Androgen Receptor Binding (Rat Prostate Cytosol)

Final Report

DATA REQUIREMENT(S): OPPTS 890.1150 (2009)

AUTHOR(S): [redacted]

STUDY COMPLETION DATE: January 27, 2012

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STATEMENT OF DATA CONFIDENTIALITY CLAIMS

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Study Number: 9070-100107ARB

Study Title: Androgen Receptor Binding (Rat Prostate Cytosol)

I, the undersigned, hereby declare that this study was performed in accordance with the Environmental Protection Agency (EPA) Good Laboratory Practice (GLP) regulations (Title 40 Part 160 with the exception of section 160.113. Dose concentrations of test and control substances will not be verified using analytical methods.

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 were noted in this report, with the full write-ups included in the study binder.

[redacted]

Study Director

27 Jan 2012

Date

FLAGGING STATEMENT

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QUALITY ASSURANCE STATEMENT

Study Title: Androgen Receptor Binding (Rat Prostate Cytosol)

Study Number: 9070-100107ARB

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
27Jun11	Draft protocol audit	27Jun11	27Jun11
20Sep11 and 21Sep11	In-process assay audit	26Sep11	26Sep11
16Dec11	Data binder audit	16Dec11	16Dec11
26Jan12	Draft report audit	26Jan12	26Jan12

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

[redacted] 27 Jan 2012

Date

Quality Assurance Auditor
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Kalamazoo, MI 49008

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

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

Study Dates

Study initiation date: July 06, 2011
Experimental start date: September 20, 2011
Experimental termination date: October 07, 2011
Study termination date: January 27, 2012

Deviations from the Protocol

See Appendix 3. There were six deviations however they did not impact the integrity of the data in this report.

Other

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

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

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1.0 EXECUTIVE SUMMARY

1.1 Study Design

The objective of this study was to evaluate the ability of oxybenzone, octylmethoxycinnamate, octylsalate and octocrylene to interact with the androgen receptors (ARs) isolated from rat prostates.

Preliminary assessments of precipitation were conducted in order to identify a suitable top concentration of oxybenzone, octylmethoxycinnamate, octylsalate and octocrylene for use in the binding assays. The concentrations assessed were: 10^{-4} and 10^{-3} M.

The final concentrations of each test article assessed in the binding assays were: 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3} M for the first valid independent run (20-September-2011) and 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} for the second and third valid independent runs (22-September-2011 and 06-October-2011).

All concentrations were tested in replicates of 3. In addition, solvent control tubes (6 replicates) were prepared to assess total binding. These replicates included the radioligand, cytosol (containing the ARs) and solvent but without the competitor R1881. The total binding tubes allowed for the identification of maximal binding of [^{3}H]-R1881. Non-specific binding (NSB) was also assessed in replicates of 6 by determining the [^{3}H]-R1881 bound in the presence of 100-fold excess unlabeled R1881. NSB was subtracted from the data, normalized to total binding and presented as % specific binding. Finally, 30 µL of [^{3}H]-R1881 was added to scintillation vials (n=6) in order to determine both total radioligand added and to calculate the percentage of total radioligand added to the tube that is bound to ARs. The duration of incubation at approximately 4°C was 16-20 hours. A complete concentration response curve for the positive control R1881 and the weak positive control (wPC) dexamethasone was run each time the binding assay was performed.

1.2 Results

The suitable top concentration of oxybenzone, octylmethoxycinnamate, octylsalate and octocrylene for use in all three valid independent runs was (20-September-2011, 22-September-2011 and 06-October-2011) was 10^{-4} M as precipitation was seen with each test article at 10^{-3} M.

In the first valid independent run (20-September-2011), the mean specific binding was > 75% at every soluble concentration tested for octylmethoxycinnamate, resulting in a classification of "non-binder." The mean specific binding for oxybenzone, octylsalate and octocrylene was 64.9%, 53.9% and 54.2% of control at 10^{-4}, respectively, and > 75% at all lower concentrations. The mean specific binding for these three test articles at 10^{-3} M was not assessed because precipitation was observed at this concentration. These data result in oxybenzone, octylsalate and octocrylene being classified as "equivocal" for this run. The

weak positive control dexamethasone had a $LogIC_{50}$ of -4.4 M while the $LogIC_{50}$ of R1881 was -8.9 M.

In the second valid independent run (22-September-2011), the mean specific binding was > 75% at every soluble concentration tested for octylmethoxycinnamate, resulting in a classification of "non-binder." The mean specific binding for oxybenzone, octylsalate and octocrylene were 62.6%, 52.9% and 50.4% of control at 10^{-4}, respectively. These data result in oxybenzone, octylsalate and octocrylene being classified as "equivocal" for this run. The weak positive control dexamethasone had a $LogIC_{50}$ of -4.3 M while the $LogIC_{50}$ of R1881 was -10.1 M.

Finally, in the third valid independent run (06-October-2011), the mean specific binding was > 75% at every soluble concentration tested for octylmethoxycinnamate, resulting in the classification as a "non-binder." The mean specific binding for oxybenzone and octocrylene were 61.2% and 51.3% of control at 10^{-4}, respectively. These data result in oxybenzone and octocrylene being classified as "equivocal" for this run. The mean specific binding for octylsalate was 38.4% of control at 10^{-4} M, resulting in a classification of "binder" with a $LogIC_{50}$ of -4.8 M and an RBA of 0.5. The weak positive control dexamethasone had a $LogIC_{50}$ of -4.5 M while the $LogIC_{50}$ of R1881 was -9.0 M.

The mean relative binding affinity, or RBA (calculated by dividing the $LogIC_{50}$ of the control/test material by the $LogIC_{50}$ of the positive control R1881) was 0.5 for dexamethasone. As oxybenzone, octylmethoxycinnamate, octylsalate and octocrylene were not classified as an overall "binder" (mean specific binding $\geq$ 50%), the RBA could not be calculated.

1.3 Conclusion

Octylmethoxycinnamate was classified as a "non-binder" in all three independent runs and thus has a final classification of "non-binder." Oxybenzone and octocrylene were classified as "equivocal" in all three independent runs and thus have a final classification of "equivocal." Finally, octylsalate was classified as "equivocal" in the first and second valid independent run, and classified as a "binder" in the third valid independent run, resulting in a final classification of "equivocal."

2.0 INTRODUCTION

2.1 Purpose

The objective of this study was to evaluate the ability of oxybenzone, octylmethoxycinnamate, octylsalate and octocrylene to interact with the androgen receptors (ARs) isolated from rat prostates. The AR contains a highly specific hormone-binding domain (HBD) that is relatively well conserved across species. Upon binding endogenous androgens to the HBD, the AR enters the nucleus and binds to specific sites in the genome

called androgen response elements (AREs). Once bound to the ARE, the AR forms a homodimer with another AR, thereby controlling gene expression.

This assay was used to provide information on the ability of a compound to interact with the androgen receptors (ARs) isolated from rat prostates. This assay is not intended to be used to show that the interaction is, specifically, one-site competitive binding, or to precisely characterize the strength of the binding interaction. It therefore may not be appropriate to use in quantitative structure-activity relationship (SAR) model development for androgen receptor binding without further refinement. This assay is intended to be used as one part of a screening program that includes other assays, to detect substances that can potentially interact with the androgen hormonal system.

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 were used by the US EPA to identify substances that have the potential to interact with the androgen, estrogen or thyroid system. Results of the Tier 1 screening battery, along with other scientifically relevant information, are to be used in a weight-of-evidence determination of a substance's potential to interact with these systems. The fact that a substance may interact with a hormone system does not mean that when the substance is used, it will cause adverse effects in humans or ecological systems. The Tier 1 battery is intended for screening purposes only and should not be used for endocrine classification or risk assessment.

2.2 Regulatory Citations

OPPTS 890.1150: Androgen Receptor Binding (Rat Prostate Cytosol). 2009 (now referred to as OCSPP though the guideline is still titled as OPPTS).

3.0 MATERIALS AND METHODS

All materials and methods described in this report are in reference to the three valid independent runs (20-September-2011, 22-September-2011 and 06-October-2011) only.

3.1 Test Substance

3.1.1 Test substance details

Test Substance Name:	2-hydroxy-4-methoxybenzophenone (Oxybenzone)
Test Substance Manufacturer:	Ivy Fine Chemicals
CAS Number:	131-57-7
Description:	Light yellow solid
Solvent Used:	DMSO
Batch/Lot Number:	20100801
Expiry Date:	01-Aug-2012
Purity:	99.92%
Molecular Formula:	$C_{14}H_{12}O_3$
Molecular Weight:	228.25
Storage Conditions:	Room Temp. (eg. ambient)

A certificate of analysis for the test substance is presented in Appendix 4.

Test Substance Name:	2-ethylhexyl p-methoxycinnamate, octyl 4-methoxycinnamate (Octylmethoxycinnamate)
Test Substance Manufacturer:	Acros Organics
CAS Number:	5466-77-3
Description:	Clear colorless liquid
Solvent Used:	DMSO
Batch/Lot Number:	A0293319
Expiry Date:	Not Provided
Purity:	99.8%
Molecular Formula:	$C_{18}H_{26}O_3$
Molecular Weight:	290.39
Storage Conditions:	Room Temp. (eg. ambient)

A certificate of analysis for the test substance is presented in Appendix 4.

Test Substance Name:	Octyl salicylate, 2-ethylhexyl salicylate (Octylsalate)
Test Substance Manufacturer:	Sigma-Aldrich
CAS Number:	118-60-5
Description:	Colorless liquid
Solvent Used:	DMSO
Batch/Lot Number:	44698PJ
Expiry Date:	Not Provided
Purity:	99.6%
Molecular Formula:	$C_{15}H_{22}O_3$
Molecular Weight:	250.33
Storage Conditions:	Room Temp. (eg. ambient)

A certificate of analysis for the test substance is presented in Appendix 4.

Test Substance Name:	2-ethylhexyl-2-cyano-3,3-diphenylacrylate (Octocrylene)
Test Substance Manufacturer:	Sigma-Aldrich
CAS Number:	6197-30-4
Description:	Yellow viscous liquid
Solvent Used:	DMSO
Batch/Lot Number:	01697MJ
Expiry Date:	Not Provided
Purity:	99.2%
Molecular Formula:	$C_{24}H_{27}NO_2$
Molecular Weight:	361.48
Storage Conditions:	Room Temp. (eg. ambient)

A certificate of analysis for the test substance is presented in Appendix 4.

The reference compound R1881 (CAS# 965-93-5) was purchased from Sigma Aldrich (St. Louis, MO) and was 98% pure. The catalog number was R0908 and the lot number was 060M4638.

The weak positive control dexamethasone (CAS# 50-02-2) was purchased from Sigma Aldrich (St. Louis, MO) and was 98.9% and 99%pure. The catalog number was D1756 and the lot numbers were 1419230 and 077K1050, respectively.

The radioligand [^{3}H]-R1881 was purchased from Perkin-Elmer (Boston, MA) and had a specific activity (SA) of 85.1 Ci/mol on the certification date (24-February-2011). The catalog number was NET590 and the lot number was 653698. The $SA_{adjusted}$ was 82.4 Ci/mol for the first two valid independent runs (20-September-2011 and 22-September-2011) and 82.2 Ci/mol for the third valid independent run (06-October-2011).

3.1.2 Vehicle selection

Dimethyl sulfoxide (DMSO) is one of the recommended solvents according to the EPA guideline (OPPTS 890.1150) and was selected as a suitable vehicle for oxybenzone, octylmethoxycinnamate, octylsalate and octocrylene. Oxybenzone, octylmethoxycinnamate, octylsalate and octocrylene solutions with a concentration of up to 10^{-4} M (the limit concentration for the assay) can be prepared while limiting the final concentration of DMSO in the assay medium to ~3.2% (v/v). R1881 and dexamethasone were prepared fresh on the day of the assay for the first two runs. For the third valid independent run, stocks were prepared on 29-September-2011, then frozen as aliquots and thawed on the day of the assay for use. The test substance was prepared on 20-September-2011for the first two valid independent runs, and on the day of the assay for the third valid independent run.

3.1.3 Test Substance Preparation

Vehicle (DMSO) was kept at the same concentration for the controls and for the test substance. DMSO was tested as a vehicle control with the reference chemical and reference

controls for the run as well. All concentrations of oxybenzone, octylmethoxycinnamate, octylsalate and octocrylene were kept at approximately 3.2% final DMSO concentration. The dose concentrations of oxybenzone, octylmethoxycinnamate, octylsalate and octocrylene were not verified using analytical methods.

Serial dilutions of test chemicals were prepared in DMSO to yield the final concentrations indicated below:

Example Dilution Procedure for oxybenzone, octylmethoxycinnamate, octylsalate and octocrylene.

Tube #	Volume of stock to add for diluted concentration	Volume of solvent to add	Total volume of diluted test chemical	Diluted test chemical concentration	*Final test chemical concentration in AR assay tube
TC1	Use 300 µl of stock test chemical (100 mM)	700 µl	1 ml	3×10^{-2} M	1×10^{-3} M
TC2	Use 100 µl of dilution TC1 (50 mM)	900 µl	1 ml	3×10^{-3} M	1×10^{-4} M
TC3	Use 100 µl of dilution TC2 (5 mM)	900 µl	1 ml	3×10^{-4} M	1×10^{-5} M
TC4	Use 100 µl of dilution TC3 (500 µM)	900 µl	1 ml	3×10^{-5} M	1×10^{-6} M
TC5	Use 100 µl of dilution TC4 (50 µM)	900 µl	1 ml	3×10^{-6} M	1×10^{-7} M
TC6	Use 100 µl of dilution TC5 (5 µM)	900 µl	1 ml	3×10^{-7} M	1×10^{-8} M
TC7	Use 100 µl of dilution TC6 (500 nM)	900 µl	1 ml	3×10^{-8} M	1×10^{-9} M
TC8	Use 100 µl of dilution TC7 (50 nM)	900 µl	1 ml	3×10^{-9} M	1×10^{-10} M

*Final concentration of oxybenzone, octylmethoxycinnamate, octylsalate and octocrylene in assay tube when 10 µl of diluted concentration is used in a total volume of 300 µl.

3.1.4 Positive and Weak Positive Reference Control Preparation

The positive control, R1881, strongly binds ARs and was included to ensure that the run was properly performed and to allow an assessment of variability in the conduct of the assay across time. Final concentrations of unlabeled R1881 ranged from 1×10^{-6} to 1×10^{-11} M as described below. Fresh 10 mM R1881 stock was diluted 1:10 (1 mM R1881 solution) and then serial dilutions of the reference standard were performed in DMSO (final concentration of approximately 3.2%).

Example Dilution Procedure for R1881

Tube #	Volume of stock to add for diluted concentration	Volume of solvent to add	Total volume of R1881	Diluted R1881 concentration	Final R1881 concentration in AR assay tube
N/A	Use 100 µl of stock R1881 (10 mM)	900 µl	1 ml	1×10^{-3} M	
NSB1	Use 30 µl of stock R1881 (1 mM)	970 µl	1 ml	3×10^{-5} M	1×10^{-6}
S2	Use 100 µl of dilution NSB1 (30 µM)	900 µl	1 ml	3×10^{-6} M	1×10^{-7}
S3	Use 100 µl of dilution S2 (3 µM)	900 µl	1 ml	3×10^{-7} M	1×10^{-8}
S4	Use 100 µl of dilution S3 (300 nM)	900 µl	1 ml	3×10^{-8} M	1×10^{-9}
S5	Use 100 µl of dilution S4 (30 nM)	900 µl	1 ml	3×10^{-9} M	1×10^{-10}
S6	Use 100 µl of dilution S5 (3 nM)	900 µl	1 ml	3×10^{-10} M	1×10^{-11}

The weak positive control was dexamethasone. A 30 mM stock was prepared in DMSO and serially diluted as described below. The concentration range tested for the weak positive control was from 1 x 10^{-3} to 1 x 10^{-10} M with DMSO kept at approximately 3.2%**.**

Example Dilution Procedure for Dexamethasone

Tube #	Volume of stock to add for diluted concentration	Volume of solvent to add	Total volume of diluted positive control	Weak Positive Control Concentration	
				Diluted	Final in AR assay tube
P1	Use stock positive control (30 mM)		1 ml	3 x 10^{-2} M	1 x 10^{-3} M
P2	Use 100 µl of stock positive control (30 mM)	900 µl	1 ml	3 x 10^{-3} M	1 x 10^{-4} M
P3	Use 100 µl of P2 (3 mM)	900 µl	1 ml	3 x 10^{-4} M	1 x 10^{-5} M
P4	Use 100 µl of P3 (300 µM)	900 µl	1 ml	3 x 10^{-5} M	1 x 10^{-6} M
P5	Use 100 µl of P4 (30 µM)	900 µl	1 ml	3 x 10^{-6} M	1 x 10^{-7} M
P6	Use 100 µl of P5 (3 µM)	900 µl	1 ml	3 x 10^{-7} M	1 x 10^{-8} M
P7	Use 100 µl of P6 (300 nM)	900 µl	1 ml	3 x 10^{-8} M	1 x 10^{-9} M
P8	Use 100 µl of P7 (30 nM)	900 µl	1 ml	3 x 10^{-9} M	1 x 10^{-10} M

3.2 Solubility/Precipitation Assay

The limit of test chemical solubility was determined by visual observation. Compound solubility was determined in solvent. In addition, the solutions were watched closely when added to the experiment tube (as the test compound may precipitate upon addition to the assay tube mixtures).

3.3 Rat Prostate Cytosol

Cytosol was collected, processed, and validated per EPA guideline and CeeTox SOP 2055 for use on this study. Related data was maintained separate from this study and the pertinent information is available in Appendix 2.

3.4 Stock Solution Preparation

A 200 mM EDTA stock solution was prepared and stored at 4±2°C. A 1 M sodium molybdate solution was also prepared along with a 1 M Tris buffer (pH adjusted to 7.4). These solutions were then used to prepare Low-salt TEDG Buffer (10 mM Tris, 1 mM sodium molybdate, 1.5 mM EDTA, 10% glycerol and 1 mM DTT [added immediately before use], pH 7.4 [cooled to 4±2°C before adjusting to pH 7.4 and stored at 4±2°C up to 3 months]).

A 600 µM stock solution of triamcinolone acetonide was prepared in 100% ethanol and diluted/aliquoted into 60 µM solutions and stored at -20±2°C.

The 60% hydroxyapatite (HAP) slurry was prepared one day before use. The HAP was gently mixed with 50 mM buffer in a graduated cylinder, and refrigerated for approximately 2 hours at 4±2°C. The HAP was then washed three times as follows. The supernatant was removed and the HAP was resuspended again in 50 mM Tris buffer (4±2°C). The slurry was mixed gently and allowed to settle for approximately 2 hours at 4±2°C. After the third wash, the HAP slurry settled overnight (at least 8 to 10 hours at 4±2°C).

The next day (day of use), the volume of HAP on the graduated cylinder was noted. The supernatant was removed and the HAP was resuspended to a final volume of 60% HAP and 40% cold 50 mM Tris buffer. The HAP slurry was well-suspended and ice-cold when used in the separation procedure.

3.5 Assays

3.5.1 Working Assay Buffer Preparation

Summary Table of Assay Conditions

		Competitive Binding Assay Protocol
Source of receptor		Rat prostate cytosol
Concentration of radioligand		1 nM
Concentration of receptor		Sufficient to bind 10-15% of radioligand
Concentration of test substance (as serial dilutions)		100 pM to 1 mM
Temperature		4±2°C
Incubation time		16-20 hours
Composition of assay buffer	Tris	10 mM (pH 7.4)
	EDTA	1.5 mM
	Glycerol	10% (v/v)
	Protease Inhibitor	0.5% (v/v)
	DTT	1 mM
	Sodium Molybdate	1 mM

On the day of assay, the Working Assay Buffer, or TEDG+PI buffer (10 mM Tris, 1 mM sodium molybdate, 1.5 mM EDTA, 10% glycerol and 1 mM DTT, 0.5% Protease Inhibitor (v/v), pH 7.4) was prepared using the TEDG buffer.

3.5.2 [^{3}H]-R1881 Preparation

[^{3}H]-R1881 was prepared on the day of assay. The specific activity was adjusted for decay over time prior to performing dilutions. The specific activity was calculated on the day of the assay using the following equation:

$SA_{adjusted}$ (Fraction Isotope Remaining) = SA * $e^{-Kdecay*Time}$

SA is the specific activity on the packaging date.
Kdecay is the decay constant for tritium (equal to 1.54 x 10^{-4}/day).

Time = days since the date on the stock bottle from the manufacturer.

The [^{3}H]-R1881 was diluted with TEDG + PI buffer so that each assay tube contained 1 nM final concentration of [^{3}H]-R1881 using the following procedure:

The specific activity was converted from Ci/mmole to nM. If SA = X Ci/mmole, and Y = concentration of radiolabel, then X Ci/mmole was converted to nM and the SA activity adjusted for decay over time by the following conversion:

(Y mCi/ml / X Ci/mmole) * 1 Ci/1000 mCi * 10^6 nmole/mmole * 1000 ml/L = (Y/X) * 10^6 nM

A 10 nM diluted stock of the [^{3}H]-R1881 was prepared so that 30 µl in a total volume of 300 µl per assay tube will give a final concentration of 1 nM. The 10 nM [^{3}H]-R1881 was kept on ice until standards, test chemicals, and assay tubes were prepared.

3.5.3 Assay Preparations

Siliconized 12 x 75 mm tubes were used for the assay. 30 µl of 10 nM [^{3}H]-R1881 (1 x 10^{-8} M) and 50 µl triamcinolone acetonide (60 µM working solution) were added to all tubes. For the 3 tubes at the beginning of assay and at the end of assay, 100X inert R1881 (30 µl of 1 µM) was also added. These were the nonspecific binding tubes. The tubes were placed in a speed-vac and dried. An aliquot of cytosol was thawed on ice and diluted to the pre-determined optimal protein concentration.

3.5.4 Individual Tubes

For the assay tubes, 10 µl of each concentration of test chemical and control was added, followed by 300 µl of the diluted cytosol. The temperature of the tubes and contents were kept at 4±2°C prior to the addition of the cytosol. The assay tubes were vortexed after additions and incubated at 4±2°C for 16 to 20 hours on a rotator.

3.5.5 Separation of Bound [^{3}H]-R1881 From Free [^{3}H]-R1881

The AR assay tubes were removed from the rotator and placed in an ice-water bath. A repeating pipette was used to add approximately 500 µl of ice cold HAP slurry (60% in 50 mM Tris buffer) to fresh new 12 x 75 mm siliconized assay tubes. 100 µl of each incubation tube was transferred to the appropriate labelled tubes containing the HAP. The tubes were vortexed for approximately 10 seconds at approximately 5 minute intervals for a total of approximately 20 minutes with tubes remaining in the ice-water bath between vortexing. Following the vortexing step, approximately 2 ml of the cold 50 mM Tris buffer was added, quickly vortexed, and centrifuged at 4±2°C for approximately 10 minutes at 700 x g. After centrifugation, the supernatant containing the free [^{3}H]-R1881 was immediately decanted and discarded. The HAP pellet contained the androgen receptor bound [^{3}H]-R1881. Approximately 2 ml of ice-cold 50 mM Tris buffer was added to each tube and vortexed to resuspend the pellet. The tubes were centrifuged again at 4±2°C for approximately 10

minutes at approximately 700 x g. The supernatant was quickly decanted and discarded. The wash and centrifugation steps were repeated three more times. After the final wash, the supernatant was decanted. The assay tubes were allowed to drain briefly for approximately 30 seconds.

3.5.6 Extraction and Quantification of [^{3}H]-R1881 Bound to AR.

Approximately 2 ml of absolute ethanol was added to each assay tube. The tubes were allowed to sit at room temperature for approximately 15 to 20 minutes, vortexing for approximately 10 seconds at approximately 5-minute intervals. The assay tubes were centrifuged for approximately 10 minutes at approximately 700 x g. The supernatant was decanted into a 20 ml scintillation vial containing approximately 14 ml scintillation cocktail (Perkin Elmer Opti-Fluor, cat# 6013199, lot# 47-11241). The vial was capped and shaken. The vials were placed in a scintillation counter (Perkin Elmer Tri-Carb 2910TR Liquid Scintillation Analyzer Model B2910) and each vial was counted for at least one minute with quench correction for determination of DPMs per vial.

Standards (^{3}H, ^{14}C and background) were used to verify accurate counting, and the liquid scintillation analyzer has an enhanced Instrument Performance Assessment (IPA) for monitoring efficiencies, backgrounds, E2/B and Chi-square values for ^{3}H and ^{14}C over the life of the instrument. The most recent IPA time and date stamped data are available on demand for reporting purposes. Each IPA printout includes instrument model, serial number, software version number and calibration standard information.

3.6 Competitive Binding Data Analysis and Interpretation

3.6.1 Analysis and Considerations

The competitive binding assay was functioning correctly if all of the following criteria had been met, according to OPPTS 890.1150:

Increasing concentrations of unlabeled R1881 displaced [^{3}H]-R1881 from the receptor in a manner consistent with one-site competitive binding. Specifically, the curve fitted to the radioinert R1881 data points using non-linear regression descended from 90% to 10% over approximately an 81-fold increase in the concentration of the test chemicals.

Ligand depletion was minimal. Specifically, the ratio of total binding in the absence of competitor to the total amount of [^{3}H]-R1881 added per assay tube was no greater than 15%.

The parameter values (top, bottom, and slope) for R1881 and the concurrent positive control (dexamethasone) were within the tolerance bounds outlined in the OPPTS guideline and are provided below.

The solvent control substance did not alter the sensitivity or reliability of the assay. Specifically, the acceptable limit of ethanol concentration in the assay tube was 3%; the

acceptable limit of DMSO concentration was ≤ 10%. All tubes must have contained equal amounts of solvent.

The test chemical was tested over a concentration range that fully defined the top of the curve (i.e. a range that showed that a top plateau was achieved), and the top was within 25 percentage points of either the solvent control or the value for the lowest concentration of the R1881 standard for that run.

Upper and Lower Limits for Parameters in Competitive Binding Assay Curves for the Standards (Radioinert R1881 and dexamethasone)

Chemical	Parameter	Lower Limit	Upper Limit
R1881 (Standard Curve)	Slope	-1.2	-0.8
	Top (%)	82	114
	Bottom (%)	-2	+2
Dexamethasone (Weak Positive)	Slope	-1.4	-0.6
	Top (%)	87	106
	Bottom (%)	-12	+12

3.6.2 Classification

The classification of a chemical as a binder or non-binder was made on the basis of the average results of three non-concurrent runs, each of which meet the performance criteria and taken together, were consistent with each other, as per OPPTS guideline 890.1150. Each run was classified as "binder," "non-binder," or "equivocal."

A run was classified as "binder" with the ARs if the lowest point on the fitted response curve within the range of the data was less than 50%.

"Percent" refers to binding of the radiolabeled R1881. Thus, "less than 50%" means that less than 50% of the radiolabeled R1881 was bound, or equivalently, that more than 50% of the radiolabeled R1881 had been displaced from the receptor. In other words, a run was classified as "binder" if a Log(IC_{50}) was obtained.

A run was classified as a "non-binder" if the lowest point on the fitted response curve within the range of the data was above 75%.

A run was classified as "equivocal" if the average lowest point on the fitted response curves within the range of the data was above 50% but below 75%.

After each run was classified, the chemical was classified by assigning the following values to each run and averaging across runs:
Binder: 2
Equivocal: 1
Non-binder: 0

Chemical classification, based on the average of all the runs performed for a chemical:
Binder: average ≥ 1.5
Equivocal: 0.5 ≤ average < 1.5
Non-binder: average < 0.5

For example, if a chemical was tested in three runs in one lab and is determined to be interactive in 2 runs and equivocal in 1 run, to classify this chemical one would average 2, 2, and 1 = ~1.67 and the chemical would be considered a "binder" because the average was greater than 1.5.

4.0 RESULTS AND DISCUSSION

4.1 Concentration Range for the Test Substance

In order to identify a suitable top concentration for use in the binding assays, preliminary assessments of precipitation were conducted as described in Sections 3.2. The final concentrations of oxybenzone, octylmethoxycinnamate, octylsalate and octocrylene to assess precipitation were 10^{-4} and 10^{-3} M.

The suitable top concentration of oxybenzone, octylmethoxycinnamate, octylsalate and octocrylene for use in all three valid independent runs was (20-September-2011, 22-September-2011 and 06-October-2011) was 10^{-4} M as precipitation was seen with each test article at 10^{-3} M.

The final concentrations of each test article assessed in the binding assays were: 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3} M for the first valid independent run (20-September-2011) and 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} for the second and third valid independent runs (22-September-2011 and 06-October-2011).

4.2 Binding Assay Acceptance Criteria

In all three valid independent runs of the assay, increasing concentrations of unlabeled R1881 displaced [^{3}H]-R1881 from the receptor in a manner consistent with one-site competitive binding, and the ligand depletion was held below 15%. Also, the solvent did not alter the assay sensitivity or reliability. Finally, the data were within the acceptable ranges specified in Section 3.6.1.

4.3 Results

The suitable top concentration of oxybenzone, octylmethoxycinnamate, octylsalate and octocrylene for use in all three valid independent runs was (20-September-2011, 22-September-2011 and 06-October-2011) was 10^{-4} M as precipitation was seen with each test article at 10^{-3} M.

Four independent runs of the binding assay were conducted because in the third run (29-September-2011), the DPM values were substantially different from typical data. The data is located in the study binder but is not included in the analysis of this report.

In the first valid independent run (20-September-2011), the mean specific binding was > 75% at every soluble concentration tested for octylmethoxycinnamate, resulting in a classification of "non-binder." The mean specific binding for oxybenzone, octylsalate and octocrylene was 64.9%, 53.9% and 54.2% of control at 10^{-4}, respectively, and > 75% at all lower concentrations. The mean specific binding for these three test articles at 10^{-3} M was not assessed because precipitation was observed at this concentration. These data result in oxybenzone, octylsalate and octocrylene being classified as "equivocal" for this run. The weak positive control dexamethasone had a $LogIC_{50}$ of -4.4 M while the $LogIC_{50}$ of R1881 was -8.9 M.

In the second valid independent run (22-September-2011), the mean specific binding was > 75% at every soluble concentration tested for octylmethoxycinnamate, resulting in a classification of "non-binder." The mean specific binding for oxybenzone, octylsalate and octocrylene were 62.6%, 52.9% and 50.4% of control at 10^{-4}, respectively. These data result in oxybenzone, octylsalate and octocrylene being classified as "equivocal" for this run. The weak positive control dexamethasone had a $LogIC_{50}$ of -4.3 M while the $LogIC_{50}$ of R1881 was -10.1 M.

Finally, in the third valid independent run (06-October-2011), the mean specific binding was > 75% at every soluble concentration tested for octylmethoxycinnamate, resulting in the classification as a "non-binder." The mean specific binding for oxybenzone and octocrylene were 61.2% and 51.3% of control at 10^{-4}, respectively. These data result in oxybenzone and octocrylene being classified as "equivocal" for this run. The mean specific binding for octylsalate was 38.4% of control at 10^{-4} M, resulting in a classification of "binder" with a $LogIC_{50}$ of -4.8 M and an RBA of 0.5. The weak positive control dexamethasone had a $LogIC_{50}$ of -4.5 M while the $LogIC_{50}$ of R1881 was -9.0 M.

The mean relative binding affinity, or RBA (calculated by dividing the $LogIC_{50}$ of the control/test material by the $LogIC_{50}$ of the positive control R1881) was 0.5 for dexamethasone. As oxybenzone, octylmethoxycinnamate, octylsalate and octocrylene were not classified as an overall "binder" (mean specific binding $\geq$ 50%), the RBA could not be calculated.

5.0 CONCLUSIONS

Octylmethoxycinnamate was classified as a "non-binder" in all three independent runs and thus has a final classification of "non-binder." Oxybenzone and octocrylene were classified as "equivocal" in all three independent runs and thus have a final classification of "equivocal." Finally, octylsalate was classified as "equivocal" in the first and second valid independent run, and classified as a "binder" in the third valid independent run, resulting in a final classification of "equivocal."

6.0 REFERENCES

Endocrine Disruptor Screening Program Test Guidelines. *OPPTS 890.1150: Androgen Receptor Binding (Rat Prostate Cytosol).* EPA 640-C-09-003. October, 2009.

TABLES SECTION

TABLE 1 Results of 1st Valid Binding Assay – Controls – September 20, 2011

Test Material	Concentration (Log[M])	Specific Binding (%)	Standard Deviation	Standard Error of Mean	% Coefficient of Variation
R1881 (NSB)	-6	0.0	1.7	0.7	N/A
	-7	3.1	1.0	0.6	31.1
	-8	13.1	1.5	0.9	11.6
	-9	55.5	1.5	0.9	2.7
	-10	92.6	2.9	1.7	3.1
	-11	96.0	3.1	1.8	3.3
Dexamethasone	-3	2.9	2.9	1.6	98.3
	-4	29.4	2.3	1.3	7.9
	-5	75.6	4.5	2.6	5.9
	-6	96.5	2.2	1.3	2.3
	-7	102.4	3.3	1.9	3.3
	-8	98.7	2.5	1.5	2.6
	-9	98.5	2.2	1.3	2.2
	-10	93.5	5.0	2.9	5.4

TABLE 2 Results of 1st Valid Binding Assay – Test Articles – September 20, 2011

Test Material	Concentration (Log[M])	Specific Binding (%)	Standard Deviation	Standard Error of Mean	% Coefficient of Variation
Oxybenzone	-3	29.3	0.8	0.5	2.9
	-4	64.9	2.4	1.4	3.6
	-5	92.1	4.6	2.7	5.0
	-6	102.1	2.8	1.6	2.8
	-7	99.5	4.7	2.7	4.7
	-8	99.9	1.4	0.8	1.4
	-9	98.6	1.2	0.7	1.2
	-10	99.2	4.5	2.6	4.5
Octyl-methoxycinnamate	-3	81.1	3.2	1.8	3.9
	-4	84.5	3.6	2.1	4.3
	-5	92.8	1.8	1.0	1.9
	-6	96.4	6.5	3.8	6.8
	-7	100.7	7.4	4.3	7.3
	-8	100.2	2.3	1.3	2.2
	-9	98.7	1.7	1.0	1.7
	-10	99.8	1.2	0.7	1.2
Octylsalate	-3	31.7	3.1	1.8	9.9
	-4	53.9	5.1	2.9	9.5
	-5	85.4	4.6	2.6	5.4
	-6	89.1	4.9	2.8	5.5
	-7	90.0	1.1	0.6	1.2
	-8	94.3	7.9	4.6	8.4
	-9	101.3	2.7	1.6	2.7
	-10	101.9	4.0	2.3	4.0
Octocrylene	-3	45.4	1.2	0.7	2.6
	-4	54.2	4.4	2.6	8.2
	-5	82.3	1.8	1.0	2.1
	-6	99.0	4.4	2.6	4.5
	-7	98.4	3.9	2.3	4.0
	-8	102.6	0.6	0.3	0.6
	-9	99.2	2.0	1.1	2.0
	-10	100.7	2.6	1.5	2.6

Red lettering indicates where significant precipitation of test material was observed.

TABLE 3 1st Valid Run - Upper and Lower Parameters in Competitive Assay Binding Curves for the Standards – September 20, 2011

Parameter	Unit	R1881	Dexamethasone
Bottom Plateau Level	% binding	1	-2
Top Plateau Level	% binding	98	99
Hill Slope	$Log_{10}(M)^{-1}$	-1.0	-0.9

TABLE 4 Results of 2nd Valid Binding Assay – Controls – September 22, 2011

Test Material	Concentration (Log[M])	Specific Binding (%)	Standard Deviation	Standard Error of Mean	% Coefficient of Variation
R1881 (NSB)	-6	0.0	2.3	0.9	N/A
	-7	1.8	1.6	0.9	88.1
	-8	3.8	0.6	0.4	16.5
	-9	12.3	0.9	0.5	7.3
	-10	53.2	1.8	1.0	3.3
	-11	98.4	2.7	1.6	2.8
Dexamethasone	-3	5.1	2.0	1.2	39.9
	-4	33.0	0.4	0.3	1.3
	-5	80.9	1.0	0.6	1.2
	-6	97.1	2.4	1.4	2.5
	-7	96.4	2.7	1.5	2.8
	-8	99.5	1.2	0.7	1.2
	-9	101.1	3.3	1.9	3.3
	-10	97.1	0.9	0.5	0.9

TABLE 5 Results of 2nd Binding Assay – Test Articles – September 22, 2011

Test Material	Concentration (Log[M])	Specific Binding (%)	Standard Deviation	Standard Error of Mean	% Coefficient of Variation
Oxybenzone	-4	62.6	0.7	0.4	1.1
	-5	92.6	5.4	3.1	5.8
	-6	95.2	4.0	2.3	4.2
	-7	92.0	7.9	4.6	8.6
	-8	92.2	4.5	2.6	4.9
	-9	93.8	4.3	2.5	4.6
	-10	87.5	10.0	5.8	11.4
	-11	95.0	4.1	2.4	4.3
Octyl-methoxycinnamate	-4	81.9	3.9	2.2	4.7
	-5	94.3	0.9	0.5	0.9
	-6	97.5	2.6	1.5	2.6
	-7	97.1	5.4	3.1	5.6
	-8	96.5	1.3	0.8	1.4
	-9	95.9	3.5	2.0	3.7
	-10	94.0	5.7	3.3	6.0
	-11	94.1	0.7	0.4	0.8
Octylsalate	-4	52.9	2.4	1.4	4.5
	-5	92.1	3.6	2.1	3.9
	-6	96.5	4.0	2.3	4.2
	-7	98.0	1.5	0.9	1.6
	-8	95.7	2.5	1.4	2.6
	-9	96.7	2.8	1.6	2.9
	-10	92.4	10.0	5.8	10.8
	-11	92.3	1.6	0.9	1.7
Octocrylene	-4	50.4	11.7	6.7	23.1
	-5	74.6	2.2	1.3	2.9
	-6	91.2	1.5	0.9	1.6
	-7	96.5	3.5	2.0	3.6
	-8	94.2	1.9	1.1	2.0
	-9	99.5	4.0	2.3	4.0
	-10	97.1	3.4	2.0	3.5
	-11	98.1	4.4	2.6	4.5

TABLE 6 Results of 2nd Binding Assay - Upper and Lower Parameters in Competitive Assay Binding Curves for the Standards – September 22, 2011

Parameter	Unit	R1881	Dexamethasone
Bottom Plateau Level	% binding	1	0
Top Plateau Level	% binding	114	99
Hill Slope	$Log_{10}(M)^{-1}$	-0.9	-1.0

TABLE 7 Results of 3rd Binding Assay – Controls – October 06, 2011

Test Material	Concentration (Log[M])	Specific Binding (%)	Standard Deviation	Standard Error of Mean	% Coefficient of Variation
R1881 (NSB)	-6	0.0	1.3	0.5	N/A
	-7	2.8	0.4	0.2	14.7
	-8	7.5	1.8	1.0	24.0
	-9	49.3	0.8	0.5	1.7
	-10	86.1	3.5	2.0	4.1
	-11	95.8	6.4	3.7	6.7
Dexamethasone	-3	0.5	1.1	0.7	211.4
	-4	19.2	0.4	0.2	2.0
	-5	70.8	3.3	1.9	4.6
	-6	88.1	4.5	2.6	5.1
	-7	93.7	2.2	1.3	2.4
	-8	95.8	2.6	1.5	2.7
	-9	92.9	3.9	2.3	4.2
	-10	95.6	1.6	0.9	1.7

TABLE 8 Results of 3rd Binding Assay – Test Articles – October 06, 2011

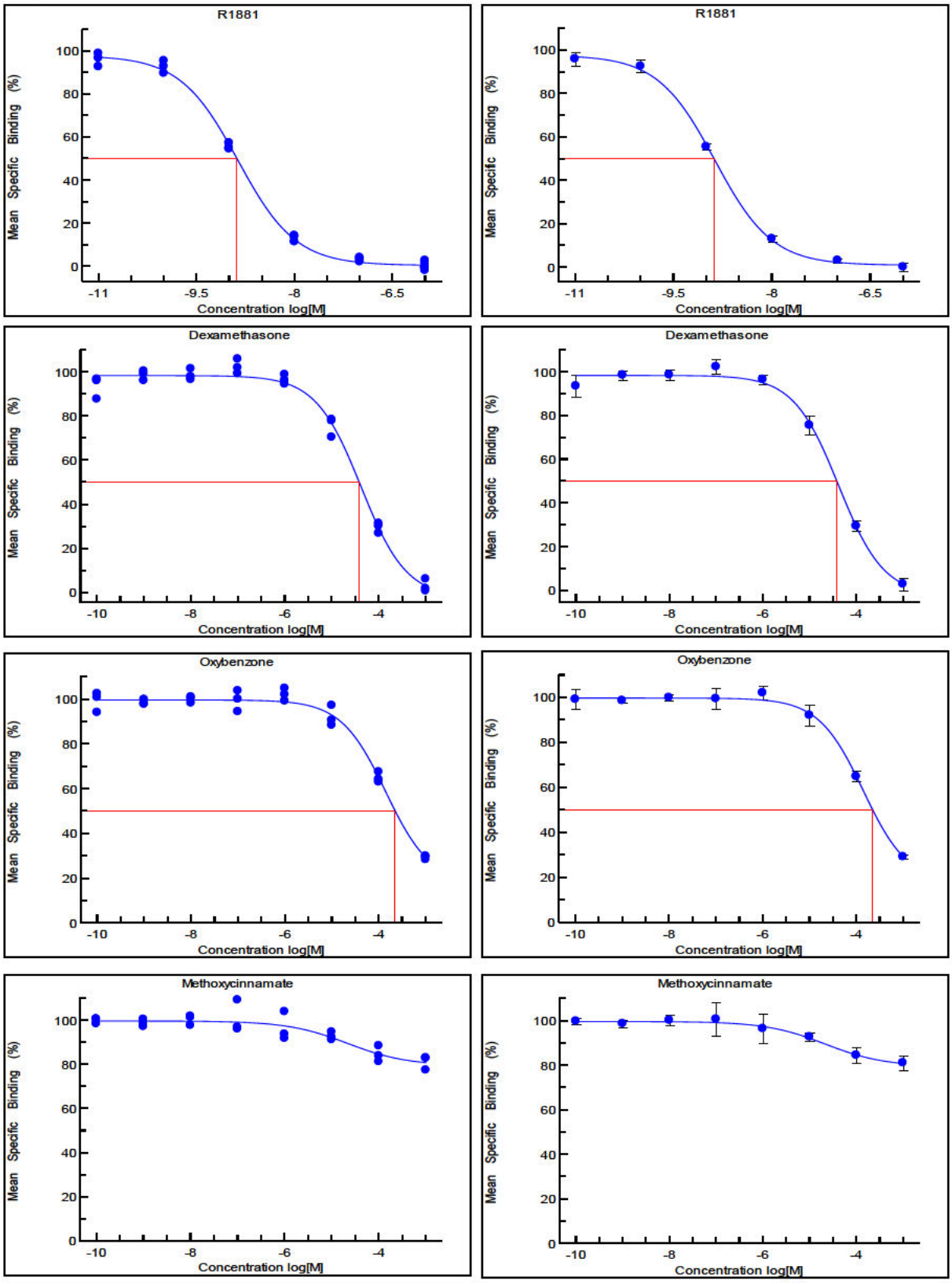
Test Material	Concentration (Log[M])	Specific Binding (%)	Standard Deviation	Standard Error of Mean	% Coefficient of Variation
Oxybenzone	-4	61.2	6.8	3.9	11.2
	-5	92.0	5.2	3.0	5.7
	-6	96.2	0.9	0.5	1.0
	-7	97.5	3.8	2.2	3.9
	-8	103.4	3.6	2.1	3.5
	-9	100.4	2.7	1.6	2.7
	-10	100.8	0.9	0.5	0.9
	-11	101.2	4.8	2.8	4.7
Octyl-methoxycinnamate	-4	89.3	2.4	1.4	2.6
	-5	96.4	3.5	2.0	3.6
	-6	99.0	0.3	0.2	0.3
	-7	95.7	8.5	4.9	8.9
	-8	100.3	2.6	1.5	2.6
	-9	97.7	1.6	0.9	1.6
	-10	100.9	3.1	1.8	3.0
	-11	100.9	5.1	2.9	5.0
Octylsalate	-4	38.4	21.4	12.3	55.6
	-5	84.0	5.5	3.2	6.6
	-6	97.3	3.2	1.8	3.3
	-7	91.6	3.3	1.9	3.6
	-8	73.1	16.3	9.4	22.2
	-9	89.4	5.2	3.0	5.8
	-10	93.0	2.6	1.5	2.8
	-11	97.4	2.6	1.5	2.7
Octocrylene	-4	51.3	1.5	0.9	3.0
	-5	84.8	2.2	1.3	2.6
	-6	98.6	6.9	4.0	7.0
	-7	97.3	7.2	4.1	7.4
	-8	100.0	1.8	1.0	1.8
	-9	99.0	3.5	2.0	3.5
	-10	97.4	0.3	0.2	0.3
	-11	96.4	7.7	4.4	8.0

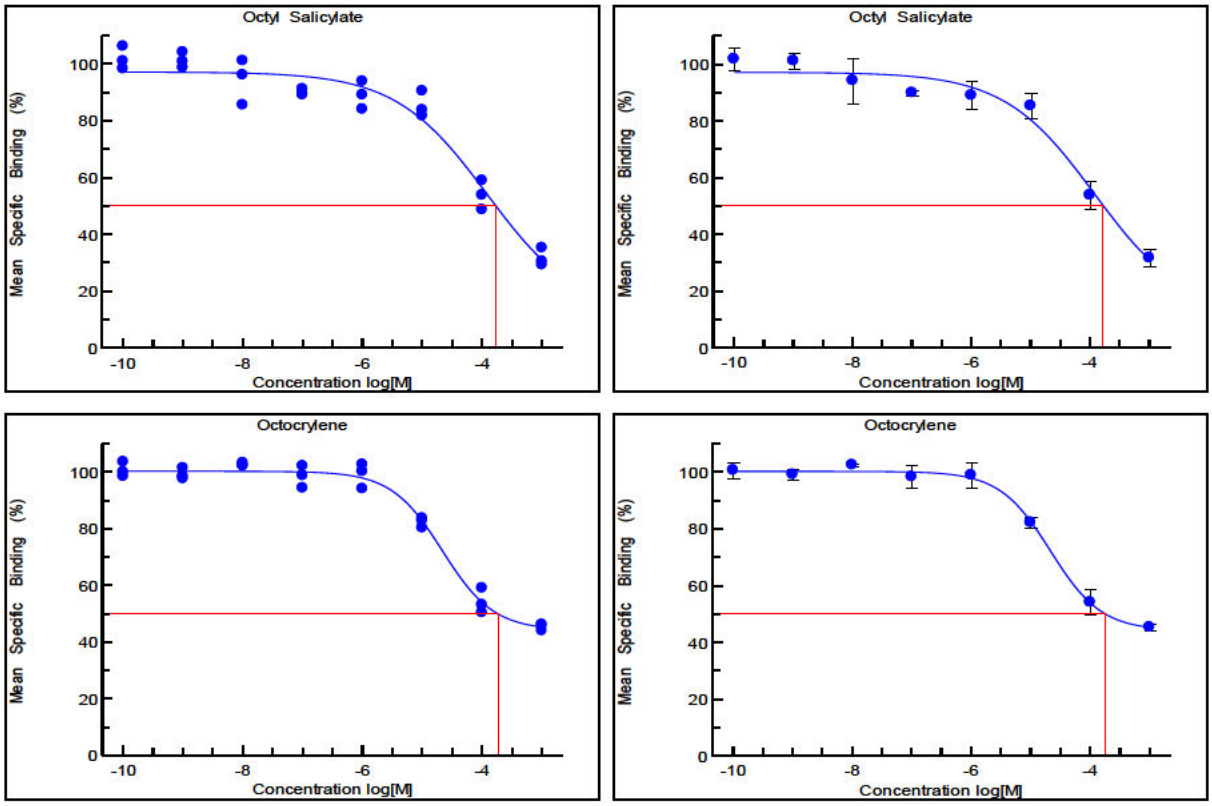
TABLE 9 Results of 3rd Binding Assay - Upper and Lower Parameters in Competitive Assay Binding Curves for the Standards – October 06, 2011

Parameter	Unit	R1881	Dexamethasone
Bottom Plateau Level	% binding	0	-3
Top Plateau Level	% binding	96	94
Hill Slope	$Log_{10}(M)^{-1}$	-1.0	-1.0

FIGURES SECTION

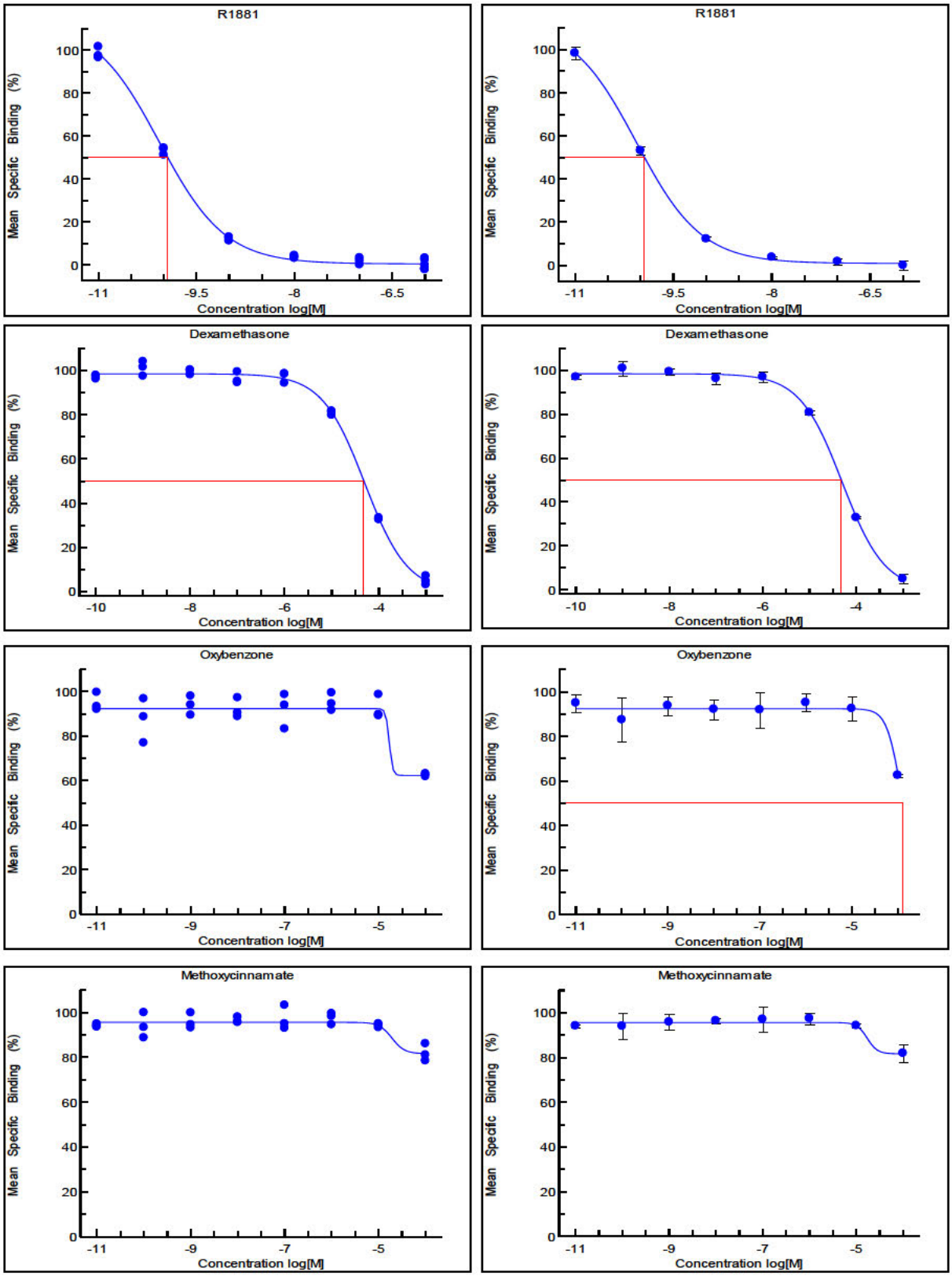
FIGURE 1 1st Valid Run % Specific Binding for Test Articles and Controls – September 20, 2011

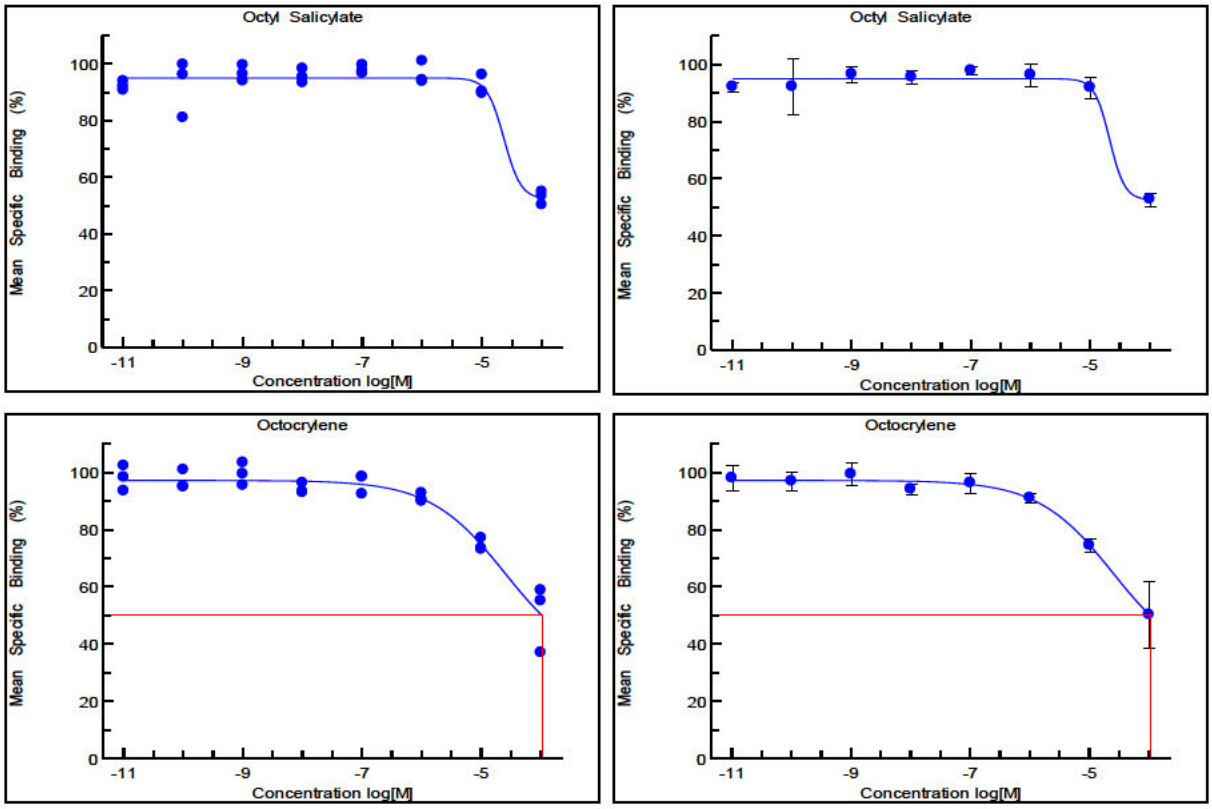




The graphs on the left show individual replicates while graphs on the right show mean data (Means±Standard Deviation) from the first independent run of the assay (n=3).

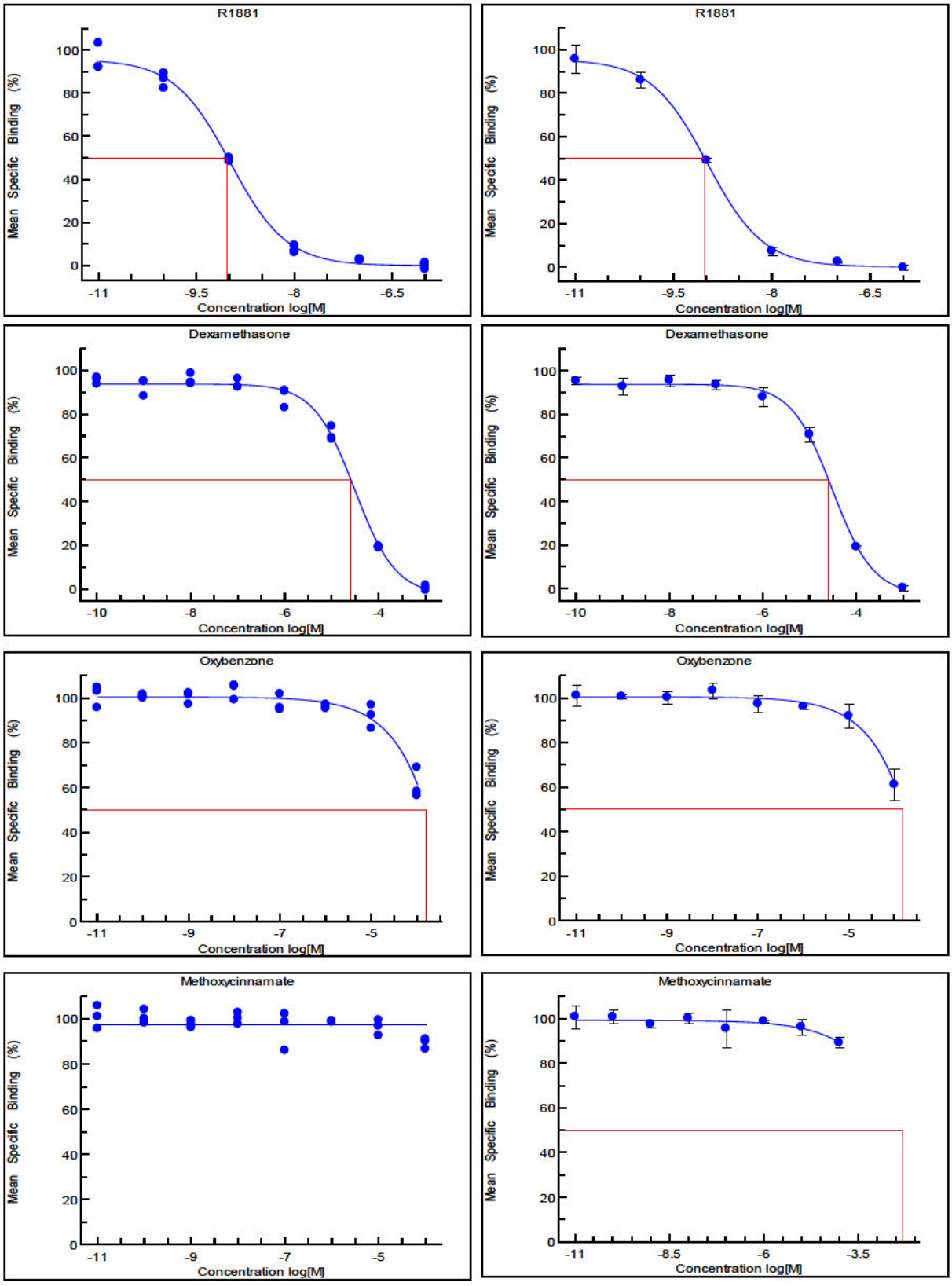
FIGURE 2 2nd Valid Run % Specific Binding for Test Articles and Controls – September 22, 2011

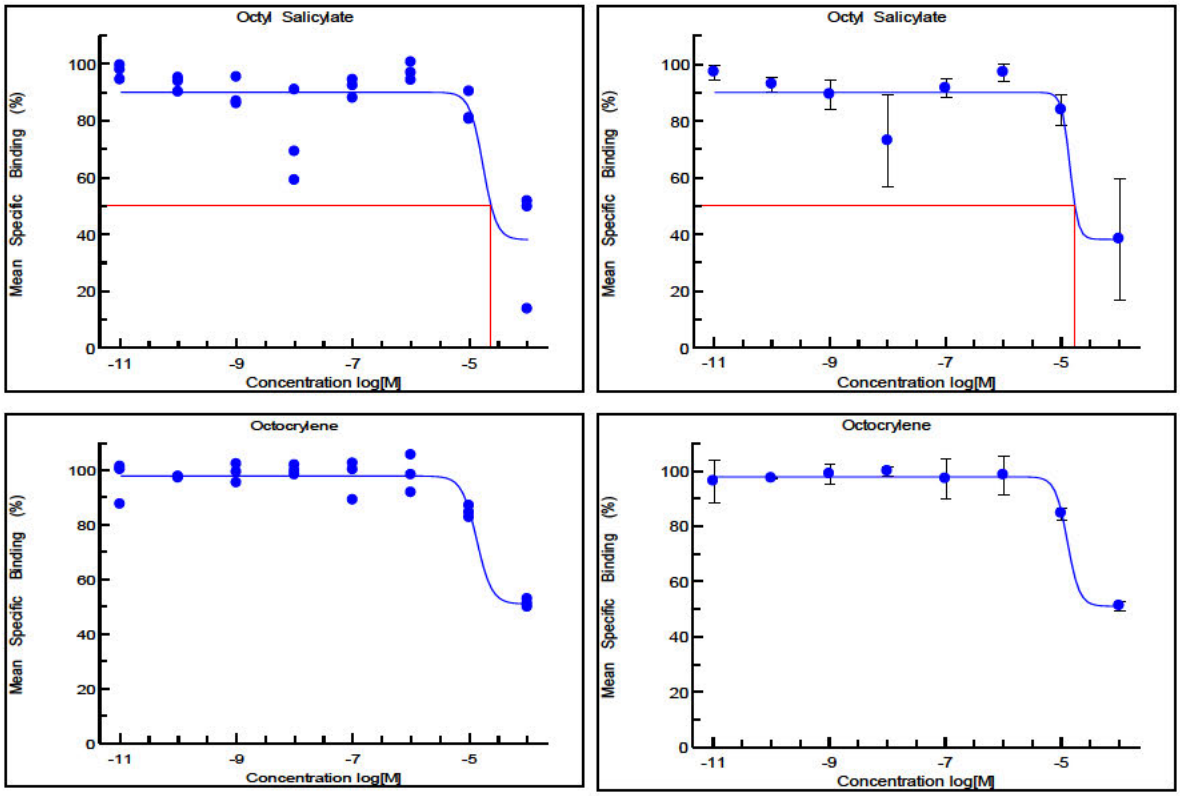




The graphs on the left show individual replicates while graphs on the right show mean data (Means±Standard Deviation) from the third independent run of the assay (n=3).

FIGURE 3 3rd Valid Run % Specific Binding for Controls and Test Articles – October 06, 2011





The graphs on the left show individual replicates while graphs on the right show mean data (Means±Standard Deviation) from the third independent run of the assay (n=3).

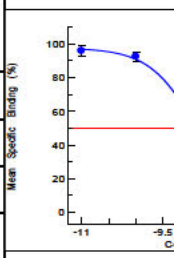
APPENDICES SECTION

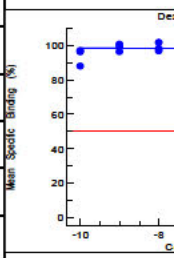
APPENDIX 1 Raw and Normalized Data Valid Run 1 – September 20, 2011

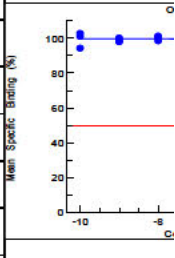
1	Experiment Date:	20-Sep-11		Study Number:	9070-100107ARB				Assays Conducted by:			[redacted]	
2	Test substance:	Oxybenzone											
3	10/27/2011 11:08												
4													
5			ug protein/assay tube =										
6													

Tube	Sample Type	DPM (1mL)	Specific Binding DPM (100 uL) - NSB	Total Specific Binding (300 uL)	Mean
1	Total Activity (Master Mix)	56542	–	56542.0	55901.7
2		55731	–	55731.0	
3		55020	–	55020.0	
4		57201	–	57201.0	
5		55440	–	55440.0	
6		55476	–	55476.0	
7	Total Binding (Solvent Control)	2095	1838.7	5516	5167.5
8		1949	1692.7	5078	
9		2038	1781.7	5345	
10		1930	1673.7	5021	
11		1982	1725.7	5177	
12		1879	1622.7	4868	

DPM (1mL) from LSC	Tube	Sample Type	Concentration log[M]	Specific Binding DPM (1mL) NSB	Total Specific Binding (3mL)	Specific Binding (%)	Residual	Squared Residual	Mean Specific Binding (%)	Standard Deviation	SEM	% CV	% Ligand Bound vs. Total Activity
242.0	13	R1881 (NSB)	-6	-14.3	-43.0	-0.8	-1.6	2.4	0.0	1.7	0.7	1.5E+17	1.3
223.0	14		-6	-33.3	-100.0	-1.9	-2.7	7.1					1.2
245.0	15		-6	-11.3	-34.0	-0.7	-1.4	1.9					1.3
282.0	16		-6	25.7	77.0	1.5	0.8	0.6					1.5
302.0	17		-6	45.7	137.0	2.7	1.9	3.7					1.6
244.0	18		-6	-12.3	-37.0	-0.7	-1.4	2.1					1.3
292.0	19	R1881	-7	35.7	107.0	2.1	0.1	0.0	3.1	1.0	0.6	31.1	1.6
325.0	20		-7	68.7	206.0	4.0	2.0	4.1					1.7
313.0	21		-7	56.7	170.0	3.3	1.3	1.7					1.7
452.0	22	R1881	-8	195.7	587.0	11.4	-1.1	1.2	13.1	1.5	0.9	11.6	2.4
501.0	23		-8	244.7	734.0	14.2	1.7	3.0					2.7
492.0	24		-8	235.7	707.0	13.7	1.2	1.5					2.6
1201.0	25	R1881	-9	944.7	2834.0	54.8	-1.4	2.0	55.5	1.5	0.9	2.7	6.4
1195.0	26		-9	938.7	2816.0	54.5	-1.8	3.1					6.4
1242.0	27		-9	985.7	2957.0	57.2	1.0	0.9					6.7
1800.0	28	R1881	-10	1543.7	4631.0	89.6	-1.3	1.8	92.6	2.9	1.7	3.1	9.7
1899.0	29		-10	1642.7	4928.0	95.4	4.4	19.6					10.2
1854.0	30		-10	1597.7	4793.0	92.8	1.8	3.3					9.9
1852.0	31	R1881	-11	1595.7	4787.0	92.6	-4.6	21.0	96.0	3.1	1.8	3.3	9.9
1921.0	32		-11	1664.7	4994.0	96.6	-0.6	0.3					10.3
1958.0	33		-11	1701.7	5105.0	98.8	1.6	2.5					10.5
269.0	37	Dexamethasone	-3	12.7	38.0	0.7	-2.4	5.7	2.9	2.9	1.6	98.3	1.4
362.0	38		-3	105.7	317.0	6.1	3.0	9.1					1.9
288.0	39		-3	31.7	95.0	1.8	-1.3	1.6					1.5
719.0	40	Dexamethasone	-4	462.7	1388.0	26.9	-2.1	4.4	29.4	2.3	1.3	7.9	3.9
775.0	41		-4	518.7	1556.0	30.1	1.2	1.4					4.2
796.0	42		-4	539.7	1619.0	31.3	2.4	5.7					4.3
1597.0	43	Dexamethasone	-5	1340.7	4022.0	77.8	1.3	1.8	75.6	4.5	2.6	5.9	8.6
1608.0	44		-5	1351.7	4055.0	78.5	2.0	3.9					8.6
1469.0	45		-5	1212.7	3638.0	70.4	-6.1	37.0					7.9
1959.0	46	Dexamethasone	-6	1702.7	5108.0	98.8	3.7	13.7	96.5	2.2	1.3	2.3	10.5
1914.0	47		-6	1657.7	4973.0	96.2	1.1	1.2					10.3
1884.0	48		-6	1627.7	4883.0	94.5	-0.6	0.4					10.1
2012.0	49	Dexamethasone	-7	1755.7	5267.0	101.9	3.8	14.4	102.4	3.3	1.9	3.3	10.8
2080.0	50		-7	1823.7	5471.0	105.9	7.7	59.9					11.2
1966.0	51		-7	1709.7	5129.0	99.3	1.1	1.3					10.6
2004.0	52	Dexamethasone	-8	1747.7	5243.0	101.5	2.9	8.7	98.7	2.5	1.5	2.6	10.8
1920.0	53		-8	1663.7	4991.0	96.6	-1.9	3.7					10.3
1943.0	54		-8	1686.7	5060.0	97.9	-0.6	0.4					10.4
1986.0	55	Dexamethasone	-9	1729.7	5189.0	100.4	1.8	3.4	98.5	2.2	1.3	2.2	10.7
1963.0	56		-9	1706.7	5120.0	99.1	0.5	0.3					10.5
1912.0	57		-9	1655.7	4967.0	96.1	-2.4	6.0					10.3
1923.0	58	Dexamethasone	-10	1666.7	5000.0	96.8	-1.8	3.3	93.5	5.0	2.9	5.4	10.3
1767.0	59		-10	1510.7	4532.0	87.7	-10.9	118.2					9.5
1911.0	60		-10	1654.7	4964.0	96.1	-2.5	6.3					10.3
771.0	61	Oxybenzone	-3	514.7	1544.0	29.9	0.6	0.3	29.3	0.8	0.5	2.9	4.1
767.0	62		-3	510.7	1532.0	29.6	0.3	0.1					4.1
744.0	63		-3	487.7	1463.0	28.3	-1.0	1.0					4.0
1362.0	64	Oxybenzone	-4	1105.7	3317.0	64.2	-0.6	0.3	64.9	2.4	1.4	3.6	7.3
1342.0	65		-4	1085.7	3257.0	63.0	-1.7	3.0					7.2
1420.0	66		-4	1163.7	3491.0	67.6	2.8	7.8					7.6
1779.0	67	Oxybenzone	-5	1522.7	4568.0	88.4	-4.6	21.3	92.1	4.6	2.7	5.0	9.5
1932.0	68		-5	1675.7	5027.0	97.3	4.3	18.2					10.4
1817.0	69		-5	1560.7	4682.0	90.6	-2.4	5.8					9.8
2063.0	70	Oxybenzone	-6	1806.7	5420.0	104.9	5.9	34.7	102.1	2.8	1.6	2.8	11.1
2015.0	71		-6	1758.7	5276.0	102.1	3.1	9.6					10.8
1966.0	72		-6	1709.7	5129.0	99.3	0.3	0.1					10.6
1884.0	73	Oxybenzone	-7	1627.7	4883.0	94.5	-5.3	28.1	99.5	4.7	2.7	4.7	10.1
2045.0	74		-7	1788.7	5366.0	103.8	4.0	16.4					11.0
1980.0	75		-7	1723.7	5171.0	100.1	0.3	0.1					10.6
1986.0	76	Oxybenzone	-8	1729.7	5189.0	100.4	0.5	0.3	99.9	1.4	0.8	1.4	10.7
1950.0	77		-8	1693.7	5081.0	98.3	-1.6	2.5					10.5
1997.0	78		-8	1740.7	5222.0	101.1	1.2	1.4					10.7
1941.0	79	Oxybenzone	-9	1684.7	5054.0	97.8	-2.1	4.4	98.6	1.2	0.7	1.2	10.4
1946.0	80		-9	1689.7	5069.0	98.1	-1.8	3.3					10.4
1978.0	81		-9	1721.7	5165.0	100.0	0.0	0.0					10.6
2023.0	82	Oxybenzone	-10	1766.7	5300.0	102.6	2.7	7.1	99.2	4.5	2.6	4.5	10.9
1877.0	83		-10	1620.7	4862.0	94.1	-5.8	33.8					10.1
1994.0	84		-10	1737.7	5213.0	100.9	1.0	0.9					10.7





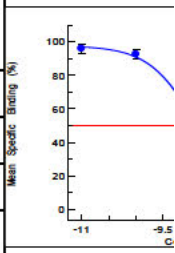


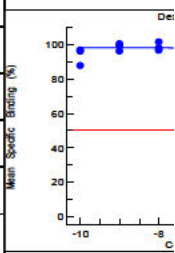
APPENDIX 1 (continued) Raw and Normalized Data Run 1 – September 20, 2011

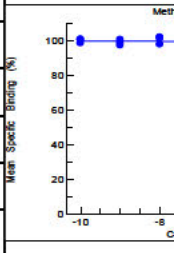
1	Experiment Date:	20-Sep-11		Study Number:	9070-100107ARB				Assays Conducted by:			[redacted]		
2	Test substance:	Methoxycinnamate												
3	10/27/2011 11:08													
4														
5			ug protein/assay tube =											
6														

Tube	Sample Type	DPM (1mL)	Specific Binding DPM (100 uL) - NSB	Total Specific Binding (300 uL)	Mean
1	Total Activity (Master Mix)	56542	--	56542.0	55901.7
2		55731	--	55731.0	
3		55020	--	55020.0	
4		57201	--	57201.0	
5		55440	--	55440.0	
6		55476	--	55476.0	
7	Total Binding (Solvent Control)	2095	1838.7	5516	5167.5
8		1949	1692.7	5078	
9		2038	1781.7	5345	
10		1930	1673.7	5021	
11		1982	1725.7	5177	
12		1879	1622.7	4868	

DPM (1mL) from LSC	Tube	Sample Type	Concentration log[M]	Specific Binding DPM (1mL) NSB	Total Specific Binding (3mL)	Specific Binding (%)	Residual	Squared Residual	Mean Specific Binding (%)	Standard Deviation	SEM	% CV	% Ligand Bound vs. Total Activity
242.0	13	R1881 (NSB)	-6	-14.3	-43.0	-0.8	-1.6	2.4	0.0	1.7	0.7	1.5E+17	1.3
223.0	14		-6	-33.3	-100.0	-1.9	-2.7	7.1					1.2
245.0	15		-6	-11.3	-34.0	-0.7	-1.4	1.9					1.3
282.0	16		-6	25.7	77.0	1.5	0.8	0.6					1.5
302.0	17		-6	45.7	137.0	2.7	1.9	3.7					1.6
244.0	18		-6	-12.3	-37.0	-0.7	-1.4	2.1					1.3
292.0	19	R1881	-7	35.7	107.0	2.1	0.1	0.0	3.1	1.0	0.6	31.1	1.6
325.0	20		-7	68.7	206.0	4.0	2.0	4.1					1.7
313.0	21		-7	56.7	170.0	3.3	1.3	1.7					1.7
452.0	22	R1881	-8	195.7	587.0	11.4	-1.1	1.2	13.1	1.5	0.9	11.6	2.4
501.0	23		-8	244.7	734.0	14.2	1.7	3.0					2.7
492.0	24		-8	235.7	707.0	13.7	1.2	1.5					2.6
1201.0	25	R1881	-9	944.7	2834.0	54.8	-1.4	2.0	55.5	1.5	0.9	2.7	6.4
1195.0	26		-9	938.7	2816.0	54.5	-1.8	3.1					6.4
1242.0	27		-9	985.7	2957.0	57.2	1.0	0.9					6.7
1800.0	28	R1881	-10	1543.7	4631.0	89.6	-1.3	1.8	92.6	2.9	1.7	3.1	9.7
1899.0	29		-10	1642.7	4928.0	95.4	4.4	19.6					10.2
1854.0	30		-10	1597.7	4793.0	92.8	1.8	3.3					9.9
1852.0	31	R1881	-11	1595.7	4787.0	92.6	-4.6	21.0	96.0	3.1	1.8	3.3	9.9
1921.0	32		-11	1664.7	4994.0	96.6	-0.6	0.3					10.3
1958.0	33		-11	1701.7	5105.0	98.8	1.6	2.5					10.5
269.0	37	Dexamethasone	-3	12.7	38.0	0.7	-2.4	5.7	2.9	2.9	1.6	98.3	1.4
362.0	38		-3	105.7	317.0	6.1	3.0	9.1					1.9
288.0	39		-3	31.7	95.0	1.8	-1.3	1.6					1.5
719.0	40	Dexamethasone	-4	462.7	1388.0	26.9	-2.1	4.4	29.4	2.3	1.3	7.9	3.9
775.0	41		-4	518.7	1556.0	30.1	1.2	1.4					4.2
796.0	42		-4	539.7	1619.0	31.3	2.4	5.7					4.3
1597.0	43	Dexamethasone	-5	1340.7	4022.0	77.8	1.3	1.8	75.6	4.5	2.6	5.9	8.6
1608.0	44		-5	1351.7	4055.0	78.5	2.0	3.9					8.6
1469.0	45		-5	1212.7	3638.0	70.4	-6.1	37.0					7.9
1959.0	46	Dexamethasone	-6	1702.7	5108.0	98.8	3.7	13.7	96.5	2.2	1.3	2.3	10.5
1914.0	47		-6	1657.7	4973.0	96.2	1.1	1.2					10.3
1884.0	48		-6	1627.7	4883.0	94.5	-0.6	0.4					10.1
2012.0	49	Dexamethasone	-7	1755.7	5267.0	101.9	3.8	14.4	102.4	3.3	1.9	3.3	10.8
2080.0	50		-7	1823.7	5471.0	105.9	7.7	59.9					11.2
1966.0	51		-7	1709.7	5129.0	99.3	1.1	1.3					10.6
2004.0	52	Dexamethasone	-8	1747.7	5243.0	101.5	2.9	8.7	98.7	2.5	1.5	2.6	10.8
1920.0	53		-8	1663.7	4991.0	96.6	-1.9	3.7					10.3
1943.0	54		-8	1686.7	5060.0	97.9	-0.6	0.4					10.4
1986.0	55	Dexamethasone	-9	1729.7	5189.0	100.4	1.8	3.4	98.5	2.2	1.3	2.2	10.7
1963.0	56		-9	1706.7	5120.0	99.1	0.5	0.3					10.5
1912.0	57		-9	1655.7	4967.0	96.1	-2.4	6.0					10.3
1923.0	58	Dexamethasone	-10	1666.7	5000.0	96.8	-1.8	3.3	93.5	5.0	2.9	5.4	10.3
1767.0	59		-10	1510.7	4532.0	87.7	-10.9	118.2					9.5
1911.0	60		-10	1654.7	4964.0	96.1	-2.5	6.3					10.3
1686.0	61	Methoxycinnamate	-3	1429.7	4289.0	83.0	2.0	4.2	81.1	3.2	1.8	3.9	9.0
1682.0	62		-3	1425.7	4277.0	82.8	1.8	3.3					9.0
1590.0	63		-3	1333.7	4001.0	77.4	-3.5	12.4					8.5
1779.0	64	Methoxycinnamate	-4	1522.7	4568.0	88.4	3.6	13.0	84.5	3.6	2.1	4.3	9.5
1656.0	65		-4	1399.7	4199.0	81.3	-3.5	12.5					8.9
1701.0	66		-4	1444.7	4334.0	83.9	-0.9	0.9					9.1
1887.0	67	Methoxycinnamate	-5	1630.7	4892.0	94.7	2.4	6.0	92.8	1.8	1.0	1.9	10.1
1827.0	68		-5	1570.7	4712.0	91.2	-1.0	1.1					9.8
1848.0	69		-5	1591.7	4775.0	92.4	0.2	0.0					9.9
2046.0	70	Methoxycinnamate	-6	1789.7	5369.0	103.9	6.3	40.1	96.4	6.5	3.8	6.8	11.0
1870.0	71		-6	1613.7	4841.0	93.7	-3.9	15.1					10.0
1837.0	72		-6	1580.7	4742.0	91.8	-5.8	33.7					9.9
1910.0	73	Methoxycinnamate	-7	1653.7	4961.0	96.0	-3.3	10.9	100.7	7.4	4.3	7.3	10.3
2137.0	74		-7	1880.7	5642.0	109.2	9.9	97.6					11.5
1924.0	75		-7	1667.7	5003.0	96.8	-2.5	6.2					10.3
2010.0	76	Methoxycinnamate	-8	1753.7	5261.0	101.8	2.1	4.4	100.2	2.3	1.3	2.2	10.8
1938.0	77		-8	1681.7	5045.0	97.6	-2.1	4.3					10.4
1999.0	78		-8	1742.7	5228.0	101.2	1.5	2.1					10.7
1954.0	79	Methoxycinnamate	-9	1697.7	5093.0	98.6	-1.2	1.5	98.7	1.7	1.0	1.7	10.5
1986.0	80		-9	1729.7	5189.0	100.4	0.6	0.4					10.7
1929.0	81		-9	1672.7	5018.0	97.1	-2.7	7.2					10.4
1987.0	82	Methoxycinnamate	-10	1730.7	5192.0	100.5	0.7	0.4	99.8	1.2	0.7	1.2	10.7
1951.0	83		-10	1694.7	5084.0	98.4	-1.4	2.0					10.5
1989.0	84		-10	1732.7	5198.0	100.6	0.8	0.6					10.7





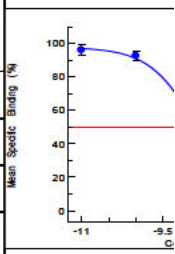


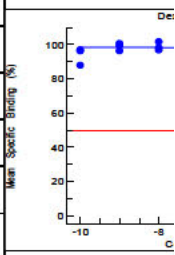
APPENDIX 1 (continued) Raw and Normalized Data Run 1 – September 20, 2011

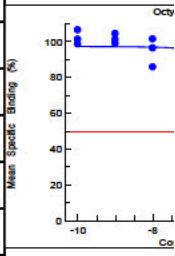
1	Experiment Date:	20-Sep-11		Study Number:	9070-100107ARB				Assays Conducted by:			[redacted]		
2	Test substance:	Octyl Salicylate												
3	10/27/2011 11:08													
4														
5			ug protein/assay tube =											
6														

Tube	Sample Type	DPM (1mL)	Specific Binding DPM (100 uL) - NSB	Total Specific Binding (300 uL)	Mean
1	Total Activity (Master Mix)	56542	--	56542.0	55901.7
2		55731	--	55731.0	
3		55020	--	55020.0	
4		57201	--	57201.0	
5		55440	--	55440.0	
6		55476	--	55476.0	
7	Total Binding (Solvent Control)	2095	1838.7	5516	5167.5
8		1949	1692.7	5078	
9		2038	1781.7	5345	
10		1930	1673.7	5021	
11		1982	1725.7	5177	
12		1879	1622.7	4868	

DPM (1mL) from LSC	Tube	Sample Type	Concentration log[M]	Specific Binding DPM (1mL) NSB	Total Specific Binding (3mL)	Specific Binding (%)	Residual	Squared Residual	Mean Specific Binding (%)	Standard Deviation	SEM	% CV	% Ligand Bound vs. Total Activity
242.0	13	R1881 (NSB)	-6	-14.3	-43.0	-0.8	-1.6	2.4	0.0	1.7	0.7	1.5E+17	1.3
223.0	14		-6	-33.3	-100.0	-1.9	-2.7	7.1					1.2
245.0	15		-6	-11.3	-34.0	-0.7	-1.4	1.9					1.3
282.0	16		-6	25.7	77.0	1.5	0.8	0.6					1.5
302.0	17		-6	45.7	137.0	2.7	1.9	3.7					1.6
244.0	18		-6	-12.3	-37.0	-0.7	-1.4	2.1					1.3
292.0	19	R1881	-7	35.7	107.0	2.1	0.1	0.0	3.1	1.0	0.6	31.1	1.6
325.0	20		-7	68.7	206.0	4.0	2.0	4.1					1.7
313.0	21		-7	56.7	170.0	3.3	1.3	1.7					1.7
452.0	22	R1881	-8	195.7	587.0	11.4	-1.1	1.2	13.1	1.5	0.9	11.6	2.4
501.0	23		-8	244.7	734.0	14.2	1.7	3.0					2.7
492.0	24		-8	235.7	707.0	13.7	1.2	1.5					2.6
1201.0	25	R1881	-9	944.7	2834.0	54.8	-1.4	2.0	55.5	1.5	0.9	2.7	6.4
1195.0	26		-9	938.7	2816.0	54.5	-1.8	3.1					6.4
1242.0	27		-9	985.7	2957.0	57.2	1.0	0.9					6.7
1800.0	28	R1881	-10	1543.7	4631.0	89.6	-1.3	1.8	92.6	2.9	1.7	3.1	9.7
1899.0	29		-10	1642.7	4928.0	95.4	4.4	19.6					10.2
1854.0	30		-10	1597.7	4793.0	92.8	1.8	3.3					9.9
1852.0	31	R1881	-11	1595.7	4787.0	92.6	-4.6	21.0	96.0	3.1	1.8	3.3	9.9
1921.0	32		-11	1664.7	4994.0	96.6	-0.6	0.3					10.3
1958.0	33		-11	1701.7	5105.0	98.8	1.6	2.5					10.5
269.0	37	Dexamethasone	-3	12.7	38.0	0.7	-2.4	5.7	2.9	2.9	1.6	98.3	1.4
362.0	38		-3	105.7	317.0	6.1	3.0	9.1					1.9
288.0	39		-3	31.7	95.0	1.8	-1.3	1.6					1.5
719.0	40	Dexamethasone	-4	462.7	1388.0	26.9	-2.1	4.4	29.4	2.3	1.3	7.9	3.9
775.0	41		-4	518.7	1556.0	30.1	1.2	1.4					4.2
796.0	42		-4	539.7	1619.0	31.3	2.4	5.7					4.3
1597.0	43	Dexamethasone	-5	1340.7	4022.0	77.8	1.3	1.8	75.6	4.5	2.6	5.9	8.6
1608.0	44		-5	1351.7	4055.0	78.5	2.0	3.9					8.6
1469.0	45		-5	1212.7	3638.0	70.4	-6.1	37.0					7.9
1959.0	46	Dexamethasone	-6	1702.7	5108.0	98.8	3.7	13.7	96.5	2.2	1.3	2.3	10.5
1914.0	47		-6	1657.7	4973.0	96.2	1.1	1.2					10.3
1884.0	48		-6	1627.7	4883.0	94.5	-0.6	0.4					10.1
2012.0	49	Dexamethasone	-7	1755.7	5267.0	101.9	3.8	14.4	102.4	3.3	1.9	3.3	10.8
2080.0	50		-7	1823.7	5471.0	105.9	7.7	59.9					11.2
1966.0	51		-7	1709.7	5129.0	99.3	1.1	1.3					10.6
2004.0	52	Dexamethasone	-8	1747.7	5243.0	101.5	2.9	8.7	98.7	2.5	1.5	2.6	10.8
1920.0	53		-8	1663.7	4991.0	96.6	-1.9	3.7					10.3
1943.0	54		-8	1686.7	5060.0	97.9	-0.6	0.4					10.4
1986.0	55	Dexamethasone	-9	1729.7	5189.0	100.4	1.8	3.4	98.5	2.2	1.3	2.2	10.7
1963.0	56		-9	1706.7	5120.0	99.1	0.5	0.3					10.5
1912.0	57		-9	1655.7	4967.0	96.1	-2.4	6.0					10.3
1923.0	58	Dexamethasone	-10	1666.7	5000.0	96.8	-1.8	3.3	93.5	5.0	2.9	5.4	10.3
1767.0	59		-10	1510.7	4532.0	87.7	-10.9	118.2					9.5
1911.0	60		-10	1654.7	4964.0	96.1	-2.5	6.3					10.3
783.0	61	Octyl Salicylate	-3	526.7	1580.0	30.6	-0.5	0.2	31.7	3.1	1.8	9.9	4.2
864.0	62		-3	607.7	1823.0	35.3	4.2	17.7					4.6
761.0	63		-3	504.7	1514.0	29.3	-1.8	3.1					4.1
1272.0	64	Octyl Salicylate	-4	1015.7	3047.0	59.0	2.6	6.8	53.9	5.1	2.9	9.5	6.8
1096.0	65		-4	839.7	2519.0	48.7	-7.6	57.9					5.9
1184.0	66		-4	927.7	2783.0	53.9	-2.5	6.2					6.4
1700.0	67	Octyl Salicylate	-5	1443.7	4331.0	83.8	3.4	11.8	85.4	4.6	2.6	5.4	9.1
1816.0	68		-5	1559.7	4679.0	90.5	10.2	103.3					9.7
1665.0	69		-5	1408.7	4226.0	81.8	1.4	2.0					8.9
1705.0	70	Octyl Salicylate	-6	1448.7	4346.0	84.1	-7.9	63.0	89.1	4.9	2.8	5.5	9.1
1792.0	71		-6	1535.7	4607.0	89.2	-2.9	8.3					9.6
1874.0	72		-6	1617.7	4853.0	93.9	1.9	3.5					10.1
1828.0	73	Octyl Salicylate	-7	1571.7	4715.0	91.2	-4.7	21.7	90.0	1.1	0.6	1.2	9.8
1799.0	74		-7	1542.7	4628.0	89.6	-6.3	40.2					9.7
1792.0	75		-7	1535.7	4607.0	89.2	-2.9	8.3					9.6
1731.0	76	Octyl Salicylate	-8	1474.7	4424.0	85.6	-11.4	129.9	94.3	7.9	4.6	8.4	9.3
1913.0	77		-8	1656.7	4970.0	96.2	-0.8	0.7					10.3
1999.0	78		-8	1742.7	5228.0	101.2	4.2	17.3					10.7
1994.0	79	Octyl Salicylate	-9	1737.7	5213.0	100.9	3.6	12.7	101.3	2.7	1.6	2.7	10.7
2051.0	80		-9	1794.7	5384.0	104.2	6.9	47.3					11.0
1957.0	81		-9	1700.7	5102.0	98.7	1.4	2.0					10.5
2087.0	82	Octyl Salicylate	-10	1830.7	5492.0	106.3	8.9	78.9	101.9	4.0	2.3	4.0	11.2
1997.0	83		-10	1740.7	5222.0	101.1	3.7	13.4					10.7
1950.0	84		-10	1693.7	5081.0	98.3	0.9	0.9					10.5



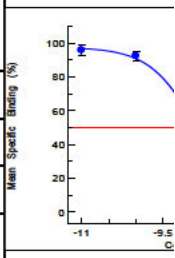


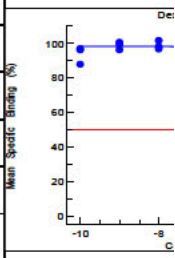


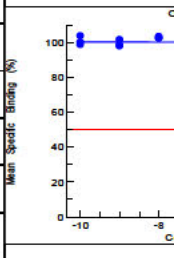
Experiment Date:	20-Sep-11		Study Number:	9070-100107ARB					Assays Conducted by:		[redacted]		
Test substance:	Octocrylene												
10/27/2011 11:08													
		ug protein/assay tube =											

Tube	Sample Type	DPM (1mL)	Specific Binding DPM (100 uL) - NSB	Total Specific Binding (300 uL)	Mean
1	Total Activity (Master Mix)	56542	–	56542.0	55901.7
2		55731	–	55731.0	
3		55020	–	55020.0	
4		57201	–	57201.0	
5		55440	–	55440.0	
6		55476	–	55476.0	
7	Total Binding (Solvent Control)	2095	1838.7	5516	5167.5
8		1949	1692.7	5078	
9		2038	1781.7	5345	
10		1930	1673.7	5021	
11		1982	1725.7	5177	
12		1879	1622.7	4868	

DPM (1mL) from LSC	Tube	Sample Type	Concentration log[M]	Specific Binding DPM (1mL) NSB	Total Specific Binding (3mL)	Specific Binding (%)	Residual	Squared Residual	Mean Specific Binding (%)	Standard Deviation	SEM	% CV	% Ligand Bound vs. Total Activity
242.0	13	R1881 (NSB)	-6	-14.3	-43.0	-0.8	-1.6	2.4	0.0	1.7	0.7	1.5E+17	1.3
223.0	14		-6	-33.3	-100.0	-1.9	-2.7	7.1					1.2
245.0	15		-6	-11.3	-34.0	-0.7	-1.4	1.9					1.3
282.0	16		-6	25.7	77.0	1.5	0.8	0.6					1.5
302.0	17		-6	45.7	137.0	2.7	1.9	3.7					1.6
244.0	18		-6	-12.3	-37.0	-0.7	-1.4	2.1					1.3
292.0	19	R1881	-7	35.7	107.0	2.1	0.1	0.0	3.1	1.0	0.6	31.1	1.6
325.0	20		-7	68.7	206.0	4.0	2.0	4.1					1.7
313.0	21		-7	56.7	170.0	3.3	1.3	1.7					1.7
452.0	22	R1881	-8	195.7	587.0	11.4	-1.1	1.2	13.1	1.5	0.9	11.6	2.4
501.0	23		-8	244.7	734.0	14.2	1.7	3.0					2.7
492.0	24		-8	235.7	707.0	13.7	1.2	1.5					2.6
1201.0	25	R1881	-9	944.7	2834.0	54.8	-1.4	2.0	55.5	1.5	0.9	2.7	6.4
1195.0	26		-9	938.7	2816.0	54.5	-1.8	3.1					6.4
1242.0	27		-9	985.7	2957.0	57.2	1.0	0.9					6.7
1800.0	28	R1881	-10	1543.7	4631.0	89.6	-1.3	1.8	92.6	2.9	1.7	3.1	9.7
1899.0	29		-10	1642.7	4928.0	95.4	4.4	19.6					10.2
1854.0	30		-10	1597.7	4793.0	92.8	1.8	3.3					9.9
1852.0	31	R1881	-11	1595.7	4787.0	92.6	-4.6	21.0	96.0	3.1	1.8	3.3	9.9
1921.0	32		-11	1664.7	4994.0	96.6	-0.6	0.3					10.3
1958.0	33		-11	1701.7	5105.0	98.8	1.6	2.5					10.5
269.0	37	Dexamethasone	-3	12.7	38.0	0.7	-2.4	5.7	2.9	2.9	1.6	98.3	1.4
362.0	38		-3	105.7	317.0	6.1	3.0	9.1					1.9
288.0	39		-3	31.7	95.0	1.8	-1.3	1.6					1.5
719.0	40	Dexamethasone	-4	462.7	1388.0	26.9	-2.1	4.4	29.4	2.3	1.3	7.9	3.9
775.0	41		-4	518.7	1556.0	30.1	1.2	1.4					4.2
796.0	42		-4	539.7	1619.0	31.3	2.4	5.7					4.3
1597.0	43	Dexamethasone	-5	1340.7	4022.0	77.8	1.3	1.8	75.6	4.5	2.6	5.9	8.6
1608.0	44		-5	1351.7	4055.0	78.5	2.0	3.9					8.6
1469.0	45		-5	1212.7	3638.0	70.4	-6.1	37.0					7.9
1959.0	46	Dexamethasone	-6	1702.7	5108.0	98.8	3.7	13.7	96.5	2.2	1.3	2.3	10.5
1914.0	47		-6	1657.7	4973.0	96.2	1.1	1.2					10.3
1884.0	48		-6	1627.7	4883.0	94.5	-0.6	0.4					10.1
2012.0	49	Dexamethasone	-7	1755.7	5267.0	101.9	3.8	14.4	102.4	3.3	1.9	3.3	10.8
2080.0	50		-7	1823.7	5471.0	105.9	7.7	59.9					11.2
1966.0	51		-7	1709.7	5129.0	99.3	1.1	1.3					10.6
2004.0	52	Dexamethasone	-8	1747.7	5243.0	101.5	2.9	8.7	98.7	2.5	1.5	2.6	10.8
1920.0	53		-8	1663.7	4991.0	96.6	-1.9	3.7					10.3
1943.0	54		-8	1686.7	5060.0	97.9	-0.6	0.4					10.4
1986.0	55	Dexamethasone	-9	1729.7	5189.0	100.4	1.8	3.4	98.5	2.2	1.3	2.2	10.7
1963.0	56		-9	1706.7	5120.0	99.1	0.5	0.3					10.5
1912.0	57		-9	1655.7	4967.0	96.1	-2.4	6.0					10.3
1923.0	58	Dexamethasone	-10	1666.7	5000.0	96.8	-1.8	3.3	93.5	5.0	2.9	5.4	10.3
1767.0	59		-10	1510.7	4532.0	87.7	-10.9	118.2					9.5
1911.0	60		-10	1654.7	4964.0	96.1	-2.5	6.3					10.3
1051.0	61	Octocrylene	-3	794.7	2384.0	46.1	0.6	0.4	45.4	1.2	0.7	2.6	5.6
1049.0	62		-3	792.7	2378.0	46.0	0.5	0.2					5.6
1015.0	63		-3	758.7	2276.0	44.0	-1.5	2.2					5.4
1274.0	64	Octocrylene	-4	1017.7	3053.0	59.1	5.1	25.7	54.2	4.4	2.6	8.2	6.8
1125.0	65		-4	868.7	2606.0	50.4	-3.6	12.8					6.0
1172.0	66		-4	915.7	2747.0	53.2	-0.9	0.7					6.3
1685.0	67	Octocrylene	-5	1428.7	4286.0	82.9	0.3	0.1	82.3	1.8	1.0	2.1	9.0
1698.0	68		-5	1441.7	4325.0	83.7	1.1	1.2					9.1
1640.0	69		-5	1383.7	4151.0	80.3	-2.3	5.1					8.8
2025.0	70	Octocrylene	-6	1768.7	5306.0	102.7	4.6	21.3	99.0	4.4	2.6	4.5	10.9
1877.0	71		-6	1620.7	4862.0	94.1	-4.0	15.8					10.1
1983.0	72		-6	1726.7	5180.0	100.2	2.2	4.7					10.6
2016.0	73	Octocrylene	-7	1759.7	5279.0	102.2	1.9	3.6	98.4	3.9	2.3	4.0	10.8
1882.0	74		-7	1625.7	4877.0	94.4	-5.9	34.6					10.1
1958.0	75		-7	1701.7	5105.0	98.8	-1.5	2.1					10.5
2035.0	76	Octocrylene	-8	1778.7	5336.0	103.3	2.8	7.7	102.6	0.6	0.3	0.6	10.9
2016.0	77		-8	1759.7	5279.0	102.2	1.9	3.6					10.8
2020.0	78		-8	1763.7	5291.0	102.4	1.9	3.7					10.8
1954.0	79	Octocrylene	-9	1697.7	5093.0	98.6	-1.9	3.8	99.2	2.0	1.1	2.0	10.5
2004.0	80		-9	1747.7	5243.0	101.5	1.0	0.9					10.8
1939.0	81		-9	1682.7	5048.0	97.7	-2.8	7.9					10.4
2041.0	82	Octocrylene	-10	1784.7	5354.0	103.6	3.1	9.7	100.7	2.6	1.5	2.6	11.0
1953.0	83		-10	1696.7	5090.0	98.5	-2.0	4.0					10.5
1979.0	84		-10	1722.7	5168.0	100.0	-0.5	0.2					10.6





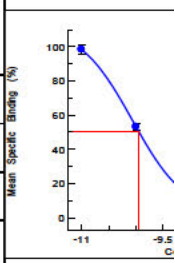


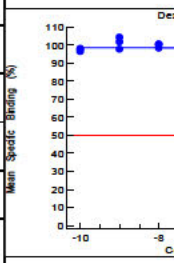
APPENDIX 1 Raw and Normalized Data Valid Run 2 – September 22, 2011

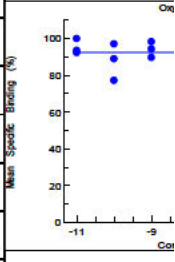
1	Experiment Date:	22-Sep-11		Study Number:	9070-100107ARB				Assays Conducted by:			[redacted]	
2	Test substance:	Oxybenzone											
3	10/27/2011 11:33												
4													
5			ug protein/assay tube =										

Tube	Sample Type	DPM (1mL)	Specific Binding DPM (100 uL) - NSB	Total Specific Binding (300 uL)	Mean
1	Total Activity (Master Mix)	63276	–	63276.0	61919.5
2		65781	–	65781.0	
3		58416	–	58416.0	
4		64532	–	64532.0	
5		59920	–	59920.0	
6		59592	–	59592.0	
7	Total Binding (Solvent Control)	2485	2190.2	6571	5982.5
8		2328	2033.2	6100	
9		2363	2068.2	6205	
10		2084	1789.2	5368	
11		2325	2030.2	6091	
12		2149	1854.2	5563	

DPM (1mL) from LSC	Tube	Sample Type	Concentration log[M]	Specific Binding DPM (1mL) NSB	Total Specific Binding (3mL)	Specific Binding (%)	Residual	Squared Residual	Mean Specific Binding (%)	Standard Deviation	SEM	% CV	% Ligand Bound vs. Total Activity
254.0	13	R1881 (NSB)	-6	-40.8	-122.5	-2.0	-2.8	8.1	0.0	2.3	0.9	2.5E+17	1.2
257.0	14		-6	-37.8	-113.5	-1.9	-2.7	7.3					1.2
262.0	15		-6	-32.8	-98.5	-1.6	-2.4	6.0					1.3
359.0	16		-6	64.2	192.5	3.2	2.4	5.8					1.7
344.0	17		-6	49.2	147.5	2.5	1.7	2.8					1.7
293.0	18		-6	-1.8	-5.5	-0.1	-0.9	0.8					1.4
333.0	19	R1881	-7	38.2	114.5	1.9	0.9	0.8	1.8	1.6	0.9	88.1	1.6
361.0	20		-7	66.2	198.5	3.3	2.3	5.3					1.7
298.0	21		-7	3.2	9.5	0.2	-0.9	0.7					1.4
358.0	22	R1881	-8	63.2	189.5	3.2	0.6	0.3	3.8	0.6	0.4	16.5	1.7
371.0	23		-8	76.2	228.5	3.8	1.2	1.5					1.8
383.0	24		-8	88.2	264.5	4.4	1.8	3.3					1.9
520.0	25	R1881	-9	225.2	675.5	11.3	-1.5	2.2	12.3	0.9	0.5	7.3	2.5
547.0	26		-9	252.2	756.5	12.6	-0.1	0.0					2.7
554.0	27		-9	259.2	777.5	13.0	0.2	0.0					2.7
1316.0	28	R1881	-10	1021.2	3063.5	51.2	-1.9	3.7	53.2	1.8	1.0	3.3	6.4
1380.0	29		-10	1085.2	3255.5	54.4	1.3	1.7					6.7
1373.0	30		-10	1078.2	3234.5	54.1	0.9	0.9					6.7
2320.0	31	R1881	-11	2025.2	6075.5	101.6	3.1	9.5	98.4	2.7	1.6	2.8	11.2
2234.0	32		-11	1939.2	5817.5	97.2	-1.2	1.5					10.8
2220.0	33		-11	1925.2	5775.5	96.5	-1.9	3.7					10.8
391.0	37	Dexamethasone	-3	96.2	288.5	4.8	-0.2	0.1	5.1	2.0	1.2	39.9	1.9
438.0	38		-3	143.2	429.5	7.2	2.1	4.5					2.1
358.0	39		-3	63.2	189.5	3.2	-1.9	3.6					1.7
962.0	40	Dexamethasone	-4	667.2	2001.5	33.5	0.5	0.3	33.0	0.4	0.3	1.3	4.7
950.0	41		-4	655.2	1965.5	32.9	-0.1	0.0					4.6
945.0	42		-4	650.2	1950.5	32.6	-0.3	0.1					4.6
1886.0	43	Dexamethasone	-5	1591.2	4773.5	79.8	-1.2	1.5	80.9	1.0	0.6	1.2	9.1
1914.0	44		-5	1619.2	4857.5	81.2	0.2	0.0					9.3
1924.0	45		-5	1629.2	4887.5	81.7	0.7	0.5					9.3
2262.0	46	Dexamethasone	-6	1967.2	5901.5	98.6	2.2	5.0	97.1	2.4	1.4	2.5	11.0
2175.0	47		-6	1880.2	5640.5	94.3	-2.1	4.5					10.5
2256.0	48		-6	1961.2	5883.5	98.3	1.9	3.7					10.9
2180.0	49	Dexamethasone	-7	1885.2	5655.5	94.5	-3.9	15.4	96.4	2.7	1.5	2.8	10.6
2192.0	50		-7	1897.2	5691.5	95.1	-3.3	11.1					10.6
2278.0	51		-7	1983.2	5949.5	99.4	1.0	1.0					11.0
2290.0	52	Dexamethasone	-8	1995.2	5985.5	100.1	1.4	1.8	99.5	1.2	0.7	1.2	11.1
2251.0	53		-8	1956.2	5868.5	98.1	-0.6	0.4					10.9
2296.0	54		-8	2001.2	6003.5	100.4	1.7	2.7					11.1
2371.0	55	Dexamethasone	-9	2076.2	6228.5	104.1	5.4	29.1	101.1	3.3	1.9	3.3	11.5
2239.0	56		-9	1944.2	5832.5	97.5	-1.2	1.5					10.8
2320.0	57		-9	2025.2	6075.5	101.6	2.8	8.0					11.2
2247.0	58	Dexamethasone	-10	1952.2	5856.5	97.9	-0.8	0.7	97.1	0.9	0.5	0.9	10.9
2213.0	59		-10	1918.2	5754.5	96.2	-2.5	6.4					10.7
2233.0	60		-10	1938.2	5814.5	97.2	-1.5	2.3					10.8
1546.0	61	Oxybenzone	-4	1251.2	3753.5	62.7	0.2	0.0	62.6	0.7	0.4	1.1	7.5
1554.0	62		-4	1259.2	3777.5	63.1	0.6	0.3					7.5
1528.0	63		-4	1233.2	3699.5	61.8	-0.7	0.5					7.4
2084.0	64	Oxybenzone	-5	1789.2	5367.5	89.7	-2.8	8.1	92.6	5.4	3.1	5.8	10.1
2074.0	65		-5	1779.2	5337.5	89.2	-3.3	11.2					10.0
2264.0	66		-5	1969.2	5907.5	98.7	6.2	38.3					11.0
2180.0	67	Oxybenzone	-6	1885.2	5655.5	94.5	1.9	3.6	95.2	4.0	2.3	4.2	10.6
2279.0	68		-6	1984.2	5952.5	99.5	6.9	47.1					11.0
2122.0	69		-6	1827.2	5481.5	91.6	-1.0	1.0					10.3
1955.0	70	Oxybenzone	-7	1660.2	4980.5	83.3	-9.4	88.0	92.0	7.9	4.6	8.6	9.5
2168.0	71		-7	1873.2	5619.5	93.9	1.3	1.7					10.5
2263.0	72		-7	1968.2	5904.5	98.7	6.1	36.7					11.0
2101.0	73	Oxybenzone	-8	1806.2	5418.5	90.6	-2.1	4.3	92.2	4.5	2.6	4.9	10.2
2235.0	74		-8	1940.2	5820.5	97.3	4.7	21.7					10.8
2066.0	75		-8	1771.2	5313.5	88.8	-3.8	14.6					10.0
2170.0	76	Oxybenzone	-9	1875.2	5625.5	94.0	1.4	2.0	93.8	4.3	2.5	4.6	10.5
2079.0	77		-9	1784.2	5352.5	89.5	-3.2	10.0					10.1
2250.0	78		-9	1955.2	5865.5	98.0	5.4	29.3					10.9
2064.0	79	Oxybenzone	-10	1769.2	5307.5	88.7	-3.9	15.3	87.5	10.0	5.8	11.4	10.0
2226.0	80		-10	1931.2	5793.5	96.8	4.2	17.7					10.8
1830.0	81		-10	1535.2	4605.5	77.0	-15.7	245.0					8.9
2283.0	82	Oxybenzone	-11	1988.2	5964.5	99.7	7.1	49.9	95.0	4.1	2.4	4.3	11.1
2156.0	83		-11	1861.2	5583.5	93.3	0.7	0.5					10.4
2131.0	84		-11	1836.2	5508.5	92.1	-0.6	0.3					10.3



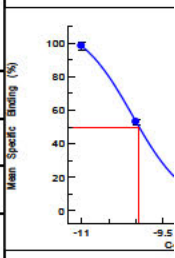


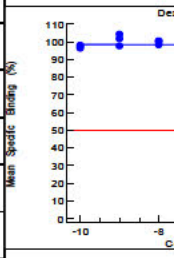


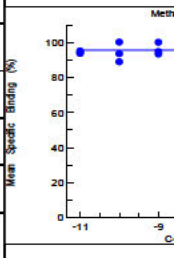
Experiment Date:	22-Sep-11		Study Number:	9070-100107ARB					Assays Conducted by:		[redacted]		
Test substance:	Methoxycinnamate												
10/27/2011 11:33													
		ug protein/assay tube =											

Tube	Sample Type	DPM (1mL)	Specific Binding DPM (100 uL) - NSB	Total Specific Binding (300 uL)	Mean
1	Total Activity (Master Mix)	63276	–	63276.0	61919.5
2		65781	–	65781.0	
3		59416	–	59416.0	
4		64532	–	64532.0	
5		59920	–	59920.0	
6		59592	–	59592.0	
7	Total Binding (Solvent Control)	2485	2190.2	6571	5982.5
8		2328	2033.2	6100	
9		2363	2068.2	6205	
10		2084	1789.2	5368	
11		2325	2030.2	6091	
12		2149	1854.2	5563	

DPM (1mL) from LSC	Tube	Sample Type	Concentration log[M]	Specific Binding DPM (1mL) NSB	Total Specific Binding (3mL)	Specific Binding (%)	Residual	Squared Residual	Mean Specific Binding (%)	Standard Deviation	SEM	% CV	% Ligand Bound vs. Total Activity
254.0	13	R1881 (NSB)	-6	-40.8	-122.5	-2.0	-2.8	8.1	0.0	2.3	0.9	2.5E+17	1.2
257.0	14		-6	-37.8	-113.5	-1.9	-2.7	7.3					1.2
262.0	15		-6	-32.8	-98.5	-1.6	-2.4	6.0					1.3
359.0	16		-6	64.2	192.5	3.2	2.4	5.8					1.7
344.0	17		-6	49.2	147.5	2.5	1.7	2.8					1.7
293.0	18		-6	-1.8	-5.5	-0.1	-0.9	0.8					1.4
333.0	19	R1881	-7	38.2	114.5	1.9	0.9	0.8	1.8	1.6	0.9	88.1	1.6
361.0	20		-7	66.2	198.5	3.3	2.3	5.3					1.7
298.0	21		-7	3.2	9.5	0.2	-0.9	0.7					1.4
358.0	22	R1881	-8	63.2	189.5	3.2	0.6	0.3	3.8	0.6	0.4	16.5	1.7
371.0	23		-8	76.2	228.5	3.8	1.2	1.5					1.8
383.0	24		-8	88.2	264.5	4.4	1.8	3.3					1.9
520.0	25	R1881	-9	225.2	675.5	11.3	-1.5	2.2	12.3	0.9	0.5	7.3	2.5
547.0	26		-9	252.2	756.5	12.6	-0.1	0.0					2.7
554.0	27		-9	259.2	777.5	13.0	0.2	0.0					2.7
1316.0	28	R1881	-10	1021.2	3063.5	51.2	-1.9	3.7	53.2	1.8	1.0	3.3	6.4
1380.0	29		-10	1085.2	3255.5	54.4	1.3	1.7					6.7
1373.0	30		-10	1078.2	3234.5	54.1	0.9	0.9					6.7
2320.0	31	R1881	-11	2025.2	6075.5	101.6	3.1	9.5	98.4	2.7	1.6	2.8	11.2
2234.0	32		-11	1939.2	5817.5	97.2	-1.2	1.5					10.8
2220.0	33		-11	1925.2	5775.5	96.5	-1.9	3.7					10.8
391.0	37	Dexamethasone	-3	96.2	288.5	4.8	-0.2	0.1	5.1	2.0	1.2	39.9	1.9
438.0	38		-3	143.2	429.5	7.2	2.1	4.5					2.1
358.0	39		-3	63.2	189.5	3.2	-1.9	3.6					1.7
962.0	40	Dexamethasone	-4	667.2	2001.5	33.5	0.5	0.3	33.0	0.4	0.3	1.3	4.7
950.0	41		-4	655.2	1965.5	32.9	-0.1	0.0					4.6
945.0	42		-4	650.2	1950.5	32.6	-0.3	0.1					4.6
1886.0	43	Dexamethasone	-5	1591.2	4773.5	79.8	-1.2	1.5	80.9	1.0	0.6	1.2	9.1
1914.0	44		-5	1619.2	4857.5	81.2	0.2	0.0					9.3
1924.0	45		-5	1629.2	4887.5	81.7	0.7	0.5					9.3
2262.0	46	Dexamethasone	-6	1967.2	5901.5	98.6	2.2	5.0	97.1	2.4	1.4	2.5	11.0
2175.0	47		-6	1880.2	5640.5	94.3	-2.1	4.5					10.5
2256.0	48		-6	1961.2	5883.5	98.3	1.9	3.7					10.9
2180.0	49	Dexamethasone	-7	1885.2	5655.5	94.5	-3.9	15.4	96.4	2.7	1.5	2.8	10.6
2192.0	50		-7	1897.2	5691.5	95.1	-3.3	11.1					10.6
2278.0	51		-7	1983.2	5949.5	99.4	1.0	1.0					11.0
2290.0	52	Dexamethasone	-8	1995.2	5985.5	100.1	1.4	1.8	99.5	1.2	0.7	1.2	11.1
2251.0	53		-8	1956.2	5868.5	98.1	-0.6	0.4					10.9
2296.0	54		-8	2001.2	6003.5	100.4	1.7	2.7					11.1
2371.0	55	Dexamethasone	-9	2076.2	6228.5	104.1	5.4	29.1	101.1	3.3	1.9	3.3	11.5
2239.0	56		-9	1944.2	5832.5	97.5	-1.2	1.5					10.8
2320.0	57		-9	2025.2	6075.5	101.6	2.8	8.0					11.2
2247.0	58	Dexamethasone	-10	1952.2	5856.5	97.9	-0.8	0.7	97.1	0.9	0.5	0.9	10.9
2213.0	59		-10	1918.2	5754.5	96.2	-2.5	6.4					10.7
2233.0	60		-10	1938.2	5814.5	97.2	-1.5	2.3					10.8
1860.0	61	Methoxycinnamate	-4	1565.2	4695.5	78.5	-3.5	11.9	81.9	3.9	2.2	4.7	9.0
2012.0	62		-4	1717.2	5151.5	86.1	4.2	17.4					9.7
1912.0	63		-4	1617.2	4851.5	81.1	-0.8	0.7					9.3
2189.0	64	Methoxycinnamate	-5	1894.2	5682.5	95.0	0.8	0.6	94.3	0.9	0.5	0.9	10.6
2155.0	65		-5	1860.2	5580.5	93.3	-0.9	0.9					10.4
2180.0	66		-5	1885.2	5655.5	94.5	0.3	0.1					10.6
2181.0	67	Methoxycinnamate	-6	1886.2	5658.5	94.6	-1.3	1.6	97.5	2.6	1.5	2.6	10.6
2280.0	68		-6	1985.2	5955.5	99.5	3.7	13.7					11.0
2255.0	69		-6	1960.2	5880.5	98.3	2.5	6.0					10.9
2189.0	70	Methoxycinnamate	-7	1894.2	5682.5	95.0	0.8	0.6	97.1	5.4	3.1	5.6	10.6
2149.0	71		-7	1854.2	5562.5	93.0	-2.9	8.2					10.4
2354.0	72		-7	2059.2	6177.5	103.3	7.4	55.0					11.4
2201.0	73	Methoxycinnamate	-8	1906.2	5718.5	95.6	-0.3	0.1	96.5	1.3	0.8	1.4	10.7
2205.0	74		-8	1910.2	5730.5	95.8	-0.1	0.0					10.7
2249.0	75		-8	1954.2	5862.5	98.0	2.1	4.6					10.9
2287.0	76	Methoxycinnamate	-9	1992.2	5976.5	99.9	4.1	16.4	95.9	3.5	2.0	3.7	11.1
2153.0	77		-9	1858.2	5574.5	93.2	-2.7	7.1					10.4
2181.0	78		-9	1886.2	5658.5	94.6	-1.3	1.6					10.6
2064.0	79	Methoxycinnamate	-10	1769.2	5307.5	88.7	-7.1	50.8	94.0	5.7	3.3	6.0	10.0
2289.0	80		-10	1994.2	5982.5	100.0	4.2	17.3					11.1
2157.0	81		-10	1862.2	5586.5	93.4	-2.5	6.1					10.5
2160.0	82	Methoxycinnamate	-11	1865.2	5595.5	93.5	-2.3	5.4	94.1	0.7	0.4	0.8	10.5
2188.0	83		-11	1893.2	5679.5	94.9	-0.9	0.8					10.6
2169.0	84		-11	1874.2	5622.5	94.0	-1.9	3.5					10.5



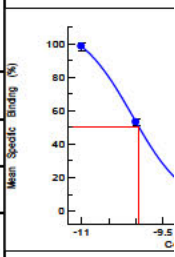


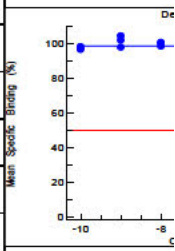


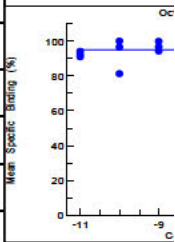
1	Experiment Date:	22-Sep-11		Study Number:	9070-100107ARB				Assays Conducted by:			[redacted]	
2	Test substance:	Octyl Salicylate											
3	10/27/2011 11:33												
4													
5			ug protein/assay tube =										
6													

Tube	Sample Type	DPM (1mL)	Specific Binding DPM (100 uL) - NSB	Total Specific Binding (300 uL)	Mean
1	Total Activity (Master Mix)	63276	–	63276.0	61919.5
2		65781	–	65781.0	
3		58416	–	58416.0	
4		64532	–	64532.0	
5		59920	–	59920.0	
6		59592	–	59592.0	
7	Total Binding (Solvent Control)	2485	2190.2	6571	5982.5
8		2328	2033.2	6100	
9		2363	2068.2	6205	
10		2084	1789.2	5368	
11		2325	2030.2	6091	
12		2149	1854.2	5563	

DPM (1mL) from LSC	Tube	Sample Type	Concentration log[M]	Specific Binding DPM (1mL) NSB	Total Specific Binding (3mL)	Specific Binding (%)	Residual	Squared Residual	Mean Specific Binding (%)	Standard Deviation	SEM	% CV	% Ligand Bound vs. Total Activity
254.0	13	R1881 (NSB)	-6	-40.8	-122.5	-2.0	-2.8	8.1	0.0	2.3	0.9	2.5E+17	1.2
257.0	14		-6	-37.8	-113.5	-1.9	-2.7	7.3					1.2
262.0	15		-6	-32.8	-98.5	-1.6	-2.4	6.0					1.3
359.0	16		-6	64.2	192.5	3.2	2.4	5.8					1.7
344.0	17		-6	49.2	147.5	2.5	1.7	2.8					1.7
293.0	18		-6	-1.8	-5.5	-0.1	-0.9	0.8					1.4
333.0	19	R1881	-7	38.2	114.5	1.9	0.9	0.8	1.8	1.6	0.9	88.1	1.6
361.0	20		-7	66.2	198.5	3.3	2.3	5.3					1.7
298.0	21		-7	3.2	9.5	0.2	-0.9	0.7					1.4
358.0	22	R1881	-8	63.2	189.5	3.2	0.6	0.3	3.8	0.6	0.4	16.5	1.7
371.0	23		-8	76.2	228.5	3.8	1.2	1.5					1.8
383.0	24		-8	88.2	264.5	4.4	1.8	3.3					1.9
520.0	25	R1881	-9	225.2	675.5	11.3	-1.5	2.2	12.3	0.9	0.5	7.3	2.5
547.0	26		-9	252.2	756.5	12.6	-0.1	0.0					2.7
554.0	27		-9	259.2	777.5	13.0	0.2	0.0					2.7
1316.0	28	R1881	-10	1021.2	3063.5	51.2	-1.9	3.7	53.2	1.8	1.0	3.3	6.4
1380.0	29		-10	1085.2	3255.5	54.4	1.3	1.7					6.7
1373.0	30		-10	1078.2	3234.5	54.1	0.9	0.9					6.7
2320.0	31	R1881	-11	2025.2	6075.5	101.6	3.1	9.5	98.4	2.7	1.6	2.8	11.2
2234.0	32		-11	1939.2	5817.5	97.2	-1.2	1.5					10.8
2220.0	33		-11	1925.2	5775.5	96.5	-1.9	3.7					10.8
391.0	37	Dexamethasone	-3	96.2	288.5	4.8	-0.2	0.1	5.1	2.0	1.2	39.9	1.9
438.0	38		-3	143.2	429.5	7.2	2.1	4.5					2.1
358.0	39		-3	63.2	189.5	3.2	-1.9	3.6					1.7
962.0	40	Dexamethasone	-4	667.2	2001.5	33.5	0.5	0.3	33.0	0.4	0.3	1.3	4.7
950.0	41		-4	655.2	1965.5	32.9	-0.1	0.0					4.6
945.0	42		-4	650.2	1950.5	32.6	-0.3	0.1					4.6
1886.0	43	Dexamethasone	-5	1591.2	4773.5	79.8	-1.2	1.5	80.9	1.0	0.6	1.2	9.1
1914.0	44		-5	1619.2	4857.5	81.2	0.2	0.0					9.3
1924.0	45		-5	1629.2	4887.5	81.7	0.7	0.5					9.3
2262.0	46	Dexamethasone	-6	1967.2	5901.5	98.6	2.2	5.0	97.1	2.4	1.4	2.5	11.0
2175.0	47		-6	1880.2	5640.5	94.3	-2.1	4.5					10.5
2256.0	48		-6	1961.2	5883.5	98.3	1.9	3.7					10.9
2180.0	49	Dexamethasone	-7	1885.2	5655.5	94.5	-3.9	15.4	96.4	2.7	1.5	2.8	10.6
2192.0	50		-7	1897.2	5691.5	95.1	-3.3	11.1					10.6
2278.0	51		-7	1983.2	5949.5	99.4	1.0	1.0					11.0
2290.0	52	Dexamethasone	-8	1995.2	5985.5	100.1	1.4	1.8	99.5	1.2	0.7	1.2	11.1
2251.0	53		-8	1956.2	5868.5	98.1	-0.6	0.4					10.9
2296.0	54		-8	2001.2	6003.5	100.4	1.7	2.7					11.1
2371.0	55	Dexamethasone	-9	2076.2	6228.5	104.1	5.4	29.1	101.1	3.3	1.9	3.3	11.5
2239.0	56		-9	1944.2	5832.5	97.5	-1.2	1.5					10.8
2320.0	57		-9	2025.2	6075.5	101.6	2.8	8.0					11.2
2247.0	58	Dexamethasone	-10	1952.2	5856.5	97.9	-0.8	0.7	97.1	0.9	0.5	0.9	10.9
2213.0	59		-10	1918.2	5754.5	96.2	-2.5	6.4					10.7
2233.0	60		-10	1938.2	5814.5	97.2	-1.5	2.3					10.8
1362.0	61	Octyl Salicylate	-4	1067.2	3201.5	53.5	0.6	0.3	52.9	2.4	1.4	4.5	6.6
1391.0	62		-4	1096.2	3288.5	55.0	2.0	4.1					6.7
1299.0	63		-4	1004.2	3012.5	50.4	-2.6	6.7					6.3
2214.0	64	Octyl Salicylate	-5	1919.2	5757.5	96.2	4.2	17.3	92.1	3.6	2.1	3.9	10.7
2096.0	65		-5	1801.2	5403.5	90.3	-1.8	3.1					10.2
2083.0	66		-5	1788.2	5364.5	89.7	-2.4	5.8					10.1
2168.0	67	Octyl Salicylate	-6	1873.2	5619.5	93.9	-1.3	1.8	96.5	4.0	2.3	4.2	10.5
2311.0	68		-6	2016.2	6048.5	101.1	5.8	34.0					11.2
2177.0	69		-6	1882.2	5646.5	94.4	-0.9	0.8					10.5
2283.0	70	Octyl Salicylate	-7	1988.2	5964.5	99.7	4.4	19.6	98.0	1.5	0.9	1.6	11.1
2240.0	71		-7	1945.2	5835.5	97.5	2.3	5.2					10.9
2224.0	72		-7	1929.2	5787.5	96.7	1.5	2.2					10.8
2197.0	73	Octyl Salicylate	-8	1902.2	5706.5	95.4	0.1	0.0	95.7	2.5	1.4	2.6	10.6
2257.0	74		-8	1962.2	5886.5	98.4	3.1	9.8					10.9
2158.0	75		-8	1863.2	5589.5	93.4	-1.8	3.4					10.5
2282.0	76	Octyl Salicylate	-9	1987.2	5961.5	99.6	4.4	19.2	96.7	2.8	1.6	2.9	11.1
2218.0	77		-9	1923.2	5769.5	96.4	1.2	1.4					10.7
2170.0	78		-9	1875.2	5625.5	94.0	-1.2	1.5					10.5
1912.0	79	Octyl Salicylate	-10	1617.2	4851.5	81.1	-14.2	201.0	92.4	10.0	5.8	10.8	9.3
2286.0	80		-10	1991.2	5973.5	99.8	4.6	21.0					11.1
2215.0	81		-10	1920.2	5760.5	96.3	1.0	1.0					10.7
2105.0	82	Octyl Salicylate	-11	1810.2	5430.5	90.8	-4.5	20.2	92.3	1.6	0.9	1.7	10.2
2169.0	83		-11	1874.2	5622.5	94.0	-1.3	1.7					10.5
2133.0	84		-11	1838.2	5514.5	92.2	-3.1	9.6					10.3



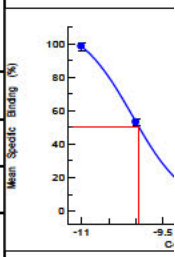


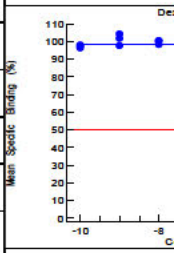


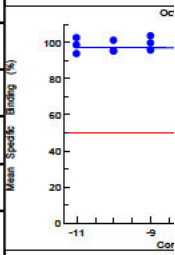
Experiment Date:	22-Sep-11		Study Number:	9070-100107ARB					Assays Conducted by:		[redacted]		
Test substance:	Octocrylene												
10/27/2011 11:33													
		ug protein/assay tube =											

Tube	Sample Type	DPM (1mL)	Specific Binding DPM (100 uL) - NSB	Total Specific Binding (300 uL)	Mean
1	Total Activity (Master Mix)	63276	–	63276.0	61919.5
2		65781	–	65781.0	
3		58416	–	58416.0	
4		64532	–	64532.0	
5		59920	–	59920.0	
6		59592	–	59592.0	
7	Total Binding (Solvent Control)	2485	2190.2	6571	5982.5
8		2328	2033.2	6100	
9		2363	2068.2	6205	
10		2084	1789.2	5368	
11		2325	2030.2	6091	
12		2149	1854.2	5563	

DPM (1mL) from LSC	Tube	Sample Type	Concentration log[M]	Specific Binding DPM (1mL) NSB	Total Specific Binding (3mL)	Specific Binding (%)	Residual	Squared Residual	Mean Specific Binding (%)	Standard Deviation	SEM	% CV	% Ligand Bound vs. Total Activity
254.0	13	R1881 (NSB)	-6	-40.8	-122.5	-2.0	-2.8	8.1	0.0	2.3	0.9	2.5E+17	1.2
257.0	14		-6	-37.8	-113.5	-1.9	-2.7	7.3					1.2
262.0	15		-6	-32.8	-98.5	-1.6	-2.4	6.0					1.3
359.0	16		-6	64.2	192.5	3.2	2.4	5.8					1.7
344.0	17		-6	49.2	147.5	2.5	1.7	2.8					1.7
293.0	18		-6	-1.8	-5.5	-0.1	-0.9	0.8					1.4
333.0	19	R1881	-7	38.2	114.5	1.9	0.9	0.8	1.8	1.6	0.9	88.1	1.6
361.0	20		-7	66.2	198.5	3.3	2.3	5.3					1.7
298.0	21		-7	3.2	9.5	0.2	-0.9	0.7					1.4
358.0	22	R1881	-8	63.2	189.5	3.2	0.6	0.3	3.8	0.6	0.4	16.5	1.7
371.0	23		-8	76.2	228.5	3.8	1.2	1.5					1.8
383.0	24		-8	88.2	264.5	4.4	1.8	3.3					1.9
520.0	25	R1881	-9	225.2	675.5	11.3	-1.5	2.2	12.3	0.9	0.5	7.3	2.5
547.0	26		-9	252.2	756.5	12.6	-0.1	0.0					2.7
554.0	27		-9	259.2	777.5	13.0	0.2	0.0					2.7
1316.0	28	R1881	-10	1021.2	3063.5	51.2	-1.9	3.7	53.2	1.8	1.0	3.3	6.4
1380.0	29		-10	1085.2	3255.5	54.4	1.3	1.7					6.7
1373.0	30		-10	1078.2	3234.5	54.1	0.9	0.9					6.7
2320.0	31	R1881	-11	2025.2	6075.5	101.6	3.1	9.5	98.4	2.7	1.6	2.8	11.2
2234.0	32		-11	1939.2	5817.5	97.2	-1.2	1.5					10.8
2220.0	33		-11	1925.2	5775.5	96.5	-1.9	3.7					10.8
391.0	37	Dexamethasone	-3	96.2	288.5	4.8	-0.2	0.1	5.1	2.0	1.2	39.9	1.9
438.0	38		-3	143.2	429.5	7.2	2.1	4.5					2.1
358.0	39		-3	63.2	189.5	3.2	-1.9	3.6					1.7
962.0	40	Dexamethasone	-4	667.2	2001.5	33.5	0.5	0.3	33.0	0.4	0.3	1.3	4.7
950.0	41		-4	655.2	1965.5	32.9	-0.1	0.0					4.6
945.0	42		-4	650.2	1950.5	32.6	-0.3	0.1					4.6
1886.0	43	Dexamethasone	-5	1591.2	4773.5	79.8	-1.2	1.5	80.9	1.0	0.6	1.2	9.1
1914.0	44		-5	1619.2	4857.5	81.2	0.2	0.0					9.3
1924.0	45		-5	1629.2	4887.5	81.7	0.7	0.5					9.3
2262.0	46	Dexamethasone	-6	1967.2	5901.5	98.6	2.2	5.0	97.1	2.4	1.4	2.5	11.0
2175.0	47		-6	1880.2	5640.5	94.3	-2.1	4.5					10.5
2256.0	48		-6	1961.2	5883.5	98.3	1.9	3.7					10.9
2180.0	49	Dexamethasone	-7	1885.2	5655.5	94.5	-3.9	15.4	96.4	2.7	1.5	2.8	10.6
2192.0	50		-7	1897.2	5691.5	95.1	-3.3	11.1					10.6
2278.0	51		-7	1983.2	5949.5	99.4	1.0	1.0					11.0
2290.0	52	Dexamethasone	-8	1995.2	5985.5	100.1	1.4	1.8	99.5	1.2	0.7	1.2	11.1
2251.0	53		-8	1956.2	5868.5	98.1	-0.6	0.4					10.9
2296.0	54		-8	2001.2	6003.5	100.4	1.7	2.7					11.1
2371.0	55	Dexamethasone	-9	2076.2	6228.5	104.1	5.4	29.1	101.1	3.3	1.9	3.3	11.5
2239.0	56		-9	1944.2	5832.5	97.5	-1.2	1.5					10.8
2320.0	57		-9	2025.2	6075.5	101.6	2.8	8.0					11.2
2247.0	58	Dexamethasone	-10	1952.2	5856.5	97.9	-0.8	0.7	97.1	0.9	0.5	0.9	10.9
2213.0	59		-10	1918.2	5754.5	96.2	-2.5	6.4					10.7
2233.0	60		-10	1938.2	5814.5	97.2	-1.5	2.3					10.8
1468.0	61	Octocrylene	-4	1173.2	3519.5	58.8	8.5	72.0	50.4	11.7	6.7	23.1	7.1
1034.0	62		-4	739.2	2217.5	37.1	-13.3	176.2					5.0
1395.0	63		-4	1100.2	3300.5	55.2	4.8	23.3					6.8
1754.0	64	Octocrylene	-5	1459.2	4377.5	73.2	-1.5	2.3	74.6	2.2	1.3	2.9	8.5
1833.0	65		-5	1538.2	4614.5	77.1	2.4	6.0					8.9
1761.0	66		-5	1466.2	4398.5	73.5	-1.2	1.3					8.5
2104.0	67	Octocrylene	-6	1809.2	5427.5	90.7	-0.3	0.1	91.2	1.5	0.9	1.6	10.2
2146.0	68		-6	1851.2	5553.5	92.8	1.8	3.4					10.4
2089.0	69		-6	1794.2	5382.5	90.0	-1.0	1.0					10.1
2259.0	70	Octocrylene	-7	1964.2	5892.5	98.5	2.5	6.1	96.5	3.5	2.0	3.6	10.9
2258.0	71		-7	1963.2	5889.5	98.4	2.4	5.8					10.9
2139.0	72		-7	1844.2	5532.5	92.5	-3.5	12.6					10.4
2150.0	73	Octocrylene	-8	1855.2	5565.5	93.0	-4.1	17.1	94.2	1.9	1.1	2.0	10.4
2156.0	74		-8	1861.2	5583.5	93.3	-3.8	14.7					10.4
2217.0	75		-8	1922.2	5766.5	96.4	-0.8	0.6					10.7
2279.0	76	Octocrylene	-9	1984.2	5952.5	99.5	2.1	4.4	99.5	4.0	2.3	4.0	11.0
2200.0	77		-9	1905.2	5715.5	95.5	-1.9	3.5					10.7
2358.0	78		-9	2063.2	6189.5	103.5	6.1	36.6					11.4
2191.0	79	Octocrylene	-10	1896.2	5688.5	95.1	-2.4	5.6	97.1	3.4	2.0	3.5	10.6
2309.0	80		-10	2014.2	6042.5	101.0	3.5	12.6					11.2
2191.0	81		-10	1896.2	5688.5	95.1	-2.4	5.6					10.6
2337.0	82	Octocrylene	-11	2042.2	6126.5	102.4	4.9	24.4	98.1	4.4	2.6	4.5	11.3
2161.0	83		-11	1866.2	5598.5	93.6	-3.9	15.1					10.5
2257.0	84		-11	1962.2	5886.5	98.4	0.9	0.9					10.9





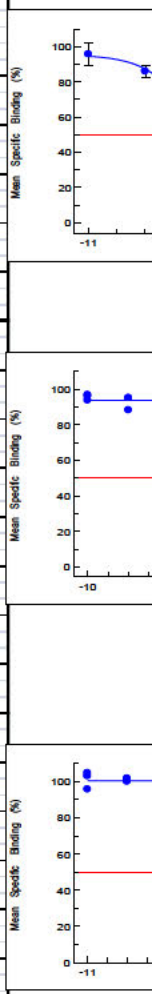


APPENDIX 1 Raw and Normalized Data Valid Run 3 – October 06, 2011

Experiment Date:	6-Oct-11		Study Number:	9070-100107ARB					Assays Conducted by:	[redacted]			
Test substance:	Oxybenzone												
1/27/2012 13:57													
		ug protein/assay tube =											

Tube	Sample Type	DPM (1mL)	Specific Binding DPM (100 uL) - NSB	Total Specific Binding (300 uL)	Mean
1	Total Activity (Master Mix)	59644	--	59644.0	57405.7
2		57430	--	57430.0	
3		55671	--	55671.0	
4		58179	--	58179.0	
5		58770	--	58770.0	
6		54740	--	54740.0	
7	Total Binding (Solvent Control)	2161	1914.0	5742	5886.5
8		2188	1941.0	5823	
9		2221	1974.0	5922	
10		2197	1950.0	5850	
11		2248	2001.0	6003	
12		2240	1993.0	5979	

DPM (1mL) from LSC	Tube	Sample Type	Concentration log[M]	Specific Binding DPM (1mL) - NSB	Total Specific Binding (3mL)	Specific Binding (%)	Residual	Squared Residual	Mean Specific Binding (%)	Standard Deviation	SEM	% CV	% Ligand Bound vs. Total Activity
260.0	13	R1881 (NSB)	-6	13.0	39.0	0.7	0.3	0.1	0.0	1.3	0.5	#DIV/0!	1.4
271.0	14		-6	24.0	72.0	1.2	0.9	0.8					1.4
218.0	15		-6	-29.0	-87.0	-1.5	-1.8	3.2					1.1
246.0	16		-6	-1.0	-3.0	-0.1	-0.4	0.1					1.3
272.0	17		-6	25.0	75.0	1.3	1.0	0.9					1.4
215.0	18		-6	-32.0	-96.0	-1.6	-1.9	3.8					1.1
306.0	19	R1881	-7	59.0	177.0	3.0	1.9	3.4	2.8	0.4	0.2	14.7	1.6
308.0	20		-7	61.0	183.0	3.1	2.0	3.8					1.6
293.0	21		-7	46.0	138.0	2.3	1.2	1.4					1.5
381.0	22	R1881	-8	134.0	402.0	6.8	-2.1	4.4	7.5	1.8	1.0	24.0	2.0
367.0	23		-8	120.0	360.0	6.1	-2.8	7.9					1.9
434.0	24		-8	187.0	561.0	9.5	0.6	0.4					2.3
1197.0	25	R1881	-9	950.0	2850.0	48.4	-0.1	0.0	49.3	0.8	0.5	1.7	6.3
1216.0	26		-9	969.0	2907.0	49.4	0.9	0.8					6.4
1230.0	27		-9	983.0	2949.0	50.1	1.6	2.5					6.4
1862.0	28	R1881	-10	1615.0	4845.0	82.3	-5.1	26.4	86.1	3.5	2.0	4.1	9.7
1999.0	29		-10	1752.0	5256.0	89.3	1.8	3.4					10.4
1950.0	30		-10	1703.0	5109.0	86.8	-0.6	0.4					10.2
2273.0	31	R1881	-11	2026.0	6078.0	103.3	8.3	69.3	95.8	6.4	3.7	6.7	11.9
2057.0	32		-11	1810.0	5430.0	92.2	-2.7	7.2					10.7
2053.0	33		-11	1806.0	5418.0	92.0	-2.9	8.3					10.7
281.0	37	Dexamethasone	-3	34.0	102.0	1.7	1.5	2.2	0.5	1.1	0.7	211.4	1.5
236.0	38		-3	-11.0	-33.0	-0.6	-0.8	0.6					1.2
256.0	39		-3	9.0	27.0	0.5	0.2	0.0					1.3
625.0	40	Dexamethasone	-4	378.0	1134.0	19.3	-0.6	0.3	19.2	0.4	0.2	2.0	3.3
632.0	41		-4	385.0	1155.0	19.6	-0.2	0.0					3.3
617.0	42		-4	370.0	1110.0	18.9	-1.0	0.9					3.2
1710.0	43	Dexamethasone	-5	1463.0	4389.0	74.6	4.7	22.5	70.8	3.3	1.9	4.6	8.9
1592.0	44		-5	1345.0	4035.0	68.5	-1.3	1.6					8.3
1608.0	45		-5	1359.0	4077.0	69.3	-0.6	0.3					8.4
1875.0	46	Dexamethasone	-6	1628.0	4884.0	83.0	-7.9	62.6	88.1	4.5	2.6	5.1	9.8
2021.0	47		-6	1774.0	5322.0	90.4	-0.5	0.2					10.6
2031.0	48		-6	1784.0	5352.0	90.9	0.0	0.0					10.6
2061.0	49	Dexamethasone	-7	1814.0	5442.0	92.4	-1.3	1.6	93.7	2.2	1.3	2.4	10.8
2059.0	50		-7	1812.0	5436.0	92.3	-1.4	1.9					10.8
2136.0	51		-7	1889.0	5667.0	96.3	2.6	6.5					11.2
2103.0	52	Dexamethasone	-8	1856.0	5568.0	94.6	0.6	0.3	95.8	2.6	1.5	2.7	11.0
2091.0	53		-8	1844.0	5532.0	94.0	0.0	0.0					10.9
2185.0	54		-8	1938.0	5814.0	98.8	4.8	22.6					11.4
2114.0	55	Dexamethasone	-9	1867.0	5601.0	95.1	1.1	1.2	92.9	3.9	2.3	4.2	11.0
2114.0	56		-9	1867.0	5601.0	95.1	1.1	1.2					11.0
1980.0	57		-9	1733.0	5199.0	88.3	-5.7	32.7					10.3
2147.0	58	Dexamethasone	-10	1900.0	5700.0	96.8	2.8	7.8	95.6	1.6	0.9	1.7	11.2
2132.0	59		-10	1885.0	5655.0	96.1	2.0	4.1					11.1
2087.0	60		-10	1840.0	5520.0	93.8	-0.3	0.1					10.9
1354.0	61	Oxybenzone	-4	1107.0	3321.0	56.4	-5.0	25.0	61.2	6.8	3.9	11.2	7.1
1602.0	62		-4	1355.0	4065.0	69.1	7.6	58.3					8.4
1390.0	63		-4	1143.0	3429.0	58.3	-3.2	10.0					7.3
2150.0	64	Oxybenzone	-5	1903.0	5709.0	97.0	6.3	39.3	92.0	5.2	3.0	5.7	11.2
2061.0	65		-5	1814.0	5442.0	92.4	1.7	3.0					10.8
1945.0	66		-5	1698.0	5094.0	86.5	-4.2	17.5					10.2
2131.0	67	Oxybenzone	-6	1884.0	5652.0	96.0	-2.2	4.6	96.2	0.9	0.5	1.0	11.1
2155.0	68		-6	1908.0	5724.0	97.2	-0.9	0.9					11.3
2119.0	69		-6	1872.0	5616.0	95.4	-2.8	7.6					11.1
2109.0	70	Oxybenzone	-7	1862.0	5586.0	94.9	-5.1	26.5	97.5	3.8	2.2	3.9	11.0
2125.0	71		-7	1878.0	5634.0	95.7	-4.3	18.8					11.1
2246.0	72		-7	1999.0	5997.0	101.9	1.8	3.4					11.7
2322.0	73	Oxybenzone	-8	2075.0	6225.0	105.8	5.2	27.4	103.4	3.6	2.1	3.5	12.1
2195.0	74		-8	1948.0	5844.0	99.3	-1.2	1.5					11.5
2313.0	75		-8	2066.0	6198.0	105.3	4.8	22.8					12.1
2254.0	76	Oxybenzone	-9	2007.0	6021.0	102.3	1.7	2.7	100.4	2.7	1.6	2.7	11.8
2156.0	77		-9	1909.0	5727.0	97.3	-3.3	11.2					11.3
2241.0	78		-9	1994.0	5982.0	101.6	1.0	1.0					11.7
2210.0	79	Oxybenzone	-10	1963.0	5889.0	100.0	-0.6	0.4	100.8	0.9	0.5	0.9	11.5
2222.0	80		-10	1975.0	5925.0	100.7	0.0	0.0					11.6
2244.0	81		-10	1997.0	5991.0	101.8	1.1	1.2					11.7
2126.0	82	Oxybenzone	-11	1879.0	5637.0	95.8	-4.9	24.1	101.2	4.8	2.8	4.7	11.1
2303.0	83		-11	2056.0	6168.0	104.8	4.1	16.9					12.0
2270.0	84		-11	2023.0	6069.0	103.1	2.4	5.9					11.9



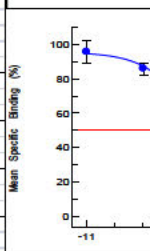
APPENDIX 1 (continued) Raw and Normalized Data Valid Run 3 – October 06, 2011

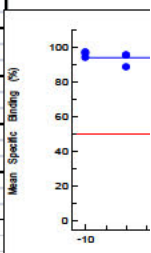
Experiment Date: 6-Oct-11 | Study Number: 9070-100107ARB | Assays Conducted by: [redacted]
Test substance: Methoxycinnamate
1/27/2012 13:59

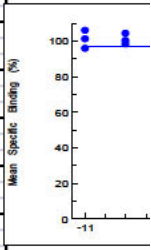
ug protein/assay tube =

Tube	Sample Type	DPM (1mL)	Specific Binding DPM (100 uL) - NSB	Total Specific Binding (300 uL)	Mean
1	Total Activity (Master Mix)	59644	--	59644.0	57405.7
2		57430	--	57430.0	
3		55671	--	55671.0	
4		58179	--	58179.0	
5		58770	--	58770.0	
6		54740	--	54740.0	
7	Total Binding (Solvent Control)	2161	1914.0	5742	5886.5
8		2188	1941.0	5823	
9		2221	1974.0	5922	
10		2197	1950.0	5850	
11		2248	2001.0	6003	
12		2240	1993.0	5979	

DPM (1mL) from LSC	Tube	Sample Type	Concentration log[M]	Specific Binding DPM (1mL) - NSB	Total Specific Binding (3mL)	Specific Binding (%)	Residual	Squared Residual	Mean Specific Binding (%)	Standard Deviation	SEM	% CV	% Ligand Bound vs. Total Activity
260.0	13	R1881 (NSB)	-6	13.0	39.0	0.7	0.3	0.1	0.0	1.3	0.5	#DIV/0!	1.4
271.0	14		-6	24.0	72.0	1.2	0.9	0.8					1.4
218.0	15		-6	-29.0	-87.0	-1.5	-1.8	3.2					1.1
246.0	16		-6	-1.0	-3.0	-0.1	-0.4	0.1					1.3
272.0	17		-6	25.0	75.0	1.3	1.0	0.9					1.4
215.0	18		-6	-32.0	-96.0	-1.6	-1.9	3.8					1.1
306.0	19	R1881	-7	59.0	177.0	3.0	1.9	3.4	2.8	0.4	0.2	14.7	1.6
308.0	20		-7	61.0	183.0	3.1	2.0	3.8					1.6
293.0	21		-7	46.0	138.0	2.3	1.2	1.4					1.5
381.0	22	R1881	-8	134.0	402.0	6.8	-2.1	4.4	7.5	1.8	1.0	24.0	2.0
367.0	23		-8	120.0	360.0	6.1	-2.8	7.9					1.9
434.0	24		-8	187.0	561.0	9.5	0.6	0.4					2.3
1197.0	25	R1881	-9	950.0	2850.0	48.4	-0.1	0.0	49.3	0.8	0.5	1.7	6.3
1216.0	26		-9	969.0	2907.0	49.4	0.9	0.8					6.4
1230.0	27		-9	983.0	2949.0	50.1	1.6	2.5					6.4
1862.0	28	R1881	-10	1615.0	4845.0	82.3	-5.1	26.4	86.1	3.5	2.0	4.1	9.7
1999.0	29		-10	1752.0	5256.0	89.3	1.8	3.4					10.4
1950.0	30		-10	1703.0	5109.0	86.8	-0.6	0.4					10.2
2273.0	31	R1881	-11	2026.0	6078.0	103.3	8.3	69.3	95.8	6.4	3.7	6.7	11.9
2057.0	32		-11	1810.0	5430.0	92.2	-2.7	7.2					10.7
2053.0	33		-11	1806.0	5418.0	92.0	-2.9	8.3					10.7
281.0	37	Dexamethasone	-3	34.0	102.0	1.7	1.5	2.2	0.5	1.1	0.7	211.4	1.5
236.0	38		-3	-11.0	-33.0	-0.6	-0.8	0.6					1.2
256.0	39		-3	9.0	27.0	0.5	0.2	0.0					1.3
625.0	40	Dexamethasone	-4	378.0	1134.0	19.3	-0.6	0.3	19.2	0.4	0.2	2.0	3.3
632.0	41		-4	385.0	1155.0	19.6	-0.2	0.0					3.3
617.0	42		-4	370.0	1110.0	18.9	-1.0	0.9					3.2
1710.0	43	Dexamethasone	-5	1463.0	4389.0	74.6	4.7	22.5	70.8	3.3	1.9	4.6	8.9
1592.0	44		-5	1345.0	4035.0	68.5	-1.3	1.6					8.3
1606.0	45		-5	1359.0	4077.0	69.3	-0.6	0.3					8.4
1875.0	46	Dexamethasone	-6	1628.0	4884.0	83.0	-7.9	62.6	88.1	4.5	2.6	5.1	9.8
2021.0	47		-6	1774.0	5322.0	90.4	-0.5	0.2					10.6
2031.0	48		-6	1784.0	5352.0	90.9	0.0	0.0					10.6
2061.0	49	Dexamethasone	-7	1814.0	5442.0	92.4	-1.3	1.6	93.7	2.2	1.3	2.4	10.8
2059.0	50		-7	1812.0	5436.0	92.3	-1.4	1.9					10.8
2136.0	51		-7	1889.0	5667.0	96.3	2.6	6.5					11.2
2103.0	52	Dexamethasone	-8	1856.0	5568.0	94.6	0.6	0.3	95.8	2.6	1.5	2.7	11.0
2091.0	53		-8	1844.0	5532.0	94.0	0.0	0.0					10.9
2185.0	54		-8	1938.0	5814.0	98.8	4.8	22.6					11.4
2114.0	55	Dexamethasone	-9	1867.0	5601.0	95.1	1.1	1.2	92.9	3.9	2.3	4.2	11.0
2114.0	56		-9	1867.0	5601.0	95.1	1.1	1.2					11.0
1980.0	57		-9	1733.0	5199.0	88.3	-5.7	32.7					10.3
2147.0	58	Dexamethasone	-10	1900.0	5700.0	96.8	2.8	7.8	95.6	1.6	0.9	1.7	11.2
2132.0	59		-10	1885.0	5655.0	96.1	2.0	4.1					11.1
2087.0	60		-10	1840.0	5520.0	93.8	-0.3	0.1					10.9
1947.0	61	Methoxycinnamate	-4	1700.0	5100.0	86.6	-10.9	118.1	89.3	2.4	1.4	2.6	10.2
2015.0	62		-4	1768.0	5304.0	90.1	-7.4	54.8					10.5
2035.0	63		-4	1788.0	5364.0	91.1	-6.4	40.7					10.6
2148.0	64	Methoxycinnamate	-5	1901.0	5703.0	96.9	-0.6	0.4	96.4	3.5	2.0	3.6	11.2
2066.0	65		-5	1819.0	5457.0	92.7	-4.8	23.1					10.8
2201.0	66		-5	1954.0	5862.0	99.6	2.1	4.3					11.5
2194.0	67	Methoxycinnamate	-6	1947.0	5841.0	99.2	1.7	3.0	99.0	0.3	0.2	0.3	11.5
2191.0	68		-6	1944.0	5832.0	99.1	1.6	2.5					11.5
2182.0	69		-6	1935.0	5805.0	98.6	1.1	1.2					11.4
2184.0	70	Methoxycinnamate	-7	1937.0	5811.0	98.7	1.2	1.5	95.7	8.5	4.9	8.9	11.4
2254.0	71		-7	2007.0	6021.0	102.3	4.8	22.8					11.8
1935.0	72		-7	1688.0	5064.0	86.0	-11.5	131.8					10.1
2265.0	73	Methoxycinnamate	-8	2018.0	6054.0	102.8	5.3	28.5	100.3	2.6	1.5	2.6	11.8
2164.0	74		-8	1917.0	5751.0	97.7	0.2	0.0					11.3
2216.0	75		-8	1969.0	5907.0	100.3	2.8	8.1					11.6
2196.0	76	Methoxycinnamate	-9	1949.0	5847.0	99.3	1.8	3.3	97.7	1.6	0.9	1.6	11.5
2134.0	77		-9	1887.0	5661.0	96.2	-1.3	1.8					11.2
2160.0	78		-9	1913.0	5739.0	97.5	0.0	0.0					11.3
2174.0	79	Methoxycinnamate	-10	1927.0	5781.0	98.2	0.7	0.5	100.9	3.1	1.8	3.0	11.4
2292.0	80		-10	2045.0	6135.0	104.2	6.7	45.1					12.0
2212.0	81		-10	1965.0	5895.0	100.1	2.6	7.0					11.6
2126.0	82	Methoxycinnamate	-11	1879.0	5637.0	95.8	-1.7	3.0	100.9	5.1	2.9	5.0	11.1
2230.0	83		-11	1983.0	5949.0	101.1	3.6	12.6					11.7
2325.0	84		-11	2078.0	6234.0	105.9	8.4	70.5					12.2







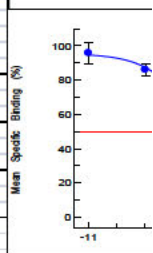
APPENDIX 1 (continued) Raw and Normalized Data Valid Run 3 – October 06, 2011

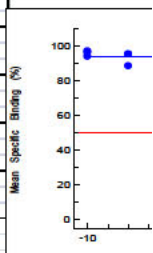
Experiment Date: 6-Oct-11 | Study Number: 9070-100107ARB | Assays Conducted by: [redacted]
Test substance: Octyl Salicylate
1/27/2012 14:00

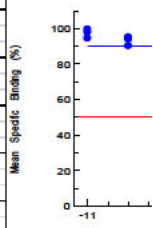
ug protein/assay tube =

Tube	Sample Type	DPM (1mL)	Specific Binding DPM (100 uL) - NSB	Total Specific Binding (300 uL)	Mean
1	Total Activity (Master Mix)	59644	--	59644.0	57405.7
2		57430	--	57430.0	
3		55671	--	55671.0	
4		58179	--	58179.0	
5		58770	--	58770.0	
6		54740	--	54740.0	
7	Total Binding (Solvent Control)	2161	1914.0	5742	5886.5
8		2188	1941.0	5823	
9		2221	1974.0	5922	
10		2197	1950.0	5850	
11		2248	2001.0	6003	
12		2240	1993.0	5979	

DPM (1mL) from LSC	Tube	Sample Type	Concentration log[M]	Specific Binding DPM (1mL) - NSB	Total Specific Binding (3mL)	Specific Binding (%)	Residual	Squared Residual	Mean Specific Binding (%)	Standard Deviation	SEM	% CV	% Ligand Bound vs. Total Activity
260.0	13	R1881 (NSB)	-6	13.0	39.0	0.7	0.3	0.1	0.0	1.3	0.5	#DIV/0!	1.4
271.0	14		-6	24.0	72.0	1.2	0.9	0.8					1.4
218.0	15		-6	-29.0	-87.0	-1.5	-1.8	3.2					1.1
246.0	16		-6	-1.0	-3.0	-0.1	-0.4	0.1					1.3
272.0	17		-6	25.0	75.0	1.3	1.0	0.9					1.4
215.0	18		-6	-32.0	-96.0	-1.6	-1.9	3.8					1.1
306.0	19	R1881	-7	59.0	177.0	3.0	1.9	3.4	2.8	0.4	0.2	14.7	1.6
308.0	20		-7	61.0	183.0	3.1	2.0	3.8					1.6
293.0	21		-7	46.0	138.0	2.3	1.2	1.4					1.5
381.0	22	R1881	-8	134.0	402.0	6.8	-2.1	4.4	7.5	1.8	1.0	24.0	2.0
367.0	23		-8	120.0	360.0	6.1	-2.8	7.9					1.9
434.0	24		-8	187.0	561.0	9.5	0.6	0.4					2.3
1197.0	25	R1881	-9	950.0	2850.0	48.4	-0.1	0.0	49.3	0.8	0.5	1.7	6.3
1216.0	26		-9	969.0	2907.0	49.4	0.9	0.8					6.4
1230.0	27		-9	983.0	2949.0	50.1	1.6	2.5					6.4
1862.0	28	R1881	-10	1615.0	4845.0	82.3	-5.1	26.4	86.1	3.5	2.0	4.1	9.7
1999.0	29		-10	1752.0	5256.0	89.3	1.8	3.4					10.4
1950.0	30		-10	1703.0	5109.0	86.8	-0.6	0.4					10.2
2273.0	31	R1881	-11	2026.0	6078.0	103.3	8.3	69.3	95.8	6.4	3.7	6.7	11.9
2057.0	32		-11	1810.0	5430.0	92.2	-2.7	7.2					10.7
2053.0	33		-11	1806.0	5418.0	92.0	-2.9	8.3					10.7
281.0	37	Dexamethasone	-3	34.0	102.0	1.7	1.5	2.2	0.5	1.1	0.7	211.4	1.5
236.0	38		-3	-11.0	-33.0	-0.6	-0.8	0.6					1.2
256.0	39		-3	9.0	27.0	0.5	0.2	0.0					1.3
625.0	40	Dexamethasone	-4	378.0	1134.0	19.3	-0.6	0.3	19.2	0.4	0.2	2.0	3.3
632.0	41		-4	385.0	1155.0	19.6	-0.2	0.0					3.3
617.0	42		-4	370.0	1110.0	18.9	-1.0	0.9					3.2
1710.0	43	Dexamethasone	-5	1463.0	4389.0	74.6	4.7	22.5	70.8	3.3	1.9	4.6	8.9
1592.0	44		-5	1345.0	4035.0	68.5	-1.3	1.6					8.3
1606.0	45		-5	1359.0	4077.0	69.3	-0.6	0.3					8.4
1875.0	46	Dexamethasone	-6	1628.0	4884.0	83.0	-7.9	62.6	88.1	4.5	2.6	5.1	9.8
2021.0	47		-6	1774.0	5322.0	90.4	-0.5	0.2					10.6
2031.0	48		-6	1784.0	5352.0	90.9	0.0	0.0					10.6
2061.0	49	Dexamethasone	-7	1814.0	5442.0	92.4	-1.3	1.6	93.7	2.2	1.3	2.4	10.8
2059.0	50		-7	1812.0	5436.0	92.3	-1.4	1.9					10.8
2136.0	51		-7	1889.0	5667.0	96.3	2.6	6.5					11.2
2103.0	52	Dexamethasone	-8	1856.0	5568.0	94.6	0.6	0.3	95.8	2.6	1.5	2.7	11.0
2091.0	53		-8	1844.0	5532.0	94.0	0.0	0.0					10.9
2185.0	54		-8	1938.0	5814.0	98.8	4.8	22.6					11.4
2114.0	55	Dexamethasone	-9	1867.0	5601.0	95.1	1.1	1.2	92.9	3.9	2.3	4.2	11.0
2114.0	56		-9	1867.0	5601.0	95.1	1.1	1.2					11.0
1980.0	57		-9	1733.0	5199.0	88.3	-5.7	32.7					10.3
2147.0	58	Dexamethasone	-10	1900.0	5700.0	96.8	2.8	7.8	95.6	1.6	0.9	1.7	11.2
2132.0	59		-10	1885.0	5655.0	96.1	2.0	4.1					11.1
2087.0	60		-10	1840.0	5520.0	93.8	-0.3	0.1					10.9
1262.0	61	Octyl Salicylate	-4	1015.0	3045.0	51.7	13.3	177.3	38.4	21.4	12.3	55.6	6.6
517.0	62		-4	270.0	810.0	13.8	-24.7	607.8					2.7
1223.0	63		-4	976.0	2928.0	49.7	11.3	128.3					6.4
1836.0	64	Octyl Salicylate	-5	1589.0	4767.0	81.0	-3.0	8.9	84.0	5.5	3.2	6.6	9.6
1828.0	65		-5	1581.0	4743.0	80.6	-3.4	11.5					9.6
2020.0	66		-5	1773.0	5319.0	90.4	6.4	40.9					10.6
2221.0	67	Octyl Salicylate	-6	1974.0	5922.0	100.6	10.3	106.2	97.3	3.2	1.8	3.3	11.6
2097.0	68		-6	1850.0	5550.0	94.3	4.0	15.9					11.0
2148.0	69		-6	1901.0	5703.0	96.9	6.6	43.4					11.2
2100.0	70	Octyl Salicylate	-7	1853.0	5559.0	94.4	4.1	17.1	91.6	3.3	1.9	3.6	11.0
2060.0	71		-7	1813.0	5439.0	92.4	2.1	4.4					10.8
1974.0	72		-7	1727.0	5181.0	88.0	-2.3	5.2					10.3
1407.0	73	Octyl Salicylate	-8	1160.0	3480.0	59.1	-31.2	972.2	73.1	16.3	9.4	22.2	7.4
1605.0	74		-8	1358.0	4074.0	69.2	-21.1	444.8					8.4
2031.0	75		-8	1784.0	5352.0	90.9	0.6	0.4					10.6
1936.0	76	Octyl Salicylate	-9	1689.0	5067.0	86.1	-4.2	17.8	89.4	5.2	3.0	5.8	10.1
2119.0	77		-9	1872.0	5616.0	95.4	5.1	26.1					11.1
1951.0	78		-9	1704.0	5112.0	86.8	-3.5	11.9					10.2
2016.0	79	Octyl Salicylate	-10	1769.0	5307.0	90.2	-0.1	0.0	93.0	2.6	1.5	2.8	10.5
2088.0	80		-10	1841.0	5523.0	93.8	3.5	12.4					10.9
2114.0	81		-10	1867.0	5601.0	95.1	4.9	23.5					11.0
2170.0	82	Octyl Salicylate	-11	1923.0	5769.0	98.0	7.7	59.4	97.4	2.6	1.5	2.7	11.3
2201.0	83		-11	1954.0	5862.0	99.6	9.3	86.2					11.5
2101.0	84		-11	1854.0	5562.0	94.5	4.2	17.5					11.0





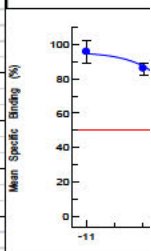


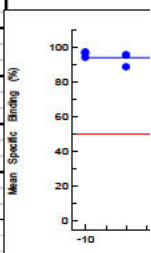
APPENDIX 1 (continued) Raw and Normalized Data Valid Run 3 – October 06, 2011

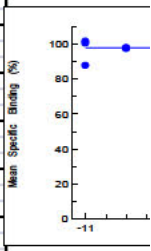
Experiment Date:	6-Oct-11	Study Number:	9070-100107ARB	Assays Conducted by:	[redacted]
Test substance:	Octocrylene				
1/27/2012 14:00					
	ug protein/assay tube =				

Tube	Sample Type	DPM (1mL)	Specific Binding DPM (100 uL) - NSB	Total Specific Binding (300 uL)	Mean
1	Total Activity (Master Mix)	59644	--	59644.0	57405.7
2		57430	--	57430.0	
3		55671	--	55671.0	
4		58179	--	58179.0	
5		58770	--	58770.0	
6		54740	--	54740.0	
7	Total Binding (Solvent Control)	2161	1914.0	5742	5886.5
8		2188	1941.0	5823	
9		2221	1974.0	5922	
10		2197	1950.0	5850	
11		2248	2001.0	6003	
12		2240	1993.0	5979	

DPM (1mL) from LSC	Tube	Sample Type	Concentration log[M]	Specific Binding DPM (1mL) - NSB	Total Specific Binding (3mL)	Specific Binding (%)	Residual	Squared Residual	Mean Specific Binding (%)	Standard Deviation	SEM	% CV	% Ligand Bound vs. Total Activity
260.0	13	R1881 (NSB)	-6	13.0	39.0	0.7	0.3	0.1	0.0	1.3	0.5	#DIV/0!	1.4
271.0	14		-6	24.0	72.0	1.2	0.9	0.8					1.4
218.0	15		-6	-29.0	-87.0	-1.5	-1.8	3.2					1.1
246.0	16		-6	-1.0	-3.0	-0.1	-0.4	0.1					1.3
272.0	17		-6	25.0	75.0	1.3	1.0	0.9					1.4
215.0	18		-6	-32.0	-96.0	-1.6	-1.9	3.8					1.1
306.0	19	R1881	-7	59.0	177.0	3.0	1.9	3.4	2.8	0.4	0.2	14.7	1.6
308.0	20		-7	61.0	183.0	3.1	2.0	3.8					1.6
293.0	21		-7	46.0	138.0	2.3	1.2	1.4					1.5
381.0	22	R1881	-8	134.0	402.0	6.8	-2.1	4.4	7.5	1.8	1.0	24.0	2.0
367.0	23		-8	120.0	360.0	6.1	-2.8	7.9					1.9
434.0	24		-8	187.0	561.0	9.5	0.6	0.4					2.3
1197.0	25	R1881	-9	950.0	2850.0	48.4	-0.1	0.0	49.3	0.8	0.5	1.7	6.3
1216.0	26		-9	969.0	2907.0	49.4	0.9	0.8					6.4
1230.0	27		-9	983.0	2949.0	50.1	1.6	2.5					6.4
1862.0	28	R1881	-10	1615.0	4845.0	82.3	-5.1	26.4	86.1	3.5	2.0	4.1	9.7
1999.0	29		-10	1752.0	5256.0	89.3	1.8	3.4					10.4
1950.0	30		-10	1703.0	5109.0	86.8	-0.6	0.4					10.2
2273.0	31	R1881	-11	2026.0	6078.0	103.3	8.3	69.3	95.8	6.4	3.7	6.7	11.9
2057.0	32		-11	1810.0	5430.0	92.2	-2.7	7.2					10.7
2053.0	33		-11	1806.0	5418.0	92.0	-2.9	8.3					10.7
281.0	37	Dexamethasone	-3	34.0	102.0	1.7	1.5	2.2	0.5	1.1	0.7	211.4	1.5
236.0	38		-3	-11.0	-33.0	-0.6	-0.8	0.6					1.2
256.0	39		-3	9.0	27.0	0.5	0.2	0.0					1.3
625.0	40	Dexamethasone	-4	378.0	1134.0	19.3	-0.6	0.3	19.2	0.4	0.2	2.0	3.3
632.0	41		-4	385.0	1155.0	19.6	-0.2	0.0					3.3
617.0	42		-4	370.0	1110.0	18.9	-1.0	0.9					3.2
1710.0	43	Dexamethasone	-5	1463.0	4389.0	74.6	4.7	22.5	70.8	3.3	1.9	4.6	8.9
1592.0	44		-5	1345.0	4035.0	68.5	-1.3	1.6					8.3
1606.0	45		-5	1359.0	4077.0	69.3	-0.6	0.3					8.4
1875.0	46	Dexamethasone	-6	1628.0	4884.0	83.0	-7.9	62.6	88.1	4.5	2.6	5.1	9.8
2021.0	47		-6	1774.0	5322.0	90.4	-0.5	0.2					10.6
2031.0	48		-6	1784.0	5352.0	90.9	0.0	0.0					10.6
2061.0	49	Dexamethasone	-7	1814.0	5442.0	92.4	-1.3	1.6	93.7	2.2	1.3	2.4	10.8
2059.0	50		-7	1812.0	5436.0	92.3	-1.4	1.9					10.8
2136.0	51		-7	1889.0	5667.0	96.3	2.6	6.5					11.2
2103.0	52	Dexamethasone	-8	1856.0	5568.0	94.6	0.6	0.3	95.8	2.6	1.5	2.7	11.0
2091.0	53		-8	1844.0	5532.0	94.0	0.0	0.0					10.9
2185.0	54		-8	1938.0	5814.0	98.8	4.8	22.6					11.4
2114.0	55	Dexamethasone	-9	1867.0	5601.0	95.1	1.1	1.2	92.9	3.9	2.3	4.2	11.0
2114.0	56		-9	1867.0	5601.0	95.1	1.1	1.2					11.0
1980.0	57		-9	1733.0	5199.0	88.3	-5.7	32.7					10.3
2147.0	58	Dexamethasone	-10	1900.0	5700.0	96.8	2.8	7.8	95.6	1.6	0.9	1.7	11.2
2132.0	59		-10	1885.0	5655.0	96.1	2.0	4.1					11.1
2087.0	60		-10	1840.0	5520.0	93.8	-0.3	0.1					10.9
1250.0	61	Octocrylene	-4	1003.0	3009.0	51.1	-0.2	0.0	51.3	1.5	0.9	3.0	6.5
1285.0	62		-4	1038.0	3114.0	52.9	1.6	2.6					6.7
1225.0	63		-4	978.0	2934.0	49.8	-1.4	2.1					6.4
1870.0	64	Octocrylene	-5	1623.0	4869.0	82.7	-2.0	4.1	84.8	2.2	1.3	2.6	9.8
1905.0	65		-5	1658.0	4974.0	84.5	-0.3	0.1					10.0
1955.0	66		-5	1708.0	5124.0	87.0	2.3	5.3					10.2
2319.0	67	Octocrylene	-6	2072.0	6216.0	105.6	7.5	56.0	98.6	6.9	4.0	7.0	12.1
2177.0	68		-6	1930.0	5790.0	98.4	0.2	0.1					11.4
2049.0	69		-6	1802.0	5406.0	91.8	-6.3	39.4					10.7
2259.0	70	Octocrylene	-7	2012.0	6036.0	102.5	4.4	19.5	97.3	7.2	4.1	7.4	11.8
2213.0	71		-7	1966.0	5898.0	100.2	2.1	4.3					11.6
1996.0	72		-7	1749.0	5247.0	89.1	-9.0	80.7					10.4
2246.0	73	Octocrylene	-8	1999.0	5997.0	101.9	3.8	14.1	100.0	1.8	1.0	1.8	11.7
2176.0	74		-8	1929.0	5787.0	98.3	0.2	0.0					11.4
2206.0	75		-8	1959.0	5877.0	99.8	1.7	3.0					11.5
2254.0	76	Octocrylene	-9	2007.0	6021.0	102.3	4.2	17.3	99.0	3.5	2.0	3.5	11.8
2196.0	77		-9	1949.0	5847.0	99.3	1.2	1.5					11.5
2119.0	78		-9	1872.0	5616.0	95.4	-2.7	7.4					11.1
2159.0	79	Octocrylene	-10	1912.0	5736.0	97.4	-0.7	0.5	97.4	0.3	0.2	0.3	11.3
2153.0	80		-10	1906.0	5718.0	97.1	-1.0	1.0					11.3
2164.0	81		-10	1917.0	5751.0	97.7	-0.4	0.2					11.3
2236.0	82	Octocrylene	-11	1989.0	5967.0	101.4	3.2	10.5	96.4	7.7	4.4	8.0	11.7
1965.0	83		-11	1718.0	5154.0	87.6	-10.6	111.6					10.3
2214.0	84		-11	1967.0	5901.0	100.2	2.1	4.5					11.6







APPENDIX 2 Rat Prostate Cytosol Preparation and Information

First Run – September 20, 2011

Supplier	Charles River Laboratories
Strain	Sprague-Dawley
Age	90 days
Days after castration	< 1
Protein Concentration	6.2 mg/mL
Method of Determination	Bradford Method
Supplier and Product	Bio-Rad Dye Reagent Concentrate
Catalog Number	500-0006
Batch/Lot Number	210007463
Method of Transport	FedEx – priority overnight
Conditions of Transport	Dry Ice

Second Run – September 22, 2011

Supplier	Charles River Laboratories
Strain	Sprague-Dawley
Age	90 days
Days after castration	< 1
Protein Concentration	5.7 mg/mL
Method of Determination	Bradford Method
Supplier and Product	Bio-Rad Dye Reagent Concentrate
Catalog Number	500-0006
Batch/Lot Number	210007463
Method of Transport	FedEx – priority overnight
Conditions of Transport	Dry Ice

Third Run – October 06, 2011

Supplier	Charles River Laboratories
Strain	Sprague-Dawley
Age	90 days
Days after castration	< 1
Protein Concentration	8.8 mg/mL
Method of Determination	Bradford Method
Supplier and Product	Bio-Rad Dye Reagent Concentrate
Catalog Number	500-0006
Batch/Lot Number	210007463
Method of Transport	FedEx – priority overnight
Conditions of Transport	Dry Ice

Isolation Procedure

- □ Make sure the homogenizer probe is pre-chilled before use, by placing it in a beaker of low-salt TEDG buffer on ice.

- □ Inspected prostate tissue for healthy appearance (no fibrous, inflamed, edematous or infected appearance) and discard any tissues that appear compromised; trim excess fascia if necessary.

- □ Weighed prostate tissues or use recorded tissue weights; calculate total weight and add to a beaker of low-salt TEDG buffer in ice bath, at 10 ml of buffer/g tissue.

- □ Mince with a scalpel blade and fine scissors until all pieces are small 1-2 mm cubes.

- □ Homogenize tissues at 4°C with a pre-chilled using a Polytron homogenizer. For a Polytron PT2100, use setting 3, and 3 short 4 sec bursts of power spaced at 20 sec intervals and place probe into ice cold TEDG buffer to cool it down between bursts.

- □ Transfer homogenates to pre-cooled centrifuge tubes, balance, and centrifuged at 30,000 x g for 30 minutes in a centrifuge cooled to 4°C. For centrifugation using a Sorvall RC5B centrifuge, use an SS-34 rotor and set centrifuge speed to 16,000 RPM.

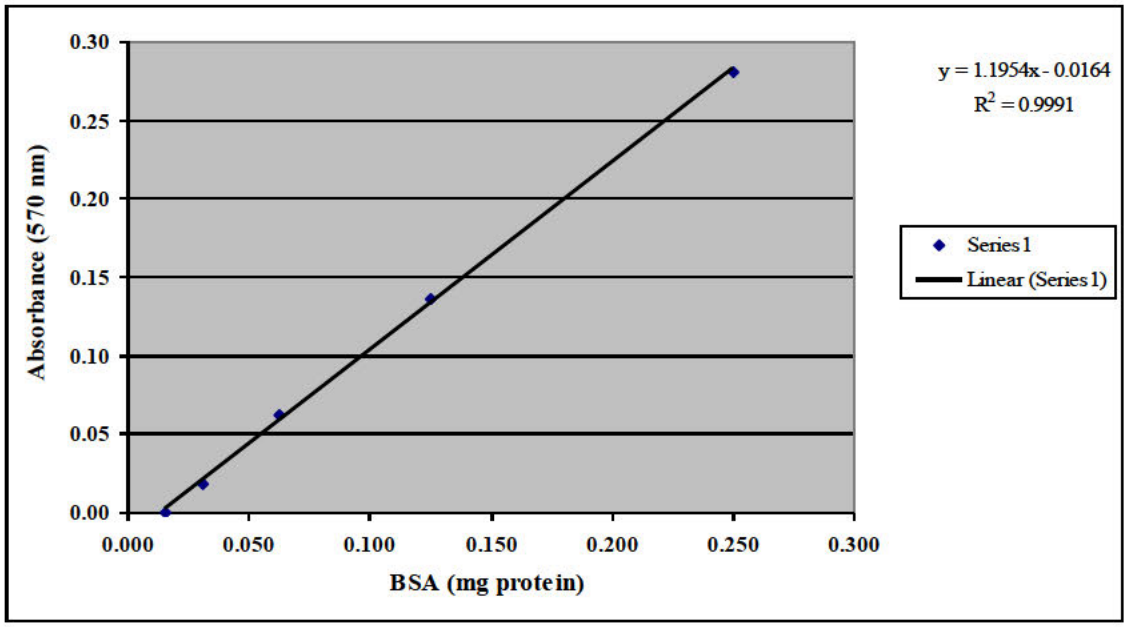
- □ The resulting supernatant contains the low-salt cytosolic receptor. Pool the supernatant from all rats.

- ☐ Aliquot into labeled tubes at a volume determined to reduce waste, as cytosolic supernatant cannot be refrozen once thawed (aliquoting multiple volumes allows flexibility). *Discard after 6 months unless revalidation run is performed.*

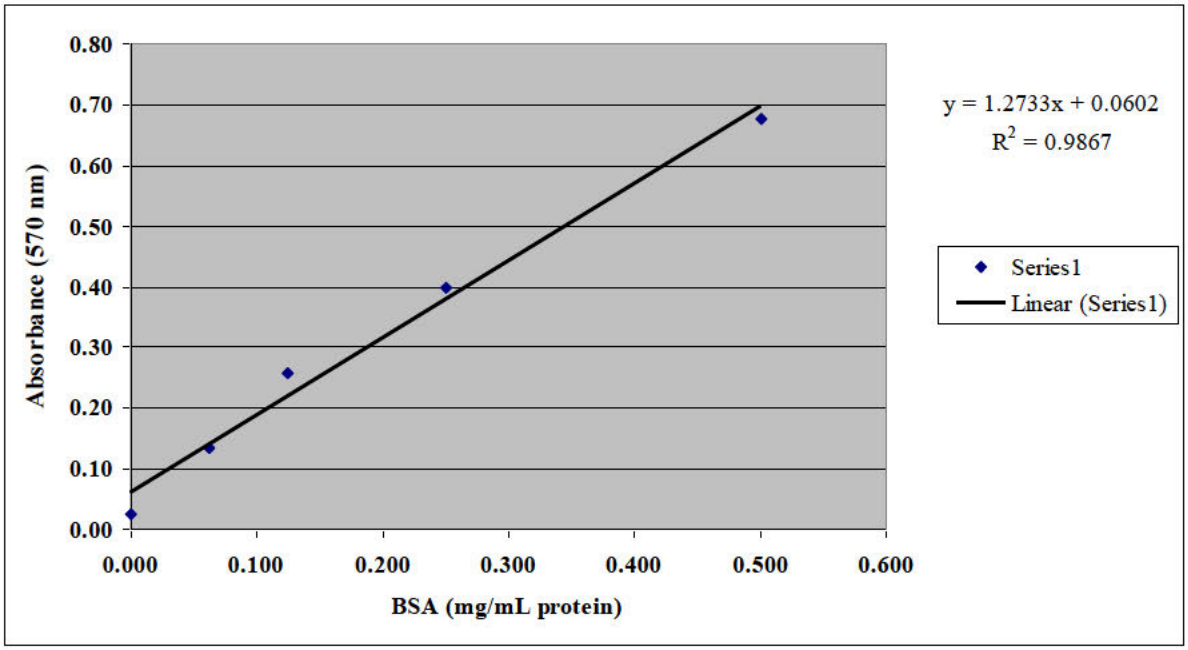
- ☐ Determined protein content for each batch of cytosol using a method that is compatible with buffers that contain DTT. Typical protein values are 1 to 4 mg/ml.

Note: *Some protein kits are not compatible with the DTT in the TEDG buffer. Be sure to use a protein assay that is compatible with DTT (e.g., BioRad Protein Assay Kit).*

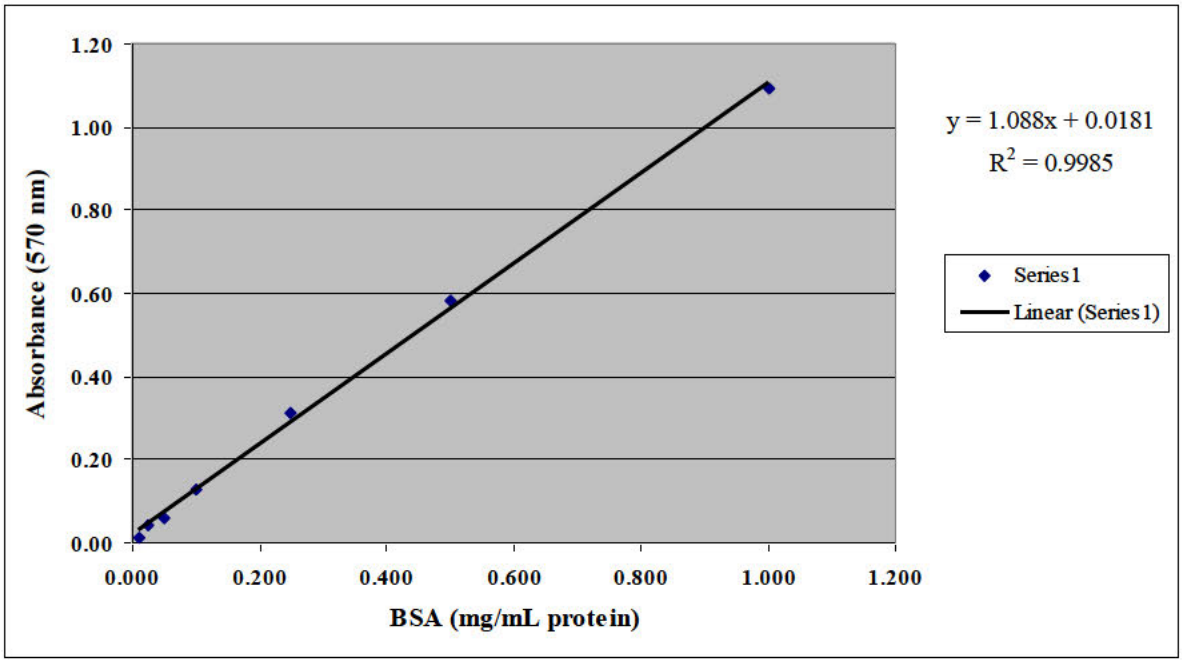
Calibration Curve – First Run – September 20, 2011



Calibration Curve – Second Run – September 22, 2011



Calibration Curve – Third Run – October 06, 2011



Raw Data Plate Map – First Run – September 20, 2011

	1	2	3	4	5	6	7	8	9	10	11	12
A	buffer bkg	buffer blank	water blank	0.5	0.5	0.5	3x cyto	3x cyto	3x cyto	buffer bkg	buffer blank	water blank
B	buffer bkg	buffer blank	water blank	0.25	0.25	0.25	5x cyto	5x cyto	5x cyto	buffer bkg	buffer blank	water blank
C	buffer bkg	buffer blank	water blank	0.125	0.125	0.125	10x cyto	10x cyto	10x cyto	buffer bkg	buffer blank	water blank
D	buffer bkg	buffer blank	water blank	0.06	0.06	0.06	20x cyto	20x cyto	20x cyto	buffer bkg	buffer blank	water blank
E	buffer bkg	buffer blank	water blank	0.5	0.5	0.5	40x cyto	40x cyto	40x cyto	buffer bkg	buffer blank	water blank
F	buffer bkg	buffer blank	water blank	0.25	0.25	0.25	80x cyto	80x cyto	80x cyto	buffer bkg	buffer blank	water blank
G	buffer bkg	buffer blank	water blank	0.125	0.125	0.125	5x water	5x water	5x water	buffer bkg	buffer blank	water blank
H	buffer bkg	buffer blank	water blank	0.06	0.06	0.06	40x water	40x water	40x water	buffer bkg	buffer blank	water blank
				BSA standards (mg/mL)	BSA standards (mg/mL)	BSA standards (mg/mL)	cytosol samples	cytosol samples	cytosol samples			

Raw Data– First Run – September 20, 2011

Plate Seq#: 8072

Comment: Acquired: Thursday, June 03, 2010 4:11 PM Temperature Min/Max: 0.0/0.0°C

Absorbance-A File Report: C:\Fusion data files\MTT_(null)_06-03-10_1142.TXT

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.056	0.412	0.414	0.839	0.922	0.943	1.544	1.711	1.369	0.044	0.042	0.045
B	0.050	0.416	0.414	0.629	0.695	0.707	1.154	1.226	1.194	0.048	0.042	0.047
C	0.053	0.414	0.428	0.517	0.574	0.573	0.841	0.881	0.876	0.044	0.043	0.040
D	0.057	0.416	0.416	0.447	0.465	0.476	0.630	0.688	0.662	0.046	0.052	0.042
E	0.054	0.416	0.419	0.839	0.909	0.902	0.516	0.553	0.547	0.044	0.043	0.044
F	0.044	0.412	0.426	0.670	0.701	0.707	0.459	0.493	0.467	0.045	0.042	0.045
G	0.055	0.412	0.411	0.530	0.558	0.569	1.462	1.288	1.986	0.043	0.040	0.042
H	0.060	0.421	0.420	0.451	0.478	0.480	0.528	0.543	0.544	0.042	0.041	0.040

Raw Data Plate Map– second Run – September 22, 2011

	1	2	3	4	5	6	7	8	9	10	11	12
A	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty
B	empty	empty	empty	3x cyto	3x cyto	3x cyto	empty	empty	2	2	2	empty
C	empty	empty	empty	5x cyto	5x cyto	5x cyto	empty	empty	1	1	1	empty
D	empty	empty	empty	10x cyto	10x cyto	10x cyto	empty	empty	0.5	0.5	0.5	empty
E	empty	empty	empty	20x cyto	20x cyto	20x cyto	empty	empty	0.25	0.25	0.25	empty
F	empty	empty	empty	40x cyto	40x cyto	40x cyto	empty	empty	0.125	0.125	0.125	empty
G	empty	empty	empty	80x cyto	80x cyto	80x cyto	empty	empty	0.06	0.06	0.06	empty
H	empty	empty	empty	empty	empty	empty	empty	empty	0	0	0	empty
					cytosol samples	cytosol samples	cytosol samples		BSA standards (mg/mL)	BSA standards (mg/mL)	BSA standards (mg/mL)	

Raw Data –Second Run – September 22, 2011

Plate Seq#: 8784

Comment: Acquired: Wednesday, March 30, 2011 5:25 PM Temperature Min/Max: 0.0/0.0⁻C

Absorbance-A File Report: C:\Fusion data files\MTT_(null)_03-30-11_1551.TXT

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.04	0.039	0.04	0.038	0.039	0.038	0.037	0.038	0.038	0.04	0.04	0.039
B	0.402	0.388	0.394	1.116	1.089	1.09	0.399	0.407	1.336	1.355	1.302	0.41
C	0.403	0.405	0.405	0.89	1.047	1.017	0.413	0.404	1.153	1.157	1.129	0.403
D	0.391	0.407	0.401	0.894	0.937	0.879	0.403	0.399	1.109	1.045	0.977	0.362
E	0.369	0.36	0.375	0.679	0.691	0.692	0.387	0.401	0.767	0.763	0.764	0.398
F	0.401	0.395	0.402	0.608	0.57	0.568	0.403	0.401	0.644	0.614	0.61	0.361
G	0.4	0.416	0.414	0.462	0.495	0.492	0.412	0.403	0.533	0.516	0.488	0.366
H	0.039	0.039	0.043	0.038	0.038	0.04	0.043	0.037	0.039	0.039	0.039	0.039

Raw Data Plate Map – Third Run – October 06, 2011

	1	2	3	4	5	6	7	8	9	10	11	12
A	neat cyto	neat cyto	neat cyto	water	empty	empty	empty	empty	empty	2	2	2
B	2X cyto	2X cyto	2X cyto	water	empty	empty	empty	empty	empty	1	1	1
C	3X cyto	3X cyto	3X cyto	water	empty	empty	empty	empty	empty	0.5	0.5	0.5
D	10X cyto	10X cyto	10X cyto	water	empty	empty	empty	empty	empty	0.25	0.25	0.25
E	20X cyto	20X cyto	20X cyto	water	empty	empty	empty	empty	empty	0.1	0.1	0.1
F	40X cyto	40X cyto	40X cyto	water	empty	empty	empty	empty	empty	0.05	0.05	0.05
G	buffer	buffer	buffer	water	empty	empty	empty	empty	empty	0.025	0.025	0.025
H	water	water	water	water	empty	empty	empty	empty	empty	0.01	0.01	0.01

Raw Data – Third Run – October 06, 2011

Plate Seq#: 9198

Comment: Acquired: Sunday, September 25, 2011 5:28 PM Temperature Min/Max: 0.0/0.0°C

Absorbance-A File Report: C:\Fusion data files\MTT_(null)_09-25-11_1732.TXT

	1	2	3	4	5	6	7	8	9	10	11	12
A	3.310	3.310	3.310	0.140	0.044	0.041	0.040	0.041	0.042	2.065	2.125	2.071
B	2.708	2.611	2.590	0.142	0.041	0.041	0.042	0.049	0.043	1.300	1.177	1.230
C	2.344	2.348	2.336	0.141	0.042	0.041	0.041	0.041	0.041	0.658	0.724	0.794
D	0.848	0.969	0.847	0.315	0.042	0.042	0.042	0.039	0.042	0.455	0.457	0.457
E	0.526	0.512	0.514	0.139	0.050	0.044	0.038	0.040	0.038	0.253	0.279	0.280
F	0.354	0.342	0.356	0.143	0.043	0.041	0.039	0.040	0.040	0.200	0.203	0.209
G	0.138	0.145	0.139	0.142	0.043	0.040	0.037	0.040	0.038	0.176	0.181	0.196
H	0.144	0.144	0.144	0.143	0.042	0.042	0.041	0.039	0.039	0.159	0.158	0.156

CeeTox
in vitro models to predict toxicity

I verify that the three saturation binding assays performed on the rat prostate cytosol batch isolated on 30-March-2011 were acceptable according to the Endocrine Disruptor Screening Program (EDSP) Test Guideline *OPPTS 890.1150: Androgen Receptor Binding (Rat Prostate Cytosol),* EPA 640-C-09-003, October, 2009, for use in the competitive binding assays. This cytosol preparation was also shown to be acceptable per OPPTS 890.1150 based upon the reference control results shown in this study report.

[redacted]

27 OCT 2011
Date

Senior Scientist/Endocrine Group Leader
CeeTox, Inc.

[redacted]

27 Oct 2011
Date

Director of Project Management
CeeTox, Inc.

CeeTox
In vitro models to predict toxicity

I verify that the three saturation binding assays performed on the rat prostate cytosol batch isolated on 23-July-2011 were acceptable according to the Endocrine Disruptor Screening Program (EDSP) Test Guideline *OPPTS 890.1150: Androgen Receptor Binding (Rat Prostate Cytosol)*, EPA 640-C-09-003, October, 2009, for use in the competitive binding assays. This cytosol preparation was also shown to be acceptable per OPPTS 890.1150 based upon the reference control results shown in this study report.

[redacted]

27 OCT 2011
Date

Senior Scientist/Endocrine Group Leader
CeeTox, Inc.

[redacted]

27 Oct 2011
Date

Director of Project Management
CeeTox, Inc.

CeeTox

I verify that the three saturation binding assays performed on the rat prostate cytosol batch isolated on 24-September-2011 were acceptable according to the Endocrine Disruptor Screening Program (EDSP) Test Guideline *OPPTS 890.1150: Androgen Receptor Binding (Rat Prostate Cytosol),* EPA 640-C-09-003, October, 2009, for use in the competitive binding assays. This cytosol preparation was also shown to be acceptable per OPPTS 890.1150 based upon the reference control results shown in this study report.

[redacted]

27 OCT 2011
Date

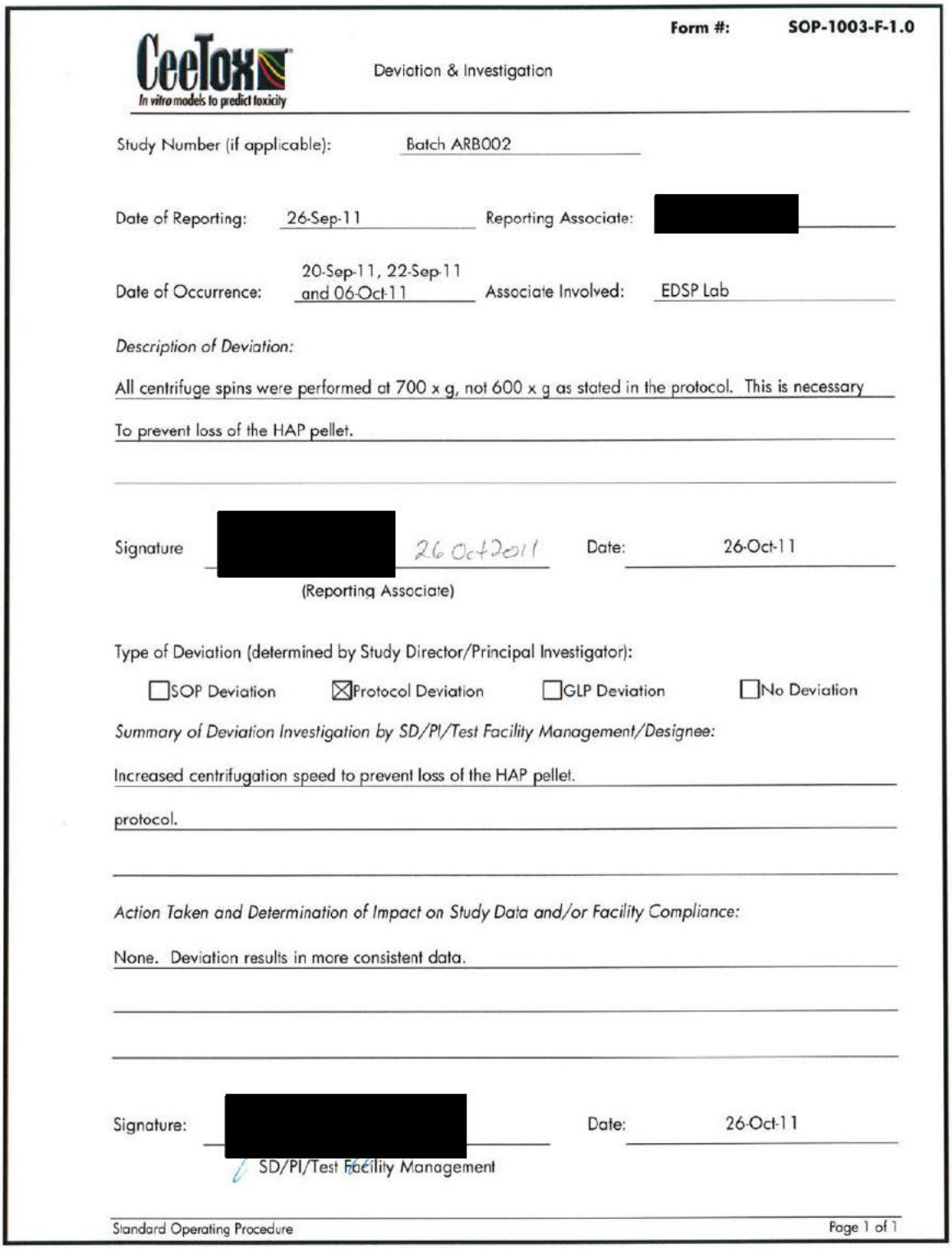
Senior Scientist/Endocrine Group Leader
CeeTox, Inc.

[redacted]

27 Oct 2011
Date

Director of Project Management
CeeTox, Inc.

APPENDIX 3 Deviation Forms



CeeTox
In vitro models to predict toxicity

Form #: SOP-1003-F-1.0

Deviation & Investigation

Study Number (if applicable): Batch ARB002

Date of Reporting: 26-Sep-11 Reporting Associate: [redacted]

Date of Occurrence: 20-Sep-11, 22-Sep-11 and 06-Oct-11 Associate Involved: EDSP Lab

Description of Deviation:

All centrifuge spins were performed at 700 x g, not 600 x g as stated in the protocol. This is necessary To prevent loss of the HAP pellet.

Signature [redacted] 26 Oct 2011 Date: 26-Oct-11

(Reporting Associate)

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

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

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

Increased centrifugation speed to prevent loss of the HAP pellet. protocol.

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

None. Deviation results in more consistent data.

Signature: [redacted] Date: 26-Oct-11

SD/PI/Test Facility Management

Standard Operating Procedure Page 1 of 1

Form #: SOP-1003-F-1.0

CeeTox
In vitro models to predict toxicity

Deviation & Investigation

Study Number (if applicable): Batch ARB002

Date of Reporting: 20-Sep-11 Reporting Associate: [redacted]

Date of Occurrence: 20-Sep-11, 22-Sep-11 and 06-Oct-11 Associate Involved: EDSP Lab

Description of Deviation:

150 mL of 60% HAP was measured and washed three times at 2 hour intervals with 50 mM Tris. The HAP was allowed to settle overnight at 4°C because it settles much better and the results are more accurate.

Signature [redacted] 26 Oct 2011 Date: 26-Oct-11

(Reporting Associate)

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

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

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

Washed 3x then let HAP settle overnight instead of wash once, settle overnight, was twice and use the next day.

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

None. Deviation results in more consistent data.

Signature: [redacted] Date: 26-Oct-11

SD/PI/Test Facility Management

Standard Operating Procedure Page 1 of 1

Form #: SOP-1003-F-1.0

CeeTox
In vitro models to predict toxicity

Deviation & Investigation

Study Number (if applicable): __9070-100107ARB_____

Date of Reporting: __27 Sep 2011___ Reporting Associate: _QA Auditor_ in process audit_____

Date of Occurrence: __20 Sep_2011___ Associate Involved: _n/a_____

Description of Deviation:

The temperatures for refrigerators 1, 2, 3, 7, 9 and freezers 4, 5, 6, 8 were not recorded on September 20th 2011. The impact of this deviation for this study is specific to Refrigerator #9 that contained materials for study number 9070-100107ARB. The min/max temperatures were examined for refrigerator #9 from the previous 24 hour perid (19Sept2011) and the post 24 hour period (21Sept2011). These temperatures were documented as: min=2°C and max=7°C for the 19th and min=2°C and max=5°C for the 21st. These min/max readings fall in to the acceptable range of 0-10°C documented in SOP-4007. Thus it can be expected that the temperature remained in range for the September 20th missed temperature documentation. It was determined upon investigation that there was no impact on study number 9070-100107ARB due to the missed temperature documentation on 20September2011.

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

Facility Deviation from SOP-4007

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

The records of the temperatures of the listed refrigerators and freezers were examined. All contents of freezers were examined for signs of freeze/thaw. The min/max temperatures were examined for excursions.

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

The result of the above listed investigation concluded there was no GLP study impact due to possible temperature excursions that could have been a result of the missed temperature monitoring for the September 20th time period.

Signature: [redacted] Date: 4 Oct 2011

SD/PI/Test Facility Management

Standard Operating Procedure Page 1 of 1

Form #: SOP-1003-F-1.0

CeeTox
In vitro models to predict toxicity

Deviation & Investigation

Study Number (if applicable): 9070-100107ARB

Date of Reporting: 27-Jan-12 Reporting Associate: [redacted]

Date of Occurrence: 06-Jul-11 Associate Involved: [redacted]

Description of Deviation:

The lot number for oxybenzone in the protocol (20080801) was not the lot provided (20100801)

Signature [redacted] Date: 27-Jan-12

(Reporting Associate)

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

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

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

Incorrect lot number in the protocol.

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

None.

Signature: [redacted] Date: 27-Jan-12

SD/PI/Test Facility Management

Standard Operating Procedure Page 1 of 1

Form #: SOP-1003-F-1.0

CeeTox
In vitro models to predict toxicity

Deviation & Investigation

Study Number (if applicable): 9070-100107ARB

Date of Reporting: 04-Jan-12 Reporting Associate: [redacted]

Date of Occurrence: 20-Sep-11, 22-Sep-11 and 06-Oct-11 Associate Involved: [redacted]

Description of Deviation:

Wrong purity was used for methoxycinnamate. Used 98% instead of 99.8%.

Signature [redacted] Date: 04-Jan-12

(Reporting Associate)

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

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

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

Wrong purity was used for methoxycinnamate.

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

None. After dilutions, the difference is negligible.

Signature: [redacted] Date: 04-Jan-12

SD/PI/Test Facility Management

Standard Operating Procedure Page 1 of 1

Form #: SOP-1003-F-1.0

CeeTox
In vitro models to predict toxicity

Deviation & Investigation

Study Number (if applicable): 9070-100107ARB

Date of Reporting: 04-Jan-12 Reporting Associate: [redacted]

Date of Occurrence: 21-Sep-11 Associate Involved: [redacted]

Description of Deviation:

Sponsor was not asked to sign amendments according to the protocol.

Signature [redacted] Date: 04-Jan-12

(Reporting Associate)

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

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

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

Sponsor was notified of pending Amendments but were not asked to sign the amendments as stated in the protocol.

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

None. Sponsor signature and date will be required for all future amendments, if any, for this study.

Signature: [redacted] Date: 04-Jan-12

SD/PI/Test Facility Management

Standard Operating Procedure Page 1 of 1

APPENDIX 4 Certificate of Analysis

IVYCHEM

IVY FINE CHEMICALS

http://www.ivychem.com

CERTICATE OF ANALYSIS

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

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

CERTIFICATE OF ANALYSIS

[redacted]

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

Specifications

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

General Product Data

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

Lot Specific Data for Lot No.: A0293319

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

ACROS ORGANICS

[redacted]

Issued: 10-08-10

[redacted] Quality Assurance Manager

Acros Organics Geel West Zone 2, Janssen Pharmaceuticalaan 3a, B-2440 Geel, Belgium Tel +32 14/57.52.11 - Fax +32 14/58.34.34 Internet: http://www.acros.com
1 Reagent Lane, Fair Lawn, NJ 07410,USA Fax 201-796-1329

[redacted]

MRS-N TDVTech 1492

A-1

Certificate of Analysis

SIGMA-ALDRICH

Product Name	2-Ethylhexyl salicylate, ≥99%
Product Number	W514600
Product Brand	ALDRICH
CAS Number	118-60-5
Molecular Formula	$(HO)C_6H_4CO_2CH_2CH(C_2H_5)(CH_2)_3CH_3$
Molecular Weight	250.33

TEST	SPECIFICATION	LOT 44696PJ RESULTS
Appearance (Color)	Colorless	Colorless
Appearance (Form)	Liquid	Liquid
Refractive Index at 20 °C	1.500 - 1.504	1.502
Infrared spectrum	Conforms to Structure	Conforms
Purity (GC)	≥99.0 %	99.6 %
Color Test	≤100 APHA	10 APHA
Arsenic (As)	≤3.0 ppm	< 1.0 ppm
Cadmium (Cd)	≤1.0 ppm	< 1.0 ppm
Mercury (Hg)	≤1.0 ppm	< 1.0 ppm
Lead (Pb)	≤10.0 ppm	< 1.0 ppm
Specification Date:		DEC 2008
Date of QC Release:		DEC 2008
Print Date:		DEC 18 2009

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

Certificate of Analysis

SIGMA-ALDRICH

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

TEST	SPECIFICATION	LOT 01697NJ RESULTS
Appearance (Color)	Yellow	Yellow
Appearance (Form)	Viscous Liquid	Viscous Liquid
Infrared spectrum	Conforms to Structure	Conforms
Purity (GC)	≥98.5 %	99.2 %
Specification Date:		OCT 2008
Date of QC Release:		OCT 2008
Print Date:		OCT 22 2008

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

APPENDIX 5 Protocol and Protocol Amendments

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

CeeTox®
In vitro models to predict toxicity

FINAL PROTOCOL

Androgen Receptor Binding (Rat Prostate Cytosol)

Data Requirements: *OPPTS 890.1150*

Author
[redacted]

Study Number:
9070-100107ARB

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

Test Facility:
CeeTox
4717 Campus Drive
Kalamazoo, MI 49008

TEST PROTOCOL

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

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

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

Table of Contents

Signatures

[redacted]
Study Sponsor

7/6/11
Date

[redacted]
Study Monitor

7/6/11
Date

[redacted]
Study Director

06 July 2011
Date

1. Title of Study

Androgen Receptor Binding (Rat Prostate Cytosol)

2. Purpose of Study

The objective of this protocol is to describe procedures for conduct of the Androgen Receptor Binding assay using Rat Prostate Cytosol for the source of the receptor as a Tier 1 screen. The ability of a substance to compete with [^{3}H] ligand for binding in rat ventral prostate tissue homogenate will be determined.

3. Compliance Statement

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

4. Quality Assurance

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

5. Regulatory Citations

Endocrine Disruptor Screening Program, *in vitro* Androgen Receptor Binding (Rat Prostate Cytosol) EPA Test Guideline OPPTS 890.1150.

6. Test Facility

CeeTox, Inc.
4717 Campus Drive
Kalamazoo, MI 49008

7. Test & Control Substance(s)

7.1 Test Substance

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. A certificate of analysis will be obtained from the vendor and included in the report for all reference control test substances. Test substance will be either returned to the Sponsor or destroyed following finalization of the study report.

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

CAS No.	131-57-7
Source:	Ivy Fine Chemicals Corporation
Lot/Batch No.:	20080801
ILS Repository No.:	11-29
Formula:	$C_{14}H_{12}O_3$
Description:	Light yellow powder
Storage	Room Temperature

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

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

7.3 *Test Substance: Octyl Salicylate (Octylsalate)*

CAS No.	118-60-5
Source:	Sigma-Aldrich
Lot/Batch No.:	44698PJ
ILS Repository No.:	11-30

Formula:	$C_{15}H_{22}O_3$
Description:	Colorless liquid
Storage	Room Temperature

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

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

7.5 Preparation of Test Substance

Vehicle (100% ethanol or appropriate solvent) will be kept at the same concentration as the positive and negative controls for the test substances. Each test substance will be dissolved in a solvent that solubilizes the test substance. Ethanol (100%) is the preferred solvent for this protocol. If a test substance is not soluble in ethanol, water, or DMSO an appropriate solvent will be used. Any solvent used to dissolve test substances will be tested with the reference controls for the run if suitable. Dose concentrations of test and control substances will not be verified using analytical methods.

Test substance solubility will be evaluated by visual inspection for evidence of precipitation.

Test substances are prepared as stocks in ethanol or other suitable solvent at 30X above the desired final concentration and serially diluted in the same solvent. Fresh stock solutions and dilutions will be prepared on the day of use in the assay.

7.6 Reference Substances

When testing substances for their ability to bind to the androgen receptor (AR), a solvent and weak positive control will be included in each experiment (i.e. one set of standards is needed in each run on a given day). A standard curve using R1881 as a positive control will be included to allow for an assessment of variability in the conduct of the experiment across time.

A weak positive substance (Dexamethasone) will be included when possible to demonstrate the sensitivity of each experiment and to allow an assessment of variability of the conduct of the experiment across time. The supplier, CAS number and purity will be included in the report.

Ethanol (EtOH), DMSO, purified water, or appropriate vehicle controls will be included when possible to indicate whether the solvent interacts with the test system and to serve as the indicator of total binding. The vehicle control provides the base from which to measure whether the solvent has an effect on the experiment system.

The Radioactive Ligand ([^{3}H]–R1881) supplier, catalog number and batch number will be included in the report. The specific activity (SA) and date for which that SA was certified by the supplier will be included along with the concentration as received from the supplier (Ci/mmol) and the concentrations tested (nM).

For the radioinert ligand (R1881), and reference control (Dexamethasone) the supplier, batch number, catalog number, CAS number and purity will be included in the report.

8. Stock Solution Preparation

Preparation of Stock Solutions for making Low-Salt TEDG (Tris, EDTA, DTT, Glycerol) Buffer

- 200mM EDTA Stock Solution: For example, 7.444g disodium EDTA will be added to 100 ml purified water. This solution will be stored at approximately 4°C.
- 1M Sodium Molybdate Stock Solution: For example, 10 ml purified water will be added to 2.419g sodium molybdate.
- 1M Tris Buffer: For example, 147.24g Tris-HCl and 8.0g Tris base will be added to 800 ml purified H_2O. The final volume will be brought to 1 Liter. The buffer will be refrigerated to approximately 4°C and then pH to ~7.4. The buffer will be stored at approximately 4°C.

Preparation of Low-Salt TEDG Buffer (pH ~7.4)

For example to make 100 ml of low-salt TEDG buffer, the following will be added in order:

- 87.15 ml purified water

- 1.0 ml 1M Tris
- 10.0 ml glycerol
- 100 µl 1M sodium molybdate
- 750 µl 200 mM EDTA
- 0.5 ml Calbiochem Protein Inhibitor Cocktail, Set III, EDTA Free (with PMSF)
- 15.4 mg DTT (will be added immediately before use, see below)

The pH of the final solution will be checked to make sure it is ~7.4 at approximately 4°C (the solution will be adjusted with HCl (~1M) or NaOH (~1N) as necessary).

15.4 mg DTT will be added directly to 100 ml low-salt TEDG buffer the morning of the receptor isolation (final concentration = 1 mM DTT).

Preparation of 60% hydroxyapatite (HAP) slurry:

- For example to prepare 50 mM Tris Buffer:
- 50 ml of 1M Tris will be added to 950 ml purified water. This solution will be stored at approximately 4°C. The pH of the final solution will be checked to make sure it is ~7.4 at approximately 4°C (the solution will be adjusted as necessary).
- BIO-RAD HT-Gel (Bio-Rad; Hercules, CA) will be shaken until all HAP is in suspension. The evening before the receptor extraction, an appropriate volume will be poured into a graduated cylinder, the top will be sealed and placed in the refrigerator for at least 2 hours.
- The phosphate buffer supernatant will be poured off, and the volume to brought to 100 ml with 50 mM Tris. The HAP will be suspended by sealing the top and inverting several times. The HAP will be placed in the refrigerator overnight.
- The next morning, HAP will be washed two more times with fresh 50 mM Tris buffer.
- After the last wash, enough 50 mM Tris will be added to make the final solution 60% slurry.
- This will be stored at approximately 4°C until ready for use in the extraction.

Preparation of [^{3}H]-R1881 Stock Solutions:

- For example the original stock of [^{3}H]–R1881 will be diluted to 0.1 µM (i.e., 1 X 10^{-7} M) by pipetting 1 µl of the stock solution for every specific activity unit (Ci/mmol) and diluting this to 10.0 ml with ethanol. Store the [^{3}H]–R1881 stock solution and dilutions at approximately -20°C.
- A 1 x 10^{-8} M stock of [^{3}H]–R1881 will be prepared by making a 10-fold dilution of the 1 x 10^{-7} M stock.
- A copy of the Certificate of Analysis for [^{3}H]–R1881 will be maintained with the study records.

Preparation of 100X Radioinert R1881 Solutions:

- A 5mM solution of R1881 will be prepared in ethanol (or alternate solvent if used with the test substance). For example, 5.00 mg of radioinert R1881 will be weighed in a tared amber vial and 3.516 ml solvent added. The 5mM stock 1:500 will be diluted in the same solvent to get 10μM stock.
- The 1 μM radioinert R1881 stock will be prepared by diluting the 10 μM stock 1:10 in an amber vial. This will be the 1 μM radioinert R1881 stock.
- The 0.1 μM radioinert R1881 stock will be prepared by pipetting the 1 μM stock 1:10 in an amber vial. This will be the 0.1 μM radioinert R1881 stock.

Preparation of Triamcinolone Acetonide Stock and Working Solutions:

- For 600 μM solution, for example 13.04 mg of triamcinolone acetonide will be added to absolute ethanol in a total volume of 50 ml. This will be mixed thoroughly and stored at approximately -20°C.
- The desired amount of 60 μM triamcinolone acetonide working solution will be prepared for the assay by making a 1:10 dilution of the 600 μM stock in ethanol. This will be mixed thoroughly and stored at approximately -20°C.

9. Tissue Homogenate Collection and Saturation Radioligand Binding Assay

Rat prostate cytosol was prepared and its use validated per EPA guideline and CeeTox SOP for use on this study. Related data will be maintained separate from this study and available upon request.

10. Competitive Radioligand Binding Assay

For the competitive binding assay, the optimal amount of cytosolic protein added contains enough receptor to bind no more than 10-15% of the radiolabeled R1881 that has been added to the tube. Preparation of serial dilutions of radioinert R1881 for standard curve:

Serial dilutions will be prepared in 100% ethanol (or alternate solvent if required) to yield the initial R1881 concentrations listed in the table below.

Table 1. Standard Curve – Recommended Standard Curve Concentrations

Standard	Initial R1881 Concentration (M)	Final R1881 Concentration (M) in AR Assay Tube
Negative Control	0	0
0	0 (Ethanol)	0
NSB	1×10^{-5}	1×10^{-6}
S1	3×10^{-6}	1×10^{-7}
S2	3×10^{-7}	1×10^{-8}
S3	3×10^{-8}	1×10^{-9}
S4	3×10^{-9}	1×10^{-10}
S5	3×10^{-10}	1×10^{-11}

Preparation of test substance stock solutions:

Test substances will be prepared at 30X the desired final concentration (listed in Table 2). Initial stocks will be prepared in 100% ethanol (or an alternate solvent if solubility problems are encountered) at a concentration of 30 mM.

Serial dilutions of the test substances will be prepared according to the scheme in Table 2.

Day 1:

An aliquot of cytosolic protein will be thawed on ice and diluted to the appropriate concentration with cold assay buffer (TEDG buffer).

An example of how the tubes will be set up and labeled is described in Table 2 below. 30 µl of 0.01 µM [^{3}H]-R1881 (1 x 10^{-8} M) and 50 µl triamcinolone acetonide (60 µM working solution) will be added to all tubes. For 3 tubes at the beginning of assay and at the end of assay, 100X inert R1881 (30 µl of 1 µM) will also be added. These are the nonspecific binding tubes. The tubes will be placed in speed-vac and dried. When dry, they will be removed and 10 µl of test substance stocks or radioinert R1881 standards will be added. 300 µl of diluted cytosol will be added to every tube on ice. The tubes will be gently vortexed and placed in a refrigerator overnight on rotator (20±2 hr). The first wash of the HAP slurry will be prepared as described above.

Table 2. Competitive Assay Tube Layout

Replicates	Competitor	Code	Competitor Initial Concentration (M)	Cytosol (µL)	Tracer (Hot R1881) Volume (µL)	Competitor Volume (µL)	Triamcinolone Volume (µL)	Competitor Final Concentration (M)	Aliquot (µL)	HAP (500 µL)
3	Ethanol	EtOH	--	300	30	10	50	--	100	500
3	Inert R1881	NSB	3.00E-05	300	30	10	50	1.00E-06	100	500
3	Inert R1881	S	3.00E-06	300	30	10	50	1.00E-07	100	500
3	Inert R1881	S	3.00E-07	300	30	10	50	1.00E-08	100	500
3	Inert R1881	S	3.00E-08	300	30	10	50	1.00E-09	100	500
3	Inert R1881	S	3.00E-09	300	30	10	50	1.00E-10	100	500
3	Inert R1881	S	3.00E-10	300	30	10	50	1.00E-11	100	500
3	Weak Positive	WPC	3.00E-02	300	30	10	50	1.00E-03	100	500
3	Weak Positive	WPC	3.00E-03	300	30	10	50	1.00E-04	100	500
3	Weak Positive	WPC	3.00E-04	300	30	10	50	1.00E-05	100	500
3	Weak Positive	WPC	3.00E-05	300	30	10	50	1.00E-06	100	500
3	Weak Positive	WPC	3.00E-06	300	30	10	50	1.00E-07	100	500
3	Weak Positive	WPC	3.00E-07	300	30	10	50	1.00E-08	100	500
3	Weak Positive	WPC	3.00E-08	300	30	10	50	1.00E-09	100	500
3	Weak	WPC	3.00E-09	300	30	10	50	1.00E-10	100	500

	Positive									
3	Unknown	TC1	3.00E-02	300	30	10	50	1.00E-03	100	500
3	Unknown	TC1	3.00E-03	300	30	10	50	1.00E-04	100	500
3	Unknown	TC1	3.00E-04	300	30	10	50	1.00E-05	100	500
3	Unknown	TC1	3.00E-05	300	30	10	50	1.00E-06	100	500
3	Unknown	TC1	3.00E-06	300	30	10	50	1.00E-07	100	500
3	Unknown	TC1	3.00E-07	300	30	10	50	1.00E-08	100	500
3	Unknown	TC1	3.00E-08	300	30	10	50	1.00E-09	100	500
3	Unknown	TC1	3.00E-09	300	30	10	50	1.00E-10	100	500
3	Ethanol	EtOH	--	300	30	10	50	--	100	500
3	Inert R1881	NSB	1.00E-05	300	30	30	50	1.00E-06	100	500
3	None	Hot	--	--	30	--		--	--	--
3	None	Hot	--	--	30	--		--	--	--

Codes: NSB: Non-specific binding; S: standard; WPC: weak positive control; TC-1: test substance #1

Day 2:

The HAP will be washed as described in section 8 above, diluted with 50 mM Tris to yield a 60% slurry, and the contents transferred to a 100 ml Erlenmeyer flask. A stir bar will be placed in the flask and the flask placed into a beaker containing ice-water. The HAP slurry will be stirred. While constantly stirring, 500 µl of HAP slurry will be pipetted into pre-labeled, pre-cooled 12x75 mm test tubes. One HAP tube will be prepared for each incubation tube. 100 µl will be pipetted from each of the incubation tubes into the appropriate pre-labeled tubes containing HAP. Each rack of HAP tubes will be vortexed and placed into the ice-water bath. Approximately every 5 minutes for a total of approximately 20 minutes they will be vortexed. The HAP tubes will be centrifuged for 2-3 minutes at approximately 4°C and 600 x g. The tubes will be placed back into the ice-water bath. The supernatant will be aspirated from each tube. 2 ml of 50 mM Tris will be added to each tube, vortexed and centrifuged at 600 x g. The supernatant will be decanted, the tube openings blotted and 2 ml of 50 mM Tris added. The Tris washing procedure will be repeated a total of 3 or 4 times the tubes kept at approximately 4°C at all times. Following the last wash and decanting, 2 ml of ethanol will be added to each tube. The tubes will be vortexed 3 times at ~5 minute intervals and centrifuged at 600xg for ~10 minutes. The supernatants will be decanted into individual 20 ml scintillation vials. 10 ml of scintillation cocktail will be added and the samples counted using the single label DPM program with quench correction.

11. Solubility/Precipitation Assay

The limit of test substance solubility will be determined by visual observation. Solubility of the test substance will be determined in solvent. It may be necessary to warm the stock solution of the test substance for 10 to 15 minutes before making the dilutions. In addition, the solutions will be watched closely when added to the experiment tube (as the test substance may precipitate upon addition to the tubes). If solubility issues occur, appropriate documentation will be provided.

12. Competitive Binding Analyses

Estimating the IC_{50}

Plot the data for the standard curve and each test substance as the percent [^{3}H]-R1881 bound versus the molar concentration. IC_{50} estimates will be made using GraphPad PRISM.

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 nonlinear regression program (GraphPad PRISM). 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 scale.

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

$$Y = \text{Bottom} + \frac{(\text{Top} - \text{Bottom})}{1 + 10^{((\log IC50 - X)\ \text{Hill Slope} + \log[(\text{top-bottom})/(50\text{-bottom}) - 1])}}$$

Where X is the logarithm of the concentration of test substance and Y is the percent of radioligand bound to the receptor. Log IC_{50} is X at Y=50%. "Top" and "bottom" refer to the value of Y when there is minimal binding by test substance, and when there is maximal binding by test substance, respectively. A concentration-response model is fitted for each test run for each test substance.

Calculation of RBA

The relative binding affinity (RBA) for each competitor will be calculated by dividing the IC_{50} for R1881 by the IC_{50} of the competitor and expressing as a percent.

$$\%\ \text{RBA} = \frac{IC_{50}\ \text{R1881}}{IC_{50}\ \text{Test Substance}} \times 100$$

Competitive Binding Performance Criteria

The competitive binding assay is functioning correctly if all of the following criteria have been met:

Increasing concentrations of unlabeled R1881 displace [^{3}H]-R1881 from the receptor in a manner consistent with one-site competitive binding. Specifically, the curve fitted to the radioinert R1881 data points using non-linear regression descend from 90% to 10% over approximately an 81-rold increase in the concentration of radioinert R1881.

Ligand depletion is minimal.

The parameter values (top, bottom, and slope) for the standard curve (R1881) and the weak positive control are within the tolerance bounds provided in Table 3.

The solvent control substance does not alter the sensitivity or reliability of the assay.

Table 3. Guidance Values for Performance

Substance	Parameter	Lower Limit	Upper Limit
Standard Curve	Slope	-1.2	-0.8
	Top (%)	82	114
	Bottom (%)	-2.0	+2.0
Weak Positive	Slope	-1.4	-0.6
	Top (%)	87	106
	Bottom (%)	-12	+12

For all test substances, it is recommended that the top of the curve fall within 80-115% binding.

13. Data Interpretation Procedure

The classification of a test substance as a binder or non-binder is made on the basis of the average results of three runs. The data interpretation criteria are presented in Table 4.

Table 4. Data Interpretation Criteria

	Criteria	Classification
Data fit 4-parameter nonlinear regression model	Average curve across runs crosses 50%*	Binder
	Average lowest portion of the curves across runs is between 50 and 75% activity**	Equivocal
	Average lowest portion of curves across runs is greater than 75% activity**	Non-Binder
Data do not fit the model	--	

*Ordinarily, a binding curve will fall from 90% to 10% over 2 log units with a slope near -1. If the curve falls outside the range for the weak positive control (-0.6 to -1.4), the run will be classified as equivocal. Unusually steep curves may be a sign that the protein is being denatured or that solubility problems are being encountered.
**If the test substance is not soluble above 10^{-6} M and the binding curve does not cross 50%, the substance is judged to be untestable. If the curve is steeper than -2.0 the result is considered to be equivocal.

14. Test System

As per the guideline (OPPTS 890.1150) prostate glands from Sprague-Dawley male rats (60 to 90 days of age at time of kill) castrated approximately 24 hours prior to being humanely killed will be used to prepare the cytosol.

15. Study Reports

The data to be reported in the interim data summary 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 substance name, code, molecular weight, concentrations tested, notes regarding solubility).

16. Alterations of the Study Design

Alterations of this protocol may be made as the study progresses. No changes in the protocol will be made without the specific written request or consent of the Sponsor. In the event that the Sponsor authorizes a protocol change verbally, CeeTox will honor such a change. However, written authorization will be obtained thereafter. All protocol amendments and justifications will be documented, signed and dated by the Study Director, Study Monitor and Sponsor and added to the report. A copy of the protocol and all amendments will be issued to the Sponsor as well as CeeTox and placed into the study binder.

17. Data Retention and Archiving

All raw data, documentation, records, protocol, and the final report generated as a result of this study will be retained at CeeTox for 15 years. Correspondence and other documents relating to interpretation and evaluation of data other than those document contained in the final report, also shall be retained. Retention of the materials after 15 years will be subjected to a future contractual agreement between the Sponsor and CeeTox.

Study Records to be maintained:

All records that document the conduct of the laboratory experiments and results obtained, as well as the equipment and chemicals used.

Protocol and any Amendments

List of any Protocol Deviations

Final Report

CeeTox

Protocol Amendment

Study Number: 9070-100107ARB

Title of Study to be Amended: Androgen Receptor Binding (Rat Prostate Cytosol)

Reason for Amendment to Protocol: The volume of scintillation fluid was altered for consistency across all ARB assays run.

Change:
Section 10 Day 2 the last sentence currently states:

"10 ml of scintillation cocktail will be added and the samples counted using the single label DPM program with quench correction."

Section 10 Day 2 the last sentence will now state:

"14 ml of scintillation cocktail will be added and the samples counted using the single label DPM program with quench correction."

Signature

CeeTox, Inc.

[redacted] _____ 21 Sept 2011
Study Director (Project Manager) Date

CeeTox

Protocol Amendment

Study Number: 9070-100107ARB

Title of Study to be Amended: Androgen Receptor Binding (Rat Prostate Cytosol)

Reason for Amendment to Protocol: Client requested amendment

Change:
Section Data Retention and Archiving will now state:

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

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

Signature

CeeTox, Inc.

[redacted]
Study Monitor

12-6-11
Date

[redacted]
Study Director (Project Manager)

06 Dec 11
Date