	78886.0	205343.3	38.4	78703.0	0.077	0.639
Oxybenzone	-8	19095.0	19010.0	38190.0	38020.0	38105.0	120.21	0.32	76210.0	205343.3	37.1	76027.0	0.074	0.617
Oxybenzone	-8	20052.0	19466.0	40104.0	38932.0	39518.0	828.73	2.10	79036.0	205343.3	38.5	78853.0	0.077	0.640
Oxybenzone	-8	12571.0	12439.0	25142.0	24878.0	25010.0	186.68	0.75	50020.0	205343.3	24.4	49837.0	0.049	0.405
Oxybenzone	-9	19702.0	19700.0	39404.0	39400.0	39402.0	2.83	0.01	78804.0	205343.3	38.4	78621.0	0.077	0.638
Oxybenzone	-9	19416.0	19491.0	38832.0	38982.0	38907.0	106.07	0.27	77814.0	205343.3	37.9	77631.0	0.076	0.630
Oxybenzone	-9	19627.0	19679.0	39254.0	39358.0	39306.0	73.54	0.19	78612.0	205343.3	38.3	78429.0	0.076	0.637
Oxybenzone	-10	18887.0	19146.0	37774.0	38292.0	38033.0	366.28	0.96	76066.0	205343.3	37.0	75883.0	0.074	0.616
Oxybenzone	-10	19537.0	19706.0	39074.0	39412.0	39243.0	239.00	0.61	78486.0	205343.3	38.2	78303.0	0.076	0.636
Oxybenzone	-10	19640.0	19419.0	39280.0	38838.0	39059.0	312.54	0.80	78118.0	205343.3	38.0	77935.0	0.076	0.633
TA		19288.0	19269.0	38576.0	38538.0	38557.0	26.87	0.07	77114.0	205343.3	37.6	76931.0	0.075	0.624
TA		19276.0	19244.0	38552.0	38488.0	38520.0	45.25	0.12	77040.0	205343.3	37.5	76857.0	0.075	0.624
NSB		46.0	46.0	92.0	92.0	92.0	0.00	0.00	184.0	205343.3	0.1	1.0	0.000	0.000
NSB		46.0	50.0	92.0	100.0	96.0	5.66	5.89	192.0	205343.3	0.1	9.0	0.000	0.000

TA = Full Activity Control (Total Activity); NSB = Background Activity Control (Non-Specific Binding)

APPENDIX 1: Run 3: Raw and Normalized DPM Data (4OH-ASDN and Oxybenzone): Part 4 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
36.21	37.52	2.215	3.837	10.226
34.51				
41.84				
63.19	63.88	0.612	1.061	1.660
65.10				
63.36				
92.51	92.29	0.399	0.691	0.748
91.52				
92.84				
102.07	99.83	2.791	4.835	4.843
94.29				
103.14				
101.23	100.81	1.024	1.773	1.759
98.87				
102.34				
98.86	88.73	12.011	20.804	23.446
102.53				
64.80				
102.23	101.72	0.394	0.683	0.671
100.95				
101.98				
98.67	100.61	0.979	1.696	1.685
101.82				
101.34				
100.04	99.99	0.048	0.068	0.068
99.94				
0.00	0.01	0.005	0.007	113.137
0.01				

APPENDIX 2: Deviation Forms

Form #: SOP-1003-F-1.0

CeeTox
In vitro models to predict toxicity

Deviation & Investigation

Study Number (if applicable): 9070-100107AROM

Date of Reporting: 03 February 2012 Reporting Associate: [redacted]

Date of Occurrence: 28 Jun 2011, 29 Jun 2011, and 27 Jul 2011 Associate Involved: [redacted]

Description of Deviation:

The batch number listed in the study protocol for test substance 2-hydroxy-4-methoxybenzophenone (Referred to as Oxybenzone) was listed as 20080801. This batch was not utilized in this study. The actual batch number of the test substance used in this study was the current batch, 20100801.

Signature [redacted] Date: 03 FEBRUARY 2012
(Reporting Associate)

Type of Deviation (determined by Study Director/Principal Investigator):

☐ SOP Deviation X ☒ Protocol Deviation ☐ GLP Deviation ☐ No Deviation

Summary of Deviation Investigation by SD/PI/Test Facility Management/Designee:

Current bath of Oxybenzone (20100801) used instead of previous batch (20080801).

Action Taken and Determination of Impact on Study Data and/or Facility Compliance:

None. No impact on study.

Signature: [redacted] Date: 03 FEBRUARY 2012
SD/PI/Test Facility Management

Standard Operating Procedure Page 1 of 1

APPENDIX 2: Deviation Forms

Form #: SOP-1003-F-1.0

CeeTox
In vitro models to predict toxicity

Deviation & Investigation

Study Number (if applicable): 9070-100107AROM

Date of Reporting: 03 January 2012 Reporting Associate: [redacted]

Date of Occurrence: 27 July 2011 Associate Involved: [redacted]

Description of Deviation:

Purity used for preparing Octylmethoxycinnamate stocks was 98% but actual was 99.8% according to C of A.

Signature [redacted] Date: 03 FEBRUARY 2012
(Reporting Associate)

Type of Deviation (determined by Study Director/Principal Investigator):

☐ SOP Deviation X ☑ Protocol Deviation ☐ GLP Deviation ☐ No Deviation

Summary of Deviation Investigation by SD/PI/Test Facility Management/Designee:

Used the incorrect purity (off by 1.8%)

Action Taken and Determination of Impact on Study Data and/or Facility Compliance:

None. Only off by 1.8% and after serial dilutions, the difference is minute.

Signature: [redacted] Date: 03 FEBRUARY 2012
SD/PI/Test Facility Management

Standard Operating Procedure Page 1 of 1

APPENDIX 2: Deviation Forms

Form #: SOP-1003-F-1.0

CeeTox
In vitro models to predict toxicity

Deviation & Investigation

Study Number (if applicable): 9070-100107AROM

Date of Reporting: 02 February 2012 Reporting Associate: [redacted]

Date of Occurrence: 26 September 2011 Associate Involved: [redacted]

Description of Deviation:

Section 15 of study protocol indicates that sponsor will sign all protocol amendments. The protocol amendment on 26 September 2011 was not signed by the sponsor.

Signature [redacted] Date: 03 FEBRUARY 2012

(Reporting Associate)

Type of Deviation (determined by Study Director/Principal Investigator):

☐ SOP Deviation X ☑ Protocol Deviation ☐ GLP Deviation ☐ No Deviation

Summary of Deviation Investigation by SD/PI/Test Facility Management/Designee:

Protocol deviation. Section 15 of study protocol specifies that sponsor will sign all amendments.

Action Taken and Determination of Impact on Study Data and/or Facility Compliance:

The deviation has no impact on the study data. The sponsor was informed of protocol changes and received a copy of the amendment.

Signature: [redacted] Date: 03 FEBRUARY 2012

SD/PI/Test Facility Management

Standard Operating Procedure Page 1 of 1

APPENDIX 3: Certificate of Analyses (Methoxycinnamate)

CERTIFICATE OF ANALYSIS

Product 29116 **Octyl 4-methoxycinnamate,98%,stabilized**

Specifications

Appearance	CLEAR COLOURLESS TO YELLOW LIQUID
Infrared spectrometry	AUTHENTIC
Separat. techn. GC	>97.5 %
Acid value	<1 mg KOH/g
Specific abs. A (1%/1cm)	>830 (at 307 to 308 nm in methanol)
Specific gravity	(25/25°C) 1.007 to 1.012
Refractive index	1.5430 to 1.5470 (20°C, 589 nm)
Stabilizer	0.05 to 0.1 % BHT

General Product Data

Version	00
CAS No.	5466-77-3
Molecular weight	290.39
Molecular formula	C18 H26 O3
Linear formula	
Flash point (°C)	193

Lot Specific Data for Lot No.: A0293319

Appearance	CLEAR COLOURLESS LIQUID
Infrared spectrometry	AUTHENTIC
Separat. techn. GC	99.8 %
Acid value	0.1 mg KOH/g
Specific abs. A (1%/1cm)	865 (at 307 to 308 nm in methanol)
Specific gravity	(25/25°C) 1.0096
Refractive index	1.5453 (20°C, 589 nm)
Stabilizer	0.09 % BHT

ACROS ORGANICS

[redacted]

Issued: 10-08-10

[redacted]**uality Assurance Manager**

Acros Organics Geel West Zone 2, Janssen Pharmaceuticalaan 3a, B-2440 Geel, Belgium Tel +32 14/57.52.11 - Fax +32 14/59.34.34 Internet: http://www.acros.com
1 Reagent Lane, Fair Lawn, NJ 07410,USA Fax 201-796-1329

MRI-NTPATsck 1492

A-1

APPENDIX 3: Certificate of Analysis (Octocrylene)

Certificate of Analysis

SIGMA-ALDRICH

Product Name	2-Ethylhexyl 2-cyano-3,3-diphenylacrylate, 97%
Product Number	415820
Product Brand	ALDRICH
CAS Number	6197-30-4
Molecular Formula	$(C_6H_5)_2C=C(CN)CO_2CH_2CH(C_2H_5)(CH_2)_3CH_3$
Molecular Weight	361.48

TEST	SPECIFICATION	LOT 01697MJ RESULTS
Appearance (Color)	Yellow	Yellow
Appearance (Form)	Viscous Liquid	Viscous Liquid
Infrared spectrum	Conforms to Structure	Conforms
Purity (GC)	≥96.5 %	99.2 %
Specification Date:		OCT 2008
Date of QC Release:		OCT 2008
Print Date:		OCT 22 2008

[redacted]
[redacted] Supervisor
Quality Control
Milwaukee, Wisconsin USA

APPENDIX 3: Certificate of Analysis (Octylsalicylate)

Certificate of Analysis

SIGMA-ALDRICH

Product Name	2-Ethylhexyl salicylate, ≥99%
Product Number	W514500
Product Brand	ALDRICH
CAS Number	118-60-5
Molecular Formula	$(HO)C_6H_4CO_2CH_2CH(C_2H_5)(CH_2)_3CH_3$
Molecular Weight	250.33

TEST	SPECIFICATION	LOT 44698PJ RESULTS
Appearance (Color)	Colorless	Colorless
Appearance (Form)	Liquid	Liquid
Refractive index at 20 °C	1.500 - 1.504	1.502
Infrared spectrum	Conforms to Structure	Conforms
Purity (GC)	≥99.0 %	99.6 %
Color Test	≤100 APHA	10 APHA
Arsenic (As)	≤3.0 ppm	< 1.0 ppm
Cadmium (Cd)	≤1.0 ppm	< 1.0 ppm
Mercury (Hg)	≤1.0 ppm	< 1.0 ppm
Lead (Pb)	≤10.0 ppm	< 1.0 ppm
Specification Date:		DEC 2008
Date of QC Release:		DEC 2008
Print Date:		DEC 18 2008

[redacted]
[redacted] Supervisor
Quality Control
Milwaukee, Wisconsin USA

APPENDIX 3: Certificate of Analyses (Oxybenzone)

IVYCHEM

IVY FINE CHEMICALS

http://www.ivychem.com

CERTICATE OF ANALYSIS

Product Name	**2-HYDROXY-4-METHOXYBENZOPHENONE**		
Synonym	Oxybenzone		
Catalog Number	HH13-026		
CAS Number	131-57-7		
Batch Number	20100801	**Quantity**	200 KG
Manu. Date	August 2, 2010	**Expiry Date**	August 1, 2012
Date of Report	August 2, 2010	**Package**	
Quality Specifications	Specifications (In house)		

Test	Standard	Results
Appearance	Light yellow to green crystalline powder	Light yellow crystalline powder
Assay (HPLC)	98% min	99.92%
Melting Point	62 °C to 65 °C	63.8 °C to 64.8 °C
Loss on Drying	0.5% max	0.07%
Heavy Metals	<= 5 ppm	2.9 ppm
Conclusion: **Conform**		

APPENDIX 3: Certificate of Analysis (Aromatase Microsomes)

BD Biosciences – Discovery Labware
BD Gentest™ Products and Services
6 Henshaw Street
Woburn, MA 01801
Tel: 781.935.5115
Fax: 781.938.8644
bdbiosciences.com

Info_gentest@bd.com

BD

Human CYP19 + P450 Reductase SUPERSOMES™

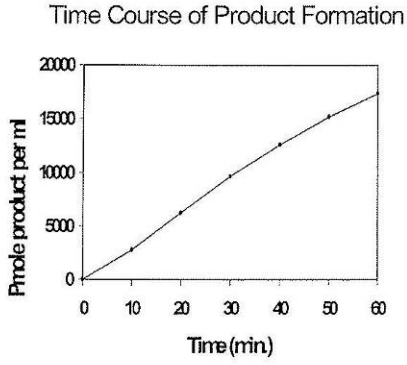
Catalog Number.....456260
Lot Number.....03897

Storage Conditions..**STORE AT -80°C**
Date Released .....2011 March
Expiration Date.....2014 February

Package Contents.....0.5 nmole cytochrome P450 in 0.5 mL
Protein Content..... 7.4 mg/mL in 100 mM potassium phosphate (pH 7.4)
Cytochrome c Reductase Activity.....290 nmole/(min x mg protein)
Cytochrome P450 Content.....1000 pmole per mL
Aromatase Activity.....6.0 pmole product/(min x pmole P450)

This activity is catalyzed by human CYP19 which is expressed from human CYP19 cDNA using a baculovirus expression system. Baculovirus infected insect cells (BTI-TN-5B1-4) were used to prepare these microsomes. These microsomes also contain cDNA-expressed human P450 reductase. A microsome preparation using wild type virus (GENTEST Catalog No. 456200 or 456201) should be used as a control for native activities.

METHOD: A 0.25 mL reaction mixture containing 25 pmole P450, 1.3 mM NADP+, 3.3 mM glucose-6-phosphate, 0.4 U/mL glucose-6-phosphate dehydrogenase, 3.3 mM magnesium chloride and 0.05 mM testosterone in 100 mM potassium phosphate (pH 7.4) was incubated at 37°C for 20 min. After incubation, the reaction was stopped by the addition of 125 uL acetonitrile and centrifuged (10,000 x g) for 3 minutes. 50 uL of the supernatant was injected into a 4.6 x 250 mm 5 µm C18 HPLC column and eluted isocratically at 45°C with a mobile phase of 60% water and 40% acetonitrile and at a flow rate of 1.5 mL per min. The product was detected by its absorbance at 200 nm and quantitated by comparing the absorbance to a standard curve of (beta)-estradiol.



ADVICE

- Thaw rapidly in a 37°C water bath. Keep on ice until use
- Aliquot to minimize freeze-thawing cycles. Less than 20% of the catalytic activity is lost after 6 freeze thaw cycles.
- Metabolite production is linear with respect to enzyme concentration up to at least 50 pmole P450 per mL.
- Metabolite production with testosterone is approximately linear for 40 minutes (see graph above).

THIS PRODUCT IS SUPPLIED FOR LABORATORY RESEARCH USE ONLY.

APPENDIX 3: Certificate of Analysis (Aromatase Microsomes)

BD Biosciences – Discovery Labware
BD Gentest™ Products and Services
6 Henshaw Street
Woburn, MA 01801
Tel: 781.935.5115
Fax: 781.938.8644
bdbiosciences.com

Info_gentest@bd.com

Human CYP19 + P450 Reductase SUPERSOMES™

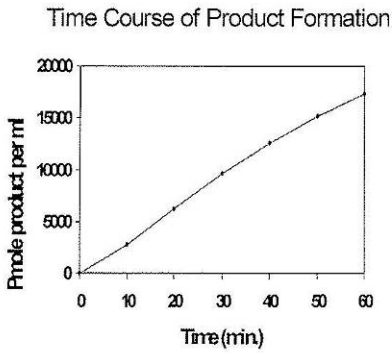
Catalog Number.....456260
Lot Number.....19701

Storage Conditions..**STORE AT -80°C**
Date Released .....2011 July
Expiration Date.....2014 July

Package Contents.....0.5 nmole cytochrome P450 in 0.5 mL
Protein Content..... 3.7 mg/mL in 100 mM potassium phosphate (pH 7.4)
Cytochrome c Reductase Activity.....540 nmole/(min x mg protein)
Cytochrome P450 Content.....1000 pmole per mL
Aromatase Activity.....5.7 pmole product/(min x pmole P450)

This activity is catalyzed by human CYP19 which is expressed from human CYP19 cDNA using a baculovirus expression system. Baculovirus infected insect cells (BTI-TN-5B1-4) were used to prepare these microsomes. These microsomes also contain cDNA-expressed human P450 reductase. A microsome preparation using wild type virus (GENTEST Catalog No. 456200 or 456201) should be used as a control for native activities.

METHOD: A 0.25 mL reaction mixture containing 25 pmole P450, 1.3 mM NADP+, 3.3 mM glucose-6-phosphate, 0.4 U/mL glucose-6-phosphate dehydrogenase, 3.3 mM magnesium chloride and 0.05 mM testosterone in 100 mM potassium phosphate (pH 7.4) was incubated at 37°C for 20 min. After incubation, the reaction was stopped by the addition of 125 uL acetonitrile and centrifuged (10,000 x g) for 3 minutes. 50 uL of the supernatant was injected into a 4.6 x 250 mm 5 µm C18 HPLC column and eluted isocratically at 45°C with a mobile phase of 60% water and 40% acetonitrile and at a flow rate of 1.5 mL per min. The product was detected by its absorbance at 200 nm and quantitated by comparing the absorbance to a standard curve of (beta)-estradiol.



ADVICE

- Thaw rapidly in a 37°C water bath. Keep on ice until use
- Aliquot to minimize freeze-thawing cycles. Less than 20% of the catalytic activity is lost after 6 freeze thaw cycles.
- Metabolite production is linear with respect to enzyme concentration up to at least 50 pmole P450 per mL.
- Metabolite production with testosterone is approximately linear for 40 minutes (see graph above).

THIS PRODUCT IS SUPPLIED FOR LABORATORY RESEARCH USE ONLY.

APPENDIX 3: Certificate of Analysis (^{3}H-Androstenedione, ^{3}H-ASDN)

PerkinElmer
precisely

PerkinElmer Life and Analytical Sciences
549 Albany Street, Boston, MA 02118

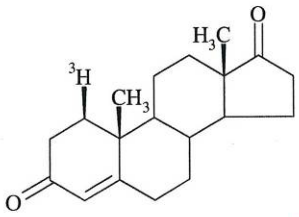
^{3}H Technical Data Certificate of Analysis

Caution: For Laboratory Use. A research chemical for research purposes only.

for EOSP Aromatase Assay rec'd. 3-23-11

NET-926 ANDROST-4-ENE-3, 17-DIONE, [1β-^{3}H(N)]-

Lot Number:	619344
Specific Activity:	26.3 Ci/mmol
	0.974 TBq/mmol
Production Date:	6 August 2010



M.W. 286
$C_{19}H_{26}O_2$

[redacted] 21 JUN 2011

37.0 MBq/mL / 0.974 TBq/mmol = 37.988 μM
1:100 dil = 380nM = 10 μCi/mL

PACKAGING: 1.0 mCi/ml (37 MBq/ml) in ethanol. Shipped on dry ice.

STABILITY AND STORAGE RECOMMENDATIONS:

When androst-4-ene-3, 17-dione, [1β-^{3}H(N)]- is stored at -20°C in its original solvent and at its original concentration, the rate of decomposition is initially 1% for 6 months from date of purification. Stability is nonlinear and not correlated to isotope half-life. Lot to lot variation may occur.

SPECIFIC ACTIVITY RANGE: 15-30 Ci/mmol (0.55-1.11 TBq/mmol)

RADIOCHEMICAL PURITY: This product was initially found to be greater than 97% when determined by the following methods. The rate of decomposition can accelerate. It is advisable to check purity prior to use:

1. High pressure liquid chromatography on a Zorbax ODS column using the following mobile phase:

   water : tetrahydrofuran : methanol (40:15:45)

2. Paper chromatography on Whatman No. 1 treated with 30% formamide in acetone using the following solvent system:

   hexane saturated with formamide.

3. Thin layer chromatography on silica gel using the following solvent system:

   toluene : ethyl acetate, (2:1).

QUALITY CONTROL: The radiochemical purity of androst-4-ene-3, 17-dione, [1β-^{3}H(N)]-is checked at appropriate intervals using the first listed chromatography method.

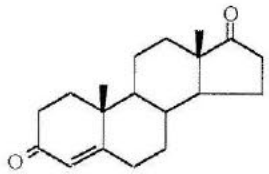
APPENDIX 3: Certificate of Analysis (Androstenedione, ASDN)

Batch Analysis

Provided by :

Steraloids, Inc.
P.O. Box 689
Newport, RI 02840

401-848-5422
E-Mail: sales@steraloids.com



Catalogue Item:	4-ANDROSTEN-3, 17-DIONE
Trivial Names:	ANDROSTENEDIONE (PURIFIED)
Catalog ID:	A6030-100
Steraloids, Inc.Batch:	L1712
Formula:	$C_{19}H_{26}O_2$
Molecular Weight:	286.41
Melting Point:	175-176°C
Rotation:	+179°(chf)
Test by TLC:	1•
Purity by HPLC:	99.8%
Visualised:	UV/pTSA,I2
Purification:	LPLC/crystallization
Storage:	Room temperature

Our steroids are for experimental and laboratory use only and are not to be used for drugs in humans or animals.

The specifics given are actual and will be for the particular batch noted.

Prepared By: [redacted]

for
Steraloids, Inc.

APR 26 2011

"TLC •" represents one spot (homogenous) when tested by thin layer chromatography.

The placing of a purchase order by the Buyer with the Seller is acceptance in full of the terms and conditions of the Seller including the acceptance that the goods as ordered by the Buyer are manufactured entirely to the Buyer's specifications as set out above.

APPENDIX 3: Certificate of Analysis (4OH-ASDN, Formestane)

Certificate of Analysis

SIGMA-ALDRICH

Product Name	Formestane, solid
Product Number	F2552
Product Brand	SIGMA
CAS Number	566-48-3
Molecular Formula	$C_{19}H_{26}O_3$
Molecular Weight	302.41

TEST	LOT 081k2133 RESULTS
APPEARANCE	WHITE POWDER
SOLUBILITY	CLEAR COLORLESS SOLUTION IN CHLOROFORM AT 50 MG/ML
SPECIFIC ROTATION	+174.3 DEG (C = 7.7 IN CHLOROFORM AT 20 DEG CELSIUS)
UV-VIS SPECTRUM	EMM = 12.6 AT LAMBDA MAX 277 NM IN ETHANOL
PURITY BY HPLC	99.6%
QC RELEASE DATE	JANUARY 2002

[redacted]

[redacted] Manager
Quality Control
St. Louis, Missouri USA

APPENDIX 4: Protocol and Protocol Amendments

4717 Campus Drive, Kalamazoo, MI 49008 (269) 353-5555 (office) (269) 544-1077 (fax) www.ceetox.com

CeeTox®

In vitro models to predict toxicity

FINAL PROTOCOL

Human Recombinant Aromatase Assay

Data Requirements: *OPPTS 890.1200*

Author

[redacted]

Study Number:
9070-100107AROM

Sponsor:
NIEHS
530 Davis Drive, MD K2-12
PO BOX 12233
Durham, NC 27713

Test Facility:
CeeTox
4717 Campus Drive
Kalamazoo, MI 49008

APPENDIX 4: Protocol and Protocol Amendments

TEST PROTOCOL

TO BE COMPLETED BY THE STUDY SPONSOR:
Study Sponsor: NIEHS/NTP [redacted] Chief Toxicology Branch)
Address: P.O. Box 12233 **Phone:** [redacted]
Research Triangle Park, NC
Study Monitor: [redacted] **E-mail:** [redacted]
Sponsor Protocol/Project No.:
Test Substance Name(s): Octyl Salicylate, 2-Ethylhexyl p-methoxycinnamate, 2-Ethylhexyl 2-cyano-3,3-diphenylacrylate, 2-Hydroxy-4-methoxybenzophenone

NIEHS/NTP Investigator
[redacted]
Telephone No.:
Facsimile No.:
E-mail:
Contract Office Technical Representative
[redacted]
(Contract No. HHSN273200900005C; NIEHS Control No. N01-ES-00005)

Study Monitor
[redacted] (ILS, Inc, Durham, NC)
Telephone No.:
Facsimile No.:
E-mail:

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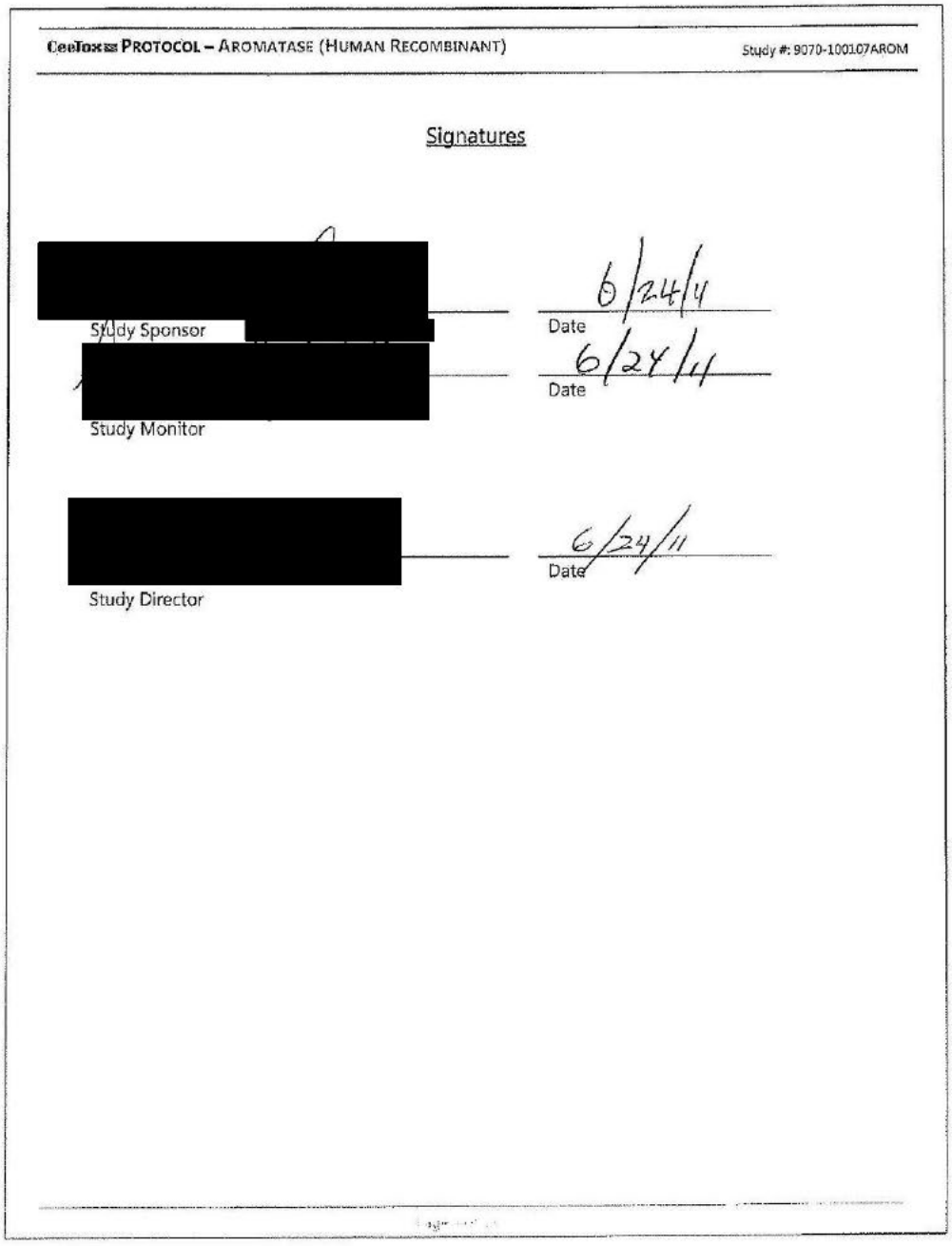

CeeTox PROTOCOL – AROMATASE (HUMAN RECOMBINANT) Study #: 9070-100107AROM

Signatures

[redacted]
Study Sponsor

6/24/14
Date

[redacted]
Study Monitor

6/24/14
Date

[redacted]
Study Director

6/24/14
Date

APPENDIX 4: Protocol and Protocol Amendments

1. Title of Study

Human Recombinant Aromatase Assay

2. Purpose of Study

The objective of this protocol is to describe procedures for conduct of the aromatase assay as a Tier 1 screening assay intended to identify substances that may affect the endocrine system (e.g., steroidogenesis) by inhibiting catalytic activity of aromatase, the enzyme responsible for the conversion of androgens to estrogens.

3. Compliance Statement

This study will be conducted in compliance with EPA GLP regulations (Title 40 Part 160) with the exception of section 160.113. Dose concentrations of test and control substances will not be verified using analytical methods.

4. Quality Assurance

This study will be subjected to periodic inspections and the draft and final reports will be reviewed by the Quality Assurance Unit of CeeTox in accordance with CeeTox SOP.

5. Regulatory Citations

Endocrine Disruptor Screening Program, *in vitro* Aromatase (Human Recombinant) EPA Test Guideline OPPTS 890.1200.

6. Test Facility

CeeTox, Inc.
4717 Campus Drive
Kalamazoo, MI 49008

7. Test & Control Substances

Test Substance(s)

Note: A certificate of analysis will be provided by the sponsor and will be stored in the study data and appended to the study report. Confirmation of the identity of the test substance, characterization and stability will be verified by the sponsor. CeeTox will obtain certificates of analysis for [^{3}H]ASDN and will store in the study data and append to the study report, along with ASDN. Test substance will be either returned to the Sponsor or destroyed following finalization of the study report.

7.1 Test Substance: 2-Hydroxy-4-Methoxybenzophenone (Oxybenzone)

CAS No. 131-57-7

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	Source:	Ivy Fine Chemicals Corporation
	Lot/Batch No.:	20080801
	ILS Repository No.:	11-29
	Formula:	$C_{14}H_{12}O_3$
	Description:	Light yellow powder
	Storage	Room Temperature

7.2 Test Substance: 2-Ethylhexyl p-methoxycinnamate (Octylmethoxycinnamate)

	CAS No.	5466-77-3
	Source:	Acros Organics
	Lot/Batch No.:	A0293319
	ILS Repository No.:	11-32
	Formula:	$C_{18}H_{26}O_3$
	Description:	Clear colorless liquid
	Storage	Room Temperature

7.3 Test Substance: Octyl Salicylate (Octylsalate)

	CAS No.	118-60-5
	Source:	Sigma-Aldrich
	Lot/Batch No.:	44698PJ
	ILS Repository No.:	11-30
	Formula:	$C_{15}H_{22}O_3$
	Description:	Colorless liquid

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Storage	Room Temperature

7.4 *Test Substance: 2-Ethylhexyl 2-Cyano-3,3-Diphenylacrylate (Octocrylene)*

CAS No.	6197-30-4
Source:	Sigma-Aldrich
Lot/Batch No.:	01697MJ
ILS Repository No.:	11-31
Formula:	$C_{24}H_{27}NO_2$
Description:	Yellow viscous liquid
Storage	Room Temperature

Preparation of Test Substance

Test substance(s) will be formulated in buffer, absolute ethanol or dimethylsulfoxide (DMSO). The total volume of solvent used in each assay will be no more than 1% (if the solvent is DMSO or ethanol) of the total assay volume in order to minimize the potential of the solvent to inhibit the enzyme. Fresh dilutions of the stock solution will be prepared on the day of use such that the target concentration of test substance can be achieved by the addition of 20 µL of the dilution to a 2 mL total assay volume. Dose concentrations of test and control substances will not be verified using analytical methods.

Positive Substance

The known aromatase inhibitor, 4-hydroxyandrostendione (4-OH ASDN), is used as the positive control. Table 1 contains identity and property information for 4-OH ASDN.

Table 1

Test Substance	CAS Number	Molecular Formula	Molecular Weight (g/mol)
4-OH ASDN	566-48-3	$C_{19}H_{26}O_3$	302.4

The 4-OH ASDN will be formulated in absolute ethanol or DMSO. Fresh dilutions of the stock solution will be prepared on the day of use. Dilutions will be prepared such that the target concentrations of control substance (Table 4) can be achieved by the addition of 20

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µL of the dilution to a 2 mL total assay volume with solvent concentrations ≤ 1%. Information on storage conditions for the control substance stock solutions will be reported.

Substrate

Substrate Name/Supplier

The substrate for the aromatase assay is androstenedione (4-Androstene-3,17-dione or ASDN). Radioinert and [^{3}H]ASDN androstenedione ([1β-^{3}H]-androstenedione, [^{3}H] ASDN) will be used. The radioinert ASDN will be ≥ 98% pure. The radiolabeled ASDN will be ≥ 95% radiochemically pure and is usually supplied at a specific activity of 20-30 Ci/mmol. The 1 mCi/ml [^{3}H]ASDN stock will be diluted to 0.3 to 0.5 Ci/mmol by the addition of buffer and radioinert ASDN. This substrate solution will have a concentration of 2 µM ASDN and a radiochemical content of about 1 µCi/ml. All applicable information regarding supplier, lot numbers and reported/measured purity for the substrates will be included in study reports.

Radiochemical Purity

The radiochemical purity of the [^{3}H]ASDN shall be greater than or equal to 95 percent. If the radiochemical purity is less than 95 percent, then a new batch of radiochemical shall be obtained.

Preparation of Substrate Solution for use in Aromatase Assay

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

The following example illustrates the preparation of a substrate solution using a stock of [^{3}H]ASDN with a specific activity of 25.3 Ci/mmol and a concentration of 1 mCi/mL:

Prepare a 1:100 dilution of radiolabeled stock in 0.1 M Sodium Phosphate Assay buffer

Prepare a 1 mg/mL solution of ASDN in ethanol and then prepare dilutions in buffer to a final concentration of 1 µg/mL.

Combine 4.6 mL of the 1 µg/mL solution of ASDN, 800 µL of the [^{3}H]ASDN and 2.6 mL buffer to make 8 mL of substrate solution (enough for 80 tubes).

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Record the weight and/or volumes of each component added to the substrate solution. After mixing well, combine 20 µL aliquots with scintillation cocktail for radiochemical content analysis. Adjust the isotope level if not within 10% of the nominal activity and test again to verify accuracy.

Add 100 µL of the substrate solution to each 2 mL assay volume to yield a final [^{3}H] ASDN concentration of 100 nM with 0.1 µCi/tube.

Microsomes

Human Recombinant Microsomes

Human Recombinant Microsomes are purchased from Gentest™ (Woburn, MA: www.gentest.com). The product name is Human CYP19 (Aromatase) and P450 reductase Supersomes ™ and the catalog number is 456260 (or equivalent microsomes). The package insert (batch data sheet) provides values for protein concentration, cytochrome c reductase activity, and aromatase activity and will be included in the report. Information regarding the stability to freeze thaw cycles is also provided on the batch data sheet. The microsomes will be stored at approximately -80°C.

Human Recombinant Microsome Preparation

Preparation of the human recombinant microsomes will involve thawing the microsomes rapidly in a approximately 37°C water bath and placing them in an ice bath and aliquoting them into individual vials based upon the protein content of the batch. This minimizes freeze-thaw cycles. The assay uses approximately 0.004 mg/mL (final concentration) of microsomal protein. After aliquoting the microsomes into individual vials, the vials that are not planned for immediate use will be returned to the approximately -80°C freezer for storage (Information regarding stability to freeze thaw cycles will be followed and is provided on the batch data sheet). All applicable information regarding supplier, lot numbers and reported/measured purity for the microsomes will be included in the study report.

Protein Assay

Protein content of the microsomes will be supplied by the vendor (Gentest™ (Woburn, MA: www.gentest.com) or vendor of equivalent microsomes) and information retained by CeeTox.

Cytochrome P450 (CYP19) Aromatase Activity

Aromatase activity of the microsome preparation will be provided by the vendor (Gentest™ (Woburn, MA: www.gentest.com) or vendor of equivalent microsomes) and verified by

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CeeTox that they have sufficient activity. Sufficient activity will be visible in the controls used in the aromatase assay when the assay is run.

Other Assay Components

Buffer

The assay buffer is 0.1M sodium phosphate buffer, pH ~7.4. Sodium phosphate monobasic and sodium phosphate dibasic will be used to prepare the buffer. Solutions of each reagent at 0.1M will be prepared in purified water and then the solutions will be combined to a final pH of ~7.4.

Propylene Glycol

Propylene glycol will be added to the assay directly as described below.

NADPH

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

8. Test System

As per the guideline (OPPTS 890.1200) recombinant microsomes (Human CYP19 + P450 Reductase SUPERSOMES™) will be used as the test system for this study.

9. Aromatase Assay Method

The reactions will be performed in 13 X 100 mm test tubes.

Each reaction tube will be labeled by applying label or writing directly on the tube.

Buffer volume will be adjusted so the total incubation volume will be 2 mL.

Propylene glycol, [^{3}H]ASDN, NADPH, and buffer (0.1 M sodium phosphate buffer, pH ~7.4) will be combined in the reaction tubes to a total volume of 980 µL.

Substance solution, positive control (or vehicle control) will be added to the mixture of propylene glycol, substrate, NADPH and buffer in a 20 µL volume prior to preincubation of that mixture. The final concentrations for the assay components are presented in Table 2.

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The reaction tubes and the microsomal suspension will be preincubated at approximately 37°C in the water bath for at least five minutes prior to initiation of the assay by the addition of 1 mL of the diluted microsomal suspension.

The total assay volume will be 2 mL, and the tubes will be incubated at approximately 37°C for ~15 min.

The reaction will be terminated by the addition of 2 mL ice-cold Methylene Chloride.

The tubes will be mixed for ca. 5s and place on ice for~ 5 minutes.

The tubes will be mixed for an additional 20-25s.

The tubes will be centrifuged for ~10 minutes at 200 x g rfc (4°C±2°C).

The Methylene Chlorine (bottom layer) will be removed and discarded.

The aqueous layers will be extracted again with ice-cold Methylene Chloride (2mL) and the Methylene Chloride (bottom layer) discarded following centrifugation as described above.

The extraction will be repeated as described for a third time.

Five hundred microliter aliquots of the aqueous layers will be transferred into two 20 mL liquid scintillation counting vials as duplicate measurements of each assay tube.

Add liquid scintillation cocktail (Opti-Fluor, Perkin Elmer) to each counting vial and shake to mix the solution. The radiochemical content of each aliquot will be determined as described below:

Table 2. Optimized Aromatase Assay Conditions

Assay Factor (units)	Human Recombinant
Microsomal Protein (mg/mL)	0.004
NADPH (mM)	0.3
[^{3}H]ASDN (nM)	100
Propylene glycol	5%
Incubation Time (min)	15

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

Results will be presented as the amount of estrone formed and activity (velocity) of the enzyme reaction. The amount of estrone formed will be determined by dividing the total amount of ^3H_2O formed by the specific activity of the [^{3}H]ASDN substrate (expressed in

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dpm/nmol). The activity of the enzyme reaction is expressed in nmol/mg-protein/min and will be calculated by dividing the amount of estrone formed by the product of mg microsomal protein used multiplied by the incubation time, i.e., 15 minutes.

10. Positive Control Assay

A run is defined as a separate independent experiment. Each run will contain tubes for full activity control, background activity control and positive control.

The minimum level of mean aromatase activity in the full activity control samples shall be 0.100 nmol/mg-protein/min.

The mean background control activity shall be ≤ 15% of the full activity control.

The concentration response curve generated for the 4-OH ASDN should meet the conditions listed in Table 3.

Table 3

	Parameter	Lower Limit	Upper Limit
Positive Control	Slope	-1.2	-0.8
	Top (%)	90	110
	Bottom (%)	-5	+6
	Log IC_{50}	-7.3	-7.0

Data available and can be added as an appendix to the report upon request

Table 4 Positive Control Study Design

Sample Type	Repetition (tubes)	Description	4-OH ASDN Conc. (M)
Full Activity Control	4	All test components. No inhibitor	N/A
Background Activity Control	4	Same as full activity control, but no NADPH	N/A
4-OH ASDN Conc. 1	3	Complete assay with 4-OH ASDN (positive control) added	$1X10^{-5}$
4-OH ASDN Conc. 2	3	same	$1X10^{-6}$
4-OH ASDN Conc. 3	3	same	$1X10^{-6.5}$
4-OH ASDN Conc. 4	3	same	$1X10^{-7}$
4-OH ASDN Conc. 5	3	same	$1X10^{-7.5}$
4-OH ASDN Conc. 6	3	same	$1X10^{-8}$
4-OH ASDN Conc. 7	3	same	$1X10^{-9}$
4-OH ASDN Conc. 8	3	same	$1X10^{-10}$

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11. Determination of the Response of Aromatase Activity to Test Substance(s)

A run is an independent experiment. [Each run will contain full activity control, background activity control, positive control, and test substances as shown in Table 4.]

Each run will test the response of aromatase activity in the presence of eight concentrations of a test substance run in triplicate (i.e., there are three tubes of each test substance concentration per run of the assay). A test substance shall be tested in three independent runs. Each run for a given test substance will be conducted entirely independently of the other runs for that test substance. There will be three (triplicate) repetitions for each concentration of a test substance. A single run of a given test substance is described in Table 5.

Three types of control samples will be included for each run. These include:

- Full enzyme (aromatase) activity controls (substrate, NADPH, propylene glycol, buffer, vehicle (used for preparation of test substance solutions) and microsomes).
- Background activity controls (all components that are in the full aromatase activity controls except NADPH).
- Positive controls (4-OH ASDN run at eight concentrations in the same manner as test substances).

Four test tubes of the full enzyme activity control and background activity controls are included with each run. The full enzyme and background activity controls sets will be split so that two tubes (of each control type) are run at the beginning and two at the end of each run. The positive control will be tested at eight concentrations in each run as indicated in Table 5. All controls are treated the same as the other samples.

The aromatase assay will be conducted as described in this protocol.

After completion of the first run, the data will be reviewed and, if necessary, the concentration of the test substance used in the second and third runs can be adjusted. The decision will be based upon the results of the first run with the following guidelines in mind:

- If insolubility (cloudiness or a precipitate) is observed at the highest concentration (10^{-3} M), then the highest concentration will be set for the second and third runs at the highest concentration that appeared soluble using mid-log concentrations; i.e., try $10^{-3.5}$M if the test substance is insoluble at 10^{-3}M as it is important to define the lower portion of the curve. Concentrations lower than 10^{-5}M for the highest concentration will not be tested.

- If the highest concentration to be tested is lowered to 10^{-4} or 10^{-5} M, then mid-log concentration(s) will be added near the lower end of the curve (higher concentrations) and around the estimated IC50 based on the results of the first run in order to keep eight concentrations in the test set.

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- The lowest concentration to be tested will be 10^{-10}M, but lower concentrations may be required to obtain the "top of the curve". That is, obtain the full enzymatic activity at the two lowest concentrations of the test substance in order to define the top of the concentration-response curve.

Table 5. Test Substance Study Design

Sample Type	Repetition	Description	Reference or Substance Conc. (M)
Full Activity Control	4	All test components plus solvent vehicle*	N/A
Background Activity Control	4	Same as full activity control, but no NADPH	N/A
Positive Control Conc1	2	Complete assay with 4-OH ASDN added	$1X10^{-5}$
Positive Control Conc2	2	same	$1X10^{-6}$
Positive Control Conc3	2	same	$1X10^{-6.5}$
Positive Control Conc4	2	same	$1X10^{-7}$
Positive Control Conc5	2	same	$1X10^{-7.5}$
Positive Control Conc6	2	same	$1X10^{-8}$
Positive Control Conc7	2	same	$1X10^{-9}$
Positive Control Conc8	2	same	$1X10^{-10}$
Test substance Conc1	3	Compete assay with test substance added	$1X10^{-3}$
Test substance Conc2	3	same	$1X10^{-4}$
Test substance Conc3	3	same	$1X10^{-5}$
Test substance Conc4	3	same	$1X10^{-6}$
Test substance Conc5	3	same	$1X10^{-7}$
Test substance Conc6	3	same	$1X10^{-8}$
Test substance Conc7	3	same	$1X10^{-9}$
Test substance Conc8	3	same	$1X10^{-10}$

N/A = not applicable

*The complete assay ("all test components") contains buffer, propylene glycol, microsomal protein, [^{3}H]ASDN and NADPH.

See Table 7 page 13 of Test Guideline

12. Data Analysis

Aromatase Activity and Percent of Control Calculations

Relevant data will be entered into the assay spreadsheet for calculations of aromatase activity and percent control. A spreadsheet will calculate the DPM/mL for each aliquot of

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the extracted aqueous incubation mixture and average DPM/mL and total DPM for each aqueous portion (after extraction). The volume (mL) of substrate solution added to the incubation multiplied by the substrates specific activity (DPM/mL) yields 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 yields the percent of the substrate that was converted to product. The total DPM remaining in the aqueous portion after extraction will be corrected for background by subtracting the average DPM present in the aqueous portion of the background activity control tubes (Table 5). This corrected DPM is then converted to nmol product formed by dividing by the substrate specific activity (DPM/nmol). The activity of the enzyme reaction will be expressed in nmol (mg product)$^{-1}$min^{-1} and will be calculated by dividing the amount of ^3H_2O formed (nmol) by the product of mg microsome protein used times the incubation time (15 minutes). Average activity in the full activity control samples will be calculated. Percent of control activity remaining in the presence of the various inhibitor concentrations, including the positive control, will be calculated by dividing the aromatase activity at a given concentration by the average full activity control and multiplying by 100.

Nominally one might expect the percent of control activity values for an inhibitor to vary between approximately 0 percent near the high inhibition concentrations and approximately 100 percent near the low inhibition concentrations. However due to experimental variation, individual observed percent of control values will sometimes extend below 0 percent or above 100 percent.

13. Model Fitting

The response curve will be fitted by weighted least squares nonlinear regression analysis with weights equal to 1/Y. Model fits will be carried out using a non-linear regression program such as Prism software (version 5.1) or xlfit (IDBS).

Concentration response trend curves will be fitted to the percent of control activity values within each of the repeat tubes at each test substance concentration. Concentration will be expressed on the log or half-log scale.

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

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

Concentration response models will be fitted for each test run for each test substance and control(s).

Y= percent of control activity in the inhibitor tube

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X= Logarithm (base 10) of the concentration

T= average DPMs across the repeat tubes with the same test substance concentration that define the Top of the curve

B= average DPMs across the repeat tubes with the same test substance concentration that define the Bottom of the curve

β= slope of the concentrations response curve (β will be negative)

Graphical and Analysis of Variance Comparisons Among Concentration Response Curve Fits

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

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

Quality Control-Analysis of Variance Comparisons of Full Enzyme Activity Control and Background Activity Control as Percent of Control.

Within each run of each test substance quadruplicate repetitions will be made of the full enzyme activity control (FEAC) and background activity control (BAC) control tubes. Half the repetitions will be carried out at the beginning of the run and half at the end. If the conditions are consistent throughout the test, the control tubes at the beginning should be equivalent to the control tubes at the end.

To assess if this is the case, control responses will be adjusted for background DPMs, divided by the average of the (background adjusted) FEAC control values, and expressed as percent of control. The average of the four BAC controls within a run must be approximately 0 percent (with an acceptable range of -5 to +6%) and the average of the four FEAC controls within a run must be approximately 100% (with an acceptable range of 90 – 110%).

Data Interpretation

Data from this assay will be used to classify substances according to their ability to inhibit aromatase. To be classed as an inhibitor, the data must fit the 4-parameter regression

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model to yield an inhibition curve and result in greater than 50% inhibition at the highest concentration. The value of the inhibition curve at each of three runs at the highest concentration should be averaged and compared with the following criteria. If the data do not fit the model the average activity of the data points at the highest concentration shall be used.

Table 6

Criteria		Classification
Data fit 4-parameter nonlinear regression model	Curve crosses 50%	Inhibitor
	Average lowest portion of curves across runs is between 50% and 75% Activity	Equivocal
	Average lowest portion of curves across runs is greater than 75%	Non-inhibitor
Data do not fit the model	---	

Statistical Software and Analysis

Concentration curves will be fitted to the data using non-linear regression analysis features in a commercial software package such as prism or xlfit. For data generated at Ceetox, basic statistical analysis will be performed on the data, which will include means of replicates, standard error of the mean, and coefficient of variation.

14. Study Reports

The data to be reported in the interim data summary and final report will include (but will not be limited to) the following information: assay date and run number, laboratory personnel involved in the study, chemical/test substance information (including but not limited to chemical name, code, molecular weight, concentrations tested, notes regarding solubility), background corrected aromatase activity (for each control and test substance repetition, percent of control activity, IC50, slope and graphs of activity versus log substance concentration.

15. Alterations of the Study Design

Alterations of this protocol may be made as the study progresses. No changes in the protocol will be made without the specific written request or consent of the Sponsor. In the event that the Sponsor authorizes a protocol change verbally, CeeTox will honor such a change. However, written authorization will be obtained thereafter. All protocol amendments and justifications will be documented, signed and dated by the Study

APPENDIX 4: Protocol and Protocol Amendments

Director, Study Monitor and Sponsor and added to the report. A copy of the protocol and all amendments will be issued to the Sponsor as well as CeeTox and placed into the study binder.

16. Data Retention and Archiving

All raw data, documentation, records, protocol, and the final report generated as a result of this study will be retained at CeeTox for 15 years. Retention of the materials after the time 15 years will be subjected to a future contractual agreement between the Sponsor and CeeTox.

Study Records to be maintained:

- All records that document the conduct of the laboratory experiments and results obtained, as well as the equipment and chemicals used.
- Protocol and protocol Amendments
- List of any Protocol Deviations
- Final Report

APPENDIX 4: Protocol and Protocol Amendments

CeeTox

Protocol Amendment

Study Number: 9070-100107AROM

Title of Study to be Amended: Human Recombinant Aromatase Assay

Reason for Amendment to Protocol: The Table of Contents had typographical errors.

Change:

The Table of Contents will now read:

Signature

CeeTox, Inc.

[redacted]

Study Director (Project Manager)

26 SEPTEMBER 2011
Date

APPENDIX 4: Protocol and Protocol Amendments

CeeTox

Protocol Amendment

Study Number: 9070-100107AROM

Title of Study to be Amended: Human Recombinant Aromatase Assay

Reason for Amendment to Protocol: Client requested amendment

Change:
Section Data Retention and Archiving will now state:

At the study closure, all study records including all original raw data and original final report, will be shipped to the sponsor at the following address:

NTP Archives
[redacted]
615 Davis Drive, Suite 300
Durham, NC 27713

Signature

CeeTox, Inc.

[redacted]
Study Monitor

12-6-11
Date

[redacted]
Study Director (Project Manager)

6 DECEMBER 2011
Date

CeeTox Study # 9070-100107AROM 6-Dec-11