Human Recombinant Aromatase Assay:

Final Report

DATA REQUIREMENT(S): OPPTS 890.1200 (2009)

AUTHOR(S): [redacted]

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STATEMENT OF DATA CONFIDENTIALITY CLAIMS

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Study Number: 9070-100794AROM

Study Title: Human Recombinant Aromatase Assay

I, the undersigned, hereby declare that this study was conducted in compliance Environmental Protection Agency Good Laboratory Practice 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]
Study Director
CeeTox, Inc.

24 APRIL 2013
Date

FLAGGING STATEMENT

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QUALITY ASSURANCE STATEMENT

Study Title: Human Recombinant Aromatase Assay

Study Number: 9070-100794AROM

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
11 January 2013	Study Protocol	11 January 2013	11 January 2013
25 February 2013	In-Process *	01 March 2013	01 March 2013
18 March 2013	Study Databook	18 March 2013	18 March 2013
18 March 2013	Draft Report	18 March 2013	18 March 2013

(*) Test substance preparation and aromatase assay.

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

[redacted]

24 Apr 2013
Date

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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
	Laboratory Manager
	Senior Scientist
	Research Associate
	Associate Scientist 1

Study Dates

Study initiation date: 13 February 2013
Experimental start date: 21 February 2013
Experimental termination date: 28 February 2013
Study completion date: 24 April 2013

Deviations from the Protocol

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

Other

All original data [including the original signed study protocol and all amendments (if any), test substance information, observations, etc.] and the original final report will be transferred to the National Toxicology Program Archives following finalization of the study report to the address below:

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 2-Phenyl-5-benzimidazolesulfonic Acid (Referred to as Avobenzone), Butyl-methoxydibenzoylmethane (Referred to as Ensulizole), 3, 3, 5-Trimethlycyclohexyl Salicylate (Referred to as Homosalate), and 2-Ethylhexyl-P-Dimethyl-Aminobenzoate (Referred to as Padimate-O or Padimate O) to act as inhibitors of aromatase activity using human CYP19 (aromatase) and P450 reductase Supersomes^{TM} purchased from Gentest^{TM} as the test system. The substrate for the assay is androstenedione (ASDN), which is converted by aromatase to estrone.

Final concentrations of Avobenzone, Homosalate, and Padimate-O tested in the aromatase assay were 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3} M. Final concentrations of Ensulizole tested in the aromatase assay were $10^{-10.5}$, $10^{-9.5}$, $10^{-8.5}$, $10^{-7.5}$, $10^{-6.5}$, $10^{-5.5}$, $10^{-4.5}$, and $10^{-3.5}$ M.

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 (21 February 2013, 25 February 2013, and 27 February 2013), increasing concentrations of Avobenzone, Ensulizole, Homosalate, and Padimate-O showed negligible decreases in aromatase activity (all $\geq$80% of control values). Avobenzone and Padimate-O was soluble in the assay buffer at concentrations of $\leq 10^{-5}$ M. Ensulizole was soluble in the assay buffer at concentrations of $\leq 10^{-3.5}$ M. Homosalate was soluble in the assay buffer at concentrations of $\leq 10^{-4}$ M. Thus, the suitable top concentrations for Avobenzone, Ensulizole, Homosalate, and Padimate-O for use in the aromatase assay were established at 10^{-5} M, $10^{-3.5}$ M, 10^{-4} M, and 10^{-5} M, respectively.

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), Avobenzone, Ensulizole, Homosalate, and Padimate-O were classified as non-inhibitors, with mean aromatase activities of 115% (± 9% SD), 102% (± 1% SD), 89% (± 2% SD), and 98% (± 2% SD), respectively, at the highest soluble test concentrations at 10^{-5} M, $10^{-3.5}$ M, 10^{-4} M, and 10^{-5} M, respectively.

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 hormone systems. 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), 2009 (now referred to as OCSPP though the guideline is still titled OPPTS).

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.

Table 1A. Test Substance Butyl-methoxydibenzoylmethane, lot L802809 (Referred to as Avobenzone)

Test Substance Name:	**Avobenzone** (Butyl-methoxydibenzoylmethane)
Manufacturer:	Universal Preserv-A-Chem Inc.
CAS Number:	70356-09-1
Description:	Off White to Yellowish Crystalline Powder
Solvent Used:	DMSO
Lot Number:	L802809
Expiry Date:	Not provided
Purity:	98.5%
Molecular Formula:	$C_{20}H_{22}O_3$
Molecular Weight:	310.39
Storage Conditions:	Room temp (e.g., ambient)

Table 1B.Test Substance 2-Phenyl-5-benzimidazolesulfonic acid, lot 05117JE (Referred to as Ensulizole)

Test Substance Name:	**Ensulizole** (2-Phenyl-5-benzimidazolesulfonic acid)
Manufacturer:	Aldrich
CAS Number:	27503-81-7
Description:	White powder
Solvent Used:	DMSO
LotNumber:	05117JE
Expiry Date:	Not provided
Purity:	99.6%
Molecular Formula:	$C_{13}H_{10}N_2O_3S$
Molecular Weight:	274.30
Storage Conditions:	Room temp (e.g., ambient)

Table 1C. Test Substance 3, 3, 5-Trimethlycyclohexyl Salicylate, lot YT0976 (Referred to as Homosalate)

Test Substance Name:	**Homosalate** (3, 3, 5-Trimethlycyclohexyl Salicylate; or Homosalate)
Manufacturer:	Spectrum
CAS Number:	118-56-9
Description:	Colorless to light yellow liquid
Solvent Used:	DMSO
Lot Number:	YT0976
Expiry Date:	Not provided
Purity:	99.3%
Molecular Formula:	$C_{16}H_{22}O_3$
Molecular Weight:	262.34
Storage Conditions:	Room temp (e.g., ambient)

Table 1D. Test Substance 2-Ethylhexyl-p-dimethyl-aminobenzoate, lot MKBF0590V (Referred to as Padimate-O or Padimate O)

Test Substance Name:	**Padimate-O** (2-Ethylhexyl-p-dimethyl-aminobenzoate)
Manufacturer:	Aldrich
CAS Number:	21245-02-3
Description:	Colorless liquid
Solvent Used:	DMSO
Lot Number:	MKBF0590V
Expiry Date:	Not provided
Purity:	98.1%
Molecular Formula:	$C_{17}H_{27}NO_2$
Molecular Weight:	277.40
Storage Conditions:	Room temp (e.g., ambient)

Note: Certificates of analysis were stored in the study data binder 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:	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-ASDNwas formulated in 100% dimethylsulfoxide (DMSO; lot RNBC5920, expires October 2014). 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 26.3Ci/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 # NET926)
CAS Number:	63-05-8
Description:	White powder, slightly crystalline
Solvent Used:	Ethanol
Batch Number:	1632499
Expiry Date:	06 June 2013
Radiochemical Purity:	>97%
Molecular Formula	$C_{19}H_{26}O_2$
Molecular Weight:	286
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 (0.1M 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 100nM and the amount of tritium added to each incubation tube was approximately 0.1 µCi.

3.3.1.1 Example Calculations

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

[^{3}H]ASDN, NET926 (Lot# 1632499; MW 286; 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 was achieved by the addition of 20 µL of the dilution to a 2 mL total assay volume. Final concentrations of Avobenzone, Homosalate, and Padimate-O tested in the aromatase assay were 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3} M. Final concentrations of Ensulizole tested in the aromatase assay were $10^{-10.5}$, $10^{-9.5}$, $10^{-8.5}$, $10^{-7.5}$, $10^{-6.5}$, $10^{-5.5}$, $10^{-4.5}$, and $10^{-3.5}$ M.

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 ™ (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 approximately -80°C.

3.4.2 Protein Assay

Protein content of the microsomes was supplied by the vendor (BD Gentest; 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; 5.7 pmol product/(min x pmol P450) for lot 19701; Appendix 3).

3.4.4 Human Recombinant Microsome Preparation

Initial preparation of the human recombinant microsomes involved thawing the microsomes rapidly in ~37°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 (0.1M 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 approximately -80°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 ^3H_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 0.1M sodium phosphate buffer, pH 7.4. Sodium phosphate monobasic (Sigma S5011, lot 070M001962V) and sodium phosphate dibasic (Sigma S5136, lot 100M01141V) 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 2AG3003) 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 (0.1M sodium phosphate, pH 7.4) and the final concentration in the assay was 0.3 mM NADPH (Calbiochem 481973, lot D00130037). 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°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°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°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 ≥0.100nmol/mg-protein/min for the assay run to be considered acceptable. In addition, the mean background control activity must be ≤ 15% (Table 24) 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., $10^{-3.5}$ M, 10^{-4} M, etc. Concentrations lower than 10^{-5} M as the highest concentration evaluated were not used for data interpretation.

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 $^{3}H_2O$ 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 (Table 24).

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

<table>
<tr><th colspan="2">Criteria</th><th>Classification</th></tr>
<tr><td rowspan="3">Data fit 4-parameter nonlinear regression model</td><td>Curve crosses 50%</td><td>Inhibitor</td></tr>
<tr><td>Average lowest portion of curves across runs is between 50% and 75% Activity</td><td>Equivocal</td></tr>
<tr><td>Average lowest portion of curves across runs is greater than 75%</td><td rowspan="2">Non-inhibitor</td></tr>
<tr><td>Data do not fit the model</td><td>----</td></tr>
</table>

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., IDBSXLfit 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

Final concentrations of Avobenzone, Homosalate, and Padimate-O tested in the aromatase assay were 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3} M. Final concentrations of Ensulizole tested in the aromatase assay were $10^{-10.5}$, $10^{-9.5}$, $10^{-8.5}$, $10^{-7.5}$, $10^{-6.5}$, $10^{-5.5}$, $10^{-4.5}$, and $10^{-3.5}$ M.

Avobenzone and Padimate-O was soluble in the assay buffer at concentrations of $\leq 10^{-5}$ M. Ensulizole was soluble in the assay buffer at concentrations of $\leq 10^{-3.5}$ M. Homosalate was soluble in the assay buffer at concentrations of $\leq 10^{4}$ M (see Table 25). Thus, the suitable top concentrations for Avobenzone, Ensulizole, Homosalate, and Padimate-O for use in the aromatase assay were established at 10^{-5} M, $10^{-3.5}$ M, 10^{-4} M, and 10^{-5} M, respectively.

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).

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 = 99.58% to 105.67%	(Guideline Range = 90% – 110%)
Bottom Curve = -0.46% to 0.15%	(Guideline Range = -5% to 6%)
Hill Slope = -0.96 to -0.88	(Guideline Range = -1.2 to -0.8)
Log IC_{50} = -7.18 to -7.07	(Guideline Range = -7.3 to -7.0)

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).

The mean aromatase activity values in the full activity control samples were at least 0.412 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 21 Feb 2013, 25 Feb 2013, and 27 Feb 2013. 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:

Avobenzone:	115% (± 9% SD) of control activity	= Non-inhibitor
Ensulizole:	102% (± 1% SD) of control activity	= Non-inhibitor
Homosalate:	89% (± 2% SD) of control activity	= Non-inhibitor
Padimate-O:	98% (± 2% SD) of control activity	= Non-inhibitor

4.5 Discussion

In three independent runs of the assay, test substances were tested at final concentrations in the range of $10^{-10.5}$ to 10^{-3} M. Final concentrations of Avobenzone, Homosalate, and Padimate-O tested in the aromatase assay were 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3} M. Final concentrations of Ensulizole tested in the aromatase assay were $10^{-10.5}$, $10^{-9.5}$, $10^{-8.5}$, $10^{-7.5}$, $10^{-6.5}$, $10^{-5.5}$, $10^{-4.5}$, and $10^{-3.5}$ M. Avobenzone, Ensulizole, Homosalate, and Padimate-O were shown to be non-inhibitors according to the EDSP guideline (Table 10, Section 3.10.5 Data Interpretation).

5.0 CONCLUSIONS

Avobenzone, Ensulizole, Homosalate, and Padimate-O were determined to be non-inhibitors, 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 Avobenzone (21 Feb 2013)

Concentration of 4OH-ASDN (M)	Aromatase Activity (% of VC)		Individual Aromatase Activity (% of VC)		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	101.54	0.556	101.14	101.93	ND
NSB	0.01	0.006	0.00	0.01	ND
10^{-5}	1.14	0.036	1.11	1.16	ND
10^{-6}	9.68	0.626	9.23	10.12	ND
$10^{-6.5}$	23.56	0.309	23.34	23.78	ND
10^{-7}	47.95	1.198	48.79	47.10	ND
$10^{-7.5}$	67.77	10.046	74.87	60.66	ND
10^{-8}	89.03	4.880	85.58	92.48	ND
10^{-9}	98.88	0.653	99.34	98.42	ND
10^{-10}	99.13	2.402	100.83	97.43	ND

Concentration of Avobenzone (M)	Aromatase Activity (% of VC)		Individual Aromatase Activity (% of VC)		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	98.46	3.036	96.32	100.61	ND
NSB	-0.01	0.039	0.02	-0.03	ND
10^{-3}	89.72	1.422	89.98	91.01	88.19
10^{-4}	103.98	8.485	94.84	111.61	105.50
10^{-5}	106.89	0.818	106.60	107.81	106.26
10^{-6}	102.25	2.497	104.24	99.45	103.07
10^{-7}	96.70	4.643	98.35	91.46	100.29
10^{-8}	99.08	1.645	97.36	100.64	99.26
10^{-9}	100.02	2.208	102.26	99.97	97.84
10^{-10}	98.90	3.187	98.33	96.03	102.33

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 Avobenzone (25 Feb 2013)

Concentration of 4OH-ASDN (M)	Aromatase Activity (% of VC)		Individual Aromatase Activity (% of VC)		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	99.36	0.393	99.08	99.63	ND
NSB	0.02	0.005	0.03	0.02	ND
10^{-5}	0.91	0.066	0.87	0.96	ND
10^{-6}	8.25	0.101	8.18	8.32	ND
$10^{-6.5}$	20.94	0.541	20.56	21.32	ND
10^{-7}	43.12	0.865	42.51	43.73	ND
$10^{-7.5}$	69.72	2.427	68.00	71.43	ND
10^{-8}	88.89	1.259	88.00	89.78	ND
10^{-9}	104.41	1.431	105.42	103.39	ND
10^{-10}	104.51	2.487	106.27	102.75	ND

Concentration of Avobenzone (M)	Aromatase Activity (% of VC)		Individual Aromatase Activity (% of VC)		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	100.64	2.446	98.92	102.37	ND
NSB	-0.02	0.060	-0.06	0.02	ND
10^{-3}	96.36	2.288	94.89	95.19	99.00
10^{-4}	110.36	1.382	108.97	111.73	110.37
10^{-5}	113.05	2.740	116.19	111.78	111.17
10^{-6}	107.39	2.687	104.69	107.41	110.07
10^{-7}	104.41	1.169	105.10	105.08	103.06
10^{-8}	101.32	3.554	100.62	105.18	98.17
10^{-9}	103.33	4.260	105.93	98.41	105.64
10^{-10}	102.54	1.598	101.66	101.57	104.38

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 Avobenzone (27 Feb 2013)

Concentration of 4OH-ASDN (M)	Aromatase Activity (% of VC)		Individual Aromatase Activity (% of VC)		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	97.69	1.264	96.80	98.59	ND
NSB	-0.18	0.000	-0.18	-0.18	ND
10^{-5}	0.78	0.007	0.79	0.78	ND
10^{-6}	7.73	0.146	7.84	7.63	ND
$10^{-6.5}$	20.97	0.759	21.51	20.44	ND
10^{-7}	43.17	0.227	43.33	43.01	ND
$10^{-7.5}$	68.03	0.775	67.48	68.58	ND
10^{-8}	89.62	2.705	91.54	87.71	ND
10^{-9}	97.63	2.693	95.72	99.53	ND
10^{-10}	99.01	3.242	101.30	96.72	ND

Concentration of Avobenzone(M)	Aromatase Activity (% of VC)		Individual Aromatase Activity (% of VC)		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	102.31	1.298	103.23	101.39	ND
NSB	0.18	0.465	-0.14	0.51	ND
10^{-3}	100.38	1.303	100.52	99.01	101.61
10^{-4}	112.51	2.360	111.77	110.61	115.15
10^{-5}	123.91	10.854	114.81	135.92	121.00
10^{-6}	100.84	1.850	98.84	101.22	102.48
10^{-7}	105.14	2.497	102.64	107.63	105.14
10^{-8}	106.35	0.332	106.68	106.02	106.34
10^{-9}	106.73	0.751	107.37	106.90	105.90
10^{-10}	106.51	2.716	104.18	105.85	109.49

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 Ensulizole (21 Feb 2013)

Concentration of 4OH-ASDN (M)	Aromatase Activity (% of VC)		Individual Aromatase Activity (% of VC)		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	101.54	0.556	101.14	101.93	ND
NSB	0.01	0.006	0.00	0.01	ND
10^{-5}	1.14	0.036	1.11	1.16	ND
10^{-6}	9.68	0.626	9.23	10.12	ND
$10^{-6.5}$	23.56	0.309	23.34	23.78	ND
10^{-7}	47.95	1.198	48.79	47.10	ND
$10^{-7.5}$	67.77	10.046	74.87	60.66	ND
10^{-8}	89.03	4.880	85.58	92.48	ND
10^{-9}	98.88	0.653	99.34	98.42	ND
10^{-10}	99.13	2.402	100.83	97.43	ND

Concentration of Ensulizole(M)	Aromatase Activity (% of VC)		Individual Aromatase Activity (% of VC)		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	98.46	3.036	96.32	100.61	ND
NSB	-0.01	0.039	0.02	-0.03	ND
$10^{-3.5}$	100.58	1.502	100.55	99.09	102.10
$10^{-4.5}$	102.13	1.681	103.89	100.54	101.97
$10^{-5.5}$	100.46	0.828	101.31	100.42	99.65
$10^{-6.5}$	100.01	2.634	97.83	102.94	99.25
$10^{-7.5}$	96.81	1.433	96.93	98.19	95.33
$10^{-8.5}$	97.51	4.543	102.19	93.12	97.21
$10^{-9.5}$	94.76	3.051	92.10	98.09	94.10
$10^{-10.5}$	99.28	3.664	95.15	102.14	100.54

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 Ensulizole (25 Feb 2013)

Concentration of 4OH-ASDN (M)	Aromatase Activity (% of VC)		Individual Aromatase Activity (% of VC)		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	99.36	0.393	99.08	99.63	ND
NSB	0.02	0.005	0.03	0.02	ND
10^{-5}	0.91	0.066	0.87	0.96	ND
10^{-6}	8.25	0.101	8.18	8.32	ND
$10^{-6.5}$	20.94	0.541	20.56	21.32	ND
10^{-7}	43.12	0.865	42.51	43.73	ND
$10^{-7.5}$	69.72	2.427	68.00	71.43	ND
10^{-8}	88.89	1.259	88.00	89.78	ND
10^{-9}	104.41	1.431	105.42	103.39	ND
10^{-10}	104.51	2.487	106.27	102.75	ND
Concentration of Ensulizole(M)	**Aromatase Activity (% of VC)**		**Individual Aromatase Activity (% of VC)**		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	100.64	2.446	98.92	102.37	ND
NSB	-0.02	0.060	-0.06	0.02	ND
$10^{-3.5}$	103.32	1.325	103.56	104.51	101.89
$10^{-4.5}$	102.03	2.062	104.24	101.67	100.17
$10^{-5.5}$	101.96	0.922	101.18	101.73	102.98
$10^{-6.5}$	105.17	0.369	105.55	104.81	105.14
$10^{-7.5}$	101.86	1.761	100.11	103.63	101.84
$10^{-8.5}$	100.29	4.676	101.62	104.16	95.10
$10^{-9.5}$	101.03	2.942	103.58	97.81	101.70
$10^{-10.5}$	94.88	4.095	96.83	97.63	90.17

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 Ensulizole (27 Feb 2013)

Concentration of 4OH-ASDN (M)	**Aromatase Activity (% of VC)**		**Individual Aromatase Activity (% of VC)**		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	97.69	1.264	96.80	98.59	ND
NSB	-0.18	0.000	-0.18	-0.18	ND
10^{-5}	0.78	0.007	0.79	0.78	ND
10^{-6}	7.73	0.146	7.84	7.63	ND
$10^{-6.5}$	20.97	0.759	21.51	20.44	ND
10^{-7}	43.17	0.227	43.33	43.01	ND
$10^{-7.5}$	68.03	0.775	67.48	68.58	ND
10^{-8}	89.62	2.705	91.54	87.71	ND
10^{-9}	97.63	2.693	95.72	99.53	ND
10^{-10}	99.01	3.242	101.30	96.72	ND

Concentration of Ensulizole(M)	**Aromatase Activity (% of VC)**		**Individual Aromatase Activity (% of VC)**		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	102.31	1.298	103.23	101.39	ND
NSB	0.18	0.465	-0.14	0.51	ND
$10^{-3.5}$	101.54	0.186	101.58	101.71	101.34
$10^{-4.5}$	104.06	1.232	102.68	104.43	105.05
$10^{-5.5}$	106.04	3.113	106.36	102.78	108.98
$10^{-6.5}$	98.06	12.944	109.78	100.23	84.16
$10^{-7.5}$	102.13	0.962	103.23	101.44	101.71
$10^{-8.5}$	103.52	1.565	102.44	102.80	105.31
$10^{-9.5}$	101.91	2.335	101.33	104.48	99.92
$10^{-10.5}$	103.77	0.915	102.71	104.33	104.26

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 Homosalate (21 Feb 2013)

Concentration of 4OH-ASDN (M)	Aromatase Activity (% of VC)		Individual Aromatase Activity (% of VC)		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	101.54	0.556	101.14	101.93	ND
NSB	0.01	0.006	0.00	0.01	ND
10^{-5}	1.14	0.036	1.11	1.16	ND
10^{-6}	9.68	0.626	9.23	10.12	ND
$10^{-6.5}$	23.56	0.309	23.34	23.78	ND
10^{-7}	47.95	1.198	48.79	47.10	ND
$10^{-7.5}$	67.77	10.046	74.87	60.66	ND
10^{-8}	89.03	4.880	85.58	92.48	ND
10^{-9}	98.88	0.653	99.34	98.42	ND
10^{-10}	99.13	2.402	100.83	97.43	ND

Concentration of Homosalate(M)	Aromatase Activity (% of VC)		Individual Aromatase Activity (% of VC)		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	98.46	3.036	96.32	100.61	ND
NSB	-0.01	0.039	0.02	-0.03	ND
10^{-3}	83.11	2.290	81.81	81.77	85.76
10^{-4}	86.74	5.722	91.72	88.00	80.49
10^{-5}	92.23	3.370	88.34	94.32	94.03
10^{-6}	95.31	3.911	95.09	99.33	91.52
10^{-7}	97.06	4.645	92.30	101.57	97.32
10^{-8}	93.62	2.593	93.88	90.91	96.08
10^{-9}	97.99	2.943	96.24	96.34	101.39
10^{-10}	100.54	0.730	99.71	101.09	100.82

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 Homosalate (25 Feb 2013)

Concentration of 4OH-ASDN (M)	Aromatase Activity (% of VC)		Individual Aromatase Activity (% of VC)		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	99.36	0.393	99.08	99.63	ND
NSB	0.02	0.005	0.03	0.02	ND
10^{-5}	0.91	0.066	0.87	0.96	ND
10^{-6}	8.25	0.101	8.18	8.32	ND
$10^{-6.5}$	20.94	0.541	20.56	21.32	ND
10^{-7}	43.12	0.865	42.51	43.73	ND
$10^{-7.5}$	69.72	2.427	68.00	71.43	ND
10^{-8}	88.89	1.259	88.00	89.78	ND
10^{-9}	104.41	1.431	105.42	103.39	ND
10^{-10}	104.51	2.487	106.27	102.75	ND

Concentration of Homosalate(M)	Aromatase Activity (% of VC)		Individual Aromatase Activity (% of VC)		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	100.64	2.446	98.92	102.37	ND
NSB	-0.02	0.060	-0.06	0.02	ND
10^{-3}	84.11	0.711	83.77	83.63	84.92
10^{-4}	90.05	2.216	92.05	90.44	87.67
10^{-5}	92.29	0.304	92.42	91.95	92.51
10^{-6}	103.34	0.790	103.58	102.46	103.99
10^{-7}	102.71	2.983	99.53	103.16	105.44
10^{-8}	100.73	1.563	102.42	100.42	99.34
10^{-9}	100.24	2.653	100.80	102.57	97.36
10^{-10}	101.60	0.947	101.02	102.70	101.09

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 Homosalate (27 Feb 2013)

Concentration of 4OH-ASDN (M)	Aromatase Activity (% of VC)		Individual Aromatase Activity (% of VC)		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	97.69	1.264	96.80	98.59	ND
NSB	-0.18	0.000	-0.18	-0.18	ND
10^{-5}	0.78	0.007	0.79	0.78	ND
10^{-6}	7.73	0.146	7.84	7.63	ND
$10^{-6.5}$	20.97	0.759	21.51	20.44	ND
10^{-7}	43.17	0.227	43.33	43.01	ND
$10^{-7.5}$	68.03	0.775	67.48	68.58	ND
10^{-8}	89.62	2.705	91.54	87.71	ND
10^{-9}	97.63	2.693	95.72	99.53	ND
10^{-10}	99.01	3.242	101.30	96.72	ND
Concentration of Homosalate(M)	**Aromatase Activity (% of VC)**		**Individual Aromatase Activity (% of VC)**		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	102.31	1.298	103.23	101.39	ND
NSB	0.18	0.465	-0.14	0.51	ND
10^{-3}	84.46	2.343	81.81	86.26	85.30
10^{-4}	90.48	1.192	90.32	89.37	91.74
10^{-5}	96.95	1.813	98.88	95.29	96.66
10^{-6}	105.93	0.807	106.10	105.05	106.63
10^{-7}	103.35	1.053	103.22	104.47	102.38
10^{-8}	102.75	2.539	102.17	100.55	105.53
10^{-9}	100.84	1.685	100.75	99.21	102.57
10^{-10}	100.41	1.520	98.69	100.94	101.59

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 Padimate-O (21 Feb 2013)

Concentration of 4OH-ASDN (M)	Aromatase Activity (% of VC)		Individual Aromatase Activity (% of VC)		
	Mean	SD	Value 1	Value 2	Value 3
TA	101.54	0.556	101.14	101.93	ND
NSB	0.01	0.006	0.00	0.01	ND
10^{-5}	1.14	0.036	1.11	1.16	ND
10^{-6}	9.68	0.626	9.23	10.12	ND
$10^{-6.5}$	23.56	0.309	23.34	23.78	ND
10^{-7}	47.95	1.198	48.79	47.10	ND
$10^{-7.5}$	67.77	10.046	74.87	60.66	ND
10^{-8}	89.03	4.880	85.58	92.48	ND
10^{-9}	98.88	0.653	99.34	98.42	ND
10^{-10}	99.13	2.402	100.83	97.43	ND
Concentration of Padimate-O(M)	**Aromatase Activity (% of VC)**		**Individual Aromatase Activity (% of VC)**		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	98.46	3.036	96.32	100.61	ND
NSB	-0.01	0.039	0.02	-0.03	ND
10^{-3}	83.49	1.628	82.98	85.32	82.19
10^{-4}	92.41	0.516	92.79	91.82	92.63
10^{-5}	96.28	1.357	96.26	94.94	97.65
10^{-6}	99.86	1.842	97.83	100.35	101.41
10^{-7}	100.68	0.855	99.74	101.41	100.89
10^{-8}	100.35	1.040	99.63	101.54	99.88
10^{-9}	97.09	2.796	99.02	93.88	98.36
10^{-10}	98.47	0.579	99.06	97.91	98.44

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 Padimate-O (25 Feb 2013)

Concentration of 4OH-ASDN (M)	Aromatase Activity (% of VC)		Individual Aromatase Activity (% of VC)		
	Mean	SD	Value 1	Value 2	Value 3
TA	99.36	0.393	99.08	99.63	ND
NSB	0.02	0.005	0.03	0.02	ND
10^{-5}	0.91	0.066	0.87	0.96	ND
10^{-6}	8.25	0.101	8.18	8.32	ND
$10^{-6.5}$	20.94	0.541	20.56	21.32	ND
10^{-7}	43.12	0.865	42.51	43.73	ND
$10^{-7.5}$	69.72	2.427	68.00	71.43	ND
10^{-8}	88.89	1.259	88.00	89.78	ND
10^{-9}	104.41	1.431	105.42	103.39	ND
10^{-10}	104.51	2.487	106.27	102.75	ND
Concentration of Padimate-O(M)	**Aromatase Activity (% of VC)**		**Individual Aromatase Activity (% of VC)**		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	100.64	2.446	98.92	102.37	ND
NSB	-0.02	0.060	-0.06	0.02	ND
10^{-3}	86.53	2.455	84.54	89.27	85.79
10^{-4}	92.06	0.941	93.12	91.76	91.30
10^{-5}	98.28	0.159	98.46	98.23	98.15
10^{-6}	100.63	2.493	98.21	103.19	100.49
10^{-7}	98.16	1.256	96.88	99.39	98.21
10^{-8}	101.46	2.450	98.64	102.94	102.81
10^{-9}	97.07	1.551	98.82	95.88	96.50
10^{-10}	101.85	2.663	103.71	98.80	103.04

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 Padimate-O (27 Feb 2013)

Concentration of 4OH-ASDN (M)	Aromatase Activity (% of VC)		Individual Aromatase Activity (% of VC)		
	Mean	SD	Value 1	Value 2	Value 3
TA	97.69	1.264	96.80	98.59	ND
NSB	-0.18	0.000	-0.18	-0.18	ND
10^{-5}	0.78	0.007	0.79	0.78	ND
10^{-6}	7.73	0.146	7.84	7.63	ND
$10^{-6.5}$	20.97	0.759	21.51	20.44	ND
10^{-7}	43.17	0.227	43.33	43.01	ND
$10^{-7.5}$	68.03	0.775	67.48	68.58	ND
10^{-8}	89.62	2.705	91.54	87.71	ND
10^{-9}	97.63	2.693	95.72	99.53	ND
10^{-10}	99.01	3.242	101.30	96.72	ND
Concentration of Padimate-O(M)	**Aromatase Activity (% of VC)**		**Individual Aromatase Activity (% of VC)**		
	Mean	**SD**	**Value 1**	**Value 2**	**Value 3**
TA	102.31	1.298	103.23	101.39	ND
NSB	0.18	0.465	-0.14	0.51	ND
10^{-3}	85.34	1.744	83.64	85.24	87.13
10^{-4}	92.90	2.738	90.03	93.21	95.48
10^{-5}	99.55	5.088	101.50	103.37	93.77
10^{-6}	106.22	0.831	106.68	105.26	106.72
10^{-7}	104.65	0.900	104.49	103.84	105.62
10^{-8}	104.51	2.054	106.78	103.99	102.77
10^{-9}	101.75	1.289	100.41	102.98	101.87
10^{-10}	100.12	3.955	103.89	100.46	96.00

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	-0.88	-0.89	-0.96	-7.07	-7.18	-7.12

Name	Top of Curve (%)			Bottom of Curve (%)		
	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3
4OH-ASDN	100.36	105.67	99.58	-0.46	-0.28	0.15

ACCEPTANCE CRITERIA

	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

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	101.14	99.08	96.80	0.00	0.03	-0.18
	101.93	99.63	98.59	0.01	0.02	-0.18
End	96.32	98.92	103.23	0.02	-0.06	-0.14
	100.61	102.37	101.39	-0.03	0.02	0.51
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: Solubility Results

Test Substance	Precipitation Code		
	Run 1	**Run 2**	**Run 3**
	Rx tubes 37^{o}C after addition of Supersomes™		
Avobenzone, 10^{-3} M	++	+++	+++
Avobenzone, 10^{-4} M	+	+	+
Avobenzone, 10^{-5} M	0	0	0
Ensulizole, $10^{-3.5}$ M	0	0	0
Homosalate, 10^{-3} M	+	+	+
Homosalate, 10^{-4} M	0	0	0
Padimate-O, 10^{-3} M	++	++	++
Padimate-O, 10^{-4} M	+	+	+
Padimate-O, 10^{-5} M	0	0	0

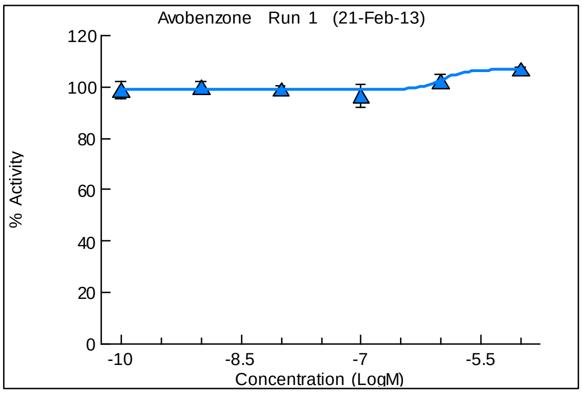
Precipitation Code (Visual):

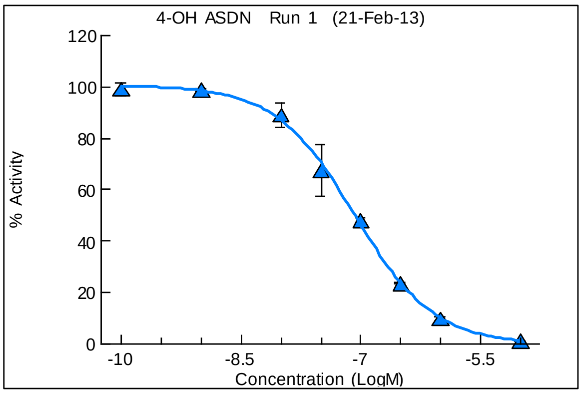
0 = Negative
+ = Small Amount
++ = Moderate Amount
+++ = Substantial Amount
ND = Not determined

FIGURES SECTION

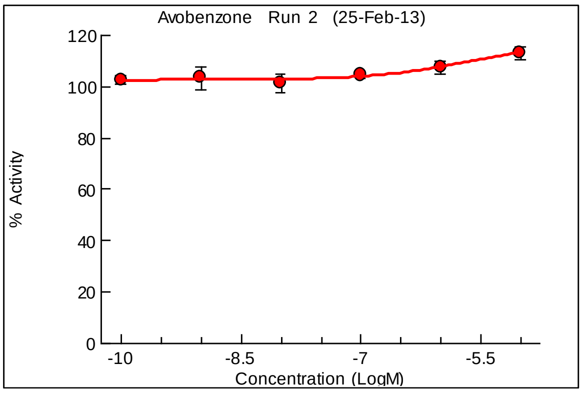
FIGURE 1: Runs 1-3: Avobenzone and 4OH-ASDN

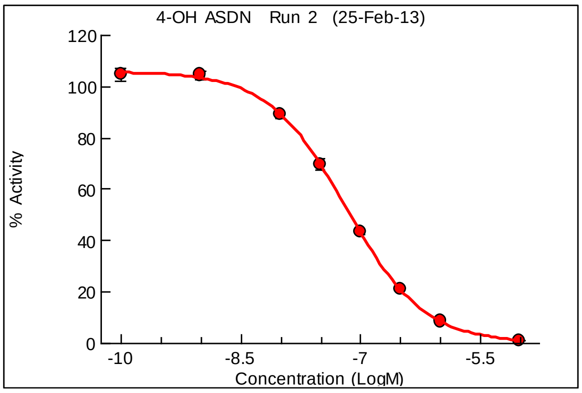
Run 1: 21 Feb 2013



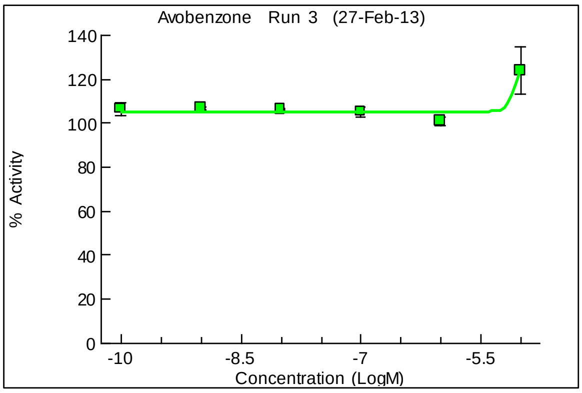


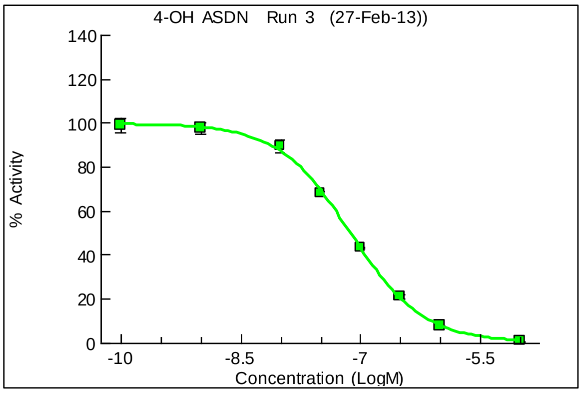
Run 2: 25 Feb 2013





Run 3: 27 Feb 2013



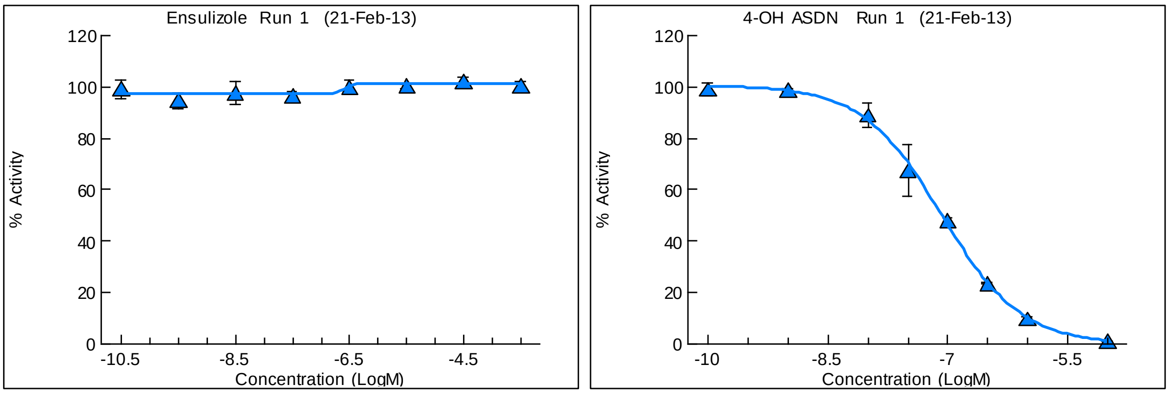


The graphs above represent the data (Means ± Standard Error of the Mean) from three independent runs of the assay (n =3/concentration for test substance; n=2/concentration for 4OH-ASDN).

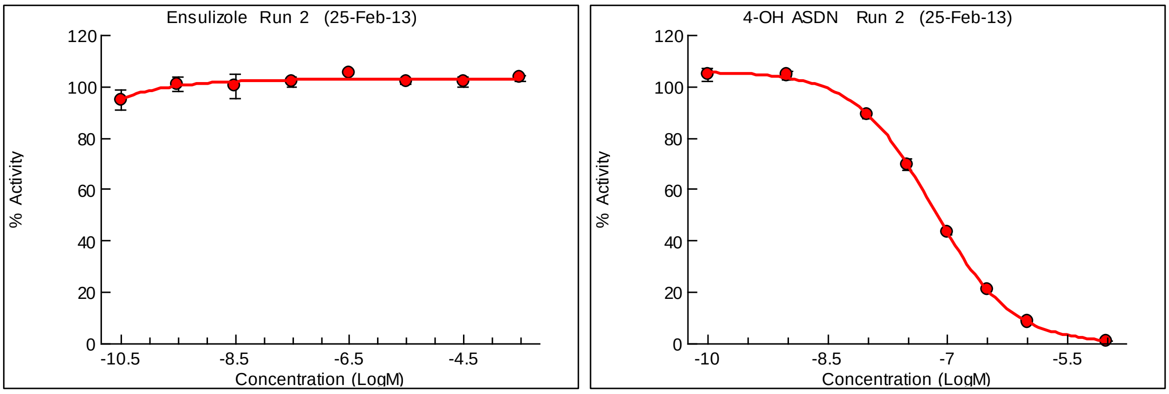
NOTE: Avobenzone soluble up to 10^{-5}M. Only soluble concentrations shown (e.g., excluding 10^{-3} M and 10^{-4} M for test substance).

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

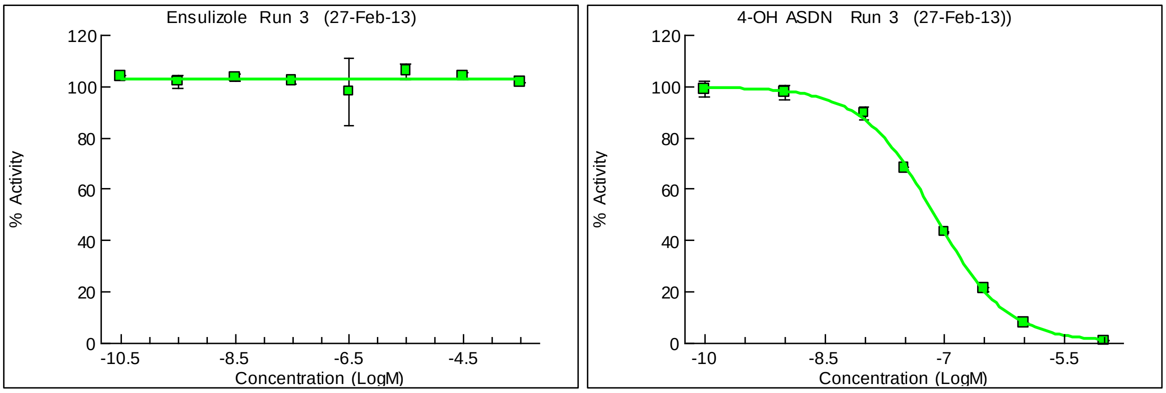
Run 1: 21 Feb 2013



Run 2: 25 Feb 2013



Run 3: 27 Feb 2013

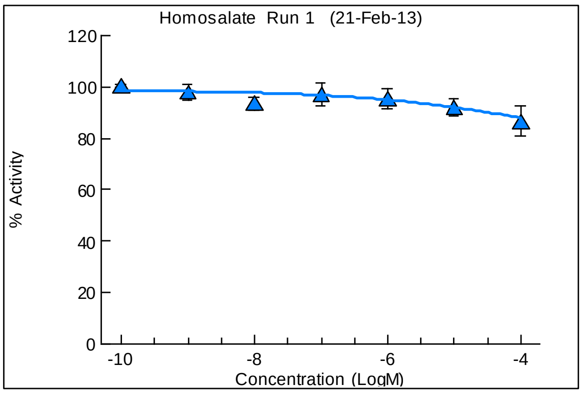


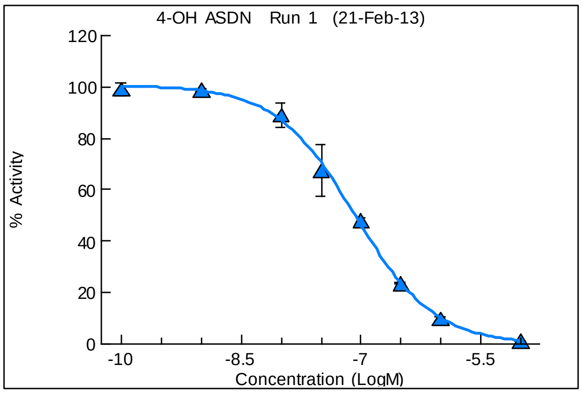
The graphs above represent the data (Means ± Standard Error of the Mean) from three independent runs of the assay (n =3/concentration for test substance; n=2/concentration for 4OH-ASDN).

NOTE: Ensulizole soluble up to $10^{-3.5}$M. All concentrations shown.

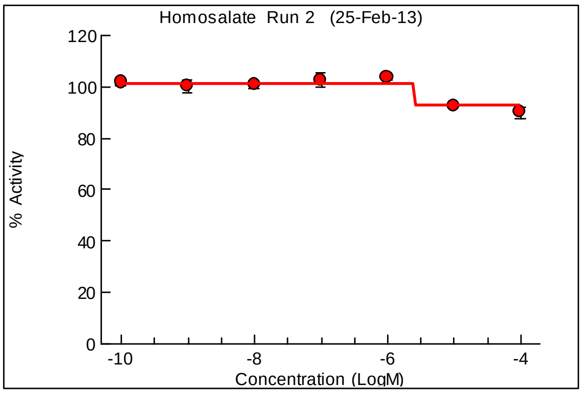
FIGURE 3: Runs 1-3: Homosalate and 4OH-ASDN

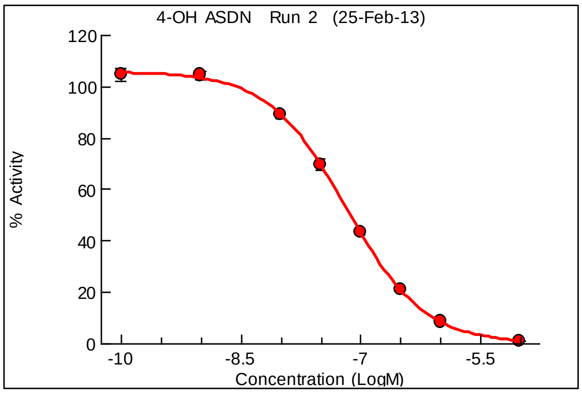
Run 1: 21 Feb 2013



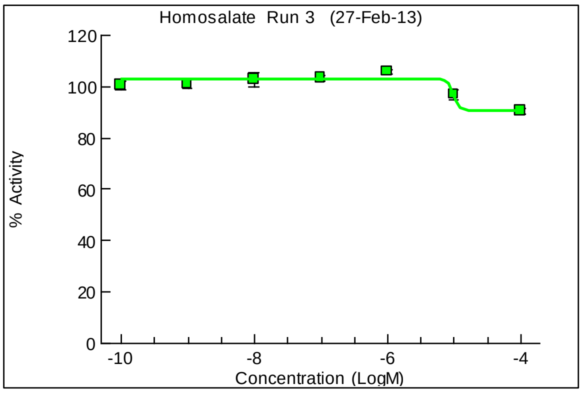


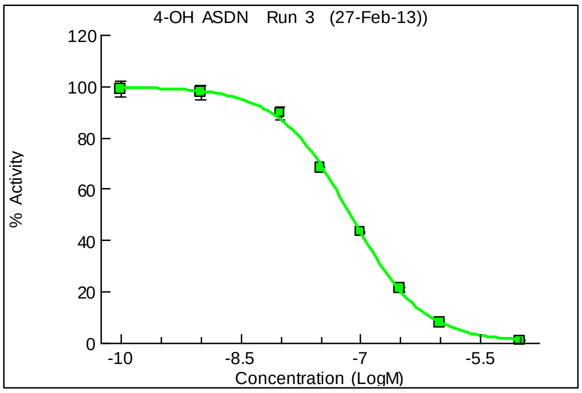
Run 2: 25 Feb 2013





Run 3: 27 Feb 2013



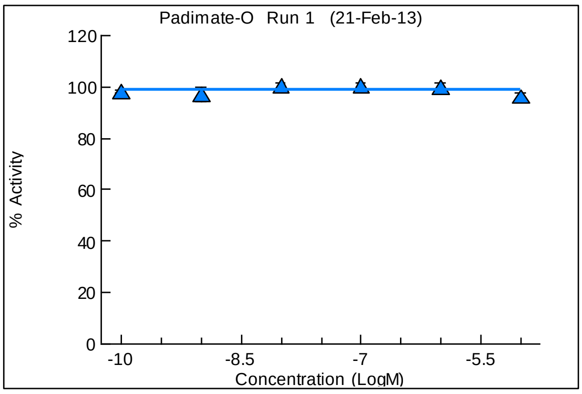


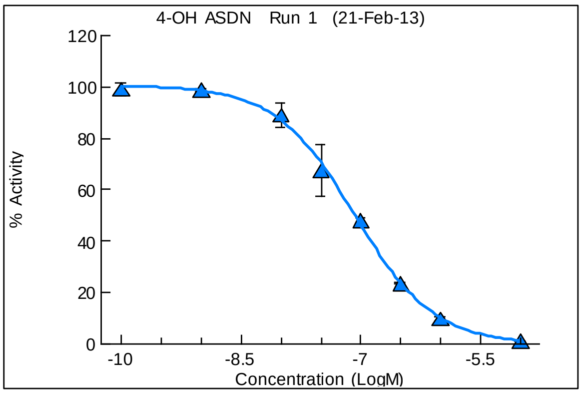
The graphs above represent the data (Means ± Standard Error of the Mean) from three independent runs of the assay (n =3/concentration for test substance; n=2/concentration for 4OH-ASDN).

NOTE: Homosalate soluble up to 10^{-4}M. Only soluble concentrations shown (e.g., excluding 10^{-3} M for test substance).

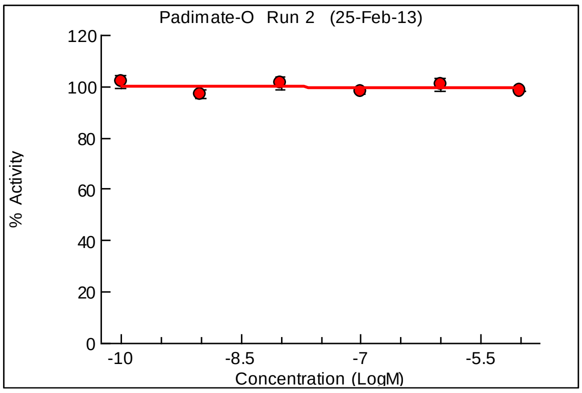
FIGURE 4: Runs 1-3: Padimate-O and 4OH-ASDN

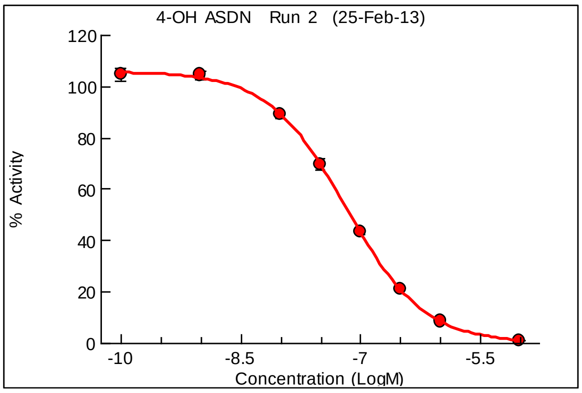
Run 1: 21 Feb 2013



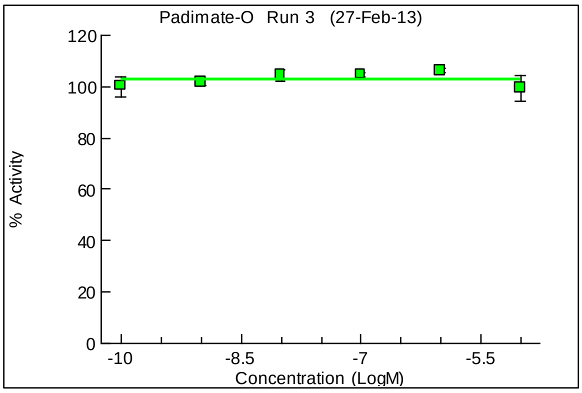


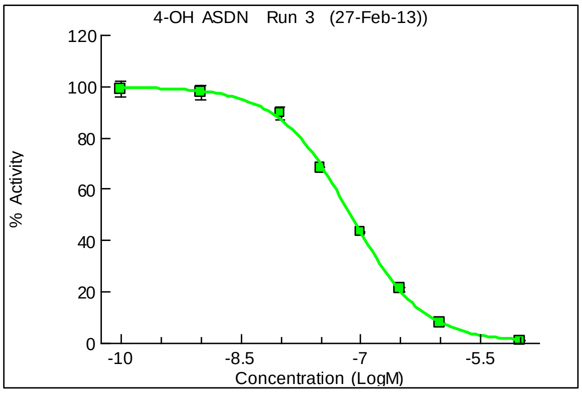
Run 2: 25 Feb 2013





Run 3: 27 Feb 2013





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: Padimate-O soluble up to 10^{-5}M. Only soluble concentrations shown (e.g., excluding 10^{-3} M and 10^{-4} M for test substance).

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

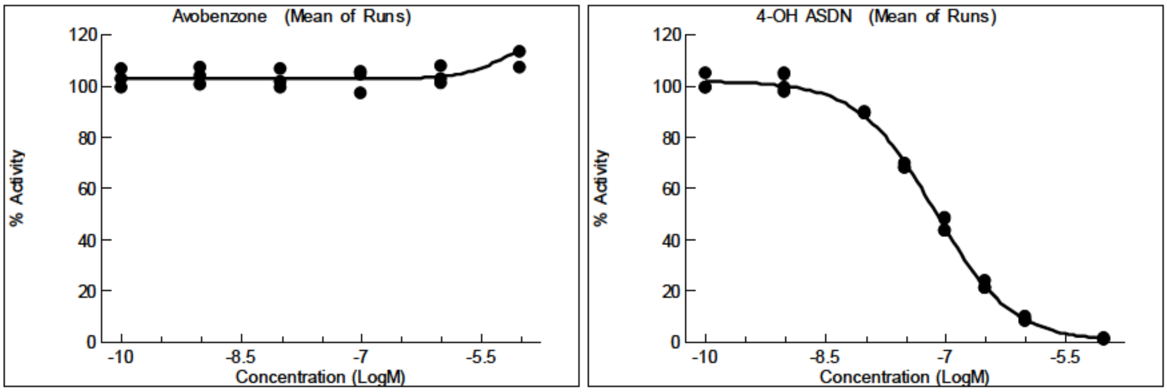


FIGURE 6: Combined Response of Runs 1-3: Avobenzone and 4OH-ASDN

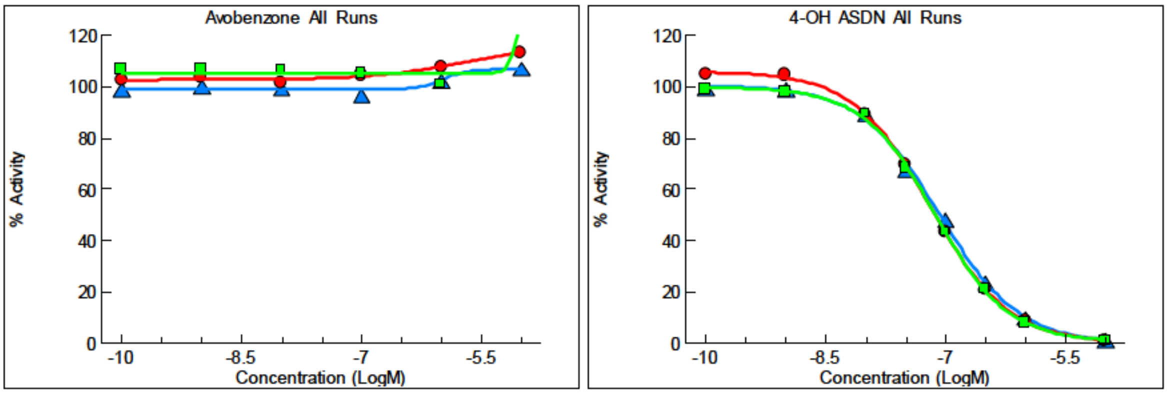
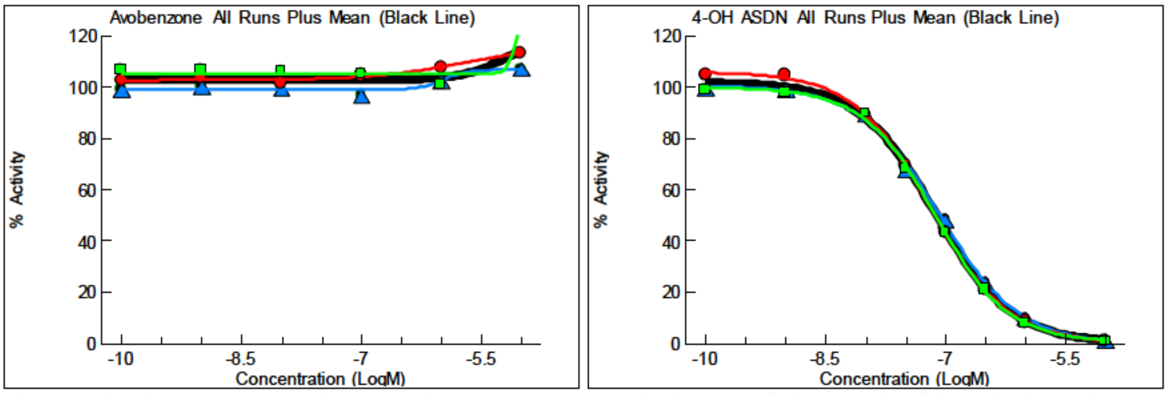


FIGURE 7: Combined Response of Mean and Runs 1-3: Avobenzone and 4OH-ASDN



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. Only soluble concentrations shown (e.g., excluding 10^{-3} M and 10^{-4} M for test substance).

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

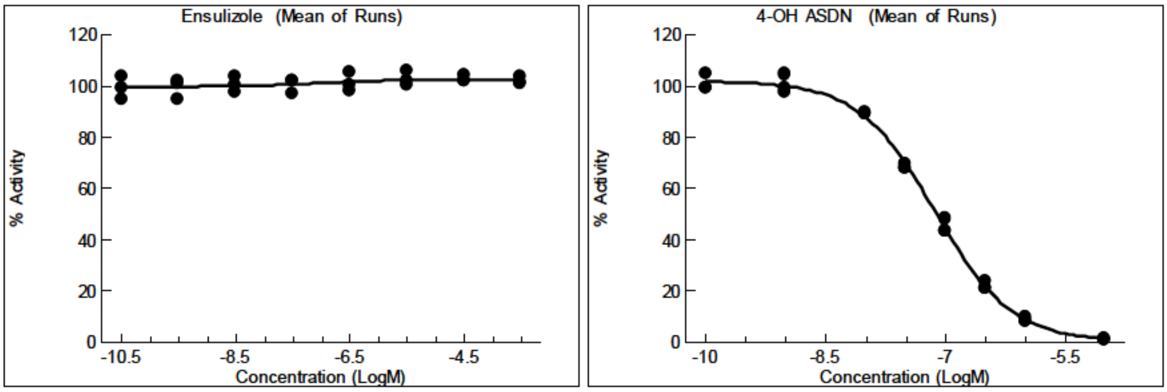


FIGURE 9: Combined Response of Runs 1-3: Ensulizole and 4OH-ASDN

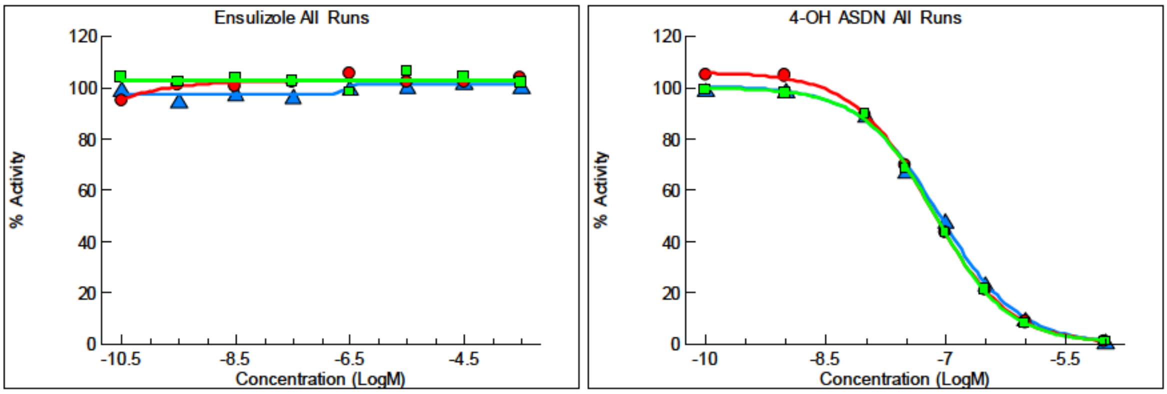
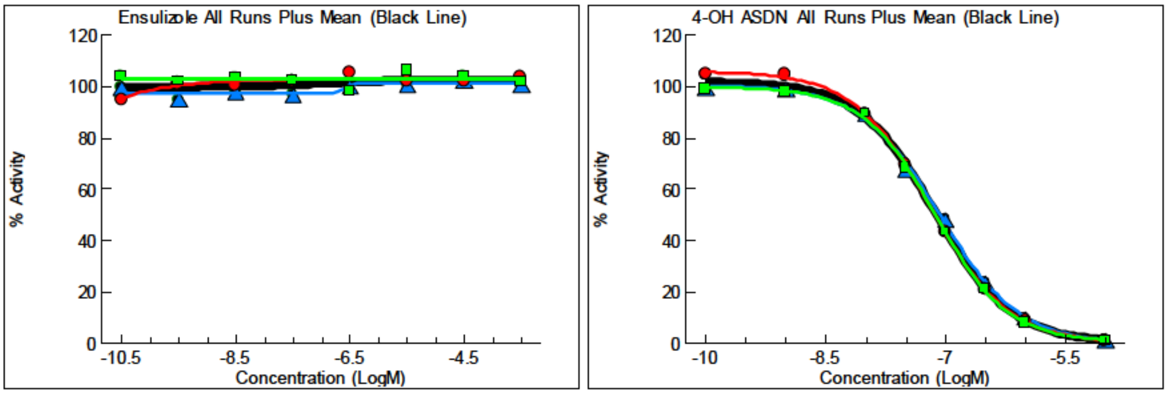


FIGURE 10: Combined Response of Mean and Runs 1-3: Ensulizole and 4OH-ASDN



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. All concentrations shown.

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

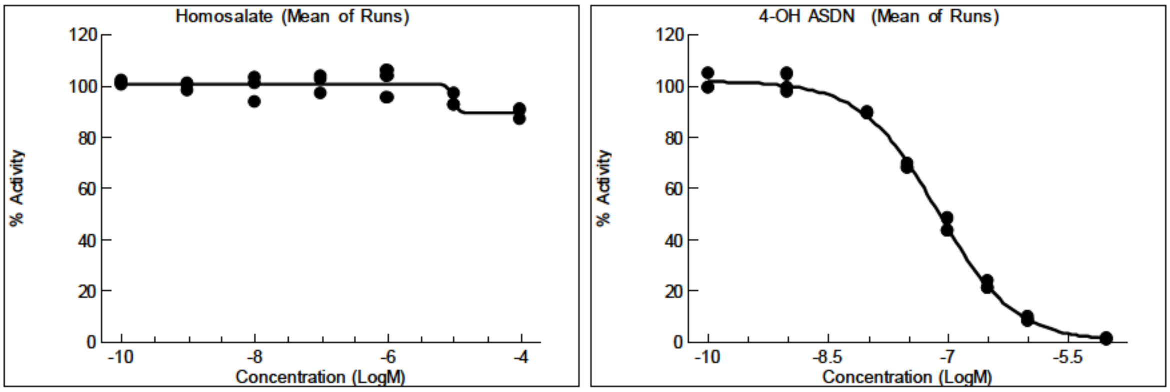


FIGURE 12: Combined Response of Runs 1-3: Homosalate and 4OH-ASDN

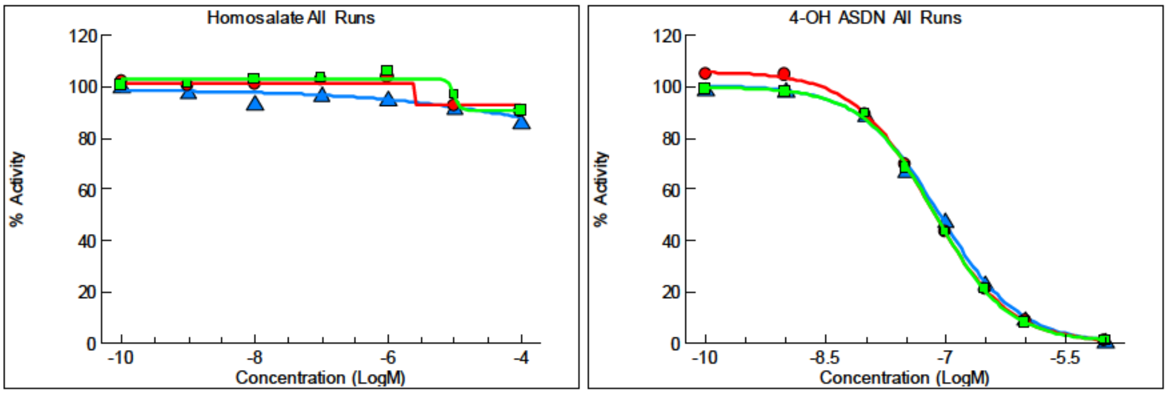
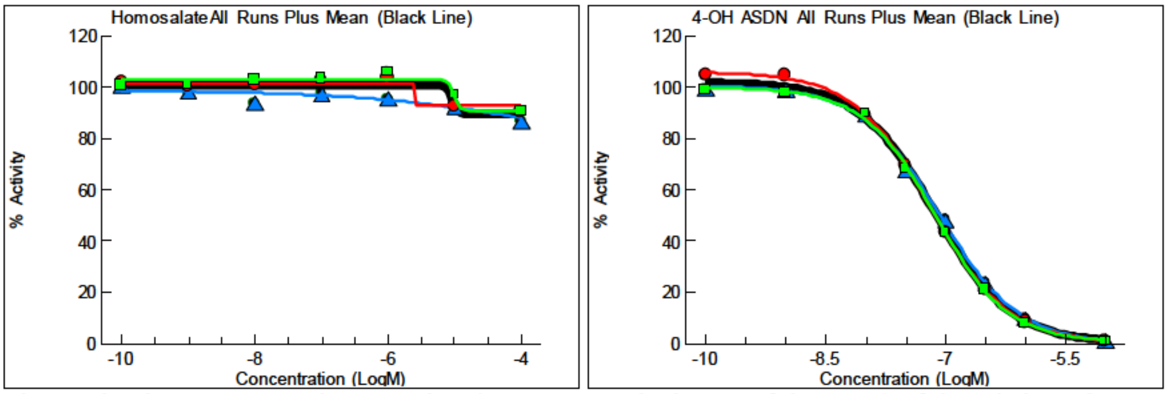


FIGURE 13: Combined Response of Mean and Runs 1-3: Homosalate and 4OH-ASDN



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. Only soluble concentrations shown (e.g., excluding 10^{-3} M for test substance).

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

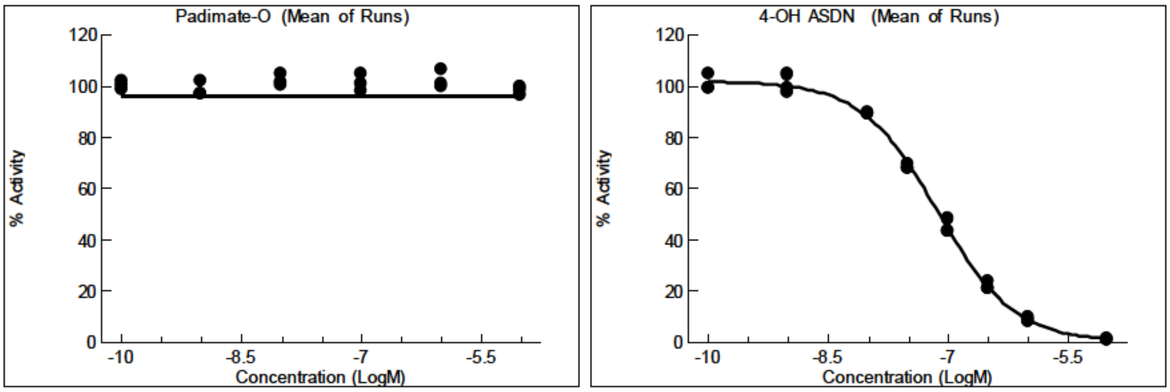


FIGURE 15: Combined Response of Runs 1-3: Padimate-O and 4OH-ASDN

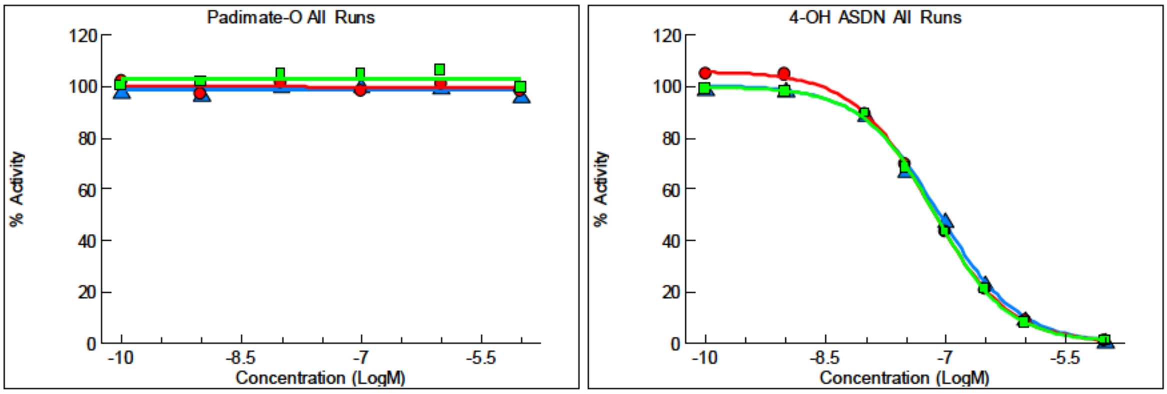
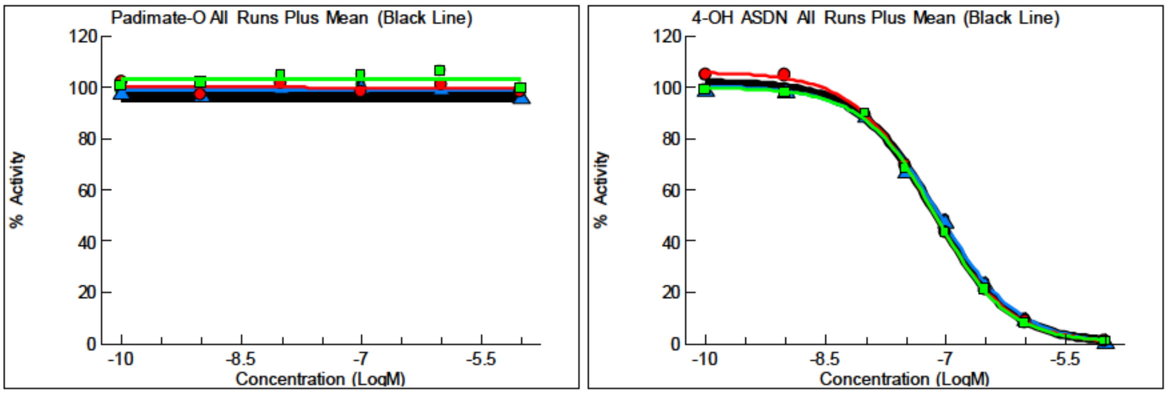


FIGURE 16: Combined Response of Mean and Runs 1-3: Padimate-O and 4OH-ASDN



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. Only soluble concentrations shown (e.g., excluding 10^{-3} M and 10^{-4} M for test substance).

APPENDICES SECTION

APPENDIX 1: Run 1: Assay Information (Avobenzone)

Experiment Date:	21-Feb-13	Study Number:	9070-100794AROM		
Test substance:	Avobenzone				
3/14/2013 16:27					
	specific activity based on decay for 4/20/10		42770.0	DPM	
	20 uL count of 3H-ASDN (mean)		41156.7	DPM	
	0.5 mL count for total activity		12768.8	DPM	
	microsomal protein/assay		0.008	mg	
	Reaction time		15	min	
	20 uL count of 3H-ASDN (DPM)		40421	41204	41845

Assays Conducted by: [redacted]					Spreadsheet locked on: 03/05/2013
					Green shaded areas: unlocked cells for data entry
Each assay contained 100 uL 3H-ASDN	205783.3	DPM	0.200	(nmoles)	
Total product 3H-H20 per assay	51075.0	DPM	0.050	(nmoles)	
Percent conversion to product (3H-H2O) (percent)	24.8				
Rate of conversion to 3H-H2O in total activity assay	0.414	nmol/(mg protein-min)			
Average activity of control Tubes	0.412	nmol/(mg protein-min)			
Average full enzyme activity controls (percent +/-SD)	100.0	2.5			
Average background activity controls (percent +/- SD)	0.0	0.0			

APPENDIX 1: Run 1: Raw and Normalized DPM Data (4OH-ASDN and Avobenzone): 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 tubesww	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
TA		12927.0	12901.0	25854.0	25802.0	25828.0	36.77	0.14	51656.0	205783.3	25.1	51445.0	0.050	0.417
TA		13098.0	12930.0	26196.0	25860.0	26028.0	237.59	0.91	52056.0	205783.3	25.3	51845.0	0.050	0.420
NSB		52.0	54.0	104.0	108.0	106.0	2.83	2.67	212.0	205783.3	0.1	1.0	0.000	0.000
NSB		53.0	55.0	106.0	110.0	108.0	2.83	2.62	216.0	205783.3	0.1	5.0	0.000	0.000
4OH-ASDN	-5	191.0	197.0	382.0	394.0	388.0	8.49	2.19	776.0	205783.3	0.4	565.0	0.001	0.005
4OH-ASDN		190.0	211.0	380.0	422.0	401.0	29.70	7.41	802.0	205783.3	0.4	591.0	0.001	0.005
4OH-ASDN	-6	1238.0	1216.0	2476.0	2432.0	2454.0	31.11	1.27	4908.0	205783.3	2.4	4697.0	0.005	0.038
4OH-ASDN		1325.0	1354.0	2650.0	2708.0	2679.0	41.01	1.53	5358.0	205783.3	2.6	5147.0	0.005	0.042
4OH-ASDN	-6.5	3043.0	2998.0	6086.0	5996.0	6041.0	63.64	1.05	12082.0	205783.3	5.9	11871.0	0.012	0.096
4OH-ASDN		3057.0	3095.0	6114.0	6190.0	6152.0	53.74	0.87	12304.0	205783.3	6.0	12093.0	0.012	0.098
4OH-ASDN	-7	6263.0	6252.0	12526.0	12504.0	12515.0	15.56	0.12	25030.0	205783.3	12.2	24819.0	0.024	0.201
4OH-ASDN		6115.0	5969.0	12230.0	11938.0	12084.0	206.48	1.71	24168.0	205783.3	11.7	23957.0	0.023	0.194
4OH-ASDN	-7.5	9660.0	9486.0	19320.0	18972.0	19146.0	246.07	1.29	38292.0	205783.3	18.6	38081.0	0.037	0.308
4OH-ASDN		7864.0	7669.0	15728.0	15338.0	15533.0	275.77	1.78	31066.0	205783.3	15.1	30855.0	0.030	0.250
4OH-ASDN	-8	11260.0	10611.0	22520.0	21222.0	21871.0	917.82	4.20	43742.0	205783.3	21.3	43531.0	0.042	0.353
4OH-ASDN		11767.0	11859.0	23534.0	23718.0	23626.0	130.11	0.55	47252.0	205783.3	23.0	47041.0	0.046	0.381
4OH-ASDN	-9	12744.0	12626.0	25488.0	25252.0	25370.0	166.88	0.66	50740.0	205783.3	24.7	50529.0	0.049	0.409
4OH-ASDN		12652.0	12483.0	25304.0	24966.0	25135.0	239.00	0.95	50270.0	205783.3	24.4	50059.0	0.049	0.405
4OH-ASDN	-10	12995.0	12754.0	25990.0	25508.0	25749.0	340.83	1.32	51498.0	205783.3	25.0	51287.0	0.050	0.415
4OH-ASDN		12631.0	12254.0	25262.0	24508.0	24885.0	533.16	2.14	49770.0	205783.3	24.2	49559.0	0.048	0.401

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 Avobenzone): Part 2 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
101.14	101.54	0.393	0.556	0.548
101.93				
0.00	0.01	0.004	0.006	94.281
0.01				
1.11	1.14	0.026	0.036	3.181
1.16				
9.23	9.68	0.442	0.626	6.465
10.12				
23.34	23.56	0.218	0.309	1.310
23.78				
48.79	47.95	0.847	1.198	2.499
47.10				
74.87	67.77	7.103	10.046	14.824
60.66				
85.58	89.03	3.450	4.880	5.481
92.48				
99.34	98.88	0.462	0.653	0.661
98.42				
100.83	99.13	1.699	2.402	2.423
97.43				

APPENDIX 1: Run 1: Raw and Normalized DPM Data (4OH-ASDN and Avobenzone): 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 tubesww	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
Avobenzone	-3	11669.0	11319.0	23338.0	22638.0	22988.0	494.97	2.15	45976.0	205783.3	22.3	45765.0	0.044	0.371
Avobenzone		11791.0	11459.0	23582.0	22918.0	23250.0	469.52	2.02	46500.0	205783.3	22.6	46289.0	0.045	0.375
Avobenzone		11365.0	11170.0	22730.0	22340.0	22535.0	275.77	1.22	45070.0	205783.3	21.9	44859.0	0.044	0.363
Avobenzone	-4	13284.0	10942.0	26568.0	21884.0	24226.0	3312.09	13.67	48452.0	205783.3	23.5	48241.0	0.047	0.391
Avobenzone		14759.0	13731.0	29518.0	27462.0	28490.0	1453.81	5.10	56980.0	205783.3	27.7	56769.0	0.055	0.460
Avobenzone		13451.0	13485.0	26902.0	26970.0	26936.0	48.08	0.18	53872.0	205783.3	26.2	53661.0	0.052	0.435
Avobenzone	-5	13705.0	13512.0	27410.0	27024.0	27217.0	272.94	1.00	54434.0	205783.3	26.5	54223.0	0.053	0.439
Avobenzone		13843.0	13682.0	27686.0	27364.0	27525.0	227.69	0.83	55050.0	205783.3	26.8	54839.0	0.053	0.444
Avobenzone		13428.0	13701.0	26856.0	27402.0	27129.0	386.08	1.42	54258.0	205783.3	26.4	54047.0	0.053	0.438
Avobenzone	-6	13095.0	13520.0	26190.0	27040.0	26615.0	601.04	2.26	53230.0	205783.3	25.9	53019.0	0.052	0.429
Avobenzone		12740.0	12657.0	25480.0	25314.0	25397.0	117.38	0.46	50794.0	205783.3	24.7	50583.0	0.049	0.410
Avobenzone		13271.0	13047.0	26542.0	26094.0	26318.0	316.78	1.20	52636.0	205783.3	25.6	52425.0	0.051	0.425
Avobenzone	-7	12698.0	12420.0	25396.0	24840.0	25118.0	393.15	1.57	50236.0	205783.3	24.4	50025.0	0.049	0.405
Avobenzone		11852.0	11513.0	23704.0	23026.0	23365.0	479.42	2.05	46730.0	205783.3	22.7	46519.0	0.045	0.377
Avobenzone		12574.0	13038.0	25148.0	26076.0	25612.0	656.20	2.56	51224.0	205783.3	24.9	51013.0	0.050	0.413
Avobenzone	-8	12225.0	12641.0	24450.0	25282.0	24866.0	588.31	2.37	49732.0	205783.3	24.2	49521.0	0.048	0.401
Avobenzone		12725.0	12974.0	25450.0	25948.0	25699.0	352.14	1.37	51398.0	205783.3	25.0	51187.0	0.050	0.415
Avobenzone		12613.0	12736.0	25226.0	25472.0	25349.0	173.95	0.69	50698.0	205783.3	24.6	50487.0	0.049	0.409
Avobenzone	-9	13266.0	12845.0	26532.0	25690.0	26111.0	595.38	2.28	52222.0	205783.3	25.4	52011.0	0.051	0.421
Avobenzone		13235.0	12296.0	26470.0	24592.0	25531.0	1327.95	5.20	51062.0	205783.3	24.8	50851.0	0.049	0.412
Avobenzone		12147.0	12841.0	24294.0	25682.0	24988.0	981.46	3.93	49976.0	205783.3	24.3	49765.0	0.048	0.403
Avobenzone	-10	12509.0	12605.0	25018.0	25210.0	25114.0	135.76	0.54	50228.0	205783.3	24.4	50017.0	0.049	0.405
Avobenzone		11900.0	12628.0	23800.0	25256.0	24528.0	1029.55	4.20	49056.0	205783.3	23.8	48845.0	0.047	0.396
Avobenzone		13034.0	13096.0	26068.0	26192.0	26130.0	87.68	0.34	52260.0	205783.3	25.4	52049.0	0.051	0.422
TA		12089.0	12512.0	24178.0	25024.0	24601.0	598.21	2.43	49202.0	205783.3	23.9	48991.0	0.048	0.397
TA		13006.0	12687.0	26012.0	25374.0	25693.0	451.13	1.76	51386.0	205783.3	25.0	51175.0	0.050	0.414
NSB		52.0	59.0	104.0	118.0	111.0	9.90	8.92	222.0	205783.3	0.1	11.0	0.000	0.000
NSB		48.0	49.0	96.0	98.0	97.0	1.41	1.46	194.0	205783.3	0.1	-17.0	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 Avobenzone): Part 4 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
89.98	89.72	0.821	1.422	1.585
91.01				
88.19				
94.84	103.98	4.899	8.485	8.160
111.61				
105.50				
106.60	106.89	0.472	0.818	0.765
107.81				
106.26				
104.24	102.25	1.442	2.497	2.442
99.45				
103.07				
98.35	96.70	2.681	4.643	4.801
91.46				
100.29				
97.36	99.08	0.950	1.645	1.660
100.64				
99.26				
102.26	100.02	1.275	2.208	2.208
99.97				
97.84				
98.33	98.90	1.840	3.187	3.223
96.03				
102.33				
96.32	98.46	2.147	3.036	3.084
100.61				
0.02	-0.01	0.028	0.039	659.966
-0.03				

APPENDIX 1: Run 1: Assay Information (Ensulizole)

Experiment Date:	21-Feb-13	Study Number:	9070-100794AROM		
Test substance:	Ensulizole				
3/14/2013 16:27					
	specific activity based on decay for 4/20/10		42770.0	DPM	
	20 uL count of 3H-ASDN (mean)		41156.7	DPM	
	0.5 mL count for total activity		12768.8	DPM	
	microsomal protein/assay		0.008	mg	
	Reaction time		15	min	
	20 uL count of 3H-ASDN (DPM)		40421	41204	41845

Assays Conducted by: [redacted]					Spreadsheet locked on: 03/05/2013
					Green shaded areas: unlocked cells for data entry
Each assay contained 100 uL 3H-ASDN	205783.3	DPM	0.200	(nmoles)	
Total product 3H-H20 per assay	51075.0	DPM	0.050	(nmoles)	
Percent conversion to product (3H-H2O) (percent)	24.8				
Rate of conversion to 3H-H2O in total activity assay	0.414	nmol/(mg protein-min)			
Average activity of control Tubes	0.412	nmol/(mg protein-min)			
Average full enzyme activity controls (percent +/-SD)	100.0	2.5			
Average background activity controls (percent +/- SD)	0.0	0.0			

APPENDIX 1: Run 1: Raw and Normalized DPM Data (4OH-ASDN and Ensulizole): 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 tubesww	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
TA		12927.0	12901.0	25854.0	25802.0	25828.0	36.77	0.14	51656.0	205783.3	25.1	51445.0	0.050	0.417
TA		13098.0	12930.0	26196.0	25860.0	26028.0	237.59	0.91	52056.0	205783.3	25.3	51845.0	0.050	0.420
NSB		52.0	54.0	104.0	108.0	106.0	2.83	2.67	212.0	205783.3	0.1	1.0	0.000	0.000
NSB		53.0	55.0	106.0	110.0	108.0	2.83	2.62	216.0	205783.3	0.1	5.0	0.000	0.000
4OH-ASDN	-5	191.0	197.0	382.0	394.0	388.0	8.49	2.19	776.0	205783.3	0.4	565.0	0.001	0.005
4OH-ASDN		190.0	211.0	380.0	422.0	401.0	29.70	7.41	802.0	205783.3	0.4	591.0	0.001	0.005
4OH-ASDN	-6	1238.0	1216.0	2476.0	2432.0	2454.0	31.11	1.27	4908.0	205783.3	2.4	4697.0	0.005	0.038
4OH-ASDN		1325.0	1354.0	2650.0	2708.0	2679.0	41.01	1.53	5358.0	205783.3	2.6	5147.0	0.005	0.042
4OH-ASDN	-6.5	3043.0	2998.0	6086.0	5996.0	6041.0	63.64	1.05	12082.0	205783.3	5.9	11871.0	0.012	0.096
4OH-ASDN		3057.0	3095.0	6114.0	6190.0	6152.0	53.74	0.87	12304.0	205783.3	6.0	12093.0	0.012	0.098
4OH-ASDN	-7	6263.0	6252.0	12526.0	12504.0	12515.0	15.56	0.12	25030.0	205783.3	12.2	24819.0	0.024	0.201
4OH-ASDN		6115.0	5969.0	12230.0	11938.0	12084.0	206.48	1.71	24168.0	205783.3	11.7	23957.0	0.023	0.194
4OH-ASDN	-7.5	9660.0	9486.0	19320.0	18972.0	19146.0	246.07	1.29	38292.0	205783.3	18.6	38081.0	0.037	0.308
4OH-ASDN		7864.0	7669.0	15728.0	15338.0	15533.0	275.77	1.78	31066.0	205783.3	15.1	30855.0	0.030	0.250
4OH-ASDN	-8	11260.0	10611.0	22520.0	21222.0	21871.0	917.82	4.20	43742.0	205783.3	21.3	43531.0	0.042	0.353
4OH-ASDN		11767.0	11859.0	23534.0	23718.0	23626.0	130.11	0.55	47252.0	205783.3	23.0	47041.0	0.046	0.381
4OH-ASDN	-9	12744.0	12626.0	25488.0	25252.0	25370.0	166.88	0.66	50740.0	205783.3	24.7	50529.0	0.049	0.409
4OH-ASDN		12652.0	12483.0	25304.0	24966.0	25135.0	239.00	0.95	50270.0	205783.3	24.4	50059.0	0.049	0.405
4OH-ASDN	-10	12995.0	12754.0	25990.0	25508.0	25749.0	340.83	1.32	51498.0	205783.3	25.0	51287.0	0.050	0.415
4OH-ASDN		12631.0	12254.0	25262.0	24508.0	24885.0	533.16	2.14	49770.0	205783.3	24.2	49559.0	0.048	0.401

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 Ensulizole): Part 2 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
101.14	101.54	0.393	0.556	0.548
101.93				
0.00	0.01	0.004	0.006	94.281
0.01				
1.11	1.14	0.026	0.036	3.181
1.16				
9.23	9.68	0.442	0.626	6.465
10.12				
23.34	23.56	0.218	0.309	1.310
23.78				
48.79	47.95	0.847	1.198	2.499
47.10				
74.87	67.77	7.103	10.046	14.824
60.66				
85.58	89.03	3.450	4.880	5.481
92.48				
99.34	98.88	0.462	0.653	0.661
98.42				
100.83	99.13	1.699	2.402	2.423
97.43				

APPENDIX 1: Run 1: Raw and Normalized DPM Data (4OH-ASDN and Ensulizole): 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 tubesww	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
Ensulizole	-3.5	12973.0	12704.0	25946.0	25408.0	25677.0	380.42	1.48	51354.0	205783.3	25.0	51143.0	0.050	0.414
Ensulizole		12941.0	12366.0	25882.0	24732.0	25307.0	813.17	3.21	50614.0	205783.3	24.6	50403.0	0.049	0.408
Ensulizole		13048.0	13023.0	26096.0	26046.0	26071.0	35.36	0.14	52142.0	205783.3	25.3	51931.0	0.050	0.421
Ensulizole	-4.5	13195.0	13331.0	26390.0	26662.0	26526.0	192.33	0.73	53052.0	205783.3	25.8	52841.0	0.051	0.428
Ensulizole		12879.0	12795.0	25758.0	25590.0	25674.0	118.79	0.46	51348.0	205783.3	25.0	51137.0	0.050	0.414
Ensulizole		13123.0	12916.0	26246.0	25832.0	26039.0	292.74	1.12	52078.0	205783.3	25.3	51867.0	0.050	0.420
Ensulizole	-5.5	12995.0	12875.0	25990.0	25750.0	25870.0	169.71	0.66	51740.0	205783.3	25.1	51529.0	0.050	0.417
Ensulizole		12862.0	12783.0	25724.0	25566.0	25645.0	111.72	0.44	51290.0	205783.3	24.9	51079.0	0.050	0.414
Ensulizole		12917.0	12532.0	25834.0	25064.0	25449.0	544.47	2.14	50898.0	205783.3	24.7	50687.0	0.049	0.411
Ensulizole	-6.5	12217.0	12769.0	24434.0	25538.0	24986.0	780.65	3.12	49972.0	205783.3	24.3	49761.0	0.048	0.403
Ensulizole		13390.0	12894.0	26780.0	25788.0	26284.0	701.45	2.67	52568.0	205783.3	25.5	52357.0	0.051	0.424
Ensulizole		12897.0	12450.0	25794.0	24900.0	25347.0	632.15	2.49	50694.0	205783.3	24.6	50483.0	0.049	0.409
Ensulizole	-7.5	12410.0	12346.0	24820.0	24692.0	24756.0	90.51	0.37	49512.0	205783.3	24.1	49301.0	0.048	0.399
Ensulizole		12371.0	12705.0	24742.0	25410.0	25076.0	472.35	1.88	50152.0	205783.3	24.4	49941.0	0.049	0.404
Ensulizole		12319.0	12030.0	24638.0	24060.0	24349.0	408.71	1.68	48698.0	205783.3	23.7	48487.0	0.047	0.393
Ensulizole	-8.5	13028.0	13067.0	26056.0	26134.0	26095.0	55.15	0.21	52190.0	205783.3	25.4	51979.0	0.051	0.421
Ensulizole		11486.0	12302.0	22972.0	24604.0	23788.0	1154.00	4.85	47576.0	205783.3	23.1	47365.0	0.046	0.384
Ensulizole		12347.0	12482.0	24694.0	24964.0	24829.0	190.92	0.77	49658.0	205783.3	24.1	49447.0	0.048	0.400
Ensulizole	-9.5	11910.0	11618.0	23820.0	23236.0	23528.0	412.95	1.76	47056.0	205783.3	22.9	46845.0	0.046	0.379
Ensulizole		12875.0	12177.0	25750.0	24354.0	25052.0	987.12	3.94	50104.0	205783.3	24.3	49893.0	0.048	0.404
Ensulizole		11959.0	12078.0	23918.0	24156.0	24037.0	168.29	0.70	48074.0	205783.3	23.4	47863.0	0.047	0.388
Ensulizole	-10.5	11739.0	12565.0	23478.0	25130.0	24304.0	1168.14	4.81	48608.0	205783.3	23.6	48397.0	0.047	0.392
Ensulizole		13139.0	12944.0	26278.0	25888.0	26083.0	275.77	1.06	52166.0	205783.3	25.3	51955.0	0.050	0.421
Ensulizole		12696.0	12978.0	25392.0	25956.0	25674.0	398.81	1.55	51348.0	205783.3	25.0	51137.0	0.050	0.414
TA		12089.0	12512.0	24178.0	25024.0	24601.0	598.21	2.43	49202.0	205783.3	23.9	48991.0	0.048	0.397
TA		13006.0	12687.0	26012.0	25374.0	25693.0	451.13	1.76	51386.0	205783.3	25.0	51175.0	0.050	0.414
NSB		52.0	59.0	104.0	118.0	111.0	9.90	8.92	222.0	205783.3	0.1	11.0	0.000	0.000
NSB		48.0	49.0	96.0	98.0	97.0	1.41	1.46	194.0	205783.3	0.1	-17.0	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 Ensulizole): Part 4 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
100.55	100.58	0.867	1.502	1.494
99.09				
102.10				
103.89	102.13	0.970	1.681	1.646
100.54				
101.97				
101.31	100.46	0.478	0.828	0.825
100.42				
99.65				
97.83	100.01	1.521	2.634	2.634
102.94				
99.25				
96.93	96.81	0.827	1.433	1.480
98.19				
95.33				
102.19	97.51	2.623	4.543	4.659
93.12				
97.21				
92.10	94.76	1.761	3.051	3.219
98.09				
94.10				
95.15	99.28	2.115	3.664	3.690
102.14				
100.54				
96.32	98.46	2.147	3.036	3.084
100.61				
0.02	-0.01	0.028	0.039	659.966
-0.03				

APPENDIX 1: Run 1: Assay Information (Homosalate)

Experiment Date:	21-Feb-13	Study Number:	9070-100794AROM		
Test substance:	Homosalate				
3/14/2013 16:27					
	specific activity based on decay for 4/20/10		42770.0	DPM	
	20 uL count of 3H-ASDN (mean)		41156.7	DPM	
	0.5 mL count for total activity		12768.8	DPM	
	microsomal protein/assay		0.008	mg	
	Reaction time		15	min	
	20 uL count of 3H-ASDN (DPM)		40421	41204	41845

Assays Conducted by:	[redacted]			Spreadsheet locked on: 03/05/2013
				Green shaded areas: unlocked cells for data entry
Each assay contained 100 uL 3H-ASDN	205783.3	DPM	0.200	(nmoles)
Total product 3H-H20 per assay	51075.0	DPM	0.050	(nmoles)
Percent conversion to product (3H-H2O) (percent)	24.8			
Rate of conversion to 3H-H2O in total activity assay	0.414	nmol/(mg protein-min)		
Average activity of control Tubes	0.412	nmol/(mg protein-min)		
Average full enzyme activity controls (percent +/-SD)	100.0	2.5		
Average background activity controls (percent +/- SD)	0.0	0.0		

APPENDIX 1: Run 1: Raw and Normalized DPM Data (4OH-ASDN and (Homosalate): 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 tubesww	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
TA		12927.0	12901.0	25854.0	25802.0	25828.0	36.77	0.14	51656.0	205783.3	25.1	51445.0	0.050	0.417
TA		13098.0	12930.0	26196.0	25860.0	26028.0	237.59	0.91	52056.0	205783.3	25.3	51845.0	0.050	0.420
NSB		52.0	54.0	104.0	108.0	106.0	2.83	2.67	212.0	205783.3	0.1	1.0	0.000	0.000
NSB		53.0	55.0	106.0	110.0	108.0	2.83	2.62	216.0	205783.3	0.1	5.0	0.000	0.000
4OH-ASDN	-5	191.0	197.0	382.0	394.0	388.0	8.49	2.19	776.0	205783.3	0.4	565.0	0.001	0.005
4OH-ASDN		190.0	211.0	380.0	422.0	401.0	29.70	7.41	802.0	205783.3	0.4	591.0	0.001	0.005
4OH-ASDN	-6	1238.0	1216.0	2476.0	2432.0	2454.0	31.11	1.27	4908.0	205783.3	2.4	4697.0	0.005	0.038
4OH-ASDN		1325.0	1354.0	2650.0	2708.0	2679.0	41.01	1.53	5358.0	205783.3	2.6	5147.0	0.005	0.042
4OH-ASDN	-6.5	3043.0	2998.0	6086.0	5996.0	6041.0	63.64	1.05	12082.0	205783.3	5.9	11871.0	0.012	0.096
4OH-ASDN		3057.0	3095.0	6114.0	6190.0	6152.0	53.74	0.87	12304.0	205783.3	6.0	12093.0	0.012	0.098
4OH-ASDN	-7	6263.0	6252.0	12526.0	12504.0	12515.0	15.56	0.12	25030.0	205783.3	12.2	24819.0	0.024	0.201
4OH-ASDN		6115.0	5969.0	12230.0	11938.0	12084.0	206.48	1.71	24168.0	205783.3	11.7	23957.0	0.023	0.194
4OH-ASDN	-7.5	9660.0	9486.0	19320.0	18972.0	19146.0	246.07	1.29	38292.0	205783.3	18.6	38081.0	0.037	0.308
4OH-ASDN		7864.0	7669.0	15728.0	15338.0	15533.0	275.77	1.78	31066.0	205783.3	15.1	30855.0	0.030	0.250
4OH-ASDN	-8	11260.0	10611.0	22520.0	21222.0	21871.0	917.82	4.20	43742.0	205783.3	21.3	43531.0	0.042	0.353
4OH-ASDN		11767.0	11859.0	23534.0	23718.0	23626.0	130.11	0.55	47252.0	205783.3	23.0	47041.0	0.046	0.381
4OH-ASDN	-9	12744.0	12626.0	25488.0	25252.0	25370.0	166.88	0.66	50740.0	205783.3	24.7	50529.0	0.049	0.409
4OH-ASDN		12652.0	12483.0	25304.0	24966.0	25135.0	239.00	0.95	50270.0	205783.3	24.4	50059.0	0.049	0.405
4OH-ASDN	-10	12995.0	12754.0	25990.0	25508.0	25749.0	340.83	1.32	51498.0	205783.3	25.0	51287.0	0.050	0.415
4OH-ASDN		12631.0	12254.0	25262.0	24508.0	24885.0	533.16	2.14	49770.0	205783.3	24.2	49559.0	0.048	0.401

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 (Homosalate): Part 2 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
101.14	101.54	0.393	0.556	0.548
101.93				
0.00	0.01	0.004	0.006	94.281
0.01				
1.11	1.14	0.026	0.036	3.181
1.16				
9.23	9.68	0.442	0.626	6.465
10.12				
23.34	23.56	0.218	0.309	1.310
23.78				
48.79	47.95	0.847	1.198	2.499
47.10				
74.87	67.77	7.103	10.046	14.824
60.66				
85.58	89.03	3.450	4.880	5.481
92.48				
99.34	98.88	0.462	0.653	0.661
98.42				
100.83	99.13	1.699	2.402	2.423
97.43				

APPENDIX 1: Run 1: Raw and Normalized DPM Data (4OH-ASDN and (Homosalate): 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 tubesww	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
Homosalate	-3	10957.0	9954.0	21914.0	19908.0	20911.0	1418.46	6.78	41822.0	205783.3	20.3	41611.0	0.040	0.337
Homosalate		10616.0	10286.0	21232.0	20572.0	20902.0	466.69	2.23	41804.0	205783.3	20.3	41593.0	0.040	0.337
Homosalate		11076.0	10839.0	22152.0	21678.0	21915.0	335.17	1.53	43830.0	205783.3	21.3	43619.0	0.042	0.353
Homosalate	-4	11646.0	11786.0	23292.0	23572.0	23432.0	197.99	0.84	46864.0	205783.3	22.8	46653.0	0.045	0.378
Homosalate		11349.0	11136.0	22698.0	22272.0	22485.0	301.23	1.34	44970.0	205783.3	21.9	44759.0	0.044	0.363
Homosalate		10194.0	10381.0	20388.0	20762.0	20575.0	264.46	1.29	41150.0	205783.3	20.0	40939.0	0.040	0.332
Homosalate	-5	11051.0	11522.0	22102.0	23044.0	22573.0	666.09	2.95	45146.0	205783.3	21.9	44935.0	0.044	0.364
Homosalate		11870.0	12223.0	23740.0	24446.0	24093.0	499.22	2.07	48186.0	205783.3	23.4	47975.0	0.047	0.389
Homosalate		11971.0	12048.0	23942.0	24096.0	24019.0	108.89	0.45	48038.0	205783.3	23.3	47827.0	0.046	0.387
Homosalate	-6	12413.0	11875.0	24826.0	23750.0	24288.0	760.85	3.13	48576.0	205783.3	23.6	48365.0	0.047	0.392
Homosalate		12952.0	12416.0	25904.0	24832.0	25368.0	758.02	2.99	50736.0	205783.3	24.7	50525.0	0.049	0.409
Homosalate		11555.0	11826.0	23110.0	23652.0	23381.0	383.25	1.64	46762.0	205783.3	22.7	46551.0	0.045	0.377
Homosalate	-7	12126.0	11452.0	24252.0	22904.0	23578.0	953.18	4.04	47156.0	205783.3	22.9	46945.0	0.046	0.380
Homosalate		13045.0	12893.0	26090.0	25786.0	25938.0	214.96	0.83	51876.0	205783.3	25.2	51665.0	0.050	0.418
Homosalate		12501.0	12354.0	25002.0	24708.0	24855.0	207.89	0.84	49710.0	205783.3	24.2	49499.0	0.048	0.401
Homosalate	-8	12206.0	11774.0	24412.0	23548.0	23980.0	610.94	2.55	47960.0	205783.3	23.3	47749.0	0.046	0.387
Homosalate		11200.0	12027.0	22400.0	24054.0	23227.0	1169.55	5.04	46454.0	205783.3	22.6	46243.0	0.045	0.375
Homosalate		12462.0	12079.0	24924.0	24158.0	24541.0	541.64	2.21	49082.0	205783.3	23.9	48871.0	0.047	0.396
Homosalate	-9	12465.0	12116.0	24930.0	24232.0	24581.0	493.56	2.01	49162.0	205783.3	23.9	48951.0	0.048	0.396
Homosalate		12573.0	12034.0	25146.0	24068.0	24607.0	762.26	3.10	49214.0	205783.3	23.9	49003.0	0.048	0.397
Homosalate		12898.0	12992.0	25796.0	25984.0	25890.0	132.94	0.51	51780.0	205783.3	25.2	51569.0	0.050	0.418
Homosalate	-10	12872.0	12592.0	25744.0	25184.0	25464.0	395.98	1.56	50928.0	205783.3	24.7	50717.0	0.049	0.411
Homosalate		12647.0	13167.0	25294.0	26334.0	25814.0	735.39	2.85	51628.0	205783.3	25.1	51417.0	0.050	0.416
Homosalate		12928.0	12818.0	25856.0	25636.0	25746.0	155.56	0.60	51492.0	205783.3	25.0	51281.0	0.050	0.415
TA		12089.0	12512.0	24178.0	25024.0	24601.0	598.21	2.43	49202.0	205783.3	23.9	48991.0	0.048	0.397
TA		13006.0	12687.0	26012.0	25374.0	25693.0	451.13	1.76	51386.0	205783.3	25.0	51175.0	0.050	0.414
NSB		52.0	59.0	104.0	118.0	111.0	9.90	8.92	222.0	205783.3	0.1	11.0	0.000	0.000
NSB		48.0	49.0	96.0	98.0	97.0	1.41	1.46	194.0	205783.3	0.1	-17.0	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 (Homosalate): Part 4 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
81.81	83.11	1.322	2.290	2.755
81.77				
85.76				
91.72	86.74	3.304	5.722	6.597
88.00				
80.49				
88.34	92.23	1.946	3.370	3.654
94.32				
94.03				
95.09	95.31	2.258	3.911	4.104
99.33				
91.52				
92.30	97.06	2.682	4.645	4.786
101.57				
97.32				
93.88	93.62	1.497	2.593	2.769
90.91				
96.08				
96.24	97.99	1.699	2.943	3.003
96.34				
101.39				
99.71	100.54	0.421	0.730	0.726
101.09				
100.82				
96.32	98.46	2.147	3.036	3.084
100.61				
0.02	-0.01	0.028	0.039	659.966
-0.03				

APPENDIX 1: Run 1: Assay Information (Padimate-O)

Experiment Date:	21-Feb-13	Study Number:	9070-100794AROM		
Test substance:	Padimate O				
3/14/2013 16:27					
	specific activity based on decay for 4/20/10		42770.0	DPM	
	20 uL count of 3H-ASDN (mean)		41156.7	DPM	
	0.5 mL count for total activity		12768.8	DPM	
	microsomal protein/assay		0.008	mg	
	Reaction time		15	min	
	20 uL count of 3H-ASDN (DPM)		40421	41204	41845

Assays Conducted by:	[redacted]				Spreadsheet locked on: 03/05/2013
					Green shaded areas: unlocked cells for data entry
Each assay contained 100 uL 3H-ASDN	205783.3	DPM	0.200	(nmoles)	
Total product 3H-H20 per assay	51075.0	DPM	0.050	(nmoles)	
Percent conversion to product (3H-H2O) (percent)	24.8				
Rate of conversion to 3H-H2O in total activity assay	0.414	nmol/(mg protein-min)			
Average activity of control Tubes	0.412	nmol/(mg protein-min)			
Average full enzyme activity controls (percent +/-SD)	100.0	2.5			
Average background activity controls (percent +/- SD)	0.0	0.0			

APPENDIX 1: Run 1: Raw and Normalized DPM Data (4OH-ASDN and Padimate-O): 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 tubesww	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
TA		12927.0	12901.0	25854.0	25802.0	25828.0	36.77	0.14	51656.0	205783.3	25.1	51445.0	0.050	0.417
TA		13098.0	12930.0	26196.0	25860.0	26028.0	237.59	0.91	52056.0	205783.3	25.3	51845.0	0.050	0.420
NSB		52.0	54.0	104.0	108.0	106.0	2.83	2.67	212.0	205783.3	0.1	1.0	0.000	0.000
NSB		53.0	55.0	106.0	110.0	108.0	2.83	2.62	216.0	205783.3	0.1	5.0	0.000	0.000
4OH-ASDN	-5	191.0	197.0	382.0	394.0	388.0	8.49	2.19	776.0	205783.3	0.4	565.0	0.001	0.005
4OH-ASDN		190.0	211.0	380.0	422.0	401.0	29.70	7.41	802.0	205783.3	0.4	591.0	0.001	0.005
4OH-ASDN	-6	1238.0	1216.0	2476.0	2432.0	2454.0	31.11	1.27	4908.0	205783.3	2.4	4697.0	0.005	0.038
4OH-ASDN		1325.0	1354.0	2650.0	2708.0	2679.0	41.01	1.53	5358.0	205783.3	2.6	5147.0	0.005	0.042
4OH-ASDN	-6.5	3043.0	2998.0	6086.0	5996.0	6041.0	63.64	1.05	12082.0	205783.3	5.9	11871.0	0.012	0.096
4OH-ASDN		3057.0	3095.0	6114.0	6190.0	6152.0	53.74	0.87	12304.0	205783.3	6.0	12093.0	0.012	0.098
4OH-ASDN	-7	6263.0	6252.0	12526.0	12504.0	12515.0	15.56	0.12	25030.0	205783.3	12.2	24819.0	0.024	0.201
4OH-ASDN		6115.0	5969.0	12230.0	11938.0	12084.0	206.48	1.71	24168.0	205783.3	11.7	23957.0	0.023	0.194
4OH-ASDN	-7.5	9660.0	9486.0	19320.0	18972.0	19146.0	246.07	1.29	38292.0	205783.3	18.6	38081.0	0.037	0.308
4OH-ASDN		7864.0	7669.0	15728.0	15338.0	15533.0	275.77	1.78	31066.0	205783.3	15.1	30855.0	0.030	0.250
4OH-ASDN	-8	11260.0	10611.0	22520.0	21222.0	21871.0	917.82	4.20	43742.0	205783.3	21.3	43531.0	0.042	0.353
4OH-ASDN		11767.0	11859.0	23534.0	23718.0	23626.0	130.11	0.55	47252.0	205783.3	23.0	47041.0	0.046	0.381
4OH-ASDN	-9	12744.0	12626.0	25488.0	25252.0	25370.0	166.88	0.66	50740.0	205783.3	24.7	50529.0	0.049	0.409
4OH-ASDN		12652.0	12483.0	25304.0	24966.0	25135.0	239.00	0.95	50270.0	205783.3	24.4	50059.0	0.049	0.405
4OH-ASDN	-10	12995.0	12754.0	25990.0	25508.0	25749.0	340.83	1.32	51498.0	205783.3	25.0	51287.0	0.050	0.415
4OH-ASDN		12631.0	12254.0	25262.0	24508.0	24885.0	533.16	2.14	49770.0	205783.3	24.2	49559.0	0.048	0.401

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 Padimate-O): Part 2 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
101.14	101.54	0.393	0.556	0.548
101.93				
0.00	0.01	0.004	0.006	94.281
0.01				
1.11	1.14	0.026	0.036	3.181
1.16				
9.23	9.68	0.442	0.626	6.465
10.12				
23.34	23.56	0.218	0.309	1.310
23.78				
48.79	47.95	0.847	1.198	2.499
47.10				
74.87	67.77	7.103	10.046	14.824
60.66				
85.58	89.03	3.450	4.880	5.481
92.48				
99.34	98.88	0.462	0.653	0.661
98.42				
100.83	99.13	1.699	2.402	2.423
97.43				

APPENDIX 1: Run 1: Raw and Normalized DPM Data (4OH-ASDN and Padimate-O): 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 tubesww	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
Padimate O	-3	10791.0	10417.0	21582.0	20834.0	21208.0	528.92	2.49	42416.0	205783.3	20.6	42205.0	0.041	0.342
Padimate O		10940.0	10863.0	21880.0	21726.0	21803.0	108.89	0.50	43606.0	205783.3	21.2	43395.0	0.042	0.351
Padimate O		10640.0	10367.0	21280.0	20734.0	21007.0	386.08	1.84	42014.0	205783.3	20.4	41803.0	0.041	0.339
Padimate O	-4	12104.0	11599.0	24208.0	23198.0	23703.0	714.18	3.01	47406.0	205783.3	23.0	47195.0	0.046	0.382
Padimate O		11829.0	11629.0	23658.0	23258.0	23458.0	282.84	1.21	46916.0	205783.3	22.8	46705.0	0.045	0.378
Padimate O		11885.0	11777.0	23770.0	23554.0	23662.0	152.74	0.65	47324.0	205783.3	23.0	47113.0	0.046	0.382
Padimate O	-5	12256.0	12331.0	24512.0	24662.0	24587.0	106.07	0.43	49174.0	205783.3	23.9	48963.0	0.048	0.397
Padimate O		11659.0	12591.0	23318.0	25182.0	24250.0	1318.05	5.44	48500.0	205783.3	23.6	48289.0	0.047	0.391
Padimate O		12470.0	12470.0	24940.0	24940.0	24940.0	0.00	0.00	49880.0	205783.3	24.2	49669.0	0.048	0.402
Padimate O	-6	12174.0	12811.0	24348.0	25622.0	24985.0	900.85	3.61	49970.0	205783.3	24.3	49759.0	0.048	0.403
Padimate O		12667.0	12959.0	25334.0	25918.0	25626.0	412.95	1.61	51252.0	205783.3	24.9	51041.0	0.050	0.413
Padimate O		12890.0	13007.0	25780.0	26014.0	25897.0	165.46	0.64	51794.0	205783.3	25.2	51583.0	0.050	0.418
Padimate O	-7	12963.0	12508.0	25926.0	25016.0	25471.0	643.47	2.53	50942.0	205783.3	24.8	50731.0	0.049	0.411
Padimate O		12903.0	12993.0	25806.0	25986.0	25896.0	127.28	0.49	51792.0	205783.3	25.2	51581.0	0.050	0.418
Padimate O		12646.0	13117.0	25292.0	26234.0	25763.0	666.09	2.59	51526.0	205783.3	25.0	51315.0	0.050	0.416
Padimate O	-8	12826.0	12618.0	25652.0	25236.0	25444.0	294.16	1.16	50888.0	205783.3	24.7	50677.0	0.049	0.410
Padimate O		13083.0	12847.0	26166.0	25694.0	25930.0	333.75	1.29	51860.0	205783.3	25.2	51649.0	0.050	0.418
Padimate O		12740.0	12766.0	25480.0	25532.0	25506.0	36.77	0.14	51012.0	205783.3	24.8	50801.0	0.049	0.411
Padimate O	-9	12270.0	13018.0	24540.0	26036.0	25288.0	1057.83	4.18	50576.0	205783.3	24.6	50365.0	0.049	0.408
Padimate O		12492.0	11489.0	24984.0	22978.0	23981.0	1418.46	5.91	47962.0	205783.3	23.3	47751.0	0.046	0.387
Padimate O		12426.0	12694.0	24852.0	25388.0	25120.0	379.01	1.51	50240.0	205783.3	24.4	50029.0	0.049	0.405
Padimate O	-10	12629.0	12670.0	25258.0	25340.0	25299.0	57.98	0.23	50598.0	205783.3	24.6	50387.0	0.049	0.408
Padimate O		12502.0	12503.0	25004.0	25006.0	25005.0	1.41	0.01	50010.0	205783.3	24.3	49799.0	0.048	0.403
Padimate O		12657.0	12484.0	25314.0	24968.0	25141.0	244.66	0.97	50282.0	205783.3	24.4	50071.0	0.049	0.406
TA		12089.0	12512.0	24178.0	25024.0	24601.0	598.21	2.43	49202.0	205783.3	23.9	48991.0	0.048	0.397
TA		13006.0	12687.0	26012.0	25374.0	25693.0	451.13	1.76	51386.0	205783.3	25.0	51175.0	0.050	0.414
NSB		52.0	59.0	104.0	118.0	111.0	9.90	8.92	222.0	205783.3	0.1	11.0	0.000	0.000
NSB		48.0	49.0	96.0	98.0	97.0	1.41	1.46	194.0	205783.3	0.1	-17.0	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 Padimate-O): Part 4 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
82.98	83.49	0.940	1.628	1.949
85.32				
82.19				
92.79	92.41	0.298	0.516	0.558
91.82				
92.63				
96.26	96.28	0.783	1.357	1.409
94.94				
97.65				
97.83	99.86	1.063	1.842	1.844
100.35				
101.41				
99.74	100.68	0.494	0.855	0.849
101.41				
100.89				
99.63	100.35	0.600	1.040	1.036
101.54				
99.88				
99.02	97.09	1.614	2.796	2.880
93.88				
98.36				
99.06	98.47	0.334	0.579	0.588
97.91				
98.44				
96.32	98.46	2.147	3.036	3.084
100.61				
0.02	-0.01	0.028	0.039	659.966
-0.03				

APPENDIX 1: Run 2: Assay Information (Avobenzone)

Experiment Date:	25-Feb-13	Study Number:	9070-100794AROM		
Test substance:	Avobenzone				
3/8/2013 1:01					
	specific activity based on decay for 4/20/10		42662.0	DPM	
	20 uL count of 3H-ASDN (mean)		41760.3	DPM	
	0.5 mL count for total activity		13015.0	DPM	
	microsomal protein/assay		0.008	mg	
	Reaction time		15	min	
	20 uL count of 3H-ASDN (DPM)		41209	41278	42794

Assays Conducted by: [redacted]

Spreadsheet locked on: 03/05/2013
Green shaded areas: unlocked cells for data entry

Each assay contained 100 uL 3H-ASDN	208801.7	DPM	0.200	(nmoles)
Total product 3H-H20 per assay	52060.0	DPM	0.050	(nmoles)
Percent conversion to product (3H-H2O) (percent)	24.9			
Rate of conversion to 3H-H2O in total activity assay	0.416	nmol/(mg protein-min)		
Average activity of control Tubes	0.413	nmol/(mg protein-min)		
Average full enzyme activity controls (percent +/-SD)	100.0	1.6		
Average background activity controls (percent +/- SD)	0.0	0.0		

APPENDIX 1: Run 2: Raw and Normalized DPM Data (4OH-ASDN and Avobenzone): 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 tubesww	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
TA		12970.0	12821.0	25940.0	25642.0	25791.0	210.72	0.82	51582.0	208801.7	24.7	51321.0	0.049	0.410
TA		13716.0	12219.0	27432.0	24438.0	25935.0	2117.08	8.16	51870.0	208801.7	24.8	51609.0	0.049	0.412
NSB		71.0	66.0	142.0	132.0	137.0	7.07	5.16	274.0	208801.7	0.1	13.0	0.000	0.000
NSB		62.0	73.0	124.0	146.0	135.0	15.56	11.52	270.0	208801.7	0.1	9.0	0.000	0.000
4OH-ASDN	-5	181.0	174.0	362.0	348.0	355.0	9.90	2.79	710.0	208801.7	0.3	449.0	0.000	0.004
4OH-ASDN		198.0	181.0	396.0	362.0	379.0	24.04	6.34	758.0	208801.7	0.4	497.0	0.000	0.004
4OH-ASDN	-6	1128.0	1120.0	2256.0	2240.0	2248.0	11.31	0.50	4496.0	208801.7	2.2	4235.0	0.004	0.034
4OH-ASDN		1164.0	1121.0	2328.0	2242.0	2285.0	60.81	2.66	4570.0	208801.7	2.2	4309.0	0.004	0.034
4OH-ASDN	-6.5	2718.0	2737.0	5436.0	5474.0	5455.0	26.87	0.49	10910.0	208801.7	5.2	10649.0	0.010	0.085
4OH-ASDN		2867.0	2786.0	5734.0	5572.0	5653.0	114.55	2.03	11306.0	208801.7	5.4	11045.0	0.011	0.088
4OH-ASDN	-7	5605.0	5535.0	11210.0	11070.0	11140.0	98.99	0.89	22280.0	208801.7	10.7	22019.0	0.021	0.176
4OH-ASDN		5658.0	5799.0	11316.0	11598.0	11457.0	199.40	1.74	22914.0	208801.7	11.0	22653.0	0.022	0.181
4OH-ASDN	-7.5	8987.0	8755.0	17974.0	17510.0	17742.0	328.10	1.85	35484.0	208801.7	17.0	35223.0	0.034	0.281
4OH-ASDN		9482.0	9149.0	18964.0	18298.0	18631.0	470.93	2.53	37262.0	208801.7	17.8	37001.0	0.035	0.295
4OH-ASDN	-8	11216.0	11707.0	22432.0	23414.0	22923.0	694.38	3.03	45846.0	208801.7	22.0	45585.0	0.044	0.364
4OH-ASDN		11924.0	11460.0	23848.0	22920.0	23384.0	656.20	2.81	46768.0	208801.7	22.4	46507.0	0.045	0.371
4OH-ASDN	-9	13924.0	13509.0	27848.0	27018.0	27433.0	586.90	2.14	54866.0	208801.7	26.3	54605.0	0.052	0.436
4OH-ASDN		13648.0	13261.0	27296.0	26522.0	26909.0	547.30	2.03	53818.0	208801.7	25.8	53557.0	0.051	0.427
4OH-ASDN	-10	13961.0	13692.0	27922.0	27384.0	27653.0	380.42	1.38	55306.0	208801.7	26.5	55045.0	0.053	0.439
4OH-ASDN		13669.0	13073.0	27338.0	26146.0	26742.0	842.87	3.15	53484.0	208801.7	25.6	53223.0	0.051	0.425

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 Avobenzone): Part 2 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
99.08	99.36	0.278	0.393	0.396
99.63				
0.03	0.02	0.004	0.005	25.713
0.02				
0.87	0.91	0.046	0.066	7.176
0.96				
8.18	8.25	0.071	0.101	1.225
8.32				
20.56	20.94	0.382	0.541	2.581
21.32				
42.51	43.12	0.612	0.865	2.007
43.73				
68.00	69.72	1.716	2.427	3.481
71.43				
88.00	88.89	0.890	1.259	1.416
89.78				
105.42	104.41	1.012	1.431	1.370
103.39				
106.27	104.51	1.759	2.487	2.380
102.75				

APPENDIX 1: Run 2: Raw and Normalized DPM Data (4OH-ASDN and Avobenzone): 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 tubesww	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
Avobenzone	-3	12350.0	12357.0	24700.0	24714.0	24707.0	9.90	0.04	49414.0	208801.7	23.7	49153.0	0.047	0.392
Avobenzone		12270.0	12515.0	24540.0	25030.0	24785.0	346.48	1.40	49570.0	208801.7	23.7	49309.0	0.047	0.394
Avobenzone		12898.0	12872.0	25796.0	25744.0	25770.0	36.77	0.14	51540.0	208801.7	24.7	51279.0	0.049	0.409
Avobenzone	-4	14125.0	14228.0	28250.0	28456.0	28353.0	145.66	0.51	56706.0	208801.7	27.2	56445.0	0.054	0.451
Avobenzone		14672.0	14397.0	29344.0	28794.0	29069.0	388.91	1.34	58138.0	208801.7	27.8	57877.0	0.055	0.462
Avobenzone		14263.0	14452.0	28526.0	28904.0	28715.0	267.29	0.93	57430.0	208801.7	27.5	57169.0	0.055	0.456
Avobenzone	-5	15128.0	15095.0	30256.0	30190.0	30223.0	46.67	0.15	60446.0	208801.7	28.9	60185.0	0.058	0.480
Avobenzone		14467.0	14614.0	28934.0	29228.0	29081.0	207.89	0.71	58162.0	208801.7	27.9	57901.0	0.055	0.462
Avobenzone		14344.0	14578.0	28688.0	29156.0	28922.0	330.93	1.14	57844.0	208801.7	27.7	57583.0	0.055	0.460
Avobenzone	-6	14002.0	13243.0	28004.0	26486.0	27245.0	1073.39	3.94	54490.0	208801.7	26.1	54229.0	0.052	0.433
Avobenzone		13884.0	14064.0	27768.0	28128.0	27948.0	254.56	0.91	55896.0	208801.7	26.8	55635.0	0.053	0.444
Avobenzone		14462.0	14175.0	28924.0	28350.0	28637.0	405.88	1.42	57274.0	208801.7	27.4	57013.0	0.055	0.455
Avobenzone	-7	13602.0	13748.0	27204.0	27496.0	27350.0	206.48	0.75	54700.0	208801.7	26.2	54439.0	0.052	0.435
Avobenzone		13714.0	13631.0	27428.0	27262.0	27345.0	117.38	0.43	54690.0	208801.7	26.2	54429.0	0.052	0.434
Avobenzone		13562.0	13261.0	27124.0	26522.0	26823.0	425.68	1.59	53646.0	208801.7	25.7	53385.0	0.051	0.426
Avobenzone	-8	13226.0	12965.0	26452.0	25930.0	26191.0	369.11	1.41	52382.0	208801.7	25.1	52121.0	0.050	0.416
Avobenzone		13775.0	13596.0	27550.0	27192.0	27371.0	253.14	0.92	54742.0	208801.7	26.2	54481.0	0.052	0.435
Avobenzone		12848.0	12709.0	25696.0	25418.0	25557.0	196.58	0.77	51114.0	208801.7	24.5	50853.0	0.049	0.406
Avobenzone	-9	13775.0	13792.0	27550.0	27584.0	27567.0	24.04	0.09	55134.0	208801.7	26.4	54873.0	0.053	0.438
Avobenzone		12712.0	12907.0	25424.0	25814.0	25619.0	275.77	1.08	51238.0	208801.7	24.5	50977.0	0.049	0.407
Avobenzone		13721.0	13770.0	27442.0	27540.0	27491.0	69.30	0.25	54982.0	208801.7	26.3	54721.0	0.052	0.437
Avobenzone	-10	13299.0	13162.0	26598.0	26324.0	26461.0	193.75	0.73	52922.0	208801.7	25.3	52661.0	0.050	0.420
Avobenzone		13487.0	12949.0	26974.0	25898.0	26436.0	760.85	2.88	52872.0	208801.7	25.3	52611.0	0.050	0.420
Avobenzone		13717.0	13448.0	27434.0	26896.0	27165.0	380.42	1.40	54330.0	208801.7	26.0	54069.0	0.052	0.432
TA		13089.0	12660.0	26178.0	25320.0	25749.0	606.70	2.36	51498.0	208801.7	24.7	51237.0	0.049	0.409
TA		13285.0	13360.0	26570.0	26720.0	26645.0	106.07	0.40	53290.0	208801.7	25.5	53029.0	0.051	0.423
NSB		55.0	59.0	110.0	118.0	114.0	5.66	4.96	228.0	208801.7	0.1	-33.0	0.000	0.000
NSB		76.0	60.0	152.0	120.0	136.0	22.63	16.64	272.0	208801.7	0.1	11.0	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 Avobenzone): Part 4 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
94.89	96.36	1.321	2.288	2.374
95.19				
99.00				
108.97	110.36	0.798	1.382	1.253
111.73				
110.37				
116.19	113.05	1.582	2.740	2.424
111.78				
111.17				
104.69	107.39	1.552	2.687	2.502
107.41				
110.07				
105.10	104.41	0.675	1.169	1.120
105.08				
103.06				
100.62	101.32	2.052	3.554	3.508
105.18				
98.17				
105.93	103.33	2.460	4.260	4.123
98.41				
105.64				
101.66	102.54	0.923	1.598	1.558
101.57				
104.38				
98.92	100.64	1.730	2.446	2.431
102.37				
-0.06	-0.02	0.042	0.060	282.843
0.02				

APPENDIX 1: Run 2: Assay Information (Ensulizole)

Experiment Date:	25-Feb-13	Study Number:	9070-100794AROM		
Test substance:	Ensulizole				
3/8/2013 1:07					
	specific activity based on decay for 4/20/10		42662.0	DPM	
	20 uL count of 3H-ASDN (mean)		41760.3	DPM	
	0.5 mL count for total activity		13015.0	DPM	
	microsomal protein/assay		0.008	mg	
	Reaction time		15	min	
	20 uL count of 3H-ASDN (DPM)		41209	41278	42794

Assays Conducted by: [redacted]				Spreadsheet locked on: 03/05/2013
				Green shaded areas: unlocked cells for data entry
Each assay contained 100 uL 3H-ASDN	208801.7	DPM	0.200 (nmoles)	
Total product 3H-H20 per assay	52060.0	DPM	0.050 (nmoles)	
Percent conversion to product (3H-H2O) (percent)	24.9			
Rate of conversion to 3H-H2O in total activity assay	0.416	nmol/(mg protein-min)		
Average activity of control Tubes	0.413	nmol/(mg protein-min)		
Average full enzyme activity controls (percent +/-SD)	100.0	1.6		
Average background activity controls (percent +/- SD)	0.0	0.0		

APPENDIX 1: Run 2: Raw and Normalized DPM Data (4OH-ASDN and Ensulizole): 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 tubesww	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
TA		12970.0	12821.0	25940.0	25642.0	25791.0	210.72	0.82	51582.0	208801.7	24.7	51321.0	0.049	0.410
TA		13716.0	12219.0	27432.0	24438.0	25935.0	2117.08	8.16	51870.0	208801.7	24.8	51609.0	0.049	0.412
NSB		71.0	66.0	142.0	132.0	137.0	7.07	5.16	274.0	208801.7	0.1	13.0	0.000	0.000
NSB		62.0	73.0	124.0	146.0	135.0	15.56	11.52	270.0	208801.7	0.1	9.0	0.000	0.000
4OH-ASDN	-5	181.0	174.0	362.0	348.0	355.0	9.90	2.79	710.0	208801.7	0.3	449.0	0.000	0.004
4OH-ASDN		198.0	181.0	396.0	362.0	379.0	24.04	6.34	758.0	208801.7	0.4	497.0	0.000	0.004
4OH-ASDN	-6	1128.0	1120.0	2256.0	2240.0	2248.0	11.31	0.50	4496.0	208801.7	2.2	4235.0	0.004	0.034
4OH-ASDN		1164.0	1121.0	2328.0	2242.0	2285.0	60.81	2.66	4570.0	208801.7	2.2	4309.0	0.004	0.034
4OH-ASDN	-6.5	2718.0	2737.0	5436.0	5474.0	5455.0	26.87	0.49	10910.0	208801.7	5.2	10649.0	0.010	0.085
4OH-ASDN		2867.0	2786.0	5734.0	5572.0	5653.0	114.55	2.03	11306.0	208801.7	5.4	11045.0	0.011	0.088
4OH-ASDN	-7	5605.0	5535.0	11210.0	11070.0	11140.0	98.99	0.89	22280.0	208801.7	10.7	22019.0	0.021	0.176
4OH-ASDN		5658.0	5799.0	11316.0	11598.0	11457.0	199.40	1.74	22914.0	208801.7	11.0	22653.0	0.022	0.181
4OH-ASDN	-7.5	8987.0	8755.0	17974.0	17510.0	17742.0	328.10	1.85	35484.0	208801.7	17.0	35223.0	0.034	0.281
4OH-ASDN		9482.0	9149.0	18964.0	18298.0	18631.0	470.93	2.53	37262.0	208801.7	17.8	37001.0	0.035	0.295
4OH-ASDN	-8	11216.0	11707.0	22432.0	23414.0	22923.0	694.38	3.03	45846.0	208801.7	22.0	45585.0	0.044	0.364
4OH-ASDN		11924.0	11460.0	23848.0	22920.0	23384.0	656.20	2.81	46768.0	208801.7	22.4	46507.0	0.045	0.371
4OH-ASDN	-9	13924.0	13509.0	27848.0	27018.0	27433.0	586.90	2.14	54866.0	208801.7	26.3	54605.0	0.052	0.436
4OH-ASDN		13648.0	13261.0	27296.0	26522.0	26909.0	547.30	2.03	53818.0	208801.7	25.8	53557.0	0.051	0.427
4OH-ASDN	-10	13961.0	13692.0	27922.0	27384.0	27653.0	380.42	1.38	55306.0	208801.7	26.5	55045.0	0.053	0.439
4OH-ASDN		13669.0	13073.0	27338.0	26146.0	26742.0	842.87	3.15	53484.0	208801.7	25.6	53223.0	0.051	0.425

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 Ensulizole): Part 2 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
99.08	99.36	0.278	0.393	0.396
99.63				
0.03	0.02	0.004	0.005	25.713
0.02				
0.87	0.91	0.046	0.066	7.176
0.96				
8.18	8.25	0.071	0.101	1.225
8.32				
20.56	20.94	0.382	0.541	2.581
21.32				
42.51	43.12	0.612	0.865	2.007
43.73				
68.00	69.72	1.716	2.427	3.481
71.43				
88.00	88.89	0.890	1.259	1.416
89.78				
105.42	104.41	1.012	1.431	1.370
103.39				
106.27	104.51	1.759	2.487	2.380
102.75				

APPENDIX 1: Run 2: Raw and Normalized DPM Data (4OH-ASDN and Ensulizole): 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 tubesww	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
Ensulizole	-3.5	13401.0	13550.0	26802.0	27100.0	26951.0	210.72	0.78	53902.0	208801.7	25.8	53641.0	0.051	0.428
Ensulizole		13628.0	13570.0	27256.0	27140.0	27198.0	82.02	0.30	54396.0	208801.7	26.1	54135.0	0.052	0.432
Ensulizole		13286.0	13234.0	26572.0	26468.0	26520.0	73.54	0.28	53040.0	208801.7	25.4	52779.0	0.051	0.421
Ensulizole	-4.5	13609.0	13520.0	27218.0	27040.0	27129.0	125.87	0.46	54258.0	208801.7	26.0	53997.0	0.052	0.431
Ensulizole		13412.0	13051.0	26824.0	26102.0	26463.0	510.53	1.93	52926.0	208801.7	25.3	52665.0	0.050	0.420
Ensulizole		13418.0	12655.0	26836.0	25310.0	26073.0	1079.04	4.14	52146.0	208801.7	25.0	51885.0	0.050	0.414
Ensulizole	-5.5	13100.0	13235.0	26200.0	26470.0	26335.0	190.92	0.72	52670.0	208801.7	25.2	52409.0	0.050	0.418
Ensulizole		13082.0	13396.0	26164.0	26792.0	26478.0	444.06	1.68	52956.0	208801.7	25.4	52695.0	0.050	0.421
Ensulizole		13407.0	13394.0	26814.0	26788.0	26801.0	18.38	0.07	53602.0	208801.7	25.7	53341.0	0.051	0.426
Ensulizole	-6.5	13911.0	13556.0	27822.0	27112.0	27467.0	502.05	1.83	54934.0	208801.7	26.3	54673.0	0.052	0.436
Ensulizole		13557.0	13719.0	27114.0	27438.0	27276.0	229.10	0.84	54552.0	208801.7	26.1	54291.0	0.052	0.433
Ensulizole		13704.0	13658.0	27408.0	27316.0	27362.0	65.05	0.24	54724.0	208801.7	26.2	54463.0	0.052	0.435
Ensulizole	-7.5	13232.0	12826.0	26464.0	25652.0	26058.0	574.17	2.20	52116.0	208801.7	25.0	51855.0	0.050	0.414
Ensulizole		13716.0	13254.0	27432.0	26508.0	26970.0	653.37	2.42	53940.0	208801.7	25.8	53679.0	0.051	0.428
Ensulizole		13433.0	13074.0	26866.0	26148.0	26507.0	507.70	1.92	53014.0	208801.7	25.4	52753.0	0.051	0.421
Ensulizole	-8.5	13221.0	13228.0	26442.0	26456.0	26449.0	9.90	0.04	52898.0	208801.7	25.3	52637.0	0.050	0.420
Ensulizole		13781.0	13327.0	27562.0	26654.0	27108.0	642.05	2.37	54216.0	208801.7	26.0	53955.0	0.052	0.431
Ensulizole		12573.0	12187.0	25146.0	24374.0	24760.0	545.89	2.20	49520.0	208801.7	23.7	49259.0	0.047	0.393
Ensulizole	-9.5	13723.0	13235.0	27446.0	26470.0	26958.0	690.14	2.56	53916.0	208801.7	25.8	53655.0	0.051	0.428
Ensulizole		12941.0	12523.0	25882.0	25046.0	25464.0	591.14	2.32	50928.0	208801.7	24.4	50667.0	0.049	0.404
Ensulizole		13255.0	13216.0	26510.0	26432.0	26471.0	55.15	0.21	52942.0	208801.7	25.4	52681.0	0.050	0.421
Ensulizole	-10.5	12639.0	12569.0	25278.0	25138.0	25208.0	98.99	0.39	50416.0	208801.7	24.1	50155.0	0.048	0.400
Ensulizole		12656.0	12760.0	25312.0	25520.0	25416.0	147.08	0.58	50832.0	208801.7	24.3	50571.0	0.048	0.404
Ensulizole		11892.0	11592.0	23784.0	23184.0	23484.0	424.26	1.81	46968.0	208801.7	22.5	46707.0	0.045	0.373
TA		13089.0	12660.0	26178.0	25320.0	25749.0	606.70	2.36	51498.0	208801.7	24.7	51237.0	0.049	0.409
TA		13285.0	13360.0	26570.0	26720.0	26645.0	106.07	0.40	53290.0	208801.7	25.5	53029.0	0.051	0.423
NSB		55.0	59.0	110.0	118.0	114.0	5.66	4.96	228.0	208801.7	0.1	-33.0	0.000	0.000
NSB		76.0	60.0	152.0	120.0	136.0	22.63	16.64	272.0	208801.7	0.1	11.0	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 Ensulizole): Part 4 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
103.56	103.32	0.765	1.325	1.282
104.51				
101.89				
104.24	102.03	1.190	2.062	2.021
101.67				
100.17				
101.18	101.96	0.532	0.922	0.904
101.73				
102.98				
105.55	105.17	0.213	0.369	0.351
104.81				
105.14				
100.11	101.86	1.017	1.761	1.729
103.63				
101.84				
101.62	100.29	2.700	4.676	4.662
104.16				
95.10				
103.58	101.03	1.698	2.942	2.912
97.81				
101.70				
96.83	94.88	2.364	4.095	4.316
97.63				
90.17				
98.92	100.64	1.730	2.446	2.431
102.37				
-0.06	-0.02	0.042	0.060	282.843
0.02				

APPENDIX 1: Run 2: Assay Information (Homosalate)

Experiment Date:	25-Feb-13	Study Number:	9070-100794AROM		
Test substance:	Homosalate				
3/8/2013 1:14					
	specific activity based on decay for 4/20/10		42662.0	DPM	
	20 uL count of 3H-ASDN (mean)		41760.3	DPM	
	0.5 mL count for total activity		13015.0	DPM	
	microsomal protein/assay		0.008	mg	
	Reaction time		15	min	
	20 uL count of 3H-ASDN (DPM)		41209	41278	42794

Assays Conducted by:	[redacted]				Spreadsheet locked on: 03/05/2013
					Green shaded areas: unlocked cells for data entry
Each assay contained 100 uL 3H-ASDN	208801.7	DPM	0.200	(nmoles)	
Total product 3H-H2O per assay	52060.0	DPM	0.050	(nmoles)	
Percent conversion to product (3H-H2O) (percent)	24.9				
Rate of conversion to 3H-H2O in total activity assay	0.416	nmol/(mg protein-min)			
Average activity of control Tubes	0.413	nmol/(mg protein-min)			
Average full enzyme activity controls (percent +/- SD)	100.0	1.6			
Average background activity controls (percent +/- SD)	0.0	0.0			

APPENDIX 1: Run 2: Raw and Normalized DPM Data (4OH-ASDN and Homosalate): 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 tubesww	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
TA		12970.0	12821.0	25940.0	25642.0	25791.0	210.72	0.82	51582.0	208801.7	24.7	51321.0	0.049	0.410
TA		13716.0	12219.0	27432.0	24438.0	25935.0	2117.08	8.16	51870.0	208801.7	24.8	51609.0	0.049	0.412
NSB		71.0	66.0	142.0	132.0	137.0	7.07	5.16	274.0	208801.7	0.1	13.0	0.000	0.000
NSB		62.0	73.0	124.0	146.0	135.0	15.56	11.52	270.0	208801.7	0.1	9.0	0.000	0.000
4OH-ASDN	-5	181.0	174.0	362.0	348.0	355.0	9.90	2.79	710.0	208801.7	0.3	449.0	0.000	0.004
4OH-ASDN		198.0	181.0	396.0	362.0	379.0	24.04	6.34	758.0	208801.7	0.4	497.0	0.000	0.004
4OH-ASDN	-6	1128.0	1120.0	2256.0	2240.0	2248.0	11.31	0.50	4496.0	208801.7	2.2	4235.0	0.004	0.034
4OH-ASDN		1164.0	1121.0	2328.0	2242.0	2285.0	60.81	2.66	4570.0	208801.7	2.2	4309.0	0.004	0.034
4OH-ASDN	-6.5	2718.0	2737.0	5436.0	5474.0	5455.0	26.87	0.49	10910.0	208801.7	5.2	10649.0	0.010	0.085
4OH-ASDN		2867.0	2786.0	5734.0	5572.0	5653.0	114.55	2.03	11306.0	208801.7	5.4	11045.0	0.011	0.088
4OH-ASDN	-7	5605.0	5535.0	11210.0	11070.0	11140.0	98.99	0.89	22280.0	208801.7	10.7	22019.0	0.021	0.176
4OH-ASDN		5658.0	5799.0	11316.0	11598.0	11457.0	199.40	1.74	22914.0	208801.7	11.0	22653.0	0.022	0.181
4OH-ASDN	-7.5	8987.0	8755.0	17974.0	17510.0	17742.0	328.10	1.85	35484.0	208801.7	17.0	35223.0	0.034	0.281
4OH-ASDN		9482.0	9149.0	18964.0	18298.0	18631.0	470.93	2.53	37262.0	208801.7	17.8	37001.0	0.035	0.295
4OH-ASDN	-8	11216.0	11707.0	22432.0	23414.0	22923.0	694.38	3.03	45846.0	208801.7	22.0	45585.0	0.044	0.364
4OH-ASDN		11924.0	11460.0	23848.0	22920.0	23384.0	656.20	2.81	46768.0	208801.7	22.4	46507.0	0.045	0.371
4OH-ASDN	-9	13924.0	13509.0	27848.0	27018.0	27433.0	586.90	2.14	54866.0	208801.7	26.3	54605.0	0.052	0.436
4OH-ASDN		13648.0	13261.0	27296.0	26522.0	26909.0	547.30	2.03	53818.0	208801.7	25.8	53557.0	0.051	0.427
4OH-ASDN	-10	13961.0	13692.0	27922.0	27384.0	27653.0	380.42	1.38	55306.0	208801.7	26.5	55045.0	0.053	0.439
4OH-ASDN		13669.0	13073.0	27338.0	26146.0	26742.0	842.87	3.15	53484.0	208801.7	25.6	53223.0	0.051	0.425

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 Homosalate): Part 2 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
99.08	99.36	0.278	0.393	0.396
99.63				
0.03	0.02	0.004	0.005	25.713
0.02				
0.87	0.91	0.046	0.066	7.176
0.96				
8.18	8.25	0.071	0.101	1.225
8.32				
20.56	20.94	0.382	0.541	2.581
21.32				
42.51	43.12	0.612	0.865	2.007
43.73				
68.00	69.72	1.716	2.427	3.481
71.43				
88.00	88.89	0.890	1.259	1.416
89.78				
105.42	104.41	1.012	1.431	1.370
103.39				
106.27	104.51	1.759	2.487	2.380
102.75				

APPENDIX 1: Run 2: Raw and Normalized DPM Data (4OH-ASDN and Homosalate): 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 tubesww	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
Homosalate	-3	10953.0	10873.0	21906.0	21746.0	21826.0	113.14	0.52	43652.0	208801.7	20.9	43391.0	0.042	0.346
Homosalate		10869.0	10920.0	21738.0	21840.0	21789.0	72.12	0.33	43578.0	208801.7	20.9	43317.0	0.041	0.346
Homosalate		10991.0	11134.0	21982.0	22268.0	22125.0	202.23	0.91	44250.0	208801.7	21.2	43989.0	0.042	0.351
Homosalate	-4	12082.0	11889.0	24164.0	23778.0	23971.0	272.94	1.14	47942.0	208801.7	23.0	47681.0	0.046	0.381
Homosalate		11852.0	11701.0	23704.0	23402.0	23553.0	213.55	0.91	47106.0	208801.7	22.6	46845.0	0.045	0.374
Homosalate		11154.0	11682.0	22308.0	23364.0	22836.0	746.70	3.27	45672.0	208801.7	21.9	45411.0	0.043	0.362
Homosalate	-5	11798.0	12270.0	23596.0	24540.0	24068.0	667.51	2.77	48136.0	208801.7	23.1	47875.0	0.046	0.382
Homosalate		12018.0	11926.0	24036.0	23852.0	23944.0	130.11	0.54	47888.0	208801.7	22.9	47627.0	0.046	0.380
Homosalate		11917.0	12173.0	23834.0	24346.0	24090.0	362.04	1.50	48180.0	208801.7	23.1	47919.0	0.046	0.382
Homosalate	-6	13660.0	13296.0	27320.0	26592.0	26956.0	514.77	1.91	53912.0	208801.7	25.8	53651.0	0.051	0.428
Homosalate		13302.0	13366.0	26604.0	26732.0	26668.0	90.51	0.34	53336.0	208801.7	25.5	53075.0	0.051	0.424
Homosalate		13626.0	13438.0	27252.0	26876.0	27064.0	265.87	0.98	54128.0	208801.7	25.9	53867.0	0.052	0.430
Homosalate	-7	12927.0	12980.0	25854.0	25960.0	25907.0	74.95	0.29	51814.0	208801.7	24.8	51553.0	0.049	0.411
Homosalate		13477.0	13372.0	26954.0	26744.0	26849.0	148.49	0.55	53698.0	208801.7	25.7	53437.0	0.051	0.427
Homosalate		13712.0	13727.0	27424.0	27454.0	27439.0	21.21	0.08	54878.0	208801.7	26.3	54617.0	0.052	0.436
Homosalate	-8	13585.0	13072.0	27170.0	26144.0	26657.0	725.49	2.72	53314.0	208801.7	25.5	53053.0	0.051	0.423
Homosalate		13288.0	12851.0	26576.0	25702.0	26139.0	618.01	2.36	52278.0	208801.7	25.0	52017.0	0.050	0.415
Homosalate		12686.0	13173.0	25372.0	26346.0	25859.0	688.72	2.66	51718.0	208801.7	24.8	51457.0	0.049	0.411
Homosalate	-9	13141.0	13097.0	26282.0	26194.0	26238.0	62.23	0.24	52476.0	208801.7	25.1	52215.0	0.050	0.417
Homosalate		13487.0	13209.0	26974.0	26418.0	26696.0	393.15	1.47	53392.0	208801.7	25.6	53131.0	0.051	0.424
Homosalate		12673.0	12672.0	25346.0	25344.0	25345.0	1.41	0.01	50690.0	208801.7	24.3	50429.0	0.048	0.403
Homosalate	-10	13249.0	13045.0	26498.0	26090.0	26294.0	288.50	1.10	52588.0	208801.7	25.2	52327.0	0.050	0.418
Homosalate		13283.0	13445.0	26566.0	26890.0	26728.0	229.10	0.86	53456.0	208801.7	25.6	53195.0	0.051	0.425
Homosalate		13282.0	13031.0	26564.0	26062.0	26313.0	354.97	1.35	52626.0	208801.7	25.2	52365.0	0.050	0.418
TA		13089.0	12660.0	26178.0	25320.0	25749.0	606.70	2.36	51498.0	208801.7	24.7	51237.0	0.049	0.409
TA		13285.0	13360.0	26570.0	26720.0	26645.0	106.07	0.40	53290.0	208801.7	25.5	53029.0	0.051	0.423
NSB		55.0	59.0	110.0	118.0	114.0	5.66	4.96	228.0	208801.7	0.1	-33.0	0.000	0.000
NSB		76.0	60.0	152.0	120.0	136.0	22.63	16.64	272.0	208801.7	0.1	11.0	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 Homosalate): Part 4 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
83.77	84.11	0.411	0.711	0.846
83.63				
84.92				
92.05	90.05	1.280	2.216	2.461
90.44				
87.67				
92.42	92.29	0.175	0.304	0.329
91.95				
92.51				
103.58	103.34	0.456	0.790	0.765
102.46				
103.99				
99.53	102.71	1.723	2.983	2.905
103.16				
105.44				
102.42	100.73	0.903	1.563	1.552
100.42				
99.34				
100.80	100.24	1.532	2.653	2.646
102.57				
97.36				
101.02	101.60	0.547	0.947	0.932
102.70				
101.09				
98.92	100.64	1.730	2.446	2.431
102.37				
-0.06	-0.02	0.042	0.060	282.843
0.02				

APPENDIX 1: Run 2: Assay Information (Padimate-O)

Experiment Date:	25-Feb-13	Study Number:	9070-100794AROM		
Test substance:	Padimate O				
3/8/2013 1:20					
	specific activity based on decay for 4/20/10		42662.0	DPM	
	20 uL count of 3H-ASDN (mean)		41760.3	DPM	
	0.5 mL count for total activity		13015.0	DPM	
	microsomal protein/assay		0.008	mg	
	Reaction time		15	min	
	20 uL count of 3H-ASDN (DPM)		41209	41278	42794

Assays Conducted by:	[redacted]				Spreadsheet locked on: 03/05/2013
					Green shaded areas: unlocked cells for data entry
Each assay contained 100 uL 3H-ASDN	208801.7	DPM	0.200	(nmoles)	
Total product 3H-H20 per assay	52060.0	DPM	0.050	(nmoles)	
Percent conversion to product (3H-H2O) (percent)	24.9				
Rate of conversion to 3H-H2O in total activity assay	0.416	nmol/(mg protein-min)			
Average activity of control Tubes	0.413	nmol/(mg protein-min)			
Average full enzyme activity controls (percent +/-SD)	100.0	1.6			
Average background activity controls (percent +/- SD)	0.0	0.0			

APPENDIX 1: Run 2: Raw and Normalized DPM Data (4OH-ASDN and Padimate-O): 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 tubesww	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
TA		12970.0	12821.0	25940.0	25642.0	25791.0	210.72	0.82	51582.0	208801.7	24.7	51321.0	0.049	0.410
TA		13716.0	12219.0	27432.0	24438.0	25935.0	2117.08	8.16	51870.0	208801.7	24.8	51609.0	0.049	0.412
NSB		71.0	66.0	142.0	132.0	137.0	7.07	5.16	274.0	208801.7	0.1	13.0	0.000	0.000
NSB		62.0	73.0	124.0	146.0	135.0	15.56	11.52	270.0	208801.7	0.1	9.0	0.000	0.000
4OH-ASDN	-5	181.0	174.0	362.0	348.0	355.0	9.90	2.79	710.0	208801.7	0.3	449.0	0.000	0.004
4OH-ASDN		198.0	181.0	396.0	362.0	379.0	24.04	6.34	758.0	208801.7	0.4	497.0	0.000	0.004
4OH-ASDN	-6	1128.0	1120.0	2256.0	2240.0	2248.0	11.31	0.50	4496.0	208801.7	2.2	4235.0	0.004	0.034
4OH-ASDN		1164.0	1121.0	2328.0	2242.0	2285.0	60.81	2.66	4570.0	208801.7	2.2	4309.0	0.004	0.034
4OH-ASDN	-6.5	2718.0	2737.0	5436.0	5474.0	5455.0	26.87	0.49	10910.0	208801.7	5.2	10649.0	0.010	0.085
4OH-ASDN		2867.0	2786.0	5734.0	5572.0	5653.0	114.55	2.03	11306.0	208801.7	5.4	11045.0	0.011	0.088
4OH-ASDN	-7	5605.0	5535.0	11210.0	11070.0	11140.0	98.99	0.89	22280.0	208801.7	10.7	22019.0	0.021	0.176
4OH-ASDN		5658.0	5799.0	11316.0	11598.0	11457.0	199.40	1.74	22914.0	208801.7	11.0	22653.0	0.022	0.181
4OH-ASDN	-7.5	8987.0	8755.0	17974.0	17510.0	17742.0	328.10	1.85	35484.0	208801.7	17.0	35223.0	0.034	0.281
4OH-ASDN		9482.0	9149.0	18964.0	18298.0	18631.0	470.93	2.53	37262.0	208801.7	17.8	37001.0	0.035	0.295
4OH-ASDN	-8	11216.0	11707.0	22432.0	23414.0	22923.0	694.38	3.03	45846.0	208801.7	22.0	45585.0	0.044	0.364
4OH-ASDN		11924.0	11460.0	23848.0	22920.0	23384.0	656.20	2.81	46768.0	208801.7	22.4	46507.0	0.045	0.371
4OH-ASDN	-9	13924.0	13509.0	27848.0	27018.0	27433.0	586.90	2.14	54866.0	208801.7	26.3	54605.0	0.052	0.436
4OH-ASDN		13648.0	13261.0	27296.0	26522.0	26909.0	547.30	2.03	53818.0	208801.7	25.8	53557.0	0.051	0.427
4OH-ASDN	-10	13961.0	13692.0	27922.0	27384.0	27653.0	380.42	1.38	55306.0	208801.7	26.5	55045.0	0.053	0.439
4OH-ASDN		13669.0	13073.0	27338.0	26146.0	26742.0	842.87	3.15	53484.0	208801.7	25.6	53223.0	0.051	0.425

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 Padimate-O): Part 2 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
99.08	99.36	0.278	0.393	0.396
99.63				
0.03	0.02	0.004	0.005	25.713
0.02				
0.87	0.91	0.046	0.066	7.176
0.96				
8.18	8.25	0.071	0.101	1.225
8.32				
20.56	20.94	0.382	0.541	2.581
21.32				
42.51	43.12	0.612	0.865	2.007
43.73				
68.00	69.72	1.716	2.427	3.481
71.43				
88.00	88.89	0.890	1.259	1.416
89.78				
105.42	104.41	1.012	1.431	1.370
103.39				
106.27	104.51	1.759	2.487	2.380
102.75				

APPENDIX 1: Run 2: Raw and Normalized DPM Data (4OH-ASDN and Padimate-O): 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 tubesww	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
Padimate O	-3	11049.0	10976.0	22098.0	21952.0	22025.0	103.24	0.47	44050.0	208801.7	21.1	43789.0	0.042	0.350
Padimate O		11686.0	11566.0	23372.0	23132.0	23252.0	169.71	0.73	46504.0	208801.7	22.3	46243.0	0.044	0.369
Padimate O		11202.0	11147.0	22404.0	22294.0	22349.0	77.78	0.35	44698.0	208801.7	21.4	44437.0	0.043	0.355
Padimate O	-4	11758.0	12489.0	23516.0	24978.0	24247.0	1033.79	4.26	48494.0	208801.7	23.2	48233.0	0.046	0.385
Padimate O		12163.0	11734.0	24326.0	23468.0	23897.0	606.70	2.54	47794.0	208801.7	22.9	47533.0	0.046	0.379
Padimate O		11899.0	11879.0	23798.0	23758.0	23778.0	28.28	0.12	47556.0	208801.7	22.8	47295.0	0.045	0.378
Padimate O	-5	12720.0	12911.0	25440.0	25822.0	25631.0	270.11	1.05	51262.0	208801.7	24.6	51001.0	0.049	0.407
Padimate O		12770.0	12802.0	25540.0	25604.0	25572.0	45.25	0.18	51144.0	208801.7	24.5	50883.0	0.049	0.406
Padimate O		12794.0	12758.0	25588.0	25516.0	25552.0	50.91	0.20	51104.0	208801.7	24.5	50843.0	0.049	0.406
Padimate O	-6	12841.0	12726.0	25682.0	25452.0	25567.0	162.63	0.64	51134.0	208801.7	24.5	50873.0	0.049	0.406
Padimate O		13322.0	13535.0	26644.0	27070.0	26857.0	301.23	1.12	53714.0	208801.7	25.7	53453.0	0.051	0.427
Padimate O		13224.0	12934.0	26448.0	25868.0	26158.0	410.12	1.57	52316.0	208801.7	25.1	52055.0	0.050	0.416
Padimate O	-7	13106.0	12116.0	26212.0	24232.0	25222.0	1400.07	5.55	50444.0	208801.7	24.2	50183.0	0.048	0.401
Padimate O		12699.0	13173.0	25398.0	26346.0	25872.0	670.34	2.59	51744.0	208801.7	24.8	51483.0	0.049	0.411
Padimate O		12964.0	12602.0	25928.0	25204.0	25566.0	511.95	2.00	51132.0	208801.7	24.5	50871.0	0.049	0.406
Padimate O	-8	13015.0	12662.0	26030.0	25324.0	25677.0	499.22	1.94	51354.0	208801.7	24.6	51093.0	0.049	0.408
Padimate O		13410.0	13382.0	26820.0	26764.0	26792.0	39.60	0.15	53584.0	208801.7	25.7	53323.0	0.051	0.426
Padimate O		13335.0	13424.0	26670.0	26848.0	26759.0	125.87	0.47	53518.0	208801.7	25.6	53257.0	0.051	0.425
Padimate O	-9	13036.0	12689.0	26072.0	25378.0	25725.0	490.73	1.91	51450.0	208801.7	24.6	51189.0	0.049	0.409
Padimate O		12436.0	12527.0	24872.0	25054.0	24963.0	128.69	0.52	49926.0	208801.7	23.9	49665.0	0.048	0.396
Padimate O		12629.0	12494.0	25258.0	24988.0	25123.0	190.92	0.76	50246.0	208801.7	24.1	49985.0	0.048	0.399
Padimate O	-10	13589.0	13402.0	27178.0	26804.0	26991.0	264.46	0.98	53982.0	208801.7	25.9	53721.0	0.051	0.429
Padimate O		12998.0	12721.0	25996.0	25442.0	25719.0	391.74	1.52	51438.0	208801.7	24.6	51177.0	0.049	0.408
Padimate O		13385.0	13432.0	26770.0	26864.0	26817.0	66.47	0.25	53634.0	208801.7	25.7	53373.0	0.051	0.426
TA		13089.0	12660.0	26178.0	25320.0	25749.0	606.70	2.36	51498.0	208801.7	24.7	51237.0	0.049	0.409
TA		13285.0	13360.0	26570.0	26720.0	26645.0	106.07	0.40	53290.0	208801.7	25.5	53029.0	0.051	0.423
NSB		55.0	59.0	110.0	118.0	114.0	5.66	4.96	228.0	208801.7	0.1	-33.0	0.000	0.000
NSB		76.0	60.0	152.0	120.0	136.0	22.63	16.64	272.0	208801.7	0.1	11.0	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 Padimate-O): Part 4 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
84.54	86.53	1.417	2.455	2.837
89.27				
85.79				
93.12	92.06	0.543	0.941	1.022
91.76				
91.30				
98.46	98.28	0.092	0.159	0.161
98.23				
98.15				
98.21	100.63	1.440	2.493	2.478
103.19				
100.49				
96.88	98.16	0.725	1.256	1.279
99.39				
98.21				
98.64	101.46	1.414	2.450	2.414
102.94				
102.81				
98.82	97.07	0.896	1.551	1.598
95.88				
96.50				
103.71	101.85	1.537	2.663	2.615
98.80				
103.04				
98.92	100.64	1.730	2.446	2.431
102.37				
-0.06	-0.02	0.042	0.060	282.843
0.02				

APPENDIX 1: Run 3: Assay Information (Avobenzone)

Experiment Date:	27-Feb-13	Study Number:	9070-100794AROM		
Test substance:	Avobenzone				
3/8/2013 1:27					
	specific activity based on decay for 4/20/10		42608.0	DPM	
	20 uL count of 3H-ASDN (mean)		41385.7	DPM	
	0.5 mL count for total activity		14910.5	DPM	
	microsomal protein/assay		0.008	mg	
	Reaction time		15	min	
	20 uL count of 3H-ASDN (DPM)		40793	41806	41558

Assays Conducted by:	[redacted]				Spreadsheet locked on: 03/05/2013	
					Green shaded areas: unlocked cells for data entry	
Each assay contained 100 uL 3H-ASDN	206928.3	DPM	0.200	(nmoles)		
Total product 3H-H2O per assay	59642.0	DPM	0.058	(nmoles)		
Percent conversion to product (3H-H2O) (percent)	28.8					
Rate of conversion to 3H-H2O in total activity assay	0.480	nmol/(mg protein-min)				
Average activity of control Tubes	0.477	nmol/(mg protein-min)				
Average full enzyme activity controls (percent +/- SD)	100.0	2.9				
Average background activity controls (percent +/- SD)	0.0	0.3				

APPENDIX 1: Run 3: Raw and Normalized DPM Data (4OH-ASDN and Avobenzone): 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 tubesww	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
TA		14766.0	14106.0	29532.0	28212.0	28872.0	933.38	3.23	57744.0	206928.3	27.9	57384.5	0.055	0.462
TA		14459.0	14943.0	28918.0	29886.0	29402.0	684.48	2.33	58804.0	206928.3	28.4	58444.5	0.056	0.471
NSB		63.0	62.0	126.0	124.0	125.0	1.41	1.13	250.0	206928.3	0.1	-109.5	0.000	-0.001
NSB		62.0	63.0	124.0	126.0	125.0	1.41	1.13	250.0	206928.3	0.1	-109.5	0.000	-0.001
4OH-ASDN	-5	211.0	202.0	422.0	404.0	413.0	12.73	3.08	826.0	206928.3	0.4	466.5	0.000	0.004
4OH-ASDN		213.0	197.0	426.0	394.0	410.0	22.63	5.52	820.0	206928.3	0.4	460.5	0.000	0.004
4OH-ASDN	-6	1275.0	1228.0	2550.0	2456.0	2503.0	66.47	2.66	5006.0	206928.3	2.4	4646.5	0.004	0.037
4OH-ASDN		1218.0	1224.0	2436.0	2448.0	2442.0	8.49	0.35	4884.0	206928.3	2.4	4524.5	0.004	0.036
4OH-ASDN	-6.5	3314.0	3242.0	6628.0	6484.0	6556.0	101.82	1.55	13112.0	206928.3	6.3	12752.5	0.012	0.103
4OH-ASDN		3130.0	3108.0	6260.0	6216.0	6238.0	31.11	0.50	12476.0	206928.3	6.0	12116.5	0.012	0.098
4OH-ASDN	-7	6594.0	6430.0	13188.0	12860.0	13024.0	231.93	1.78	26048.0	206928.3	12.6	25688.5	0.025	0.207
4OH-ASDN		6498.0	6431.0	12996.0	12862.0	12929.0	94.75	0.73	25858.0	206928.3	12.5	25498.5	0.025	0.205
4OH-ASDN	-7.5	10296.0	9887.0	20592.0	19774.0	20183.0	578.41	2.87	40366.0	206928.3	19.5	40006.5	0.039	0.322
4OH-ASDN		10274.0	10234.0	20548.0	20468.0	20508.0	56.57	0.28	41016.0	206928.3	19.8	40656.5	0.039	0.327
4OH-ASDN	-8	13700.0	13612.0	27400.0	27224.0	27312.0	124.45	0.46	54624.0	206928.3	26.4	54264.5	0.052	0.437
4OH-ASDN		13166.0	13012.0	26332.0	26024.0	26178.0	217.79	0.83	52356.0	206928.3	25.3	51996.5	0.050	0.419
4OH-ASDN	-9	14422.0	14131.0	28844.0	28262.0	28553.0	411.54	1.44	57106.0	206928.3	27.6	56746.5	0.055	0.457
4OH-ASDN		14849.0	14833.0	29698.0	29666.0	29682.0	22.63	0.08	59364.0	206928.3	28.7	59004.5	0.057	0.475
4OH-ASDN	-10	15194.0	15013.0	30388.0	30026.0	30207.0	255.97	0.85	60414.0	206928.3	29.2	60054.5	0.058	0.484
4OH-ASDN		14463.0	14385.0	28926.0	28770.0	28848.0	110.31	0.38	57696.0	206928.3	27.9	57336.5	0.055	0.462

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 Avobenzone): Part 2 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
96.80	97.69	0.894	1.264	1.294
98.59				
-0.18	-0.18	0.000	0.000	0.000
-0.18				
0.79	0.78	0.005	0.007	0.915
0.78				
7.84	7.73	0.103	0.146	1.881
7.63				
21.51	20.97	0.536	0.759	3.617
20.44				
43.33	43.17	0.160	0.227	0.525
43.01				
67.48	68.03	0.548	0.775	1.140
68.58				
91.54	89.62	1.913	2.705	3.018
87.71				
95.72	97.63	1.904	2.693	2.759
99.53				
101.30	99.01	2.292	3.242	3.274
96.72				

APPENDIX 1: Run 3: Raw and Normalized DPM Data (4OH-ASDN and Avobenzone): 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 tubesww	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
Avobenzone	-3	14930.0	15044.0	29860.0	30088.0	29974.0	161.22	0.54	59948.0	206928.3	29.0	59588.5	0.058	0.480
Avobenzone		14387.0	15142.0	28774.0	30284.0	29529.0	1067.73	3.62	59058.0	206928.3	28.5	58698.5	0.057	0.473
Avobenzone		15286.0	15012.0	30572.0	30024.0	30298.0	387.49	1.28	60596.0	206928.3	29.3	60236.5	0.058	0.485
Avobenzone	-4	17149.0	16162.0	34298.0	32324.0	33311.0	1395.83	4.19	66622.0	206928.3	32.2	66262.5	0.064	0.534
Avobenzone		16737.0	16228.0	33474.0	32456.0	32965.0	719.83	2.18	65930.0	206928.3	31.9	65570.5	0.063	0.528
Avobenzone		17256.0	17056.0	34512.0	34112.0	34312.0	282.84	0.82	68624.0	206928.3	33.2	68264.5	0.066	0.550
Avobenzone	-5	17230.0	16980.0	34460.0	33960.0	34210.0	353.55	1.03	68420.0	206928.3	33.1	68060.5	0.066	0.548
Avobenzone		20239.0	20230.0	40478.0	40460.0	40469.0	12.73	0.03	80938.0	206928.3	39.1	80578.5	0.078	0.649
Avobenzone		18230.0	17816.0	36460.0	35632.0	36046.0	585.48	1.62	72092.0	206928.3	34.8	71732.5	0.069	0.578
Avobenzone	-6	14715.0	14761.0	29430.0	29522.0	29476.0	65.05	0.22	58952.0	206928.3	28.5	58592.5	0.057	0.472
Avobenzone		15086.0	15096.0	30172.0	30192.0	30182.0	14.14	0.05	60364.0	206928.3	29.2	60004.5	0.058	0.483
Avobenzone		15258.0	15298.0	30516.0	30596.0	30556.0	56.57	0.19	61112.0	206928.3	29.5	60752.5	0.059	0.489
Avobenzone	-7	15346.0	15257.0	30692.0	30514.0	30603.0	125.87	0.41	61206.0	206928.3	29.6	60846.5	0.059	0.490
Avobenzone		16245.0	15838.0	32490.0	31676.0	32083.0	575.58	1.79	64166.0	206928.3	31.0	63806.5	0.062	0.514
Avobenzone		15811.0	15535.0	31622.0	31070.0	31346.0	390.32	1.25	62692.0	206928.3	30.3	62332.5	0.060	0.502
Avobenzone	-8	16013.0	15789.0	32026.0	31578.0	31802.0	316.78	1.00	63604.0	206928.3	30.7	63244.5	0.061	0.509
Avobenzone		15774.0	15831.0	31548.0	31662.0	31605.0	80.61	0.26	63210.0	206928.3	30.5	62850.5	0.061	0.506
Avobenzone		15693.0	16007.0	31386.0	32014.0	31700.0	444.06	1.40	63400.0	206928.3	30.6	63040.5	0.061	0.508
Avobenzone	-9	15973.0	16034.0	31946.0	32068.0	32007.0	86.27	0.27	64014.0	206928.3	30.9	63654.5	0.062	0.513
Avobenzone		16286.0	15580.0	32572.0	31160.0	31866.0	998.43	3.13	63732.0	206928.3	30.8	63372.5	0.061	0.510
Avobenzone		15811.0	15760.0	31622.0	31520.0	31571.0	72.12	0.23	63142.0	206928.3	30.5	62782.5	0.061	0.506
Avobenzone	-10	15663.0	15398.0	31326.0	30796.0	31061.0	374.77	1.21	62122.0	206928.3	30.0	61762.5	0.060	0.497
Avobenzone		15699.0	15855.0	31398.0	31710.0	31554.0	220.62	0.70	63108.0	206928.3	30.5	62748.5	0.061	0.505
Avobenzone		16222.0	16413.0	32444.0	32826.0	32635.0	270.11	0.83	65270.0	206928.3	31.5	64910.5	0.063	0.523
TA		15452.0	15325.0	30904.0	30650.0	30777.0	179.61	0.58	61554.0	206928.3	29.7	61194.5	0.059	0.493
TA		15090.0	15143.0	30180.0	30286.0	30233.0	74.95	0.25	60466.0	206928.3	29.2	60106.5	0.058	0.484
NSB		72.0	65.0	144.0	130.0	137.0	9.90	7.23	274.0	206928.3	0.1	-85.5	0.000	-0.001
NSB		176.0	156.0	352.0	312.0	332.0	28.28	8.52	664.0	206928.3	0.3	304.5	0.000	0.002

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 Avobenzone): Part 4 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
100.52	100.38	0.752	1.303	1.298
99.01				
101.61				
111.77	112.51	1.363	2.360	2.098
110.61				
115.15				
114.81	123.91	6.267	10.854	8.760
135.92				
121.00				
98.84	100.84	1.068	1.850	1.835
101.22				
102.48				
102.64	105.14	1.441	2.497	2.375
107.63				
105.14				
106.68	106.35	0.192	0.332	0.313
106.02				
106.34				
107.37	106.73	0.433	0.751	0.703
106.90				
105.90				
104.18	106.51	1.568	2.716	2.550
105.85				
109.49				
103.23	102.31	0.918	1.298	1.268
101.39				
-0.14	0.18	0.329	0.465	251.846
0.51				

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

APPENDIX 1: Run 3: Assay Information (Ensulizole)

Experiment Date:	27-Feb-13	Study Number:	9070-100794AROM	
Test substance:	Ensulizole			
3/8/2013 1:37				
	specific activity based on decay for 4/20/10	42608.0	DPM	
	20 uL count of 3H-ASDN (mean)	41385.7	DPM	
	0.5 mL count for total activity	14910.5	DPM	
	microsomal protein/assay	0.008	mg	
	Reaction time	15	min	
	20 uL count of 3H-ASDN (DPM)	40793	41806	41558

Assays Conducted by: [redacted]					Spreadsheet locked on: 03/05/2013
					Green shaded areas: unlocked cells for data entry
Each assay contained 100 uL 3H-ASDN	206928.3	DPM	0.200	(nmoles)	
Total product 3H-H20 per assay	59642.0	DPM	0.058	(nmoles)	
Percent conversion to product (3H-H2O) (percent)	28.8				
Rate of conversion to 3H-H2O in total activity assay	0.480	nmol/(mg protein-min)			
Average activity of control Tubes	0.477	nmol/(mg protein-min)			
Average full enzyme activity controls (percent +/-SD)	100.0	2.9			
Average background activity controls (percent +/- SD)	0.0	0.3			

APPENDIX 1: Run 3: Raw and Normalized DPM Data (4OH-ASDN and Ensulizole): 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 tubesww	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
TA		14766.0	14106.0	29532.0	28212.0	28872.0	933.38	3.23	57744.0	206928.3	27.9	57384.5	0.055	0.462
TA		14459.0	14943.0	28918.0	29886.0	29402.0	684.48	2.33	58804.0	206928.3	28.4	58444.5	0.056	0.471
NSB		63.0	62.0	126.0	124.0	125.0	1.41	1.13	250.0	206928.3	0.1	-109.5	0.000	-0.001
NSB		62.0	63.0	124.0	126.0	125.0	1.41	1.13	250.0	206928.3	0.1	-109.5	0.000	-0.001
4OH-ASDN	-5	211.0	202.0	422.0	404.0	413.0	12.73	3.08	826.0	206928.3	0.4	466.5	0.000	0.004
4OH-ASDN		213.0	197.0	426.0	394.0	410.0	22.63	5.52	820.0	206928.3	0.4	460.5	0.000	0.004
4OH-ASDN	-6	1275.0	1228.0	2550.0	2456.0	2503.0	66.47	2.66	5006.0	206928.3	2.4	4646.5	0.004	0.037
4OH-ASDN		1218.0	1224.0	2436.0	2448.0	2442.0	8.49	0.35	4884.0	206928.3	2.4	4524.5	0.004	0.036
4OH-ASDN	-6.5	3314.0	3242.0	6628.0	6484.0	6556.0	101.82	1.55	13112.0	206928.3	6.3	12752.5	0.012	0.103
4OH-ASDN		3130.0	3108.0	6260.0	6216.0	6238.0	31.11	0.50	12476.0	206928.3	6.0	12116.5	0.012	0.098
4OH-ASDN	-7	6594.0	6430.0	13188.0	12860.0	13024.0	231.93	1.78	26048.0	206928.3	12.6	25688.5	0.025	0.207
4OH-ASDN		6498.0	6431.0	12996.0	12862.0	12929.0	94.75	0.73	25858.0	206928.3	12.5	25498.5	0.025	0.205
4OH-ASDN	-7.5	10296.0	9887.0	20592.0	19774.0	20183.0	578.41	2.87	40366.0	206928.3	19.5	40006.5	0.039	0.322
4OH-ASDN		10274.0	10234.0	20548.0	20468.0	20508.0	56.57	0.28	41016.0	206928.3	19.8	40656.5	0.039	0.327
4OH-ASDN	-8	13700.0	13612.0	27400.0	27224.0	27312.0	124.45	0.46	54624.0	206928.3	26.4	54264.5	0.052	0.437
4OH-ASDN		13166.0	13012.0	26332.0	26024.0	26178.0	217.79	0.83	52356.0	206928.3	25.3	51996.5	0.050	0.419
4OH-ASDN	-9	14422.0	14131.0	28844.0	28262.0	28553.0	411.54	1.44	57106.0	206928.3	27.6	56746.5	0.055	0.457
4OH-ASDN		14849.0	14833.0	29698.0	29666.0	29682.0	22.63	0.08	59364.0	206928.3	28.7	59004.5	0.057	0.475
4OH-ASDN	-10	15194.0	15013.0	30388.0	30026.0	30207.0	255.97	0.85	60414.0	206928.3	29.2	60054.5	0.058	0.484
4OH-ASDN		14463.0	14385.0	28926.0	28770.0	28848.0	110.31	0.38	57696.0	206928.3	27.9	57336.5	0.055	0.462

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 Ensulizole): Part 2 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
96.80	97.69	0.894	1.264	1.294
98.59				
-0.18	-0.18	0.000	0.000	0.000
-0.18				
0.79	0.78	0.005	0.007	0.915
0.78				
7.84	7.73	0.103	0.146	1.881
7.63				
21.51	20.97	0.536	0.759	3.617
20.44				
43.33	43.17	0.160	0.227	0.525
43.01				
67.48	68.03	0.548	0.775	1.140
68.58				
91.54	89.62	1.913	2.705	3.018
87.71				
95.72	97.63	1.904	2.693	2.759
99.53				
101.30	99.01	2.292	3.242	3.274
96.72				

APPENDIX 1: Run 3: Raw and Normalized DPM Data (4OH-ASDN and Ensulizole): 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 tubesww	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
Ensulizole	-3.5	15336.0	14952.0	30672.0	29904.0	30288.0	543.06	1.79	60576.0	206928.3	29.3	60216.5	0.058	0.485
Ensulizole		15443.0	14884.0	30886.0	29768.0	30327.0	790.55	2.61	60654.0	206928.3	29.3	60294.5	0.058	0.486
Ensulizole		15222.0	14996.0	30444.0	29992.0	30218.0	319.61	1.06	60436.0	206928.3	29.2	60076.5	0.058	0.484
Ensulizole	-4.5	15389.0	15226.0	30778.0	30452.0	30615.0	230.52	0.75	61230.0	206928.3	29.6	60870.5	0.059	0.490
Ensulizole		15610.0	15525.0	31220.0	31050.0	31135.0	120.21	0.39	62270.0	206928.3	30.1	61910.5	0.060	0.499
Ensulizole		15664.0	15655.0	31328.0	31310.0	31319.0	12.73	0.04	62638.0	206928.3	30.3	62278.5	0.060	0.502
Ensulizole	-5.5	15976.0	15731.0	31952.0	31462.0	31707.0	346.48	1.09	63414.0	206928.3	30.6	63054.5	0.061	0.508
Ensulizole		15591.0	15053.0	31182.0	30106.0	30644.0	760.85	2.48	61288.0	206928.3	29.6	60928.5	0.059	0.491
Ensulizole		16309.0	16173.0	32618.0	32346.0	32482.0	192.33	0.59	64964.0	206928.3	31.4	64604.5	0.062	0.520
Ensulizole	-6.5	16478.0	16241.0	32956.0	32482.0	32719.0	335.17	1.02	65438.0	206928.3	31.6	65078.5	0.063	0.524
Ensulizole		14742.0	15148.0	29484.0	30296.0	29890.0	574.17	1.92	59780.0	206928.3	28.9	59420.5	0.057	0.479
Ensulizole		12438.0	12689.0	24876.0	25378.0	25127.0	354.97	1.41	50254.0	206928.3	24.3	49894.5	0.048	0.402
Ensulizole	-7.5	15418.0	15359.0	30836.0	30718.0	30777.0	83.44	0.27	61554.0	206928.3	29.7	61194.5	0.059	0.493
Ensulizole		15007.0	15241.0	30014.0	30482.0	30248.0	330.93	1.09	60496.0	206928.3	29.2	60136.5	0.058	0.484
Ensulizole		15175.0	15153.0	30350.0	30306.0	30328.0	31.11	0.10	60656.0	206928.3	29.3	60296.5	0.058	0.486
Ensulizole	-8.5	15251.0	15292.0	30502.0	30584.0	30543.0	57.98	0.19	61086.0	206928.3	29.5	60726.5	0.059	0.489
Ensulizole		15648.0	15003.0	31296.0	30006.0	30651.0	912.17	2.98	61302.0	206928.3	29.6	60942.5	0.059	0.491
Ensulizole		15725.0	15670.0	31450.0	31340.0	31395.0	77.78	0.25	62790.0	206928.3	30.3	62430.5	0.060	0.503
Ensulizole	-9.5	15270.0	14946.0	30540.0	29892.0	30216.0	458.21	1.52	60432.0	206928.3	29.2	60072.5	0.058	0.484
Ensulizole		15706.0	15443.0	31412.0	30886.0	31149.0	371.94	1.19	62298.0	206928.3	30.1	61938.5	0.060	0.499
Ensulizole		15049.0	14748.0	30098.0	29496.0	29797.0	425.68	1.43	59594.0	206928.3	28.8	59234.5	0.057	0.477
Ensulizole	-10.5	15416.0	15209.0	30832.0	30418.0	30625.0	292.74	0.96	61250.0	206928.3	29.6	60890.5	0.059	0.490
Ensulizole		15660.0	15444.0	31320.0	30888.0	31104.0	305.47	0.98	62208.0	206928.3	30.1	61848.5	0.060	0.498
Ensulizole		15693.0	15392.0	31386.0	30784.0	31085.0	425.68	1.37	62170.0	206928.3	30.0	61810.5	0.060	0.498
TA		15452.0	15325.0	30904.0	30650.0	30777.0	179.61	0.58	61554.0	206928.3	29.7	61194.5	0.059	0.493
TA		15090.0	15143.0	30180.0	30286.0	30233.0	74.95	0.25	60466.0	206928.3	29.2	60106.5	0.058	0.484
NSB		72.0	65.0	144.0	130.0	137.0	9.90	7.23	274.0	206928.3	0.1	-85.5	0.000	-0.001
NSB		176.0	156.0	352.0	312.0	332.0	28.28	8.52	664.0	206928.3	0.3	304.5	0.000	0.002

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 Ensulizole): Part 4 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
101.58	101.54	0.108	0.186	0.184
101.71				
101.34				
102.68	104.06	0.711	1.232	1.184
104.43				
105.05				
106.36	106.04	1.797	3.113	2.936
102.78				
108.98				
109.78	98.06	7.473	12.944	13.201
100.23				
84.16				
103.23	102.13	0.555	0.962	0.942
101.44				
101.71				
102.44	103.52	0.904	1.565	1.512
102.80				
105.31				
101.33	101.91	1.348	2.335	2.291
104.48				
99.92				
102.71	103.77	0.528	0.915	0.882
104.33				
104.26				
103.23	102.31	0.918	1.298	1.268
101.39				
-0.14	0.18	0.329	0.465	251.846
0.51				

APPENDIX 1: Run 3: Assay Information (Homosalate)

Experiment Date:	27-Feb-13	Study Number:	9070-100794AROM		
Test substance:	Homosalate				
3/8/2013 1:43					
	specific activity based on decay for 4/20/10		42608.0	DPM	
	20 uL count of 3H-ASDN (mean)		41385.7	DPM	
	0.5 mL count for total activity		14910.5	DPM	
	microsomal protein/assay		0.008	mg	
	Reaction time		15	min	
	20 uL count of 3H-ASDN (DPM)		40793	41806	41558

Assays Conducted by:	[redacted]				Spreadsheet locked on: 03/05/2013
					Green shaded areas: unlocked cells for data entry
Each assay contained 100 uL 3H-ASDN	206928.3	DPM	0.200	(nmoles)	
Total product 3H-H20 per assay	59642.0	DPM	0.058	(nmoles)	
Percent conversion to product (3H-H2O) (percent)	28.8				
Rate of conversion to 3H-H2O in total activity assay	0.480	nmol/(mg protein-min)			
Average activity of control Tubes	0.477	nmol/(mg protein-min)			
Average full enzyme activity controls (percent +/-SD)	100.0	2.9			
Average background activity controls (percent +/- SD)	0.0	0.3			

APPENDIX 1: Run 3: Raw and Normalized DPM Data (4OH-ASDN and Homosalate): 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 tubesww	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
TA		14766.0	14106.0	29532.0	28212.0	28872.0	933.38	3.23	57744.0	206928.3	27.9	57384.5	0.055	0.462
TA		14459.0	14943.0	28918.0	29886.0	29402.0	684.48	2.33	58804.0	206928.3	28.4	58444.5	0.056	0.471
NSB		63.0	62.0	126.0	124.0	125.0	1.41	1.13	250.0	206928.3	0.1	-109.5	0.000	-0.001
NSB		62.0	63.0	124.0	126.0	125.0	1.41	1.13	250.0	206928.3	0.1	-109.5	0.000	-0.001
4OH-ASDN	-5	211.0	202.0	422.0	404.0	413.0	12.73	3.08	826.0	206928.3	0.4	466.5	0.000	0.004
4OH-ASDN		213.0	197.0	426.0	394.0	410.0	22.63	5.52	820.0	206928.3	0.4	460.5	0.000	0.004
4OH-ASDN	-6	1275.0	1228.0	2550.0	2456.0	2503.0	66.47	2.66	5006.0	206928.3	2.4	4646.5	0.004	0.037
4OH-ASDN		1218.0	1224.0	2436.0	2448.0	2442.0	8.49	0.35	4884.0	206928.3	2.4	4524.5	0.004	0.036
4OH-ASDN	-6.5	3314.0	3242.0	6628.0	6484.0	6556.0	101.82	1.55	13112.0	206928.3	6.3	12752.5	0.012	0.103
4OH-ASDN		3130.0	3108.0	6260.0	6216.0	6238.0	31.11	0.50	12476.0	206928.3	6.0	12116.5	0.012	0.098
4OH-ASDN	-7	6594.0	6430.0	13188.0	12860.0	13024.0	231.93	1.78	26048.0	206928.3	12.6	25688.5	0.025	0.207
4OH-ASDN		6498.0	6431.0	12996.0	12862.0	12929.0	94.75	0.73	25858.0	206928.3	12.5	25498.5	0.025	0.205
4OH-ASDN	-7.5	10296.0	9887.0	20592.0	19774.0	20183.0	578.41	2.87	40366.0	206928.3	19.5	40006.5	0.039	0.322
4OH-ASDN		10274.0	10234.0	20548.0	20468.0	20508.0	56.57	0.28	41016.0	206928.3	19.8	40656.5	0.039	0.327
4OH-ASDN	-8	13700.0	13612.0	27400.0	27224.0	27312.0	124.45	0.46	54624.0	206928.3	26.4	54264.5	0.052	0.437
4OH-ASDN		13166.0	13012.0	26332.0	26024.0	26178.0	217.79	0.83	52356.0	206928.3	25.3	51996.5	0.050	0.419
4OH-ASDN	-9	14422.0	14131.0	28844.0	28262.0	28553.0	411.54	1.44	57106.0	206928.3	27.6	56746.5	0.055	0.457
4OH-ASDN		14849.0	14833.0	29698.0	29666.0	29682.0	22.63	0.08	59364.0	206928.3	28.7	59004.5	0.057	0.475
4OH-ASDN	-10	15194.0	15013.0	30388.0	30026.0	30207.0	255.97	0.85	60414.0	206928.3	29.2	60054.5	0.058	0.484
4OH-ASDN		14463.0	14385.0	28926.0	28770.0	28848.0	110.31	0.38	57696.0	206928.3	27.9	57336.5	0.055	0.462

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 Homosalate): Part 2 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
96.80	97.69	0.894	1.264	1.294
98.59				
-0.18	-0.18	0.000	0.000	0.000
-0.18				
0.79	0.78	0.005	0.007	0.915
0.78				
7.84	7.73	0.103	0.146	1.881
7.63				
21.51	20.97	0.536	0.759	3.617
20.44				
43.33	43.17	0.160	0.227	0.525
43.01				
67.48	68.03	0.548	0.775	1.140
68.58				
91.54	89.62	1.913	2.705	3.018
87.71				
95.72	97.63	1.904	2.693	2.759
99.53				
101.30	99.01	2.292	3.242	3.274
96.72				

APPENDIX 1: Run 3: Raw and Normalized DPM Data (4OH-ASDN and Homosalate): 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 tubesww	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
Homosalate	-3	12159.0	12269.0	24318.0	24538.0	24428.0	155.56	0.64	48856.0	206928.3	23.6	48496.5	0.047	0.391
Homosalate		12937.0	12810.0	25874.0	25620.0	25747.0	179.61	0.70	51494.0	206928.3	24.9	51134.5	0.049	0.412
Homosalate		12666.0	12799.0	25332.0	25598.0	25465.0	188.09	0.74	50930.0	206928.3	24.6	50570.5	0.049	0.407
Homosalate	-4	13562.0	13390.0	27124.0	26780.0	26952.0	243.24	0.90	53904.0	206928.3	26.0	53544.5	0.052	0.431
Homosalate		13270.0	13400.0	26540.0	26800.0	26670.0	183.85	0.69	53340.0	206928.3	25.8	52980.5	0.051	0.427
Homosalate		13586.0	13786.0	27172.0	27572.0	27372.0	282.84	1.03	54744.0	206928.3	26.5	54384.5	0.053	0.438
Homosalate	-5	14911.0	14579.0	29822.0	29158.0	29490.0	469.52	1.59	58980.0	206928.3	28.5	58620.5	0.057	0.472
Homosalate		14165.0	14260.0	28330.0	28520.0	28425.0	134.35	0.47	56850.0	206928.3	27.5	56490.5	0.055	0.455
Homosalate		14333.0	14499.0	28666.0	28998.0	28832.0	234.76	0.81	57664.0	206928.3	27.9	57304.5	0.055	0.462
Homosalate	-6	15979.0	15649.0	31958.0	31298.0	31628.0	466.69	1.48	63256.0	206928.3	30.6	62896.5	0.061	0.507
Homosalate		15727.0	15590.0	31454.0	31180.0	31317.0	193.75	0.62	62634.0	206928.3	30.3	62274.5	0.060	0.502
Homosalate		16326.0	15461.0	32652.0	30922.0	31787.0	1223.29	3.85	63574.0	206928.3	30.7	63214.5	0.061	0.509
Homosalate	-7	15300.0	15474.0	30600.0	30948.0	30774.0	246.07	0.80	61548.0	206928.3	29.7	61188.5	0.059	0.493
Homosalate		15619.0	15526.0	31238.0	31052.0	31145.0	131.52	0.42	62290.0	206928.3	30.1	61930.5	0.060	0.499
Homosalate		15485.0	15040.0	30970.0	30080.0	30525.0	629.33	2.06	61050.0	206928.3	29.5	60690.5	0.059	0.489
Homosalate	-8	15350.0	15115.0	30700.0	30230.0	30465.0	332.34	1.09	60930.0	206928.3	29.4	60570.5	0.059	0.488
Homosalate		15428.0	14555.0	30856.0	29110.0	29983.0	1234.61	4.12	59966.0	206928.3	29.0	59606.5	0.058	0.480
Homosalate		15714.0	15745.0	31428.0	31490.0	31459.0	43.84	0.14	62918.0	206928.3	30.4	62558.5	0.060	0.504
Homosalate	-9	14897.0	15146.0	29794.0	30292.0	30043.0	352.14	1.17	60086.0	206928.3	29.0	59726.5	0.058	0.481
Homosalate		14999.0	14587.0	29998.0	29174.0	29586.0	582.66	1.97	59172.0	206928.3	28.6	58812.5	0.057	0.474
Homosalate		15463.0	15121.0	30926.0	30242.0	30584.0	483.66	1.58	61168.0	206928.3	29.6	60808.5	0.059	0.490
Homosalate	-10	14742.0	14691.0	29484.0	29382.0	29433.0	72.12	0.25	58866.0	206928.3	28.4	58506.5	0.057	0.471
Homosalate		15471.0	14630.0	30942.0	29260.0	30101.0	1189.35	3.95	60202.0	206928.3	29.1	59842.5	0.058	0.482
Homosalate		15100.0	15191.0	30200.0	30382.0	30291.0	128.69	0.42	60582.0	206928.3	29.3	60222.5	0.058	0.485
TA		15452.0	15325.0	30904.0	30650.0	30777.0	179.61	0.58	61554.0	206928.3	29.7	61194.5	0.059	0.493
TA		15090.0	15143.0	30180.0	30286.0	30233.0	74.95	0.25	60466.0	206928.3	29.2	60106.5	0.058	0.484
NSB		72.0	65.0	144.0	130.0	137.0	9.90	7.23	274.0	206928.3	0.1	-85.5	0.000	-0.001
NSB		176.0	156.0	352.0	312.0	332.0	28.28	8.52	664.0	206928.3	0.3	304.5	0.000	0.002

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 Homosalate): Part 4 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
81.81	84.46	1.353	2.343	2.775
86.26				
85.30				
90.32	90.48	0.688	1.192	1.317
89.37				
91.74				
98.88	96.95	1.047	1.813	1.870
95.29				
96.66				
106.10	105.93	0.466	0.807	0.761
105.05				
106.63				
103.22	103.35	0.608	1.053	1.018
104.47				
102.38				
102.17	102.75	1.466	2.539	2.471
100.55				
105.53				
100.75	100.84	0.973	1.685	1.671
99.21				
102.57				
98.69	100.41	0.878	1.520	1.514
100.94				
101.59				
103.23	102.31	0.918	1.298	1.268
101.39				
-0.14	0.18	0.329	0.465	251.846
0.51				

APPENDIX 1: Run 3: Assay Information (Padimate-O)

Experiment Date:	27-Feb-13	Study Number:	9070-100794AROM		
Test substance:	Padimate O				
3/8/2013 1:52					
	specific activity based on decay for 4/20/10		42608.0	DPM	
	20 uL count of 3H-ASDN (mean)		41385.7	DPM	
	0.5 mL count for total activity		14910.5	DPM	
	microsomal protein/assay		0.008	mg	
	Reaction time		15	min	
	20 uL count of 3H-ASDN (DPM)		40793	41806	41558

Assays Conducted by:	[redacted]				Spreadsheet locked on: 03/05/2013
					Green shaded areas: unlocked cells for data entry
Each assay contained 100 uL 3H-ASDN		206928.3	DPM	0.200	(nmoles)
Total product 3H-H20 per assay		59642.0	DPM	0.058	(nmoles)
Percent conversion to product (3H-H2O) (percent)		28.8			
Rate of conversion to 3H-H2O in total activity assay		0.480	nmol/(mg protein-min)		
Average activity of control Tubes		0.477	nmol/(mg protein-min)		
	Average full enzyme activity controls (percent +/-SD)	100.0	2.9		
	Average background activity controls (percent +/- SD)	0.0	0.3		

APPENDIX 1: Run 3: Raw and Normalized DPM Data (4OH-ASDN and Padimate-O): 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 tubesww	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
TA		14766.0	14106.0	29532.0	28212.0	28872.0	933.38	3.23	57744.0	206928.3	27.9	57384.5	0.055	0.462
TA		14459.0	14943.0	28918.0	29886.0	29402.0	684.48	2.33	58804.0	206928.3	28.4	58444.5	0.056	0.471
NSB		63.0	62.0	126.0	124.0	125.0	1.41	1.13	250.0	206928.3	0.1	-109.5	0.000	-0.001
NSB		62.0	63.0	124.0	126.0	125.0	1.41	1.13	250.0	206928.3	0.1	-109.5	0.000	-0.001
4OH-ASDN	-5	211.0	202.0	422.0	404.0	413.0	12.73	3.08	826.0	206928.3	0.4	466.5	0.000	0.004
4OH-ASDN		213.0	197.0	426.0	394.0	410.0	22.63	5.52	820.0	206928.3	0.4	460.5	0.000	0.004
4OH-ASDN	-6	1275.0	1228.0	2550.0	2456.0	2503.0	66.47	2.66	5006.0	206928.3	2.4	4646.5	0.004	0.037
4OH-ASDN		1218.0	1224.0	2436.0	2448.0	2442.0	8.49	0.35	4884.0	206928.3	2.4	4524.5	0.004	0.036
4OH-ASDN	-6.5	3314.0	3242.0	6628.0	6484.0	6556.0	101.82	1.55	13112.0	206928.3	6.3	12752.5	0.012	0.103
4OH-ASDN		3130.0	3108.0	6260.0	6216.0	6238.0	31.11	0.50	12476.0	206928.3	6.0	12116.5	0.012	0.098
4OH-ASDN	-7	6594.0	6430.0	13188.0	12860.0	13024.0	231.93	1.78	26048.0	206928.3	12.6	25688.5	0.025	0.207
4OH-ASDN		6498.0	6431.0	12996.0	12862.0	12929.0	94.75	0.73	25858.0	206928.3	12.5	25498.5	0.025	0.205
4OH-ASDN	-7.5	10296.0	9887.0	20592.0	19774.0	20183.0	578.41	2.87	40366.0	206928.3	19.5	40006.5	0.039	0.322
4OH-ASDN		10274.0	10234.0	20548.0	20468.0	20508.0	56.57	0.28	41016.0	206928.3	19.8	40656.5	0.039	0.327
4OH-ASDN	-8	13700.0	13612.0	27400.0	27224.0	27312.0	124.45	0.46	54624.0	206928.3	26.4	54264.5	0.052	0.437
4OH-ASDN		13166.0	13012.0	26332.0	26024.0	26178.0	217.79	0.83	52356.0	206928.3	25.3	51996.5	0.050	0.419
4OH-ASDN	-9	14422.0	14131.0	28844.0	28262.0	28553.0	411.54	1.44	57106.0	206928.3	27.6	56746.5	0.055	0.457
4OH-ASDN		14849.0	14833.0	29698.0	29666.0	29682.0	22.63	0.08	59364.0	206928.3	28.7	59004.5	0.057	0.475
4OH-ASDN	-10	15194.0	15013.0	30388.0	30026.0	30207.0	255.97	0.85	60414.0	206928.3	29.2	60054.5	0.058	0.484
4OH-ASDN		14463.0	14385.0	28926.0	28770.0	28848.0	110.31	0.38	57696.0	206928.3	27.9	57336.5	0.055	0.462

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 Padimate-O): Part 2 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
96.80	97.69	0.894	1.264	1.294
98.59				
-0.18	-0.18	0.000	0.000	0.000
-0.18				
0.79	0.78	0.005	0.007	0.915
0.78				
7.84	7.73	0.103	0.146	1.881
7.63				
21.51	20.97	0.536	0.759	3.617
20.44				
43.33	43.17	0.160	0.227	0.525
43.01				
67.48	68.03	0.548	0.775	1.140
68.58				
91.54	89.62	1.913	2.705	3.018
87.71				
95.72	97.63	1.904	2.693	2.759
99.53				
101.30	99.01	2.292	3.242	3.274
96.72				

APPENDIX 1: Run 3: Raw and Normalized DPM Data (4OH-ASDN and Padimate-O): 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 tubesww	%Substrate Converted to product	Total DPM-Bkg	3H-H2O (nmole)	Aromatase Activity nmol/(mg prot.-min)
Padimate O	-3	12554.0	12419.0	25108.0	24838.0	24973.0	190.92	0.76	49946.0	206928.3	24.1	49586.5	0.048	0.399
Padimate O		12695.0	12752.0	25390.0	25504.0	25447.0	80.61	0.32	50894.0	206928.3	24.6	50534.5	0.049	0.407
Padimate O		13115.0	12891.0	26230.0	25782.0	26006.0	316.78	1.22	52012.0	206928.3	25.1	51652.5	0.050	0.416
Padimate O	-4	13485.0	13380.0	26970.0	26760.0	26865.0	148.49	0.55	53730.0	206928.3	26.0	53370.5	0.052	0.430
Padimate O		13908.0	13899.0	27816.0	27798.0	27807.0	12.73	0.05	55614.0	206928.3	26.9	55254.5	0.053	0.445
Padimate O		14119.0	14362.0	28238.0	28724.0	28481.0	343.65	1.21	56962.0	206928.3	27.5	56602.5	0.055	0.456
Padimate O	-5	15146.0	15120.0	30292.0	30240.0	30266.0	36.77	0.12	60532.0	206928.3	29.3	60172.5	0.058	0.485
Padimate O		15462.0	15358.0	30924.0	30716.0	30820.0	147.08	0.48	61640.0	206928.3	29.8	61280.5	0.059	0.494
Padimate O		14836.0	13139.0	29672.0	26278.0	27975.0	2399.92	8.58	55950.0	206928.3	27.0	55590.5	0.054	0.448
Padimate O	-6	16050.0	15751.0	32100.0	31502.0	31801.0	422.85	1.33	63602.0	206928.3	30.7	63242.5	0.061	0.509
Padimate O		15514.0	15866.0	31028.0	31732.0	31380.0	497.80	1.59	62760.0	206928.3	30.3	62400.5	0.060	0.503
Padimate O		15982.0	15830.0	31964.0	31660.0	31812.0	214.96	0.68	63624.0	206928.3	30.7	63264.5	0.061	0.510
Padimate O	-7	15546.0	15607.0	31092.0	31214.0	31153.0	86.27	0.28	62306.0	206928.3	30.1	61946.5	0.060	0.499
Padimate O		15606.0	15354.0	31212.0	30708.0	30960.0	356.38	1.15	61920.0	206928.3	29.9	61560.5	0.059	0.496
Padimate O		15805.0	15682.0	31610.0	31364.0	31487.0	173.95	0.55	62974.0	206928.3	30.4	62614.5	0.061	0.504
Padimate O	-8	15899.0	15931.0	31798.0	31862.0	31830.0	45.25	0.14	63660.0	206928.3	30.8	63300.5	0.061	0.510
Padimate O		15430.0	15575.0	30860.0	31150.0	31005.0	205.06	0.66	62010.0	206928.3	30.0	61650.5	0.060	0.497
Padimate O		15303.0	15339.0	30606.0	30678.0	30642.0	50.91	0.17	61284.0	206928.3	29.6	60924.5	0.059	0.491
Padimate O	-9	14741.0	15201.0	29482.0	30402.0	29942.0	650.54	2.17	59884.0	206928.3	28.9	59524.5	0.058	0.479
Padimate O		15368.0	15336.0	30736.0	30672.0	30704.0	45.25	0.15	61408.0	206928.3	29.7	61048.5	0.059	0.492
Padimate O		15102.0	15273.0	30204.0	30546.0	30375.0	241.83	0.80	60750.0	206928.3	29.4	60390.5	0.058	0.486
Padimate O	-10	15682.0	15291.0	31364.0	30582.0	30973.0	552.96	1.79	61946.0	206928.3	29.9	61586.5	0.060	0.496
Padimate O		14811.0	15147.0	29622.0	30294.0	29958.0	475.18	1.59	59916.0	206928.3	29.0	59556.5	0.058	0.480
Padimate O		14293.0	14342.0	28586.0	28684.0	28635.0	69.30	0.24	57270.0	206928.3	27.7	56910.5	0.055	0.458
TA		15452.0	15325.0	30904.0	30650.0	30777.0	179.61	0.58	61554.0	206928.3	29.7	61194.5	0.059	0.493
TA		15090.0	15143.0	30180.0	30286.0	30233.0	74.95	0.25	60466.0	206928.3	29.2	60106.5	0.058	0.484
NSB		72.0	65.0	144.0	130.0	137.0	9.90	7.23	274.0	206928.3	0.1	-85.5	0.000	-0.001
NSB		176.0	156.0	352.0	312.0	332.0	28.28	8.52	664.0	206928.3	0.3	304.5	0.000	0.002

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 Padimate-O): Part 4 of 4

Aromatase Activity (%)	Mean Aromatase activity (%)	±SEM	StDEV	CV(%)
83.64	85.34	1.007	1.744	2.044
85.24				
87.13				
90.03	92.90	1.581	2.738	2.948
93.21				
95.48				
101.50	99.55	2.938	5.088	5.112
103.37				
93.77				
106.68	106.22	0.480	0.831	0.782
105.26				
106.72				
104.49	104.65	0.519	0.900	0.860
103.84				
105.62				
106.78	104.51	1.186	2.054	1.965
103.99				
102.77				
100.41	101.75	0.744	1.289	1.267
102.98				
101.87				
103.89	100.12	2.284	3.955	3.951
100.46				
96.00				
103.23	102.31	0.918	1.298	1.268
101.39				
-0.14	0.18	0.329	0.465	251.846
0.51				

APPENDIX 2: Deviation Forms

CeeTox
In vitro models to predict toxicity

Deviation and Investigation

Form #: SOP-1003-F-1.2

Study Number (if applicable): 9070-100794/AROM

SOP Number (if applicable): N/A

Equipment Serial Number (if applicable): TRI-CARB 2910TR DG03117657

Date of Reporting: 27FEB2013 Reporting Associate: [redacted]

Date of Occurrence: 27FEB2013 Associate Involved: [redacted]

Description of Deviation:

2 sigma terminator was set on to minimize time of LSC counts from 10 minutes per sample when 95% confidence was achieved.

Signature [redacted] Date: 27FEB2013

Reporting Associate

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

☐ SOP Deviation ☒ Protocol Deviation ☐ GLP Deviation ☐ Facility Deviation ☐ No Deviation

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

Protocol states 10 min counts; however, counts were sufficient to allow shorter read times as stated above.

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

None taken; No impact on study.

Signature [redacted] Date: 08 MARCH 2013

SD/PI/Management Acknowledgement

Signature [redacted] Date: 08MAR2013

Quality Assurance Review

If study specific, sponsor notified on: 08 MARCH 2013 By: [redacted]

Standard Operating Procedure Page 1 of 2

APPENDIX 2: Deviation Forms

Form #: SOP-1003-F-1.2

CeeTox
In vitro models to predict toxicity

Deviation and Investigation

See Attached Documentation (email documentation is sufficient) ☒

Standard Operating Procedure

Page 2 of 2

APPENDIX 2: Deviation Forms

CeeTox
In vitro models to predict toxicity

Deviation and Investigation

Form #: SOP-1003-F-1.2

Study Number (if applicable): 9070-100794 AROM

SOP Number (if applicable): N/A

Equipment Serial Number (if applicable): TRI-CARB 2910 TR DG03117657

Date of Reporting: 25FEB2013 Reporting Associate: [redacted]

Date of Occurrence: 25FEB2013 Associate Involved: [redacted]

Description of Deviation:

2 sigma terminator was set on to minimize time of counts from 10 minutes per sample when 95% confidence was achieved

Signature [redacted] Date: 25 FEB 2013

Reporting Associate

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

☐ SOP Deviation ☒ Protocol Deviation ☐ GLP Deviation ☐ Facility Deviation ☐ No Deviation

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

Protocol states 10 min counts; however, counts were sufficient to allow shorter read times as stated above.

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

None taken; No impact on Study.

Signature [redacted] Date: 08 MARCH 2013

SD/PI/Management Acknowledgement

Signature [redacted] Date: 08MAR2013

Quality Assurance Review

If study specific, sponsor notified on: 08 MARCH 2013 By: [redacted]

Standard Operating Procedure Page 1 of 2

APPENDIX 2: Deviation Forms

Form #: SOP-1003-F-1.2

CeeTox
In vitro models to predict toxicity

Deviation and Investigation

See Attached Documentation (email documentation is sufficient) ☒

Standard Operating Procedure

Page 2 of 2

APPENDIX 2: Deviation Forms

CeeTox
In vitro models to predict toxicity

Deviation and Investigation

Form #: SOP-1003-F-1.2

Study Number (if applicable): 9070-100794 AROM

SOP Number (if applicable): N/A

Equipment Serial Number (if applicable): TRI-CARB 2910TR DG03117657

Date of Reporting: 26FEB2013 Reporting Associate: [redacted]

Date of Occurrence: 26FEB 2013 Associate Involved: [redacted]

Description of Deviation:

2 Sigma terminator was set on to minimize time of LSC counts from 10 minutes per sample when 95% Confidence was achieved.

Signature [redacted] Date: 26FEB 2013

Reporting Associate

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

☐ SOP Deviation ☒ Protocol Deviation ☐ GLP Deviation ☐ Facility Deviation ☐ No Deviation

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

Protocol states 10 min counts; however, counts were sufficient to allow shorter read times as stated above.

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

None taken; No impact on Study.

Signature [redacted] Date: 08 MARCH 2013

SD/PI/Management Acknowledgement

Signature [redacted] Date: 08MAR2013

Quality Assurance Review

If study specific, sponsor notified on: 08 MARCH 2013 By: [redacted]

Standard Operating Procedure Page 1 of 2

APPENDIX 2: Deviation Forms

Form #: SOP-1003-F-1.2

CeeTox
in vitro models to predict toxicity

Deviation and Investigation

See Attached Documentation (email documentation is sufficient) ☒

Standard Operating Procedure

Page 2 of 2

APPENDIX 3: Certificate of Analyses (Avobenzone)

This PDF File is an Exact Copy of t[redacted]
Signature:[redacted]
Date: 2-16-12

MRIGlobal

Analytical Chemistry Services for the NTP
NIEHS Contract No. HHSN273201100001C
MRI Project No.: 110730
NTP ChemTask No.: CHEM10985

Chemical Comprehensive Analysis Final Report

Avobenzone

Chemical Comprehensive Analysis of Avobenzone

MRI Assignment No.: 2003

February 16, 2012

Prepared by:

[redacted]
Study Director

Approved by:

[redacted]
Joseph W. Algaier, Ph.D.
Principal Investigator

Reviewed by:

[redacted]
Group Leader

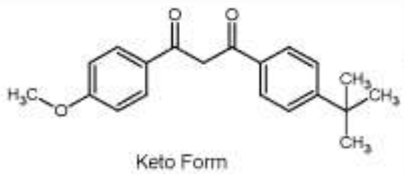
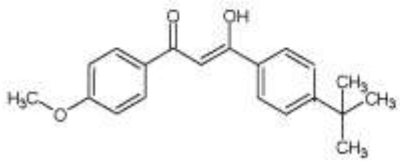
Submitted to:

[redacted]
National Institute of Environmental
Health Sciences
111 T. W. Alexander Drive, MD K2-07
P.O. Box 12233
Research Triangle Park, NC 27709-2233

APPENDIX 3: Certificate of Analyses (Avobenzone)

Chemical Comprehensive Analysis of Avobenzone

Chemical Information

CAS No.: 70356-09-1 MRI Assignment No.: 2003 ChemTask No. CHEM10985 Program Supported: TOX Analysis Dates: 2/11/11 to 12/14/11 Interim Result Date(s): 2/25/11, 4/7/11, 5/17/11	Lot No.: L802809 MRI Assigned Batch No.: 01 Amount Received: 20 Kg Sample Receipt Date: 1/5/11 Appearance: Off white to yellowish crystalline powder per CoA; confirmed by visual observation Supplier: Universal Preserv-A-Chem Inc. Supplier Purity: 98.30% per CoA Storage conditions (at Analytical Lab): Ambient, protected from light
 Keto Form  Enol Form (predominant)	Mol. Wt.: 310.39 Mol. Formula: $C_{20}H_{22}O_3$

APPENDIX 3: Certificate of Analyses (Avobenzone)

Executive Summary

The purpose of this assignment was to perform a chemical comprehensive analysis for avobenzone, Lot No. L802809, received from Universal Preserv-A-Chem Inc. Based on the results, the identity of the test article was confirmed to be avobenzone, with a purity of approximately 98.5%. Evaluation by gas chromatography with flame ionization detection of samples stored at various temperatures indicated avobenzone is stable when stored for 2 weeks, protected from light, at temperatures up to approximately 60°C. Nuclear magnetic resonance spectroscopic analysis of these samples, as well as samples exposed to light for 1 week, detected some conversion of enol to keto form under elevated temperature and light exposure.

The chemical comprehensive analysis included identity confirmation using infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy, residual solvent analysis for volatile content using gas chromatography (GC)/headspace analysis, ultraviolet/visible (UV/Vis) spectroscopy, water content using Karl Fischer titration, elemental analysis, determination of melting point, and log P, differential scanning calorimetry (DSC), and chromatographic profiling using gas chromatography (GC) with flame ionization detection (FID). Additionally, gas chromatography/mass spectrometry (GC/MS) was performed to confirm identity of the test article.

Spectra obtained for the test article using IR and NMR spectroscopy techniques were consistent with reference spectra and the proposed structure for the enol form of the test article. One absorbance maximum was observed using ultraviolet/visible spectroscopy: 358 nm, ϵ_{max} = 36241 ± 186(s). Analysis using GC/MS with electron capture ionization provided confirmation of identity based on the molecular ion (310 Da) observed, as well as comparison to a reference spectrum.

Water content determined by Karl Fischer was 0.223 ± 0.008(s) %. Elemental analysis determined 77.36% carbon, 7.39% hydrogen, and 0.02% nitrogen compared to expected values of 77.39 carbon, 7.15% hydrogen, and no nitrogen. The observed melting point range was 83.0° to 85.5°C (literature values of 83.5°C and 81° to 86°C). The determined log P was 3.10.

Differential scanning calorimetry was performed, and the observed melting point range was consistent with the melting point range from the MSDS. The results indicated a purity of 98.8 ± 0.5(d) %. Chromatographic profiling, using GC with a DB-5 column and FID, indicated 98.7% purity, with seven reportable impurities totaling 1.26% relative to the total peak area. GC/headspace analysis indicated residual solvent peak responses for methanol and cis-1,2-dichloroethene, but they were not present at levels greater than the Class 2 Mixture A Standard. There were no other Class 1 or Class 2 solvents observed to be present in the test article.

Accelerated stability was performed using GC with FID to evaluate possible degradation of the test article. The test variability limit (TVL), which is statistically determined, established that in order to be statistically significant at the 95% confidence level, the loss or gain under ambient, refrigerated, or elevated storage conditions must be greater than 3.8% relative to the sample under the frozen storage condition. The maximum variance from the frozen storage condition was +0.7%, observed for the sample stored at approximately 60°C. Using the TVL criteria,

APPENDIX 3: Certificate of Analyses (Avobenzone)

avobenzone is stable when stored for 2 weeks as the bulk chemical, protected from light, at temperatures up to approximately 60°C. An additional evaluation using ^{1}H-NMR spectroscopy of the accelerated stability samples and stability samples exposed to light exhibited decreased enol/keto ratios of the –OH and –CH_2 functional groups for the samples stored at 60°C, as well as samples exposed to fluorescent or mercury/xenon lighting. This indicates some conversion of the enol to the keto form.

APPENDIX 3: Certificate of Analyses (Avobenzone)

Quality Assurance Statement

Chemical Comprehensive Analysis of Avobenzone

ChemTask No. CHEM10985
MRI Project No. 110730
MRI Assignment No. 2003

This study was inspected by the Quality Assurance Unit of MRI (QAU) and the findings reported to the Study Director and Management as follows:

Phase inspected	Date inspected	Date reported
Protocol Audit	3/1/11	3/1/11
In-life Audit; Stability analysis	3/1/11	3/1/11
Protocol Amendment No. 1 Audit	2/8/12	2/10/12
Protocol Amendment No. 2 Audit	2/8/12	2/10/12
Protocol Amendment No. 3 Audit	2/8/12	2/10/12
Data Audit	2/9/12	2/10/12
Draft Final Report Audit	2/9/12	2/10/12

In addition to the study-specific audits/inspections cited above, inspection of applicable facilities and equipment was performed by the QAU and reports were submitted to management as follows:

Facility/equipment	Inspection date	Management submitted date
285N laboratory complex	7/13/11	7/14/11
GC facility	7/14/11	7/15/11

MIDWEST RESEARCH INSTITUTE

[redacted]

Senior Quality Assurance Officer

Approved:

[redacted]

Director, Quality and Regulatory Systems

February 16, 2012

MRIOldbd-NTP\Assignment_2003.doc iv

APPENDIX 3: Certificate of Analyses (Avobenzone)

Good Laboratory Practice Compliance Statement

Chemical Comprehensive Analysis of Avobenzone

ChemTask No. CHEM10985
MRI Project No. 110730
MRI Assignment No. 2003

All work performed at Midwest Research Institute for this assignment was conducted in compliance with the Good Laboratory Practice regulations of the U.S. Food and Drug Administration (21 *CFR* Part 58). Elemental analysis was performed by ICON Developmental Solutions, LLC, in compliance with FDA current Good Laboratory Practices (21 *CFR* Part 58).

The raw data and report will be stored in the MRI Archives.

[redacted]
Study Director

2/16/12
Date:

MRI\Iss\bd-NTP\Assignment_2003.doc

v

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NTP Analytical Chemistry Services
3040 Cornwallis Road • PO Box 12194 • Research Triangle Park, NC 27709-2194 • USA
Telephone 919.541.6730 or 919.541.5975 • Fax 919.485.2650 • www.rti.org

This pdf is an exact duplicate of the original approved report.
[redacted]
Program Information Coordinator

Analytical Chemistry Services for the NTP
NIH Contract No. HHSN273201100003C
RTI Project 0212839.200.003.080
ChemTask No. CHEM11786
CAS No. 27503-81-7

ENSULIZOLE

CHEMICAL REANALYSIS

September 5, 2012

Prepared by:
[redacted] 09-05-12
Task Leader Date

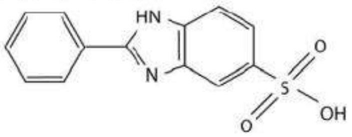
Approved by:
[redacted] 09/05/12
Reshan Fernando, Ph.D. Date
Principal Investigator

Submitted to:
[redacted]
National Institute of Environmental Health Sciences
P.O. Box 12233
111 T. W. Alexander Drive
Research Triangle Park, NC 27709-2233

APPENDIX 3: Certificate of Analysis (Ensulizole)

ENSULIZOLE

CAS No.: 27503-81-7	Study Lab: (Investigator): ILS [redacted]
RTI Chemical ID Code: N60	Lot No. (Vendor): 05117JE(Aldrich)
ChemTask No.: CHEM11786	Vendor Purity: 99.9% (by HPLC, Aldrich COA)
RTI Log Nos. (Amt. Received): Analytical: 082010-C-15 (~50 g) Reference: 082010-C-05 (~5 g)	Receipt Date: Aug 20, 2010 (Bulk receipt and reference)
Program Supported: TOX	Receipt Condition: No damage noted
Analysis Dates: May 11, 15 and 24, 2012	Submitter: [redacted] RTI)
Interim Results Date: May 29, 2012	Shipping Container: NA (in-house transfer)
	Storage Conditions: Bulk: Room temperature Reference: Freezer (~ -20 °C)

STRUCTURE	MOL. WT.	MOL. FORMULA
	274.30	$C_{13}H_{10}N_2O_3S$

EXECUTIVE SUMMARY

In support of the Toxicity Testing Program, an aliquot of ensulizole was submitted for bulk chemical reanalysis. Chemical purity of the bulk sample was determined relative to a reference standard of the same lot/batch number which had been stored at RTI under freezer conditions. Analytical results obtained by LC chromatographic method indicated that the sample had a percent relative purity of 99.6% when compared to the frozen reference standard. The FTIR spectrum of the bulk sample matched the spectrum of the frozen reference and was consistent with the structure for ensulizole.

APPENDIX 3: Certificate of Analysis (Ensulizole)

RTI INTERNATIONAL

Quality Assurance Statement

Chemical Name: Ensulizole

Task Type: Chemical Reanalysis

Chem Task Number: CHEM11786

This study/task was audited by the Regulatory and Quality Assurance (RQA) – Quality Assurance Unit and the results of the inspections and audits were reported to the task leader/study director and management as identified below. To the best of our knowledge, the reported results accurately describe the study methods and procedures used, and the reported results accurately reflect the raw data.

Inspections and Audits	Inspection and Audit Date(s)	Date Inspection/Audit Report Sent to Task Leader/ Management
Sample Preparation Inspection for HPLC Analysis	05/15/12	05/22/12
Data & Report Audit	08/24/12 & 08/28/12	08/28/12

Prepared by:

[redacted]
Quality Assurance Specialist

9-5-12
Date

Reviewed by:

[redacted]
Quality Assurance Specialist

9/5/12
Date

turning knowledge into practice

APPENDIX 3: Certificate of Analysis (Ensulizole)

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Figures

APPENDIX 3: Certificate of Analysis (Ensulizole)

ENSULIZOLE

1.0 INTRODUCTION

The objective of this work was to determine the purity and verify the identity of ensulizole to the current studies being conducted at RTI International. To accomplish this objective, a bulk chemical reanalysis was performed. The identity of the chemical was confirmed by FTIR and its purity assessed by LC.

2.0 CHEMICAL ANALYSIS

An aliquot of the bulk sample of ensulizole was received at the analytical laboratory on March 27, 2012 for chemical reanalysis (RTI log 082010-C-15). The aliquot was stored at room temperature. A frozen reference (RTI log 082010-C-05) sample was received at the analytical laboratory on May 10, 2012 and was stored at freezer temperature.

3.0 CONFIRMATION OF IDENTITY - INFRARED SPECTROMETRY (IR)

3.1 IR Parameters

System	Thermo Nicolet 6700 FTIR
Software	Omnic, Ver. 7.3
Method	KBr pellet, scan 4000 - 400 cm^{-1}

3.2 Results

Bulk Sample Frequency (1/cm)	Frozen Reference Sample Frequency (1/cm)	Assignment
3367	3372	N-H stretch
3059-2725	3059-2725	O-H, N-H, C-H stretch
1633, 1568	1630, 1567	C=C, C=N stretch
1368	1368	C-N stretch
1176	1176	C-C, SO_2 stretch
1028	1028	N-H bend
780	777	C-H, N-H bend
631	630	S-O stretch

The observed spectrum for the bulk sample matched the spectrum of the frozen reference sample, and is consistent with the structure of ensulizole (as reported in the characterization protocols development task CHEM11291). Figure 1 shows the IR spectra for the bulk and frozen samples.

APPENDIX 3: Certificate of Analysis (Ensulizole)

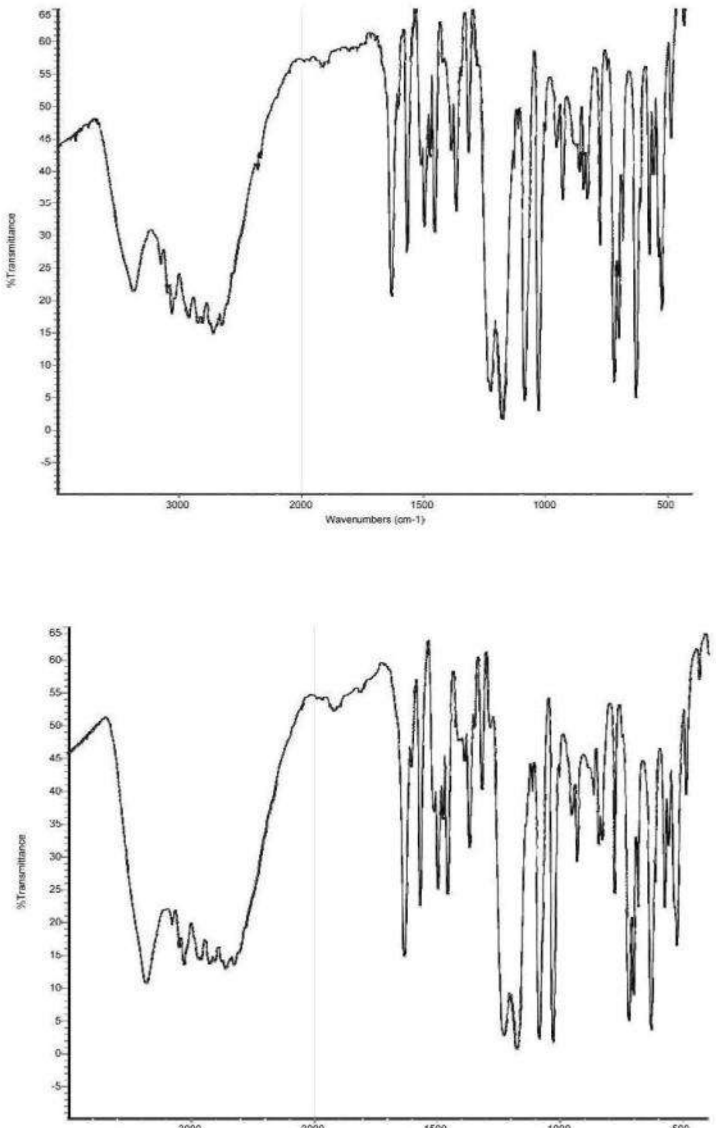

Figure 1: Infrared Spectrum of Ensulizole Frozen Reference (top spectrum) and Bulk Sample (bottom spectrum)

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4.0 DETERMINATION OF PURITY - LIQUID CHROMATOGRAPHY

This section describes the liquid chromatographic method used to estimate sample purity.

4.1 Preparation of Internal Standard (IS) Solution

A stock solution of IS was prepared by weighing 500 mg of padimate O and transferring it into a 10-mL volumetric flask. The IS was diluted to volume with mobile phase B (methanol with 0.1% formic acid). The flask was mixed by inversion. A working IS solution (WIS) was prepared as a 1 mL to 1 L dilution with mobile phase B and mixing by inversion, yielding 0.050 mg/mL working IS.

4.2 Bulk Sample and Frozen Reference Standard Solution Preparation

Triplicate solutions of the reference standard and bulk samples were prepared by transferring approximately 25 mg of compound to individual 100-mL volumetric flasks and diluting to volume with WIS and mixing by inversion. All samples were transferred to autosampler vials and analyzed by liquid chromatography.

4.3 Analysis

LC Parameters

System	Waters Alliance 2695
Software	Empower 2; Build 2154
Column	Waters XBridge C18 3.5 µm, 100 x 2.1 mm, guard column, 5 µm 2.1 x 10 mm
Column Temp	40 °C
Mobile Phases	A: 0.1% formic acid in water B: 0.1% formic acid in methanol
Flow Rate	0.25 mL/min
Gradient	Hold 90 % A for 0.67 min., 90% A to 90% B in 10 min., hold 90% B for 10 min., 90% B to 90% A in 5 min., hold 90% A for 5 min.
Injection Volume - Solvent	2 µL - Mobile Phase B
Retention Time (min)	Ensulizole - 5.73 min Padimate O (IS) - 16.59 min
Detector	Waters 2996 PDA, 312 nm

3

The suitability of the system was evaluated, and the results are shown below.

Parameter	Result	Criteria	Pass/Fail
Capacity Factor, k	2.8	$2 \geq k \leq 12$	Pass
Tailing Factor, T	1.2	$0.5 \geq T \leq 2.0$	Pass
Column Efficiency, N	29,000	$N \geq 6{,}000$ plates	Pass

4.4 Results

Calculations based on a major peak comparison technique gave the results shown in the following table.

RTI Log No.	Chemical	RRF[a]	Mean RRF[a] (%RSD)	Percent Relative Purity[b]
082010-C-15	Analytical Replicate #1 Analytical Replicate #2 Analytical Replicate #3	3.072 3.022 3.045	3.046 (0.82)	99.6
082010-C-05	Reference Replicate #1 Reference Replicate #2 Reference Replicate #3	3.034 3.083 3.054	3.057 (0.81)	--

[a] RRF = Relative Response Factor; normalized to sample concentration.
[b] Relative Purity = (Mean RRF, bulk/Mean RRF, ref.) x 100.

Based on the chromatographic results, the bulk sample had not significantly changed as compared to the frozen reference, and no significant impurities were observed. Typical chromatograms are shown in Figure 2.

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APPENDIX 3: Certificate of Analysis (Ensulizole)

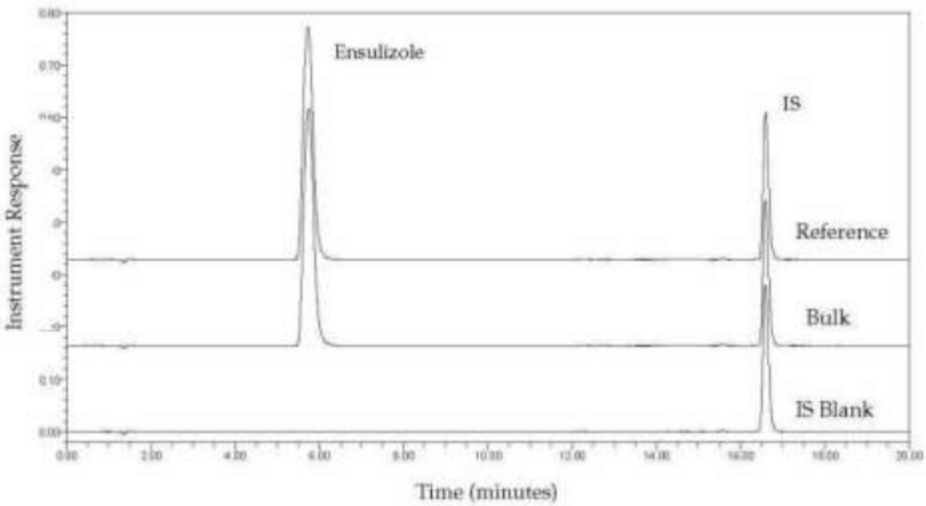

Figure 2: Example Liquid Chromatograms of Ensulizole Reference and Bulk Sample, and a Blank

5.0 REFERENCE

RTI International report "Ensulizole, Characterization Protocols Development, (CHEM11291), January 9, 2012.

6.0 ACKNOWLEDGMENTS

Personnel contributing to this task: [redacted]

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APPENDIX 3: Certificate of Analysis (Homosalate)

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Analytical Chemistry Services for the NTP
NIH Contract No. HHSN273201100003C
RTI Project 0212839.200.003.082
ChemTask No. CHEM11788
CAS No. 118-56-9

This pdf is an exact duplicate of the original approved report.

[redacted]

Program Information Coordinator

HOMOSALATE

CHEMICAL REANALYSIS

September 5, 2012

Prepared by:

[redacted] 09-05-12
Task Leader Date

Approved by:

[redacted] 09/05/12
Reshan Fernando, Ph.D. Date
Principal Investigator

Submitted to:

[redacted]

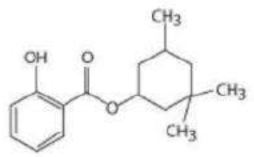
National Institute of Environmental Health Sciences
P.O. Box 12233
111 T. W. Alexander Drive
Research Triangle Park, NC 27709-2233

APPENDIX 3: Certificate of Analysis (Homosalate)

HOMOSALATE

CAS No.: 118-56-9	Study Lab: (Investigator): ILS ([redacted]
RTI Chemical ID Code: N67	Lot No. (Vendor): YT0976 (Spectrum)
ChemTask No.: CHEM11788	Vendor Purity: 99.88% (Spectrum COA)
RTI Log Nos. (Amt. Received): Analytical: 091410-A-14 (~50 g) Reference: 091410-A-05 (~5 g)	Receipt Date: Sep 14, 2010 (Bulk) Receipt Condition: No damage noted
Program Supported: TOX	Submitter: [redacted] (RTI)
Analysis Date: May 11, 21-23, 2012	Shipping Container: NA (in-house transfer)
Interim Results Date: May 29, 2012	Storage Conditions: Bulk: Room temperature Reference: Freezer (~-20 °C)

STRUCTURE



MOL. WT.
262.34

MOL. FORMULA
$C_{16}H_{22}O_3$

EXECUTIVE SUMMARY

In support of the Toxicity Testing Program, an aliquot of homosalate was submitted for bulk chemical reanalysis. Chemical purity of the bulk sample was determined relative to a reference standard of the same lot/batch number which had been stored at RTI under freezer conditions. Analytical results obtained by a GC/FID chromatographic method indicated that the sample had a percent relative purity of 99.3% when compared to the frozen reference standard. The FTIR spectrum of the bulk sample matched the spectrum of the frozen reference and was consistent with an identity of homosalate.

APPENDIX 3: Certificate of Analysis (Homosalate)

RTI INTERNATIONAL

Quality Assurance Statement

Chemical Name: **Homosalate**

Task Type: **Chemical Reanalysis**

Chem Task Number: **CHEM11788**

This study/task was audited by the Regulatory and Quality Assurance (RQA) – Quality Assurance Unit and the results of the inspections and audits were reported to the task leader/study director and management as identified below. To the best of our knowledge, the reported results accurately describe the study methods and procedures used, and the reported results accurately reflect the raw data.

Inspections and Audits	Inspection and Audit Date(s)	Date Inspection/Audit Report Sent to Task Leader/ Management
Sample Preparation Inspection	05/21/12	05/21/12
Data & Report Audit	08/16/12	08/16/12

Prepared by:

[redacted]
Quality Assurance Specialist

9/5/12
Date

Reviewed by:

[redacted]
Quality Assurance Specialist

9/5/12
Date

turning knowledge into practice

APPENDIX 3: Certificate of Analysis (Homosalate)

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APPENDIX 3: Certificate of Analysis (Homosalate)

HOMOSALATE

1.0 INTRODUCTION

The objective of this work was to determine the purity and verify the identity of homosalate in support of studies being conducted at ILS. To accomplish this objective, a chemical reanalysis was performed. The identity of the chemical was confirmed by FTIR and its purity assessed by GC.

2.0 CHEMICAL ANALYSIS

An aliquot of the bulk sample of homosalate was received on March 27, 2012 for chemical reanalysis (RTI log 091410-A-14). The aliquot was stored at room temperature. A frozen reference (RTI log 091410-A-05) sample was received May 10, 2012 and was stored at freezer temperature.

3.0 CONFIRMATION OF IDENTITY - INFRARED SPECTROMETRY (IR)

3.1 IR Parameters

System	Thermo Nicolet 6700 FTIR
Software	Omnic, Ver. 7.3
Method	NaCl disks, scan 4000 - 400 cm^{-1}

3.2 Results

Bulk Sample Frequency (1/cm)	Frozen Reference Sample Frequency (1/cm)	Assignment
3150	3150	O-H stretch
2953-2869	2953-2869	C-H stretch
1672	1672	C=C, C=0 stretch
1614	1614	C=C stretch
1585	1585	C=C stretch
1089	1089	C-C, C-O stretch
757	757	C-H bend

The observed spectrum for the bulk sample matched the spectrum of the frozen reference sample, and is consistent with the structure of homosalate (as reported in the bulk chemical comprehensive task CHEM11090). Figure 1 shows the bulk and frozen reference IR spectra.

APPENDIX 3: Certificate of Analysis (Homosalate)

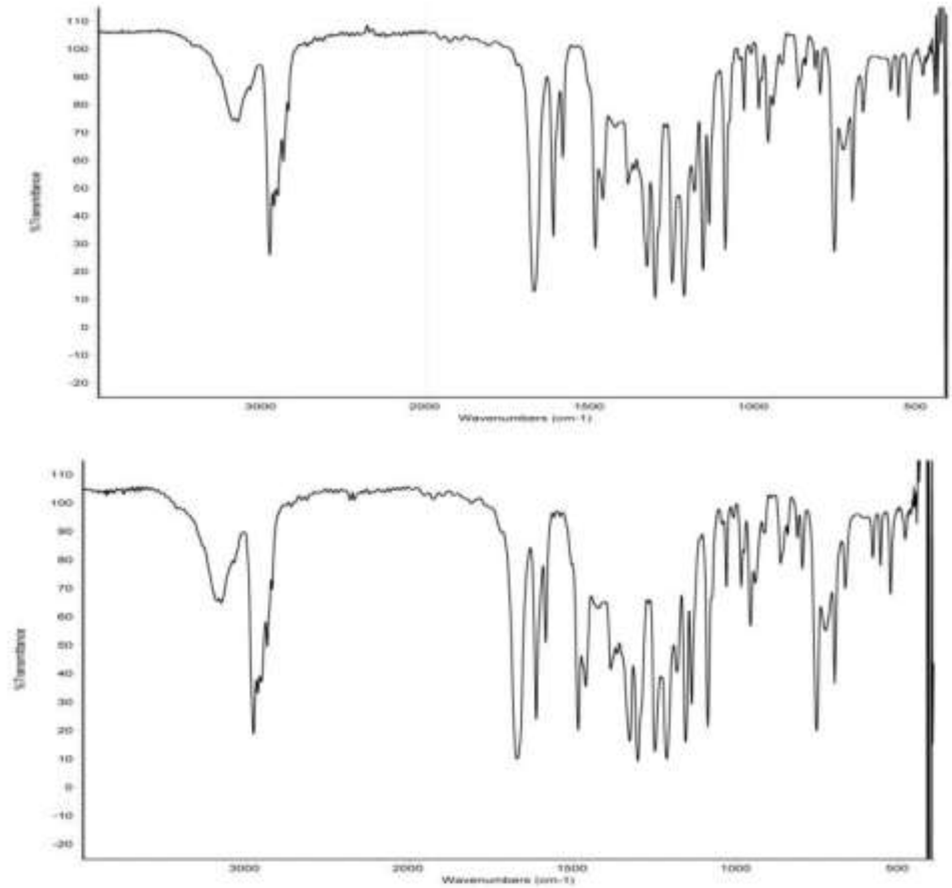


Figure 1: Infrared Spectrum of Homosalate Bulk (top spectrum)
and Frozen Reference (bottom spectrum)

2

APPENDIX 3: Certificate of Analysis (Homosalate)

4.0 DETERMINATION OF PURITY - GAS CHROMATOGRAPHY

This section describes the gas chromatographic method used to estimate sample purity.

4.1 Preparation of Internal Standard (IS) Solution

A solution of IS was prepared by weighing 115.49 mg of octanophenone and transferring it into a 200-mL volumetric flask. The IS was diluted to volume with dichloromethane. The flask was mixed by inversion. The IS solution had a concentration of 0.577 mg/mL.

4.2 Bulk Sample and Frozen Reference Standard Solution Preparation

Triplicate solutions of the reference standard and bulk samples were prepared by transferring approximately 25 mg of compound to individual 25-mL volumetric flasks and diluting to volume with IS solution and mixing by inversion. An aliquot of the bulk and reference solutions were transferred to GC vials for analysis. The samples were analyzed by gas chromatography.

4.3 Analysis

GC Parameters

Instrument	Agilent 6890N GC
Data System	Empower 2; Build 2154
Column	Phenomenex ZB-5MS (30 m x 0.25 mm ID, 0.5 µm film) with 5 m pre-guard
Carrier Gas	Helium
Flow Rate	1.5 mL/min
Oven Temperature	70 °C for 1 min., ramp to 270 °C at 20 °C/min with a 7 min hold
Retention Times	Homosalate: ~11.1 min. and 11.2 min (two peaks - cis/trans isomers) Octanophenone (IS): ~9.9 min.
Injector Type and Volume	Split (20:1), 1 µL
Injector Temperature	250 °C
Detector-Temperature	FID at 290 °C

3

APPENDIX 3: Certificate of Analysis (Homosalate)

The suitability of the system was evaluated, and the results are shown below.

Parameter	Criteria	Result	Pass/Fail
Tailing Factor, T	$0.5 \geq T \leq 2.0$	1.0	Pass
Column Efficiency, N	$\geq$ 250,000 plates	2,460,486	Pass
Precision (%RSD)	$\leq$ 5% (n=6)	0.2	Pass
Resolution	$\geq$ 40	41	Pass

4.4 Results

Calculations based on a major peak comparison technique gave the results shown in the following table. Typical chromatograms are shown in Figure 2.

RTI Log No.	Chemical	RRF[a]	Mean RRF[a] (%RSD)	Percent Relative Purity[b]
091410-A-14	Analytical Replicate #1 Analytical Replicate #2 Analytical Replicate #3	1.443 1.412 1.388	1.414 (2.0)	99.3
091410-A-05	Reference Replicate #1 Reference Replicate #2 Reference Replicate #3	1.430 1.430 1.413	1.424 (0.69)	—

[a] RRF = Relative Response Factor; normalized to sample concentration.
[b] Relative Purity = (Mean RRF, bulk/Mean RRF, ref.) x 100.

Based on the chromatographic results, the bulk sample had not significantly changed as compared to the frozen reference, and no significant impurities were observed.

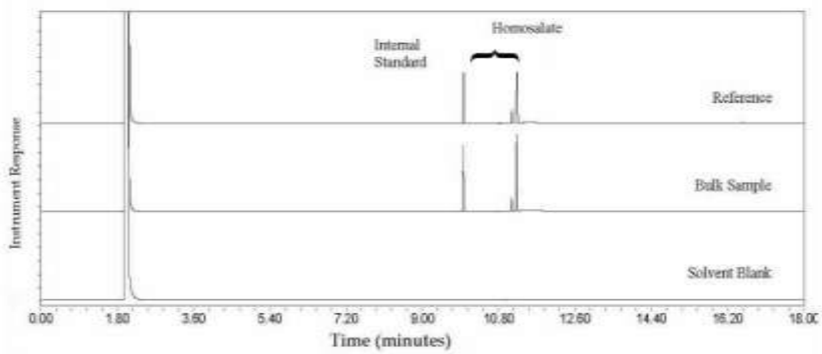


Figure 2: Example Gas Chromatograms of Homosalate Reference and Bulk Sample, and a Blank

4

APPENDIX 3: Certificate of Analysis (Homosalate)

5.0 REFERENCE

RTI International report "Homosalate, Characterization Protocols Development, (CHEM11293), January 6, 2012.

6.0 ACKNOWLEDGMENTS

Personnel contributing to this task: [redacted]

5

APPENDIX 3: Certificate of Analyses (Padimate-O)

RTI INTERNATIONAL

turning knowledge into practice

NTP Analytical Chemistry Services

3040 Cornwallis Road • PO Box 12194 • Research Triangle Park, NC 27709-2194 • USA
Telephone 919.541.6730 or 919.541.5975 • Fax 919.485.2650 • www.rti.org

This pdf is an exact duplicate of the original approved report.

[redacted]
Program Information Coordinator

Analytical Chemistry Services for the NTP
NIH Contract No. HHSN273201100003C
RTI Project 0212839.200.003.081
ChemTask No. CHEM11787
CAS No. 21245-02-3

2-ETHYLHEXYL-P-DIMETHYL-AMINOBENZOATE (PADIMATE O)

CHEMICAL REANALYSIS

September 5, 2012

Prepared by:		Approved by:	
[redacted]	09-05-12	[redacted]	09/05/12
Task Leader	Date	Reshan Fernando, Ph.D. Principal Investigator	Date

Submitted to:
[redacted]
National Institute of Environmental Health Sciences
P.O. Box 12233
111 T. W. Alexander Drive
Research Triangle Park, NC 27709-2233

APPENDIX 3: Certificate of Analyses (Padimate-O)

2-ETHYLHEXYL-P-DIMETHYL-AMINOBENZOATE (PADIMATE O)

CAS No.: 21245-02-3

RTI Chemical ID Code: L98

ChemTask No.: CHEM11787

RTI Log Nos. (Amt. Received):
Bulk Analytical: 082010-B-14 (~50 g)
Reference: 082010-B-05 (~5 g)

Program Supported: TOX

Analysis Dates: May 21-22, 24, 2012

Interim Results Date: May 30, 2012

Study Lab: (Investigator): ILS [redacted]

Lot No. (Vendor): MKBF0590V (Aldrich)

Vendor Purity: 98.3% (Aldrich COA)

Receipt Date: Aug 20, 2010 (Bulk)

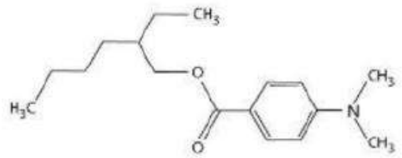
Bulk Receipt Condition: Good, room temperature

Submitter: [redacted] (RTI)

Shipping Container: NA (in-house transfer)

Storage Conditions:
Bulk: Room temperature
Reference: Freezer (~ -20 °C)

STRUCTURE



MOL. WT. 277.40

MOL. FORMULA $C_{17}H_{27}NO_2$

EXECUTIVE SUMMARY

In support of the Toxicity Testing Program, an aliquot of padimate O was submitted for bulk chemical reanalysis. Chemical purity of the bulk sample was determined relative to a reference standard of the same lot/batch number which had been stored at RTI under freezer conditions. Analytical results obtained by a GC/FID chromatographic method indicated that the sample had a percent relative purity of 98.1% when compared to the frozen reference standard. The FTIR spectrum of the bulk sample matched the spectrum of the frozen reference and was consistent with an identity of padimate O.

APPENDIX 3: Certificate of Analyses (Padimate-O)

RTI INTERNATIONAL

Quality Assurance Statement

Chemical Name: **2-Ethylhexyl-p-dimethyl-aminobenzoate (Padimate O)**

Task Type: **Chemical Reanalysis**

RTI Task Number: **0212839.200.003.081**

Chem Task Number: **CHEM11787**

This study/task was audited by the Regulatory and Quality Assurance (RQA) – Quality Assurance Unit and the results of the inspections and audits were reported to the task leader/study director and management as identified below. To the best of our knowledge, the reported results accurately describe the study methods and procedures used, and the reported results accurately reflect the raw data.

Inspections and Audits	Inspection and Audit Date(s)	Date Inspection/Audit Report Sent to Task Leader/ Management
Sample Analysis Inspection	05/15/12	05/22/12
Data & Report Audit	08/20/12	08/20/12

Prepared by:

[redacted]
Quality Assurance Specialist

9/5/12
Date

Reviewed by:

[redacted]
Quality Assurance Specialist

9/5/12
Date

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APPENDIX 3: Certificate of Analyses (Padimate-O)

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APPENDIX 3: Certificate of Analyses (Padimate-O)

2-ETHYLHEXYL-P-DIMETHYL-AMINOBENZOATE (PADIMATE O)

1.0 INTRODUCTION

The objective of this work was to determine the purity and verify the identity of 2-Ethylhexyl-p-dimethyl-aminobenzoate (padimate O) in support of studies being conducted at ILS. To accomplish this objective, a chemical reanalysis was performed. The identity of the chemical was confirmed by FTIR and its purity assessed by GC.

2.0 CHEMICAL ANALYSIS

An aliquot of the bulk sample of padimate O was received on March 27, 2012 for chemical reanalysis (RTI log 082010-B-14). The aliquot was stored at room temperature. A frozen reference (RTI log 082010-B-05) sample was received May 10, 2012 and was stored at freezer temperature.

3.0 CONFIRMATION OF IDENTITY - INFRARED SPECTROMETRY (IR)

3.1 IR Parameters

System	Thermo Nicolet 6700 FTIR
Software	Omnic, Ver. 7.3
Method	NaCl disks, scan 4000 - 400 cm^{-1}

3.2 Results

Bulk Sample Frequency (1/cm)	Frozen Reference Sample Frequency (1/cm)	Assignment
2958-2860	2958-2860	C-H Stretch
2819	2820	N-CH_3 stretch
1703	1703	C = O stretch
1609, 1527	1609, 1527	C=C Stretch
1317	1317	C-N (tertiary amine stretch)
1183	1184	C = O Stretch
1107	1107	C-O-C Stretch

The observed spectrum for the bulk sample matched the spectrum of the frozen reference sample, and is consistent with the structure of padimate O (as reported in the bulk chemical comprehensive task CHEM11089). Figure 1 shows the bulk and frozen reference IR spectra.

1

APPENDIX 3: Certificate of Analyses (Padimate-O)

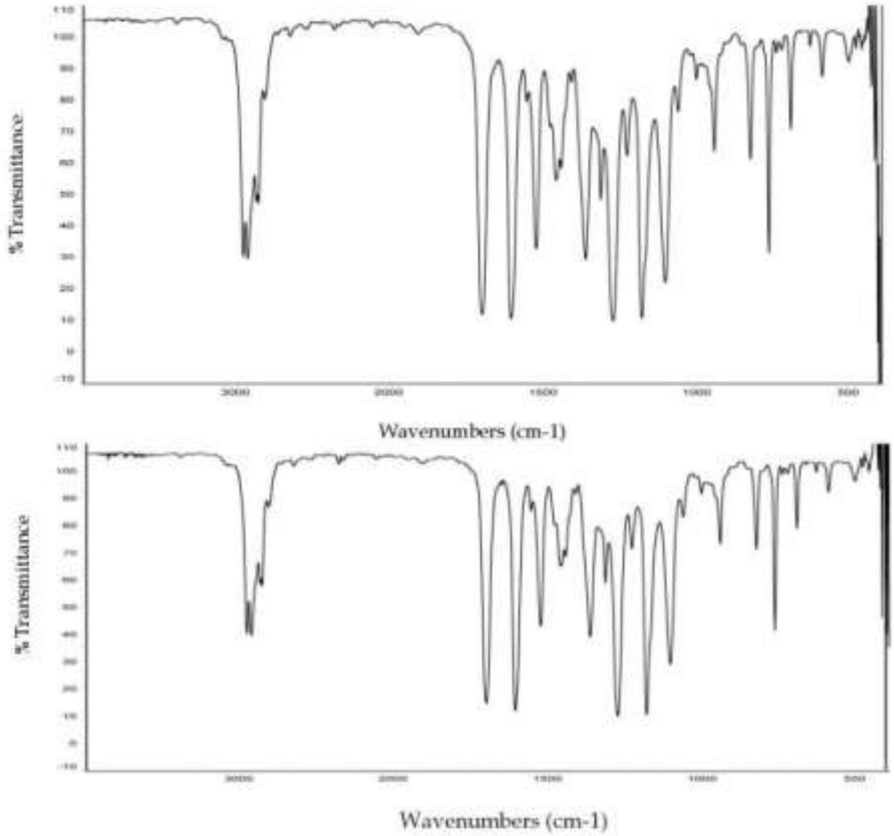


Figure 1: Infrared Spectrum of Padimate O Bulk (top spectrum) and Frozen Reference (bottom spectrum)

2

APPENDIX 3: Certificate of Analyses (Padimate-O)

4.0 DETERMINATION OF PURITY - GAS CHROMATOGRAPHY

This section describes the gas chromatographic method used to estimate sample purity.

4.1 Preparation of Internal Standard (IS) Solution

A solution of IS was prepared by weighing 103.4 mg of octanophenone and transferring it into a 200-mL volumetric flask. The IS was diluted to volume with dichloromethane. The flask was mixed by inversion. The IS solution had a concentration of 0.517 mg/mL.

4.2 Bulk Sample and Frozen Reference Standard Solution Preparation

Triplicate solutions of the reference standard and bulk samples were prepared by transferring approximately 25 mg of compound to individual 25-mL volumetric flasks and diluting to volume with IS solution and mixing by inversion. An aliquot of the bulk and reference solutions were transferred to GC vials for analysis. The samples and an IS blank was analyzed by gas chromatography.

4.3 Analysis

GC Parameters

Instrument	Agilent 6890N GC
Data System	Empower 2; Build 2154
Column	Phenomenex ZB-5MS (30 m x 0.25 mm ID, 0.5 µm film) with 5 m pre-guard
Carrier Gas	Helium
Flow Rate	1.5 mL/min
Oven Temperature	70 °C for 1 min., ramp to 270°C at 20 °C/min with a 7 min hold;
Retention Times	Padimate O: ~13.6 min. ; Octanophenone (IS): ~9.9 min.
Injector Type (ratio)	Split (20:1); 1 µL
Injector Temperature	250 °C
Detector-Temperature	FID at 290 °C

3

APPENDIX 3: Certificate of Analyses (Padimate-O)

The suitability of the system was evaluated, and the results are shown below.

Parameter	Criteria	Result	Pass/Fail
Tailing Factor, T	$0.5 \leq T \leq 2.0$	0.79	Pass
Column Efficiency, N	$\geq$ 250,000 plates	1,070,819	Pass
Precision (%RSD)	$\leq$ 5% (n=6)	0.6%	Pass
Resolution	$\geq$ 40	91.5	Pass

4.4 Results

Calculations based on a major peak comparison technique gave the results shown in the following table. Typical chromatograms are shown in Figure 2.

RTI Log No.	Chemical	RRF[a]	Mean RRF[a](%RSD)	Percent Relative Purity[b]
082010-B-14	Analytical Replicate #1 Analytical Replicate #2 Analytical Replicate #3	1.637 1.647 1.637	1.640 (0.4)	98.1
082010-B-05	Reference Replicate #1 Reference Replicate #2 Reference Replicate #3	1.661 1.645 1.711	1.672 (2.1)	--

[a] RRF = Relative Response Factor; normalized to sample concentration.
[b] Relative Purity = (Mean RRF, bulk/Mean RRF, ref.) x 100.

Based on the chromatographic results, the bulk sample had not significantly changed as compared to the frozen reference, and no significant impurities were observed.

4

APPENDIX 3: Certificate of Analyses (Padimate-O)

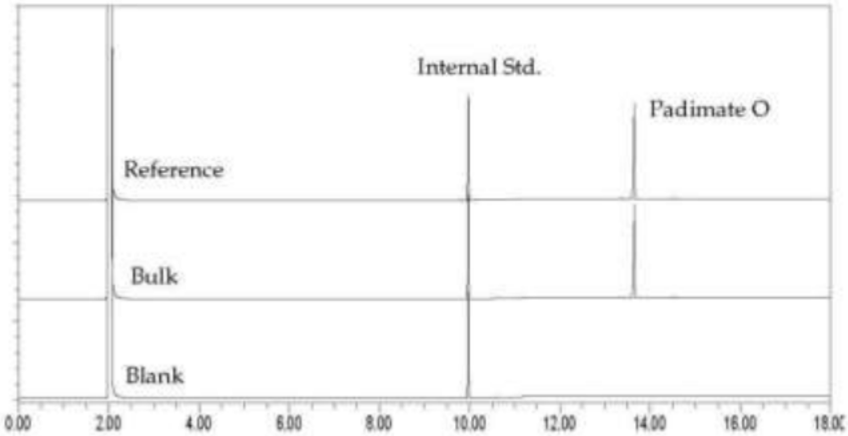


Figure 2: Example Gas Chromatograms of Padimate O Reference and Bulk Sample, and an IS Blank

5.0 REFERENCE

RTI International report "2-Ethylhexyl-p-dimethyl-aminobenzoate (Padimate O), Characterization Protocols Development, (CHEM11292), January 6, 2012.

6.0 ACKNOWLEDGMENTS

Personnel contributing to this task: [redacted]

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..................19701

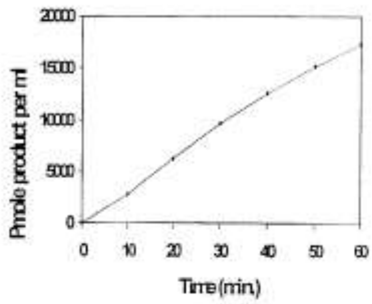
Storage Conditions..**STORE AT -80°C**
Date Released ....2011 July
Expiration Date........2014 July

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 f'om 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 (GE NTEST 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 µL 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.

Time Course of Product Formation



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

BD

INSECT CELL MICROSOMES
SAFETY INFORMATION

HAZARD WARNING:

The product was produced using baculovirus (*Autographa californica*) infected insect cells (BTI-TN-5B1-4). This virus is not known to be pathogenic to humans or other mammals.

SAFETY RECOMMENDATIONS:

When using this product, follow good laboratory safety procedures:

Do not eat, drink or smoke.

Avoid contact with skin or eyes.

Do not inhale aerosols.

Do not pipette by mouth.

Wear suitable protective clothing, gloves and eye protection.

Steam sterilize product or treat product with a 1% solution of sodium hypochlorite prior to disposal.

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

TECHNICAL DATA SHEET ^{3}H

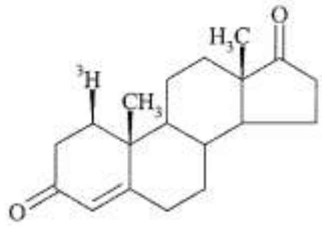
Caution: For Laboratory Use. A product for research purposes only.

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

Product Number: NET926

LOT SPECIFIC INFORMATION

Lot Number:	1632499	
Specific Activity:	26.3	Ci/mmol
	973.1	GBq/mmol
Production Date:	06 Jun 2012	



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

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 (555-1110 GBq/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:

High pressure liquid chromatography on a Zorbax ODS column using the following mobile phase:
water : tetrahydrofuran : methanol (40:15:45)

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

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.

PREPARATIVE PROCEDURE: Androst-4-ene-3, 17-dione, [1β-^{3}H(N)]- is prepared by treatment of androst-4-ene-3, 17-dione, [1β,2β-^{3}H(N)]- with potassium hydroxide under appropriate conditions (1) Purification is by HPLC.

PerkinElmer

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

REFERENCE: H. Mohler, W. Sieghart, J. C. Richards and W. Hunkeler, *Eur. J. Pharmacol.*, 102, 191 (1984).

HAZARD INFORMATION: WARNING: This product contains a chemical known to the state of California to cause cancer

PerkinElmer, Inc.
549 Albany Street
Boston, MA 02118 USA
P: (800) 762-4000 or (+1) 203-925-4602
www.perkinelmer.com/nenradiochemicals
For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

PerkinElmer®

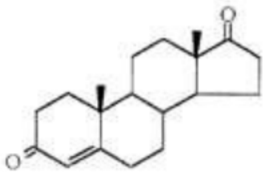
APPENDIX 3: Certificate of Analysis (Androstenedione, ASDN)

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 solely to the Buyer's specifications as set out above.

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

Certificate Of Analysis   Page 1 of 1

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

http://www.sigmaaldrich.com/catalog/CertOfAnalysisPage.do?symbol=F2552&LotNo=08...   6/27/2011

APPENDIX 4: Protocol

In vitro models to predict toxicity

PROTOCOL

Human Recombinant Aromatase Assay

Data Requirements: *OPPTS 890.1200*

Study Number:
9070-100794AROM

Sponsor:
NIEHS
National Institute of Environmental Health Sciences
PO Box 12233
Research Triangle Park, NC 27709
USA

Test Facility:
CeeTox
4717 Campus Drive
Kalamazoo, MI 49008

APPENDIX 4: Protocol

TEST PROTOCOL

TO BE COMPLETED BY THE STUDY SPONSOR:

Study Sponsor: NIEHS/NTP ([redacted] Chief Toxicology Branch)	
Address: P.O. Box 12233	
Research Triangle Park, NC	**Phone:** [redacted]
Study Monitor: [redacted]	**E-mail:** [redacted] **Phone:** [redacted]
CoStudy Monitor: N/A	**Phone:** N/A
Sponsor Protocol/Project No: N/A	
Test Substance Name(s): 2-Phenyl-5-benzimidazolesulfonic Acid (Ensulizole)	
Purity: 99.6%	
Batch or Lot#: 05117JE	
Test Substance Name(s): Butyl-methoxydibenzoylmethane (Avobenzone)	
Purity: ~98.5%	
Batch or Lot#: L802809	
Test Substance Name(s): 3, 3, 5-Trimethlycyclohexyl Salicylate (Homosalate)	
Purity: 99.3%	
Batch or Lot#: YT0976	
Test Substance Name(s): 2-Ethylhexyl-P-Dimethyl-Aminobenzoate (Padimate-O)	
Purity: 98.1%	
Batch or Lot#: MKBF0590V	
***Proposed Experimental Start Date:** January 16, 2013 (date subject to change; actual experimental start date to be provided in final report)	
***Proposed Experimental Termination Date:** February 15, 2013 (date subject to change; actual experimental termination date to be provided in final report)	

APPENDIX 4: Protocol

Sponsor
National Institute of Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, NC 27709

[redacted]
Contract Office Technical Representative
National Toxicology Program, National Institutes of Environmental Health

[redacted]
National Toxicology Program (NTP) Investigator
Telephone No.: [redacted]
Facsimile No.:
E-mail: [redacted]

Study Monitor
[redacted]
Integrated Laboratory Systems, Inc.
Telephone No.: [redacted]
Facsimile No.:
E-mail:

Project Identification
ILS Project No.: N135
Study No.: 007
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APPENDIX 4: Protocol

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CeeTox Study #: 9070-100794AROM

Signatures

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Chief, Toxicology Branch
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Date

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Contract Office Technical Representative
National Toxicology Program, NIEHS

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Date

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Integrated Laboratory Systems, Inc
Study Monitor

11Feb2013
Date

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Study Director

13 FEB 2013
Date

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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.

The results of this screen are intended to be used in conjunction with results from other Tier 1 in vitro and in vivo screening assays (OCSPP 890 test guideline series) that constitute the full screening battery under the Endocrine Disruptor Screening Program (EDSP). Results of the Tier 1 screening battery, along with other scientifically relevant information, are to be used in a weight-of-evidence assessment leading to the determination of a substance's potential to interact with the endocrine system. The Tier 1 battery is intended for screening purposes only and should not be used for endocrine classification or risk assessment.

Aromatase laboratory proficiency assays with econazole, fenarimol, nitrofen, and atrazine were conducted on three separate occasions at CeeTox according to test guideline (OPPTS 890.1200). Data for laboratory proficiency assays are maintained at CeeTox.

3. Compliance Statement

This study will be conducted in compliance with the U.S. Environmental Protection Agency Good Laboratory Practice regulations Title 40, Part 160 with the exception of section 160.113. Dose concentrations of test substance and control substances will not be verified using analytical methods.

4. Quality Assurance

This study will be subjected to periodic inspections and the draft final report will be reviewed by the Quality Assurance Unit of CeeTox in accordance with CeeTox Standard Operating Procedure (SOP).

5. Regulatory Citations

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

APPENDIX 4: Protocol and Protocol Amendments

6. Test Facility

CeeTox, Inc.
4717 Campus Drive
Kalamazoo, MI 49008

7. Experimental Design

The Aromatase (Human Recombinant) Assay will be used as the screening assay to identify substances that may affect the endocrine system by inhibiting catalytic activity of aromatase (CYP 19), the enzyme responsible for the conversion of androgens to estrogens.

8. Justification of the Test System

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

The Aromatase (Human Recombinant) Assay is a screening assay intended to identify chemicals that may affect the endocrine system by inhibiting catalytic activity of aromatase (CYP 19), the enzyme responsible for the conversion of androgens to estrogens.

9. 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 or sponsor's desingee. 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.

Test Substance:	2-Ethylhexyl-p-dimethyl-aminobenzoate (Padimate O)
CAS No.:	21245-02-3
Source:	Sigma-Aldrich
Lot/Batch No.:	MKBF0590V
Formula:	$(CH_3)_2NC_6H_4CO_2CH_2CH(C_2H_5)(CH_2)_3CH_3$
Description:	Colorless liquid
Purity:	98.1%

Test Substance:	2-Phenyl-5-benzimidazolesulfonic acid (Ensulizole)
CAS No.:	27503-81-7
Source:	Sigma-Aldrich
Lot/Batch No.:	05117JE
Formula:	$C_{13}H_{10}N_2O_3S$

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Description: White powder
Purity: 99.6%

Test Substance: 3, 3, 5-Trimethlycyclohexyl Salicylate (Homosalate)
CAS No.: 118-56-9
Source: Spectrum Chemical Mfg. Corp
Lot/Batch No.: YT0976
Formula: $C_{16}H_{22}O_3$
Description: Colorless to light yellow liquid
Purity: 99.3%

Test Substance: Butyl-methoxydibenzoylmethane (Avobenzone)
CAS No.: 70356-09-1
Source: Universal Preserv-A-Chem Inc.
Lot/Batch No.: L802809
Formula: $C_{20}H_{22}O_3$
Description: Off White to Yellowish Crystalline Powder
Purity: ~98.5%

Preparation of Test Substance(s)

The test substances will be formulated directly in dimethyl sulfoxide (DMSO). 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. 4-OH ASDN Positive Control Inhibitor

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 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 µL of the dilution to a 2 mL total assay volume with solvent concentrations ≤ 1%. The total volume of solvent used in each assay will be no more than 1% of the total assay volume in order to minimize the potential of the solvent to inhibit the enzyme. Information on storage conditions for the control substance stock solutions will be reported.

<u>Substrate</u>

Substrate Name/Supplier

The substrate for the aromatase assay will be 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 will 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 100 nM and the amount of tritium added to each incubation will be 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:

A 1:100 dilution of radiolabeled stock will be prepared in 0.1 M Sodium Phosphate Assay buffer.

A 1 mg/mL solution of ASDN will be prepared in ethanol and then dilutions in buffer to a final concentration of 1 μg/mL will be prepared.

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

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The weight and/or volumes of each component added to the substrate solution will be recorded. After mixing well, 20 µL aliquots will be combined with scintillation cocktail for radiochemical content analysis. The isotope level will be adjusted if not within 10% of the nominal activity and tested again to verify accuracy.

One hundred microliters of the substrate solution will be added to each 2 mL assay volume to yield a final [^{3}H] ASDN concentration of 100 nM with 0.1 µCi/tube.

10. Identification of the Test System

Microsomes

Human Recombinant Microsomes

Human Recombinant Microsomes will be 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 microsome tube will be appropriately labeled with catalog number, lot number, and relevant dates. The microsomes will be stored at approximately -80°C. Bias is not a factor in this test system.

Human Recombinant Microsome Preparation

Preparation of the human recombinant microsomes will involve thawing the microsomes rapidly in an 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 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 0.1 M 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.

11. 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.

Test 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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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

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.

Total assay volume will be 2 mL. Tubes will be incubated at ~ 37°C for ~15 minutes.

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

The tubes will be mixed for approximately 5 seconds 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 (~4°C).

The Methylene Chloride (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.

Liquid scintillation cocktail (Opti-Fluor, Perkin Elmer) will be added to each vial and shaken. The radiochemical content of each aliquot will be determined as described below:

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

Liquid scintillation vials will be counted for 10 minutes.

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 dpm/nmol). The activity of the enzyme reaction is expressed in nmol/mg-protein/min and

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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.

12. 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. Performance Criteria for Positive Control 4-OH ASDN

	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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13. 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. If insolubility occurs such that the highest concentration would be $10^{5.5}$M or lower than the assay will not be run.

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- 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.

- 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** (tubes)	**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.

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14. 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 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 substrate's 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 $^{3}H_2O$ 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.

15. 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:

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

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

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 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. 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	—	

Proposed Statistical Methods and Software

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

16. Final Study Report

The data to be reported in the draft report and final report will be determined per Standard Operating Procedure (SOP) and 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, and data interpretation.

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The draft report will be submitted to the Sponsor in electronic form. The final report will be submitted as one hard copy and one electronic copy.

17. 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 to document this verbal request. All protocol amendments with justifications will be documented, signed and dated by the Study Director and Sponsor's Representative. A copy of the protocol and all amendments will be issued to the Sponsor and the originals will be placed into the study binder.

18. Data Retention and Archiving

All original data [including the original signed study protocol and all amendments (if any), test substance information, observations, etc.] and the original final report will be transferred to the National Toxicology Program Archives following finalization of the study report to the address below:

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

19. Test Substance Disposition

Test substance will be either returned to the sponsor or destroyed following finalization of the study report.