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Androgen Receptor Binding (Rat Prostate Cytosol)

Final Report

DATA REQUIREMENT(S): OPPTS 890.1150 (2009)

AUTHOR(S):

STUDY COMPLETION DATE: January 27, 2012

TEST FACILITY:

CeeTox, Inc. 4717 Campus Drive Kalamazoo, MI 49008 USA

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SPONSOR(S): NIEHS 530 Davis Drive, MD K2-12 PO BOX 12233 Durham, NC 27713

STUDY MONITOR:

(ILS, Inc, Durham, NC)

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.

	/	

27 Jan 2012 Date

Study Director

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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	27 Jun11	
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.

27 Jan 2012 Date

Quality Assurance Auditor 4717 Campus Drive Kalamazoo, MI 49008

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Name	Title
	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

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 dexame thasone 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 LogIC₅₀ of -4.3 M while the LogIC₅₀ 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 LogIC₅₀ of -4.8 M and an RBA of 0.5. The weak positive control dexamethasone had a LogIC₅₀ of -4.5 M while the LogIC₅₀ of R1881 was -9.0 M.

The mean relative binding affinity, or RBA (calculated by dividing the LogIC₅₀ of the control/test material by the LogIC₅₀ 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 contificate of analysis for the ta	at substance is presented in Appendix 1

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

Octyl salicylate, 2-ethylhexyl salicylate
(Octylsalate)
Sigma-Aldrich
118-60-5
Colorless liquid
DMSO
44698PJ
Not Provided
99.6%
C ₁₅ H ₂₂ O ₃
250.33
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 [³H]-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 x 10 ⁻² M	1 x 10 ⁻³ M
TC2	Use 100 µl of dilution TC1 (50 mM)	900 µl	1 ml	3 x 10 ⁻³ M	1 x 10 ⁻⁴ M
TC3	Use 100 µl of dilution TC2 (5 mM)	900 µl	1 ml	3 x 10 ⁻⁴ M	1 x 10 ⁻⁵ M
TC4	Use 100 µl of dilution TC3 (500 µM)	900 µl	1 ml	3 x 10 ⁻⁵ M	1 x 10 ⁻⁶ M
TC5	Use 100 µl of dilution TC4 (50 µM)	900 µl	1 ml	3 x 10 ⁻⁶ M	1 x 10 ⁻⁷ M
TC6	Use 100 µl of dilution TC5 (5 µM)	900 µl	1 ml	3 x 10 ⁻⁷ M	1 x 10 ⁻⁸ M
TC7	Use 100 µl of dilution TC6 (500 nM)	900 µl	1 ml	3 x 10 ⁻⁸ M	1 x 10 ⁻⁹ M
TC8	Use 100 µl of dilution TC7 (50 nM)	900 µl	1 ml	3 x 10 ⁻⁹ M	1 x 10 ⁻¹⁰ M

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

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%).

Lampi	Example Dilution Procedure for K1681					
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 µ1	1 ml	1 x 10 ⁻³ M		
NSB1	Use 30 µl of stock R1881 (1 mM)	970 µl	1 ml	3 x 10 ⁻⁵ M	1 x 10 ⁻⁶	
S2	Use 100 µl of dilution NSB1 (30 µM)	900 µl	1 ml	3 x 10 ⁻⁶ M	1 x 10 ⁻⁷	
S 3	Use 100 µl of dilution S2 (3 µM)	900 µl	1 ml	3 x 10 ⁻⁷ M	1 x 10 ⁻⁸	
S4	Use 100 µl of dilution S3 (300 nM)	900 µl	1 ml	3 x 10 ⁻⁸ M	1 x 10 ⁻⁹	
S5	Use 100 µl of dilution S4 (30 nM)	900 µl	1 ml	3 x 10 ⁻⁹ M	1 x 10 ⁻¹⁰	
S6	Use 100 µl of dilution S5 (3 nM)	900 µl	1 ml	3 x 10 ⁻¹⁰ M	1 x 10 ⁻¹¹	

Example Dilution	Procedure fo	r R1881
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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 1×10^{-3} to 1×10^{-10} M with DMSO kept at approximately 3.2%.

Tube #	Volume of stock to add for diluted	Volume of solvent to	Total volume of	Weak Positive Control Concentration		
Tube #	concentration	add	diluted positive control	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	
Р3	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	

Example Dilution Procedure for Dexamethasone

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 $4\pm2^{\circ}$ 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 $4\pm2^{\circ}$ C before adjusting to pH 7.4 and stored at $4\pm2^{\circ}$ C up to 3 months]).

A 600 μ M stock solution of triamcinolone acetonide was prepared in 100% ethanol and diluted/aliquoted into 60 μ M 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 $4\pm2^{\circ}$ 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 ($4\pm2^{\circ}$ C). The slurry was mixed gently and allowed to settle for approximately 2 hours at $4\pm2^{\circ}$ C. After the third wash, the HAP slurry settled overnight (at least 8 to 10 hours at $4\pm2^{\circ}$ 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

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

Summary Table of Assay Conditions

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 [³H]-R1881 Preparation

[³H]-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:

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

SA is the specific activity on the packaging date. Kdecay is the decay constant for tritium (equal to 1.54×10^{-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 μ l of 10 nM [³H]-R1881 (1 x 10⁻⁸ M) and 50 μ l triamcinolone acetonide (60 μ M 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 μ l of 1 μ M) 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 predetermined optimal protein concentration.

3.5.4 Individual Tubes

For the assay tubes, 10 μ l of each concentration of test chemical and control was added, followed by 300 μ l 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 [³H]-R1881 From Free [³H]-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 μ l of ice cold HAP slurry (60% in 50 mM Tris buffer) to fresh new 12 x 75 mm siliconized assay tubes. 100 μ l 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 [³H]-R1881 was immediately decanted and discarded. The HAP pellet contained the androgen receptor bound [³H]-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 [³H]-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 (³H, ¹⁴C 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 ³H and ¹⁴C 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 [³H]-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 $\leq 10\%$. 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.

Standar as (Italionitit' Itiool and achanicinasone)				
Chemical	Parameter	Lower Limit	Upper Limit	
D1001	Slope	-1.2	-0.8	
R1881 (Standard Curve)	Top (%)	82	114	
	Bottom (%)	-2	+2	
Devemethesene	Slope	-1.4	-0.6	
Dexamethasone (Weak Positive)	Top (%)	87	106	
	Bottom (%)	-12	+12	

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

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:2Equivocal:1Non-binder:0

Chemical classification, based on the average of all the runs performed for a chemical:Binder: $average \ge 1.5$ Equivocal: $0.5 \le average < 1.5$ 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 $1 = \sim 1.67$ 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 [³H]-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 LogIC₅₀ of -4.4 M while the LogIC₅₀ 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 LogIC₅₀ of -4.3 M while the LogIC₅₀ 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 LogIC₅₀ of -4.8 M and an RBA of 0.5. The weak positive control dexamethasone had a LogIC₅₀ of -4.5 M while the LogIC₅₀ of R1881 was -9.0 M.

The mean relative binding affinity, or RBA (calculated by dividing the LogIC₅₀ of the control/test material by the LogIC₅₀ 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

Test Material	Concentration (Log[M])	Specific Binding (%)	Standard Deviation	Standard Error of Mean	% Coefficient of Variation
	-6	0.0	1.7	0.7	N/A
	-7	3.1	1.0	0.6	31.1
D1991 (NCD)	-8	13.1	1.5	0.9	11.6
R1881 (NSB)	-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
	-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
Dexamethasone	-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 1
 Results of 1st Valid Binding Assay – Controls – September 20, 2011

TABLE 2Results 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
	-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
Orreleanne	-6	102.1	2.8	1.6	2.8
Oxybenzone	-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
	-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
Octyl-	-6	96.4	6.5	3.8	6.8
methoxycinnamate	-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
	-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
Ostrilaslata	-6	89.1	4.9	2.8	5.5
Octylsalate	-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
	-3	45.4	1.2	0.7	2.6
Octocrylene	-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 31st Valid Run - Upper and Lower Parameters in Competitive AssayBinding 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

Test Material	Concentration (Log[M])	Specific Binding (%)	Standard Deviation	Standard Error of Mean	% Coefficient of Variation
	-6	0.0	2.3	0.9	N/A
	-7	1.8	1.6	0.9	88.1
D1001 (NCD)	-8	3.8	0.6	0.4	16.5
R1881 (NSB)	-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
	-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
Dexamethasone	-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 4Results 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
	-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
Oraclessor	-7	92.0	7.9	4.6	8.6
Oxybenzone	-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
	-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
Octyl-	-7	97.1	5.4	3.1	5.6
methoxycinnamate	-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
	-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
Ostvlaslata	-7	98.0	1.5	0.9	1.6
Octylsalate	-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
	-4	50.4	11.7	6.7	23.1
Oste emilene	-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
Octocrylene	-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 5Results of 2nd Binding Assay – Test Articles – September 22, 2011

TABLE 6Results of 2nd Binding Assay - Upper and Lower Parameters inCompetitive 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

Test Material	Concentration (Log[M])	Specific Binding (%)	Standard Deviation	Standard Error of Mean	% Coefficient of Variation
	-6	0.0	1.3	0.5	N/A
	-7	2.8	0.4	0.2	14.7
D1001 (NCD)	-8	7.5	1.8	1.0	24.0
R1881 (NSB)	-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 7Results of 3rd Binding Assay – Controls – October 06, 2011

Test Material	Concentration (Log[M])	Specific Binding (%)	Standard Deviation	Standard Error of Mean	% Coefficient of Variation
	-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
Ownhangana	-7	97.5	3.8	2.2	3.9
Oxybenzone	-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
	-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
Octyl-	-7	95.7	8.5	4.9	8.9
methoxycinnamate	-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
	-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
Ostvlaslata	-7	91.6	3.3	1.9	3.6
Octylsalate	-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
	-4	51.3	1.5	0.9	3.0
Ontermine	-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
Octocrylene	-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 8 Results of 3rd Binding Assay – Test Articles – October 06, 2011

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

Report Number: 9070-100107ARB

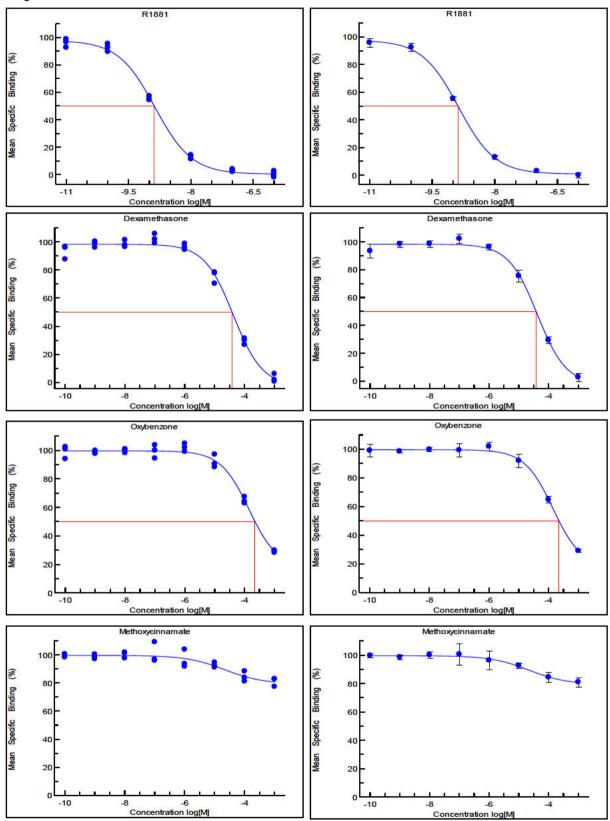
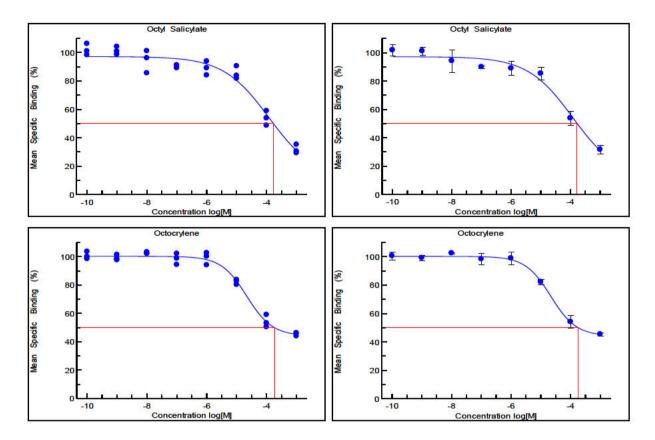


FIGURE 1 1st Valid Run % Specific Binding for Test Articles and Controls – September 20, 2011

Report Number: 9070-100107ARB

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

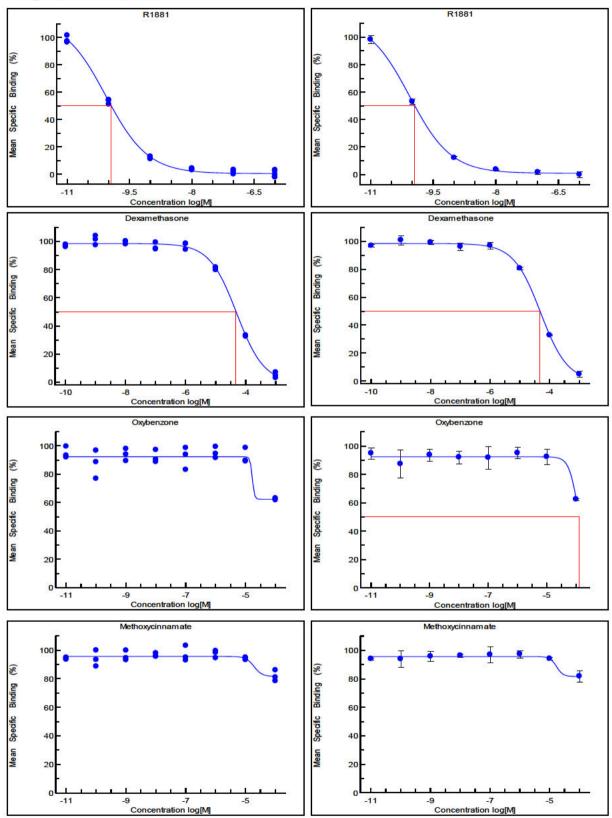
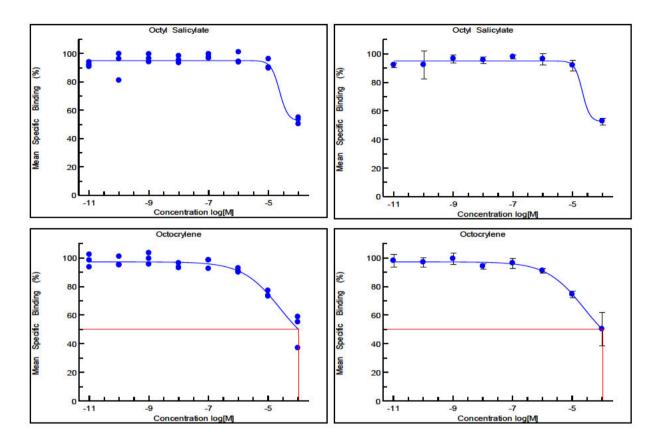


FIGURE 2 2nd Valid Run % Specific Binding for Test Articles and Controls – September 22, 2011

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

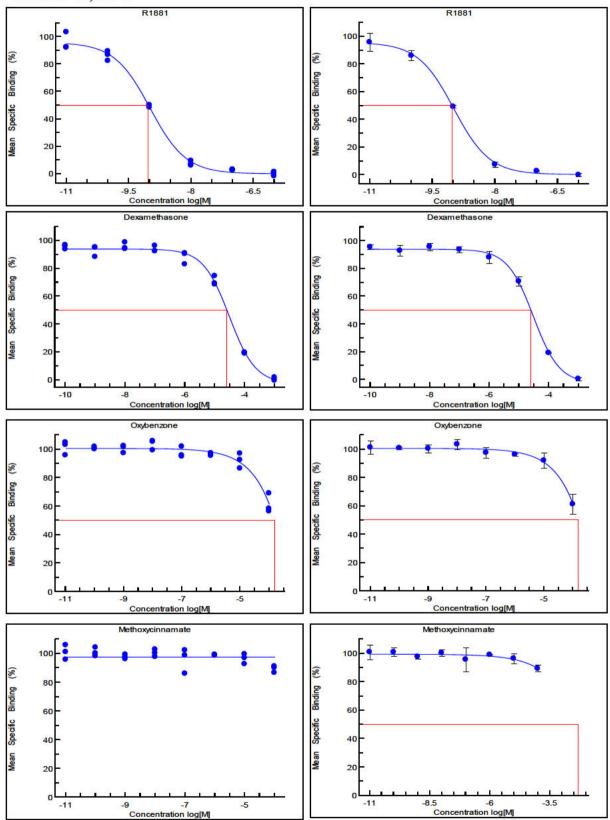
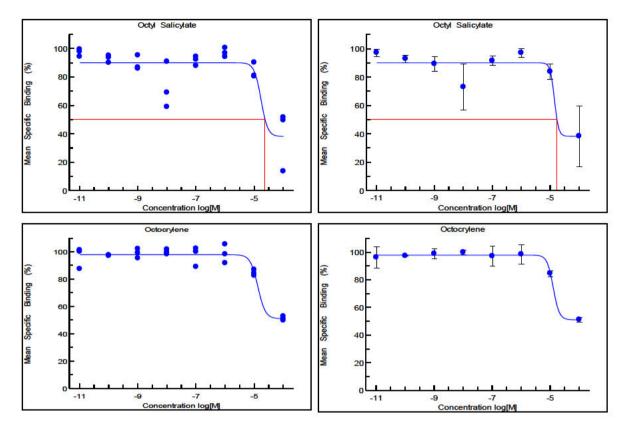


FIGURE 3 3rd Valid Run % Specific Binding for Controls and Test Articles – October 06, 2011

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

APPENDICES SECTION

A	в	с	D	E	F	G	н		J	к	L	м	N	i		
	20-Sep-11 Oxybenzone		Study Number:	9070-100107	ARB				Assays Con	ducted by:			1			
10/27/2011 11:08	2	ug protein/assay tube =														
	Tube	Sample Type	DPM (1mL)	Specific Binding DPM (100 uL) - NSB	Total Specific Binding (300 uL)	Mean										
	1 2 3 4 5 8	Total Activity (Master Mix)	56542 55731 55020 57201 55440 55476	1111	56542.0 55731.0 55020.0 57201.0 55440.0 55476.0	55901.7										
	7 8 9 10 11 12	Total Binding (Solvent Control)	2095 1949 2038 1930 1982 1879	1838.7 1692.7 1781.7 1673.7 1725.7 1622.7	5518 5078 5345 5021 5177 4868	5167.5										
			1070	10000	1000		S.									
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			
242.0 223.0	13 14	R1881 (NSB)	-6 -6	-14.3 -33.3	-43.0 -100.0	-0.8 -1.9	-1.6 -2.7	2.4 7.1	0.0	1.7	0.7	1.5E+17	1.3 1.2			
245.0 282.0	15 16		-6 -6	-11.3 25.7	-34.0 77.0	-0.7 1.5	-1.4 0.8	1.9 0.6					1.3 1.5		Г	
302.0 244.0	17 18		48 48	45.7 -12.3	137.0 -37.0	2.7 -0.7	1.9 -1.4	3.7 2.1					1.6 1.3	8	100	- E
292.0 325.0	19 20	R1881	-7 -7	35.7 68.7	107.0 206.0	2.1	0.1 2.0	0.0 4.1	3.1	1.0	0.6	31.1	1.6 1.7) Bupu	80 -	
313.0 452.0	21 22	R1881	-7 -8	56.7 195.7	170.0 587.0	3.3 11.4	1.3	1.7	13.1	1.5	0.9	11.6	1.7	Mc Br	60-	
501.0 492.0	23 24	D1004	-8 -8	244.7 235.7	734.0 707.0	14.2 13.7	1.7	3.0 1.5	55.5	15	0.0		2.7 2.6	n Spec	40-	
1201.0 1195.0	25 26 27	R1881	6 6	944.7 938.7 095.7	2834.0 2816.0 2057.0	54.8 54.5	-1.4 -1.8	2.0 3.1	55.5	1.5	0.9	2.7	6.4 6.4	Mea	20 -	
1242.0 1800.0 1899.0	27 28 29	R1881	-9 -10 -10	985.7 1543.7 1642.7	2957.0 4631.0 4928.0	57.2 89.6 95.4	1.0 -1.3 4.4	0.9 1.8 19.6	92.6	2.9	1.7	3.1	6.7 9.7 10.2	1	-11	
1859.0 1854.0 1852.0	30 31	R1881	-10 -10 -11	1597.7 1595.7	4928.0 4793.0 4787.0	92.8 92.6	4.4 1.8 -4.6	3.3	96.0	3.1	1.8	3.3	9.9	\vdash	6.0 (BRD)	
1852.0 1921.0 1958.0	31 32 33	R 1881	-11 -11 -11	1595.7 1664.7 1701.7	4/8/.0 4994.0 5105.0	92.6 96.6 98.8	-4.0 -0.6 1.6	0.3 2.5	80.0	3.1	1.8	3.3	10.3 10.5			
269.0 362.0	33 37 38	Dexamethasone	-11	12.7	38.0	0.7	-2.4	5.7 9.1	2.9	2.9	1.6	98.3	1.4	1		
288.0	39 40	Dexamethasone	2 07 4 4	31.7	95.0 1388.0	1.8	-1.3	1.6	29.4	2.3	1.3	7.9	1.5		E	
775.0 796.0	41 42		4	518.7 539.7	1556.0	30.1 31.3	1.2	1.4					4.2	9	100	•
1597.0 1608.0	43 44	Dexamethasone	-5 -5	1340.7 1351.7	4022.0 4055.0	77.8	1.3	1.8	75.6	4.5	2.6	5.9	8.6 8.6	S) Buj	80	
1469.0	45 48	Dexamethasone	-5	1212.7	3638.0	70.4	-6.1 3.7	37.0	96.5	2.2	1.3	2.3	7.9	c Brid	60-	
1914.0 1884.0	47 48		-6	1657.7 1627.7	4973.0 4883.0	96.2 94.5	1.1	1.2 0.4					10.3 10.1	Sped	40	
2012.0 2080.0	49 50	Dexamethasone	-7 -7	1755.7 1823.7	5267.0 5471.0	101.9 105.9	3.8 7.7	14.4 59.9	102.4	3.3	1.9	3.3	10.8 11.2	Mean	20-	
1966.0 2004.0	51 52	Dexamethasone	-7 -8	1709.7 1747.7	5129.0 5243.0	99.3 101.5	1.1 2.9	1.3 8.7	98.7	2.5	1.5	2.6	10.6 10.8		•	
1920.0 1943.0	53 54		-8 -8	1663.7 1686.7	4991.0 5060.0	96.6 97.9	-1.9 -0.6	3.7 0.4					10.3 10.4	⊢	-10	10
1986.0 1963.0	55 56	Dexamethasone	-9 -9	1729.7 1706.7	5189.0 5120.0	100.4 99.1	1.8 0.5	3.4 0.3	98.5	2.2	1.3	2.2	10.7 10.5			
1912.0 1923.0 1767.0	57 58 50	Dexamethasone	-9 -10 10	1655.7 1666.7 1510.7	4967.0 5000.0 4532.0	96.1 96.8 97.7	-2.4	6.0 3.3	93.5	5.0	2.9	5.4	10.3 10.3			
1767.0 1911.0 771.0	59 60	Onternet	-10 -10 -3	1510.7 1654.7	4964.0	87.7 96.1	-10.9 -2.5 0.6	118.2 6.3 0.3	29.3	0.8	0.5	2.9	9.5 10.3			
771.0 767.0 744.0	61 62 63	Oxybenzone	444	514.7 510.7 487.7	1544.0 1532.0 1463.0	29.9 29.6 28.3	0.6	0.3	28.3	U.8	0.5	2.9	4.1 4.1 4.0			
1362.0 1342.0	64 65	Oxybenzone	4	487.7 1105.7 1085.7	3317.0 3257.0	64.2 63.0	-1.0 -0.6 -1.7	0.3	64.9	2.4	1.4	3.6	4.0 7.3 7.2	1	100	
1342.0 1420.0 1779.0	66 67	Oxybenzone	4	1163.7 1522.7	3491.0 4568.0	67.6 88.4	-1.7 2.8 -4.6	7.8	92.1	4.6	2.7	5.0	7.6	8	80-	-
1932.0 1817.0	68 69	UNJUCIEURE	2 -5 -5	1675.7 1560.7	5027.0 4682.0	97.3 90.6	4.3	18.2	side: 1				10.4	Binding	60-	
2063.0 2015.0	70 71	Oxybenzone	44	1806.7 1758.7	5420.0 5276.0	104.9 102.1	5.9	34.7 9.6	102.1	2.8	1.6	2.8	11.1 10.8	actic		
1966.0 1884.0	72	Oxybenzone	-8 -7	1709.7	5129.0 4883.0	99.3 94.5	0.3	0.1	99.5	4.7	2.7	4.7	10.6	an Sp	40-	
2045.0	74 75	Sajaci Kure	-7 -7 -7	1788.7	5366.0 5171.0	103.8	4.0	16.4					11.0	Ň	20-	
1986.0	76 77	Oxybenzone	-7 -8 -8	1729.7 1693.7	5189.0 5081.0	100.4 98.3	0.5	0.3	99.9	1.4	0.8	1.4	10.7	1	-10	<u> </u>
1997.0 1941.0	78 79	Oxybenzone		1740.7	5222.0 5054.0	101.1 97.8	1.2	1.4	98.6	1.2	0.7	1.2	10.7			
1946.0	80	Service States	-0 -0	1689.7	5069.0 5165.0	98.1 100.0	-1.8	3.3		1000			10.4			
2023.0 1877.0	82 83	Oxybenzone	-10 -10	1766.7 1620.7	5300.0 4862.0	102.6 94.1	2.7 -5.8	7.1 33.8	99.2	4.5	2.6	4.5	10.9 10.1	1		
1994.0	84		-10	1737.7	5213.0	100.9	1.0	0.9					10.7	I –		

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

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

Ť	A	в	с	D	E	F	G	н		J	к	L	M	N	i		
	Experiment Date: Test substance:		mate	Study Number:	9070-100107	ARB				Assays Con	ducted by:			7	1		
3	10/27/2011 11:08	Methoxycinna	inate														
4 5 6			ug protein/assay tube =		l.												
7		Tube	Sample Type	DPM (1mL)	Specific Binding DPM (100 uL) - NSB	Total Specific Binding (300 uL)	Mean										
8 9 10 11 12 13		1 2 3 4 5 6	Total Activity (Master Mix)	58542 55731 55020 57201 55440 55478	1111	56542.0 55731.0 55020.0 57201.0 55440.0 55476.0	55901.7										
12 13 14 15 16 17 18 19 20		7 8 9 10 11 12	Total Binding (Solvent Control)	2095 1949 2038 1930 1982 1879	1838.7 1692.7 1781.7 1673.7 1725.7 1622.7	5516 5078 5345 5021 5177 4868	5167.5										
20 21 22																	
23	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			
24 25	242.0 223.0	13 14	R1881 (NSB)	-8 -6	-14.3 -33.3	-43.0 -100.0	-0.8 -1.9	-1.6 -2.7	2.4	0.0	1.7	0.7	1.5E+17	1.3			
26 27	245.0 282.0	15 16		-6 -6	-11.3 25.7	-34.0 77.0	-0.7 1.5	-1.4 0.8	1.9					1.3 1.5		Г	
26 27 28 29	302.0 244.0	17 18		-6 -6	45.7	137.0	2.7	1.9	3.7					1.6		100	•
30 31 32	292.0 325.0	19 20	R1881	-7 -7	35.7 68.7	107.0 206.0	2.1 4.0	0.1 2.0	0.0 4.1	3.1	1.0	0.6	31.1	1.6 1.7	(%) Bulpu	80-	
3	313.0 452.0	21 22	R1881	-7 -8	56.7 195.7	170.0 587.0	3.3	-1.1	1.7	13.1	1.5	0.9	11.6	1.7	Alc Bi	60-	
14 35	501.0 492.0	23 24	0.000	-8 -8	244.7 235.7	734.0 707.0	14.2 13.7	1.7 1.2	3.0 1.5					2.7 2.6	n Spe	40-	
16 17 18	1201.0 1195.0 1242.0	25 26 27	R1881	6 6	944.7 938.7 985.7	2834.0 2816.0 2957.0	54.8 54.5 57.2	-1.4 -1.8 1.0	2.0 3.1 0.9	55.5	1.5	0.9	2.7	6.4 6.4 6.7	Mear	20-	
39 40	1800.0 1899.0	28 29	R1881	-10 -10	1543.7 1642.7	4631.0 4928.0	89.6 95.4	-1.3 4.4	1.8 19.6	92.6	2.9	1.7	3.1	9.7 10.2		0 <u>-</u> -11	-9.9
41 42 43	1854.0 1852.0 1921.0	30 31 32	R1881	-10 -11 -11	1597.7 1595.7 1664.7	4793.0 4787.0 4994.0	92.8 92.6 96.6	1.8 -4.6 -0.6	3.3 21.0 0.3	96.0	3.1	1.8	3.3	9.9 9.9 10.3	ſ		
44 45 46	1958.0 269.0 362.0	33 37 38	Dexamethasone	-11 -3 -3	1701.7 12.7 105.7	5105.0 38.0 317.0	98.8 0.7 6.1	1.6 -2.4 3.0	2.5 5.7 9.1	2.9	2.9	1.6	98.3	10.5 1.4 1.9			
47 48	288.0	39 40	Dexamethasone	3	31.7	95.0 1388.0	1.8	-1.3	1.6	29.4	2.3	1.3	7.9	1.5		Г	De
9 0	775.0 796.0	41 42		4	518.7 539.7	1556.0 1619.0	30.1 31.3	1.2 2.4	1.4 5.7					4.2 4.3		100	+
51 52 53	1597.0 1608.0 1469.0	43 44 45	Dexamethasone	-\$ -\$ -\$	1340.7 1351.7 1212.7	4022.0 4055.0 3638.0	77.8 78.5 70.4	1.3 2.0 -6.1	1.8 3.9 37.0	75.6	4.5	2.6	5.9	8.6 8.6 7.9	anding (80 - - 60 -	
54 55 56	1959.0 1914.0 1884.0	46 47 48	Dexamethasone	-6 -6	1702.7 1657.7 1627.7	5108.0 4973.0 4883.0	98.8 96.2 94.5	3.7 1.1 -0.6	13.7	96.5	22	1.3	2.3	10.5 10.3	Specific	40-	
57 58	2012.0 2080.0	49 50	Dexamethasone	-8 -7 -7	1755.7 1823.7	5267.0 5471.0	101.9 105.9	3.8 7.7	0.4 14.4 59.9	102.4	3.3	1.9	3.3	10.1 10.8 11.2	Mean	20	
59 30 31	1966.0 2004.0 1920.0	51 52 53	Dexamethasone	-7 -8 -8	1709.7 1747.7 1663.7	5129.0 5243.0 4991.0	99.3 101.5 96.6	1.1 2.9 -1.9	1.3 8.7 3.7	98.7	2.5	1.5	2.6	10.6 10.8 10.3		• <u>-10</u>	-8
62 63	1943.0 1986.0	54 55	Dexamethasone	-8 -9 -9	1686.7 1729.7	5060.0 5189.0	97.9 100.4 99.1	-0.6 1.8 0.5	0.4 3.4	98.5	2.2	1.3	2.2	10.4 10.7	┢		C
34 35 36	1963.0 1912.0 1923.0	56 57 58	Dexamethasone	-9 -9 -10	1706.7 1655.7 1666.7	5120.0 4967.0 5000.0	99.1 96.1 96.8	-2.4 -1.8	0.3 6.0 3.3	93.5	5.0	2.9	5.4	10.5 10.3 10.3			
87 88 89	1767.0 1911.0 1686.0	59 60 61	Methoxycinnamate	-10 -10 -3	1510.7 1654.7 1429.7	4532.0 4964.0 4289.0	87.7 96.1 83.0	-10.9 -2.5 2.0	118.2 6.3 4.2	81.1	3.2	1.8	3.9	9.5 10.3 9.0			
70 71	1682.0 1590.0	62 63		33	1425.7 1333.7	4277.0 4001.0	82.8 77.4	1.8 -3.5	3.3 12.4		963.03		ALCONG.	9.0 8.5	\vdash	2	Met
72 73 74	1779.0 1656.0 1701.0	64 65 68	Methoxycinnamate	4 4 4	1522.7 1399.7 1444.7	4568.0 4199.0 4334.0	88.4 81.3 83.9	3.6 -3.5 -0.9	13.0 12.5 0.9	84.5	3.6	2.1	4.3	9.5 8.9 9.1	3	100	•
75 76 77	1887.0 1827.0	67 68	Methoxycinnamate	-5 -5	1630.7 1570.7	4892.0 4712.0	94.7 91.2	2.4 -1.0	6.0 1.1	92.8	1.8	1.0	1.9	10.1 9.8	inding ()	80-	
78 79	1848.0 2046.0 1870.0	69 70 71	Methoxycinnamate	-5 -8 -8	1591.7 1789.7 1613.7	4775.0 5369.0 4841.0	92.4 103.9 93.7	0.2 6.3 -3.9	0.0 40.1 15.1	96.4	6.5	3.8	6.8	9.9 11.0 10.0	pecfic B	60-	
80 81	1837.0 1910.0	72 73	Methoxycinnamate	-8 -7	1580.7 1653.7	4742.0 4961.0	91.8 96.0	-5.8 -3.3	33.7 10.9	100.7	7.4	4.3	7.3	9.9 10.3	Mean S	40	
82 83 84	2137.0 1924.0 2010.0	74 75 78	Methoxycinnamate	-7 -7 -8	1880.7 1667.7 1753.7	5642.0 5003.0 5261.0	109.2 96.8 101.8	9.9 -2.5 2.1	97.6 6.2 4.4	100.2	2.3	1.3	2.2	11.5 10.3 10.8		0 -10	-8
85 86 87	1938.0 1999.0	77 78 79		-8 -8	1681.7 1742.7	5045.0 5228.0	97.6 101.2	-2.1 1.5	4.3 2.1	LI MADE	CALTA		117 PER	10.4 10.7	\vdash	-70	-6
87 88 89 90	1954.0 1986.0 1929.0	79 80 81	Methoxycinnamate	6 6	1697.7 1729.7 1672.7	5093.0 5189.0 5018.0	98.6 100.4 97.1	-1.2 0.6 -2.7	1.5 0.4 7.2	98.7	1.7	1.0	1.7	10.5 10.7 10.4			
91	1987.0 1951.0	82 83	Methoxycinnamate	-10 -10	1730.7 1694.7	5192.0 5084.0	100.5 98.4	-2.7 0.7 -1.4	0.4	99.8	1.2	0.7	1.2	10.7 10.5	1		
92	1989.0	84	1	-10	1732.7	5198.0	100.6	0.8	0.6				L	10.7	1		

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

<u></u>		В	С	D	E	F	G	Н	1	J	К	L	м	N	1	
	tance: (20-Sep-11 Octyl Salicylate		Study Number:	9070-100107	ARB				Assays Cond	lucted by:					
10/27/2011	1 11:08		ug protein/assay tube =													
		Tube	Sample Type	DPM (1mL)	Specific Binding DPM (100 uL) - NSB	Total Specific Binding (300 uL)	Mean									
		1 2 3 4 5 6	Total Activity (Master Mix)	58542 55731 55020 57201 55440 55478		56542.0 55731.0 55020.0 57201.0 55440.0 55476.0	55901.7									
		7 8 9 10 11 12	Total Binding (Solvent Control)	2095 1949 2038 1930 1982 1879	1838.7 1692.7 1781.7 1673.7 1725.7 1622.7	5516 5078 5345 5021 5177 4868	5167.5									
		1-	·	10.0	102	1000		6								
DPN (1mL) from	om 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		
242.0 223.0		13 14	R1881 (NSB)	-6 -6	-14.3 -33.3	-43.0 -100.0	-0.8 -1.9	-1.6 -2.7	2.4 7.1	0.0	1.7	0.7	1.5E+17	1.3 1.2	1_	
245.0 282.0		15 16		-6 -6	-11.3 25.7	-34.0 77.0	-0.7	-1.4 0.8	1.9					1.3 1.5		Г
302.0		17 18		-6 -6	45.7	137.0 -37.0	2.7 -0.7	1.9	3.7					1.6 1.3		100
292.0 325.0	.0	19 20	R1881	-7 -7	35.7 68.7	107.0 206.0	2.1 4.0	0.1 2.0	0.0 4.1	3.1	1.0	0.6	31.1	1.6 1.7	s) Bup	80-
313.0 452.0	.0	21 22	R1881	-7 -8	56.7 195.7	170.0 587.0	3.3 11.4	1.3 -1.1	1.7	13.1	1.5	0.9	11.6	1.7	fic Bu	60-
501.0 492.0	.0	23 24		-8 -8	244.7 235.7	734.0 707.0	14.2 13.7	1.7 1.2	3.0 1.5					2.7 2.6	Speo	40-
1201. 1195.	5.0	25 26	R1881	9	944.7 938.7	2834.0 2816.0	54.8 54.5	-1.4 -1.8	2.0 3.1	55.5	1.5	0.9	2.7	6.4 6.4	Mear	20 -
1242	0.0	27 28	R1881	-9 -10	985.7 1543.7	2957.0 4631.0	57.2 89.6	-1.3	0.9	92.6	2.9	1.7	3.1	6.7 9.7	1	-11
1899. 1854.	1.0	29 30	10000000	-10 -10	1642.7 1597.7	4928.0 4793.0	95.4 92.8	4.4 1.8	19.6 3.3	di deserva d	0.000			10.2 9.9	╞	-11
1852 1921	1.0	31 32	R1881	-11 -11	1595.7 1664.7	4787.0 4994.0	92.6 96.6	-4.6	21.0 0.3	96.0	3.1	1.8	3.3	9.9 10.3		
1958.	.0	33 37	Dexamethasone	-11	1701.7 12.7	5105.0 38.0	98.8 0.7	1.6 -2.4	2.5 5.7	2.9	2.9	1.6	98.3	10.5 1.4		
362.0 288.0	.0	38 39	-	33	105.7 31.7	317.0 95.0	6.1 1.8	3.0 -1.3	9.1 1.6					1.9 1.5	\vdash	-
719.0	.0	40 41	Dexamethasone	4 4 -	462.7 518.7	1388.0 1556.0	26.9 30.1	-2.1	4.4	29.4	2.3	1.3	7.9	3.9 4.2		100
796.0	7.0	42	Dexamethasone	-5	539.7 1340.7	1619.0	31.3 77.8	2.4	5.7	75.6	4.5	2.6	5.9	4.3 8.6	(%) 0	80-
1608. 1469. 1959.	9.0	44 45	Deverentherene	-5 -5	1351.7 1212.7 1702.7	4055.0 3638.0 5108.0	78.5 70.4 98.8	2.0 -6.1	3.9 37.0	08.5	22	12	22	8.6 7.9 10.5	Bindin	60-
1909. 1914. 1884.	1.0	46 47	Dexamethasone	-6 -6	1657.7	4973.0 4883.0	96.2	3.7 1.1	13.7	96.5	22	1.3	2.3	10.3	Specific	40-
2012	2.0	48	Dexamethasone	-6 -7 7	1627.7 1755.7	5267.0	94.5 101.9	-0.6 3.8 7.7	0.4	102.4	3.3	1.9	3.3	10.1	Weam	20 -
2080. 1966.	3.0	50 51	Doctor	-7 -7	1823.7 1709.7	5471.0 5129.0	105.9 99.3	7.7	59.9 1.3	08.7	25	15	24	11.2 10.6		- •
2004. 1920. 1943.	0.0	52 53 54	Dexamethasone	-8 -8 -8	1747.7 1663.7 1686.7	5243.0 4991.0 5060.0	101.5 96.6 97.9	2.9 -1.9 -0.6	8.7 3.7 0.4	98.7	2.5	1.5	2.6	10.8 10.3 10.4		-10
1943. 1986. 1963.	3.0	55 56	Dexamethasone	-8 -9 -9	1686.7 1729.7 1706.7	5060.0 5189.0 5120.0	97.9 100.4 99.1	-0.6 1.8 0.5	0.4 3.4 0.3	98.5	22	1.3	2.2	10.4 10.7 10.5	1	
1912	2.0	57 58	Dexamethasone	-9	1655.7	4967.0	96.1	-2.4	6.0	93.5	50	20	E 4	10.3		
1923. 1767. 1911.	7.0	58 59 60	Dexamethasone	-10 -10 -10	1666.7 1510.7 1654.7	5000.0 4532.0 4964.0	96.8 87.7 96.1	-1.8 -10.9 -2.5	3.3 118.2 6.3	83.5	5.0	2.9	5.4	10.3 9.5 10.3		
783.0	.0	61 62	Octyl Salicylate	-3	526.7	1580.0 1823.0	30.6 35.3	-0.5	0.2	31.7	3.1	1.8	9.9	4.2	1	
864.0 761.0 1272	.0	62 63 64	Octyl Salicylate	3 3 4	607.7 504.7 1015.7	1823.0 1514.0 3047.0	30.3 29.3 59.0	4.2 -1.8 2.6	17.7 3.1 6.8	53.9	5.1	2.9	9.5	4.6 4.1 6.8		[
12/2 1096 1184	3.0	65 66	ouyi sancylate	4 4	839.7 927.7	2519.0 2783.0	48.7 53.9	-7.6 -2.5	57.9 6.2	00.8	3.1	2.8	8.0	5.9 6.4		100
1700.	0.0	67	Octyl Salicylate	-5	1443.7	4331.0	83.8	3.4	11.8	85.4	4.6	2.6	5.4	9.1	10 (%)	80-
1816. 1665. 1705.	5.0	68 69 70	Octol Salindata	-5 -5	1559.7 1408.7 1448.7	4679.0 4226.0 4346.0	90.5 81.8 84.1	10.2 1.4 -7.9	103.3 2.0 63.0	89.1	4.9	2.8	5.5	9.7 8.9 9.1	Brid	60
1792	2.0	71	Octyl Salicylate	-6 -6	1535.7	4607.0	89.2	-2.9	8.3	08.1	4.8	2.8	0.0	9.6	specific	40-
1874. 1828.	3.0	72 73 74	Octyl Salicylate	-6 -7 7	1617.7 1571.7	4853.0 4715.0	93.9 91.2	1.9 -4.7	3.5 21.7	90.0	1.1	0.6	1.2	10.1 9.8	lean S	-
1799. 1792.	2.0	74 75 78	Orth Coline	-7 -7	1542.7 1535.7	4628.0 4607.0	89.6 89.2	-6.3	40.2 8.3	04.0	7.0	4.0		9.7 9.6	2	20-
1731.	3.0	76 77	Octyl Salicylate	\$ \$	1474.7 1656.7	4424.0 4970.0	85.6 96.2	-11.4	129.9	94.3	7.9	4.6	8.4	9.3 10.3		-10
1999. 1994. 2051	1.0	78 79	Octyl Salicylate	-8 -9 0	1742.7 1737.7 1704.7	5228.0 5213.0	101.2 100.9	4.2 3.6	17.3	101.3	2.7	1.6	2.7	10.7 10.7 11.0		
2051. 1957.	7.0	80 81	0.110 5 11	-9 -9	1794.7 1700.7	5384.0 5102.0	104.2 98.7	6.9 1.4	47.3	101.0		2.3		11.0 10.5	1	
2087.	0	82	Octyl Salicylate	-10	1830.7	5492.0	106.3	8.9	78.9	101.9	4.0		4.0	11.2	1	

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

Ē	A	в	с	D	E	F	G	н			K	L	M	N	1	
	Experiment Date:	20-Sep-11	<u> </u>		9070-100107	ARB			0.4	Assays Cond		-				
3 1	Test substance: 10/27/2011 11:08	Octocrylene														
4 5 6			ug protein/assay tube =					20								
7		Tube	Sample Type	DPM (1mL)	Specific Binding DPM (100 uL) - NSB	Total Specific Binding (300 uL)	Mean									
8 9 10 11 12 13		1 2 3 4 5 6	Total Activity (Master Mix)	56542 55731 55020 57201 55440 55476		56542.0 55731.0 55020.0 57201.0 55440.0 55476.0	55901.7									
4567890		7 8 9 10 11 12	Total Binding (Solvent Control)	2095 1949 2038 1930 1982 1879	1838.7 1692.7 1781.7 1673.7 1725.7 1622.7	5516 5078 5345 5021 5177 4868	5167.5									
20				10/0	10000	1000										
23	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		
24 25	242.0 223.0	13 14	R1881 (NSB)	-6 -6	-14.3 -33.3	-43.0 -100.0	-0.8 -1.9	-1.6 -2.7	2.4 7.1	0.0	1.7	0.7	1.5E+17	1.3 1.2	1	
26 27	245.0 282.0	15 16		-6 -6	-11.3 25.7	-34.0	-0.7	-1.4	1.9					1.3		Г
8	302.0 244.0	17		-6 -6	45.7	137.0	2.7	1.9	3.7					1.6		100
30 31 32	292.0 325.0 313.0	19 20 21	R1881	-7 -7 -7 -7	35.7 68.7 56.7	107.0 206.0 170.0	2.1 4.0 3.3	0.1 2.0 1.3	0.0 4.1 1.7	3.1	1.0	0.6	31.1	1.6 1.7 1.7	%) Bulpu	60-
33	452.0 501.0	21 22 23	R1881	-7 -8 -8	195.7 244.7	587.0 734.0	11.4 14.2	-1.1 1.7	1.2	13.1	1.5	0.9	11.6	2.4 2.7	chc B	40-
4 5 6	492.0 1201.0	24 25	R1881	-8	235.7 944.7	707.0 2834.0	13.7	1.2	1.5	55.5	1.5	0.9	2.7	2.6	an Spe	
78	1195.0	28 27		-9 -9	938.7 985.7	2816.0 2957.0	54.5 57.2	-1.8	3.1					6.4 6.7	Me	20-
9 0	1800.0 1899.0	28 29	R1881	-10 -10	1543.7 1642.7	4631.0 4928.0	89.6 95.4	-1.3 4.4	1.8 19.6	92.6	2.9	1.7	3.1	9.7 10.2	1	0 <mark>-, , , ,</mark> -11 -4
1	1854.0 1852.0 1921.0	30 31 32	R1881	-10 -11 -11	1597.7 1595.7 1664.7	4793.0 4787.0 4994.0	92.8 92.6 96.6	1.8 -4.6 -0.6	3.3 21.0	96.0	3.1	1.8	3.3	9.9 9.9	ſ	
3	1958.0	33	Deverenthereses	-11 -11 -3	1701.7	5105.0 38.0	98.8	1.6	0.3 2.5 5.7	2.9	20	1.0	98.3	10.3 10.5	1	
5 6 7	362.0 288.0	37 38 39	Dexamethasone	223	12.7 105.7 31.7	317.0 95.0	0.7 6.1 1.8	-2.4 3.0 -1.3	9.1	2.8	2.9	1.6	80.3	1.4 1.9 1.5		
8	719.0 775.0	40 41	Dexamethasone	4	462.7 518.7	1388.0 1556.0	26.9 30.1	-2.1 1.2	4.4	29.4	2.3	1.3	7.9	3.9	1	100
0	796.0 1597.0	42 43	Dexamethasone	-5	539.7 1340.7	1619.0 4022.0	31.3 77.8	2.4 1.3	5.7 1.8	75.6	4.5	2.6	5.9	4.3 8.6	(%) 5	80-08
2	1608.0 1469.0	44 45		-5 -5	1351.7 1212.7	4055.0 3638.0	78.5 70.4	2.0 -6.1	3.9 37.0					8.6 7.9	Bindin	60-
4 5 6	1959.0 1914.0 1884.0	46 47 48	Dexamethasone	-6 -6 -6	1702.7 1657.7 1627.7	5108.0 4973.0 4883.0	98.8 96.2 94.5	3.7 1.1 -0.6	13.7 1.2 0.4	96.5	2.2	1.3	2.3	10.5 10.3 10.1	Specific	40-
7 8	2012.0 2080.0	49 50	Dexamethasone	-7 -7	1755.7 1823.7	5267.0 5471.0	101.9 105.9	3.8 7.7	14.4 59.9	102.4	3.3	1.9	3.3	10.8 11.2	Mean	20-
9 0	1966.0 2004.0	51 52	Dexamethasone	-7 -8	1709.7 1747.7	5129.0 5243.0	99.3 101.5	1.1 2.9	1.3 8.7	98.7	2.5	1.5	2.6	10.6 10.8	1	
1 2 3	1920.0 1943.0 1986.0	53 54 55	Dexamethasone	-8 -8 -9	1663.7 1686.7 1729.7	4991.0 5060.0 5189.0	96.6 97.9 100.4	-1.9 -0.6 1.8	3.7 0.4 3.4	98.5	2.2	1.3	22	10.3 10.4 10.7	┢	-10 -1
4	1963.0 1912.0	56 57	Dexametrasone	-9 -9	1706.7	5120.0 4967.0	99.1 96.1	0.5	0.3 6.0	60.0	~~	1.3	~~	10.7		
36 37	1923.0 1767.0	58 59	Dexamethasone	-10 -10	1666.7 1510.7	5000.0 4532.0	96.8 87.7	-1.8 -10.9	3.3 118.2	93.5	5.0	2.9	5.4	10.3 9.5	1	
18 19 10	1911.0 1051.0 1049.0	60 61 62	Octocrylene	-10 -3 -3	1654.7 794.7 792.7	4964.0 2384.0 2378.0	96.1 46.1 46.0	-2.5 0.6 0.5	6.3 0.4 0.2	45.4	1.2	0.7	2.6	10.3 5.6 5.6	1	
70 71 72	1049.0 1015.0 1274.0	63 64	Octocrylene	3 3 4	792.7 758.7 1017.7	2378.0 2276.0 3053.0	40.0 44.0 59.1	-1.5 5.1	2.2 25.7	54.2	4.4	2.6	8.2	5.4 6.8		
3	1125.0 1172.0	65 66		4	868.7 915.7	2606.0 2747.0	50.4 53.2	-3.6	12.8				-	6.0 6.3	8	100
75 76 77	1685.0 1698.0	67 68	Octocrylene	-6 -5	1428.7 1441.7	4286.0 4325.0	82.9 83.7	0.3	0.1	82.3	1.8	1.0	2.1	9.0 9.1	() Bujo	80 - -
8	1640.0 2025.0	69 70	Octocrylene	-5 -8	1383.7 1768.7	4151.0 5306.0	80.3 102.7	-2.3 4.6	5.1 21.3	99.0	4.4	2.6	4.5	8.8 10.9	dic Bn	60 -
9	1877.0 1983.0	71 72		-6 -6	1620.7 1728.7	4862.0 5180.0	94.1 100.2	-4.0	15.8 4.7					10.1	in Spe	40-
31 32 33	2016.0 1882.0 1958.0	73 74 75	Octocrylene	-7 -7 -7	1759.7 1625.7 1701.7	5279.0 4877.0 5105.0	102.2 94.4 98.8	1.9 -5.9 -1.5	3.6 34.6 2.1	98.4	3.9	2.3	4.0	10.8 10.1 10.5	Mea	20 -
83 84 85	2035.0 2016.0	76 77	Octocrylene	-7 -8 -8	1778.7 1759.7	5336.0 5279.0	103.3 102.2	-1.5 2.8 1.9	7.7	102.6	0.6	0.3	0.6	10.9	1	-10 -1
36 37	2020.0 1954.0	78 79	Octocrylene	-8 -9	1763.7 1697.7	5291.0 5093.0	102.4 98.6	1.9 -1.9	3.7 3.8	99.2	2.0	1.1	2.0	10.8 10.5	-	
38 39 90	2004.0 1939.0 2041.0	80 81 82	Octocrylene	-9 -9 -10	1747.7 1682.7 1784.7	5243.0 5048.0 5354.0	101.5 97.7 103.6	1.0 -2.8 3.1	0.9 7.9 9.7	100.7	2.6	1.5	2.6	10.8 10.4 11.0	1	
90 91 92	1953.0 1979.0	82 83 84	occurryene	-10 -10 -10	1784.7 1696.7 1722.7	5090.0 5168.0	98.5 100.0	-2.0 -0.5	4.0	100.7	2.0	1.0	2.0	10.5 10.6		

_																
1	A Experiment Date:	B 22-Sep-11	C	D Study Number:	E 9070-100107	F 7ARB	G	H		Assays Cond	K tucted by:	L	M	N		
2	Test substance: 10/27/2011 11:33	Oxybenzone													1	
4 5 6			ug protein/assay tube =													
7		Tube	Sample Type	DPM (1mL)	Specific Binding DPM (100 uL) - NSB	Total Specific Binding (300 uL)	Mean									
8 9 10		1 2 3 4 5 6	Total Activity (Master Mix)	63276 65781 58416 64532 59920 59592	n n n	63276.0 65781.0 58416.0 64532.0 59920.0 59592.0	61919.5									
11 12 13 14 15 16 17 18 19		7 8 9 10 11 12	Total Binding (Solvent Control)	2485 2328 2363 2084 2325 2149	2190.2 2033.2 2068.2 1789.2 2030.2 1854.2	6571 6100 6205 5368 6091 5563	5982.5									
20 21 22								а 						-		
23	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		
	254.0 257.0	13 14	R1881 (NSB)	-6 -6	-40.8 -37.8	-122.5 -113.5	-2.0 -1.9	-2.8 -2.7	8.1 7.3	0.0	2.3	0.9	2.5E+17	1.2 1.2	1	
26	262.0	15		-8 -8	-32.8 64.2	-98.5	-1.6	-2.4	6.0 5.8					1.3		F.
24 25 26 27 28 29	344.0	17		-6	49.2	147.5	2.5	1.7	2.8					1.7		100-5
29 30 31 32 33 34 35	293.0 333.0 361.0 298.0	18 19 20 21	R1881	-8 -7 -7 -7	-1.8 38.2 66.2 3.2	-5.5 114.5 198.5 9.5	-0.1 1.9 3.3 0.2	-0.9 0.9 2.3 -0.9	0.8 0.8 5.3 0.7	1.8	1.6	0.9	88.1	1.4 1.6 1.7 1.4	Anding (%)	80-
33	358.0	22 23	R1881	-8	63.2 76.2	189.5	3.2	0.6	0.3	3.8	0.6	0.4	16.5	1.7	Mc B	
35	371.0 383.0	24	Bioot	-8 -8	88.2	264.5	4.4	1.2 1.8	1.5 3.3	10.0				1.8 1.9	n Spe	40-
36 37 38	520.0 547.0	25 26	R1881	-9 -9	225.2 252.2	675.5 756.5	11.3 12.6	-1.5 -0.1	2.2 0.0	12.3	0.9	0.5	7.3	2.5 2.7	Mear	20-
38 39	554.0 1316.0	27 28	R1881	-9 -10	259.2 1021.2	777.5 3063.5	13.0 51.2	0.2	0.0 3.7	53.2	1.8	1.0	3.3	2.7 6.4	1	
39 40 41	1380.0 1373.0	29 30		-10 -10	1085.2 1078.2	3255.5 3234.5	54.4 54.1	1.3	1.7					6.7 6.7		-11 -9.5 C
42 43 44	2320.0 2234.0	31 32	R1881	-11 -11	2025.2 1939.2	6075.5 5817.5	101.6 97.2	3.1	9.5 1.5	98.4	2.7	1.6	2.8	11.2 10.8	1	
44 45	2220.0 391.0	33 37	Dexamethasone	-11 -3	1925.2 96.2	5775.5 288.5	96.5 4.8	-1.9 -0.2	3.7 0.1	5.1	2.0	1.2	39.9	10.8 1.9	4	
46 47	438.0 358.0	38 39		33	143.2 63.2	429.5 189.5	7.2	2.1	4.5 3.6					2.1 1.7	\vdash	De
48	962.0 950.0	40 41	Dexamethasone	4	667.2 655.2	2001.5	33.5 32.9	0.5	0.3	33.0	0.4	0.3	1.3	4.7 4.6	1	
50	945.0	42	Devametharene	4	650.2	1950.5	32.6	-0.3	0.1	80.0	10	0.8	12	4.6	8	90 - 80 -
52 53	1886.0 1914.0 1924.0	44 45	Dexamethasone	-5 -5 -5	1591.2 1619.2 1629.2	4773.5 4857.5 4887.5	79.8 81.2 81.7	-1.2 0.2 0.7	1.5 0.0 0.5	80.9	1.0	0.6	1.2	9.3 9.3	Binding	70 - 60 -
54 55 56	2262.0 2175.0	46 47	Dexamethasone	-6 -6	1967.2 1880.2	5901.5 5640.5	98.6 94.3	22	5.0 4.5	97.1	2.4	1.4	2.5	11.0 10.5	Specific	50 40
57 58	2256.0 2180.0 2192.0	48 49 50	Dexamethasone	-6 -7 -7	1961.2 1885.2 1897.2	5883.5 5655.5 5691.5	98.3 94.5 95.1	1.9 -3.9 -3.3	3.7 15.4 11.1	96.4	2.7	1.5	2.8	10.9 10.6 10.6	Mean	30 - 20 - 10 -
59 60 61	2278.0 2290.0 2251.0	51 52 53	Dexamethasone	-7 -8 -8	1983.2 1995.2 1956.2	5949.5 5985.5 5868.5	99.4 100.1 98.1	1.0 1.4 -0.6	1.0 1.8 0.4	99.5	1.2	0.7	12	11.0 11.1 10.9		-10 -8 c
62 63 64	2296.0 2371.0 2239.0	54 55 58	Dexamethasone	-8 -9 -9	2001.2 2076.2 1944.2	6003.5 6228.5 5832.5	100.4 104.1 97.5	1.7 5.4 -1.2	2.7 29.1 1.5	101.1	3.3	1.9	3.3	11.1 11.5 10.8		
65 66 67	2320.0 2247.0 2213.0	57 58 59	Dexamethasone	-9 -10 -10	2025.2 1952.2 1918.2	6075.5 5856.5 5754.5	101.6 97.9 96.2	2.8 -0.8 -2.5	8.0 0.7 6.4	97.1	0.9	0.5	0.9	11.2 10.9 10.7	1	
68 69 70 71	2233.0 1546.0 1554.0	60 61 62	Oxybenzone	-10 -4 -4	1938.2 1251.2 1259.2	5814.5 3753.5 3777.5	97.2 62.7 63.1	-1.5 0.2 0.6	2.3 0.0 0.3	62.6	0.7	0.4	1.1	10.8 7.5 7.5	L	
71 72 73	1528.0 2084.0 2074.0	63 64 65	Oxybenzone	-4 -5 -5	1233.2 1789.2 1779.2	3699.5 5367.5 5337.5	61.8 89.7 89.2	-0.7 -2.8 -3.3	0.5 8.1 11.2	92.6	5.4	3.1	5.8	7.4 10.1 10.0		100
74	2074.0 2264.0 2180.0	66 67	Oxybenzone	-5 -5	1969.2	5907.5 5655.5	98.7 94.5	6.2 1.9	38.3	95.2	4.0	2.3	4.2	11.0	(%)	80-
72 73 74 75 76 77	2279.0 2122.0	68 69	1997 A	-6 -6	1984.2 1827.2	5952.5 5481.5	99.5 91.6	6.9 -1.0	47.1 1.0					11.0 10.3	Binding	60 -
78 79 80	1955.0 2168.0 2263.0	70 71 72	Oxybenzone	-7 -7 -7	1660.2 1873.2 1968.2	4980.5 5619.5 5904.5	83.3 93.9 98.7	-9.4 1.3 6.1	88.0 1.7 36.7	92.0	7.9	4.6	8.6	9.5 10.5 11.0	Spedic	40 -
81 82 83	2101.0 2235.0 2066.0	73 74 75	Oxybenzone	\$ \$ \$	1806.2 1940.2 1771.2	5418.5 5820.5 5313.5	90.6 97.3 88.8	-2.1 4.7 -3.8	4.3 21.7 14.6	92.2	4.5	2.6	4.9	10.2 10.8 10.0	Mean	20-
84 85	2170.0 2079.0 2250.0	76 77 78	Oxybenzone	-0 -0 -0	1875.2 1784.2 1955.2	5625.5 5352.5 5865.5	94.0 89.5 98.0	1.4 -3.2 5.4	2.0 10.0 29.3	93.8	4.3	2.5	4.6	10.5 10.1 10.9		-11 -9 Co
79 80 81 82 83 84 85 86 87 88 80 90 91 92	2250.0 2064.0 2226.0 1830.0	79 80 81	Oxybenzone	-10 -10 -10	1769.2 1931.2 1535.2	5307.5 5793.5 4605.5	88.7 96.8 77.0	-3.9 4.2 -15.7	15.3 17.7 245.0	87.5	10.0	5.8	11.4	10.0 10.8 8.9	1	
90 91	2283.0 2156.0	82 83	Oxybenzone	-11 -11	1988.2 1861.2	4005.5 5964.5 5583.5 5508.5	99.7 93.3 92.1	-15.7 7.1 0.7 -0.6	49.9 0.5 0.3	95.0	4.1	2.4	4.3	11.1 10.4 10.3	1	
92	2131.0	84		-11	1836.2	0008.0	92.1	-0.0	0.3	1				10.3	1	

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

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

-	A	в	с	D	E	-	G	н			K		м	N			
1	Experiment Date:	22-Sep-11			9070-100107	ARB	6			Assays Con			m	N			
2	Test substance: 10/27/2011 11:33	Methoxycinna	mate														
4 5 6			ug protein/assay tube =		1			-									
7	- 	Tube	Sample Type	DPM (1mL)	Specific Binding DPM (100 uL) - NSB	Total Specific Binding (300 uL)	Mean										
8 9 10 11 12 13		1 2 3 4 5 6	Total Activity (Master Mix)	63276 65781 58416 64532 59920 59592		63276.0 65781.0 58416.0 64532.0 59920.0 59592.0	61919.5										
14 15 16		7 8 9 10 11 12	Total Binding (Solvent Control)	2485 2328 2363 2084 2325 2149	2190.2 2033.2 2068.2 1789.2 2030.2 1854.2	6571 6100 6205 5368 6091 5563	5982.5										
18 19 20 21 22		12		2170	1007.2	3505		52.									
23	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			
24	254.0	13	R1881 (NSB)	-8 8	-40.8	-122.5	-2.0	-2.8	8.1	0.0	2.3	0.9	2.5E+17	1.2	1		
25 26 27	257.0 262.0 359.0	14 15 16		-6 -6 -6	-37.8 -32.8 64.2	-113.5 -98.5 192.5	-1.9 -1.6 3.2	-2.7 -2.4 2.4	7.3 6.0 5.8					1.2 1.3 1.7	\vdash	Γ.	
27 28 29	369.0 344.0 293.0	10 17 18		-6	64.2 49.2 -1.8	147.5	3.2 2.5 -0.1	2.4 1.7 -0.9	5.8 2.8 0.8					1.7	0352	100-	
30	333.0 361.0	19 20	R1881	-6 -7 -7	38.2 66.2	-5.5 114.5 198.5	1.9 3.3	0.9 2.3	0.8 5.3	1.8	1.6	0.9	88.1	1.4 1.6 1.7	(%) Bulp	80-	
32 33	298.0 358.0	21 22	R1881	-7 -8	3.2 63.2	9.5 189.5	0.2 3.2	-0.9 0.6	0.7	3.8	0.6	0.4	16.5	1.4 1.7	fic Bin	60-	
34 35	371.0 383.0	23 24	1988 DOM	49 49	76.2 88.2	228.5 264.5	3.8 4.4	1.2 1.8	1.5 3.3		1000407	2.5.9555	Sensitive a	1.8 1.9	Speci	40-	
36 37 38	520.0 547.0 554.0	25 26 27	R1881	ቀ ቀ ቀ	225.2 252.2 259.2	675.5 756.5 777.5	11.3 12.6 13.0	-1.5 -0.1 0.2	2.2 0.0 0.0	12.3	0.9	0.5	7.3	2.5 2.7 2.7	Mean	20 -	
39 40	1316.0 1380.0	28 29	R1881	-10 -10	1021.2 1085.2	3063.5 3255.5	51.2 54.4	-1.9 1.3	3.7 1.7	53.2	1.8	1.0	3.3	6.4 6.7	1	-11	
41 42 43 44	1373.0 2320.0 2234.0	30 31 32	R1881	-10 -11 -11	1078.2 2025.2 1939.2	3234.5 6075.5 5817.5	54.1 101.6 97.2	0.9 3.1 -1.2	0.9 9.5 1.5	98.4	2.7	1.6	2.8	6.7 11.2 10.8	1		
45	2220.0 391.0	33 37	Dexamethasone	-11 -3	1925.2 96.2	5775.5 288.5	96.5 4.8	-1.9 -0.2	3.7 0.1	5.1	2.0	1.2	39.9	10.8 1.9	1		
48 47	438.0 358.0	38 39		\$ \$	143.2 63.2	429.5 189.5	7.2	2.1	4.5 3.6					2.1 1.7		110	De
48 49 50 51	962.0 950.0 945.0	40 41 42	Dexamethasone	4 4 4	667.2 655.2 650.2	2001.5 1965.5 1950.5	33.5 32.9 32.6	0.5 -0.1 -0.3	0.3 0.0 0.1	33.0	0.4	0.3	1.3	4.7 4.6 4.6	(9	100-0-	•••
51 52 53	1886.0 1914.0 1924.0	43 44 45	Dexamethasone	\$ \$ \$	1591.2 1619.2 1629.2	4773.5 4857.5 4887.5	79.8 81.2 81.7	-1.2 0.2 0.7	1.5 0.0 0.5	80.9	1.0	0.6	1.2	9.1 9.3 9.3	() Supu	80 - 70 -	
54 55	2262.0 2175.0	46 47	Dexamethasone	-8 -6	1967.2 1880.2	5901.5 5640.5	98.6 94.3	2.2 -2.1	5.0 4.5	97.1	24	1.4	2.5	11.0 10.5	pedic	60 - 50 - 40 -	
56 57 58	2256.0 2180.0 2192.0	48 49 50	Dexamethasone	-6 -7 -7	1961.2 1885.2 1897.2	5883.5 5655.5 5691.5	98.3 94.5 95.1	1.9 -3.9 -3.3	3.7 15.4 11.1	96.4	2.7	1.5	2.8	10.9 10.6 10.6	Mean	30 - 20 -	
59 60 61	2278.0 2290.0 2251.0	51 52 53	Dexamethasone	-7 -8 -8	1983.2 1995.2 1956.2	5949.5 5985.5 5868.5	99.4 100.1 98.1	1.0 1.4 -0.6	1.0 1.8 0.4	99.5	1.2	0.7	1.2	11.0 11.1 10.9		10- 0-1	
62 63	2296.0 2371.0	54 55	Dexamethasone	-8 -9	2001.2 2076.2	6003.5 6228.5	100.4 104.1	1.7 5.4	2.7 29.1	101.1	3.3	1.9	3.3	11.1 11.5	┢		(
64 65 66	2239.0 2320.0 2247.0	56 57 58	Dexamethasone	-9 -9 -10	1944.2 2025.2 1952.2	5832.5 6075.5 5856.5	97.5 101.6 97.9	-1.2 2.8 -0.8	1.5 8.0 0.7	97.1	0.9	0.5	0.9	10.8 11.2 10.9	1		
67 68	2213.0 2233.0	59 60	DexametridSone	-10 -10	1918.2 1938.2	5754.5 5814.5	96.2 97.2	-2.5 -1.5	6.4 2.3					10.7 10.8			
69 70 71	1860.0 2012.0 1912.0	61 62 63	Methoxycinnamate	4 4 4	1565.2 1717.2 1617.2	4695.5 5151.5 4851.5	78.5 86.1 81.1	-3.5 4.2 -0.8	11.9 17.4 0.7	81.9	3.9	2.2	4.7	9.0 9.7 9.3			Met
72	2189.0 2155.0	64 65	Methoxycinnamate	-5 -5	1894.2 1860.2	5682.5 5580.5	95.0 93.3	0.8 -0.9	0.6	94.3	0.9	0.5	0.9	10.6 10.4		100	: :
74 75 76	2180.0 2181.0 2280.0	66 67 68	Methoxycinnamate	-5 -6 -8	1885.2 1886.2 1985.2	5655.5 5658.5 5955.5	94.5 94.6 99.5	0.3 -1.3 3.7	0.1 1.6 13.7	97.5	2.6	1.5	2.6	10.6 10.6 11.0	(%) Bup	80	
76 77 78	2255.0 2189.0	69 70	Methoxycinnamate	-8 -7	1960.2 1894.2	5880.5 5682.5	98.3 95.0	2.5 0.8	6.0 0.6	97.1	5.4	3.1	5.6	10.9 10.6	dic Bud	60	
79 80 81	2149.0 2354.0 2201.0	71 72 73	Methoxycinnamate	-7 -7 -8	1854.2 2059.2 1906.2	5562.5 6177.5 5718.5	93.0 103.3 95.6	-2.9 7.4 -0.3	8.2 55.0 0.1	96.5	1.3	0.8	1.4	10.4 11.4 10.7	ean Spe	40-	
81 82 83	2205.0 2249.0	74 75	52	9 9	1910.2 1954.2	5730.5 5862.5	95.8 98.0	-0.1 2.1	0.0 4.6					10.7 10.9	×	20-	
84 85 86	2287.0 2153.0 2181.0	76 77 78	Methoxycinnamate	ዋ ዋ ዋ	1992.2 1858.2 1886.2	5976.5 5574.5 5658.5	99.9 93.2 94.6	4.1 -2.7 -1.3	16.4 7.1 1.6	95.9	3.5	2.0	3.7	11.1 10.4 10.6		-11	-9 (
87 88	2064.0 2289.0	79 80	Methoxycinnamate	-10 -10	1769.2 1994.2	5307.5 5982.5	88.7 100.0	-7.1 4.2	50.8 17.3	94.0	5.7	3.3	6.0	10.0 11.1	1		
89 90 91	2157.0 2160.0 2188.0	81 82 83	Methoxycinnamate	-10 -11 -11	1862.2 1865.2 1893.2	5586.5 5595.5 5679.5	93.4 93.5 94.9	-2.5 -2.3 -0.9	6.1 5.4 0.8	94.1	0.7	0.4	0.8	10.5 10.5 10.6			
92	2169.0	84		-11	1874.2	5622.5	94.0	-1.9	3.5					10.5	1		

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

	В	С	D	E	F	G	н	1	J	к	L	м	N	1	
Experiment Date: Test substance:	22-Sep-11		Study Number:	9070-100107	ARB				Assays Cond	lucted by:	1		12		
10/27/2011 11:33															
		ug protein/assay tube =					in and a second s								
5	Tube	Sample Type	DPM (1mL)	Specific Binding DPM (100 uL) - NSB	Total Specific Binding (300 uL)	Mean									
	1 2 3 4 5 6	Total Activity (Master Mix)	63276 65781 58416 64532 59920 59592		63276.0 65781.0 58416.0 64532.0 59920.0 59592.0	61919.5									
	7 8 9 10 11 12	Total Binding (Solvent Control)	2485 2328 2363 2084 2325 2149	2190.2 2033.2 2068.2 1789.2 2030.2 1854.2	6571 6100 6205 5368 6091 5563	5982.5									
	12		1110	Specific									Without		
DPM (1mL) from LSC	la la constante	Sample Type	Concentration log[M]	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		
254.0 257.0	13 14	R1881 (NSB)	-6 -6	-40.8 -37.8	-122.5	-2.0	-2.8	8.1 7.3	0.0	2.3	0.9	2.5E+17	1.2		
254.0 257.0 262.0 359.0 344.0 293.0	15 16 17 18		-6 -6 -6	-32.8 64.2 49.2 -1.8	-98.5 192.5 147.5 -5.5	-1.6 3.2 2.5 -0.1	-2.4 2.4 1.7 -0.9	6.0 5.8 2.8 0.8					1.3 1.7 1.7 1.4		100
333.0 361.0 298.0	19 20 21	R1881	-7 -7 -7 -7	38.2 66.2 3.2	-5.5 114.5 198.5 9.5	1.9 3.3 0.2	0.9 2.3 -0.9	0.8 5.3 0.7	1.8	1.6	0.9	88.1	1.4 1.6 1.7 1.4	Binding (%)	80-
358.0 371.0	22 23	R1881	-8 -8	63.2 76.2	189.5 228.5	3.2 3.8	0.6 1.2	0.3 1.5	3.8	0.6	0.4	16.5	1.7 1.8	Specific B	40-
383.0 520.0 547.0	24 25 28	R1881	-8 -9 -9	88.2 225.2 252.2	264.5 675.5 756.5	4.4 11.3 12.6	1.8 -1.5 -0.1	3.3 2.2 0.0	12.3	0.9	0.5	7.3	1.9 2.5 2.7	Mean S	20-
554.0 1316.0	20 27 28	R1881	-9 -10	259.2 259.2 1021.2	700.0 777.5 3063.5	12.0 13.0 51.2	-0.1 0.2 -1.9	0.0	53.2	1.8	1.0	3.3	2.7		
1380.0 1373.0	29 30		-10 -10	1085.2 1078.2	3255.5 3234.5	54.4 54.1	1.3 0.9	1.7 0.9					6.7 6.7		ના ન
2320.0 2234.0 2220.0	31 32 33	R1881	-11 -11 -11	2025.2 1939.2 1925.2	6075.5 5817.5 5775.5	101.6 97.2 96.5	3.1 -1.2 -1.9	9.5 1.5 3.7	98.4	2.7	1.6	2.8	11.2 10.8 10.8		
391.0 438.0	37 38	Dexamethasone	3 3	96.2 143.2	288.5 429.5	4.8 7.2	-0.2 2.1	0.1 4.5	5.1	2.0	1.2	39.9	1.9 2.1		
358.0 962.0 950.0	39 40 41	Dexamethasone	3 4 4	63.2 667.2 655.2	189.5 2001.5 1965.5	3.2 33.5 32.9	-1.9 0.5 -0.1	3.6 0.3 0.0	33.0	0.4	0.3	1.3	1.7 4.7 4.6		100-
945.0 1886.0	42 43	Dexamethasone	-4 -5	650.2 1591.2	1950.5 4773.5	32.6 79.8	-0.3 -1.2	0.1	80.9	1.0	0.6	1.2	4.6 9.1	(%) 50	80-
1914.0 1924.0 2262.0	44 45 48	Dexamethasone	-5 -5 -8	1619.2 1629.2 1967.2	4857.5 4887.5 5901.5	81.2 81.7 98.6	0.2 0.7 2.2	0.0 0.5 5.0	97.1	2.4	1.4	2.5	9.3 9.3 11.0	c Bride	60-
2175.0 2256.0	47 48		-6 -6	1880.2 1961.2	5640.5 5883.5	94.3 98.3	-2.1 1.9	4.5 3.7					10.5 10.9	Sped	40-
2180.0 2192.0 2278.0	49 50	Dexamethasone	-7 -7 -7	1885.2 1897.2 1983.2	5655.5 5691.5 5949.5	94.5 95.1 99.4	-3.9 -3.3	15.4 11.1	96.4	27	1.5	2.8	10.6 10.6 11.0	Mean	20
2278.0 2290.0 2251.0 2296.0	51 52 53 54	Dexamethasone	-/ -8 -8 -8	1983.2 1995.2 1956.2 2001.2	5985.5 5868.5 6003.5	99.4 100.1 98.1 100.4	1.0 1.4 -0.6 1.7	1.0 1.8 0.4 2.7	99.5	1.2	0.7	1.2	11.0 11.1 10.9 11.1		-10 -
2371.0 2239.0	55 56	Dexamethasone	-9 -9	2076.2 1944.2	6228.5 5832.5	104.1 97.5	5.4 -1.2	29.1 1.5	101.1	3.3	1.9	3.3	11.5 10.8	1	
2320.0 2247.0 2213.0	57 58 59	Dexamethasone	-9 -10 -10	2025.2 1952.2 1918.2	6075.5 5856.5 5754.5	101.6 97.9 96.2	2.8 -0.8 -2.5	8.0 0.7 6.4	97.1	0.9	0.5	0.9	11.2 10.9 10.7	1	
2233.0 1362.0 1391.0	60 61 62	Octyl Salicylate	-10 -4 -4	1938.2 1067.2 1096.2	5814.5 3201.5 3288.5	97.2 53.5 55.0	-1.5 0.6 2.0	2.3 0.3 4.1	52.9	2.4	1.4	4.5	10.8 6.6 6.7		
1299.0 2214.0 2096.0	63 64 65	Octyl Salicylate	-4 -5 -5	1004.2 1919.2 1801.2	3012.5 5757.5 5403.5	50.4 96.2 90.3	-2.6 4.2 -1.8	6.7 17.3 3.1	92.1	3.6	2.1	3.9	6.3 10.7 10.2		100-
2083.0 2168.0	66 67	Octyl Salicylate	-5 -6	1788.2 1873.2	5364.5 5619.5	89.7 93.9	-2.4 -1.3	5.8 1.8	96.5	4.0	2.3	4.2	10.1 10.5	(%) 0	80
2311.0 2177.0 2283.0	68 69 70	Octyl Salicylate	-6 -6 -7	2016.2 1882.2 1988.2	6048.5 5646.5 5964.5	101.1 94.4 99.7	5.8 -0.9 4.4	34.0 0.8 19.6	98.0	1.5	0.9	1.6	11.2 10.5 11.1	c Bridin	ea -
2240.0 2224.0	71 72		-7 -7	1945.2 1929.2	5835.5 5787.5	97.5 96.7	2.3 1.5	5.2 2.2		2000	CRASHES -	200ke	10.9 10.8	m Specifi	40-
2197.0 2257.0 2158.0	73 74 75	Octyl Salicylate	4 4 4	1902.2 1962.2 1863.2	5706.5 5886.5 5589.5	95.4 98.4 93.4	0.1 3.1 -1.8	0.0 9.8 3.4	95.7	2.5	1.4	2.6	10.6 10.9 10.5	Mea	20 -
2282.0 2218.0	76 77	Octyl Salicylate	-9 -9	1987.2 1923.2	5961.5 5769.5	99.6 96.4	4.4 1.2	19.2 1.4	96.7	2.8	1.6	2.9	11.1 10.7		-11 -1
2170.0	78 79 80	Octyl Salicylate	-9 -10 -10	1875.2 1617.2 1991.2	5625.5 4851.5 5973.5	94.0 81.1 99.8	-1.2 -14.2 4.6	1.5 201.0 21.0	92.4	10.0	5.8	10.8	10.5 9.3 11.1	1	
1912.0 2286.0 2215.0	81	1 1	-10	1920.2	5760.5	96.3	1.0	1.0					10.7		

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

Impartment Tabula Diverse Diverse Amp Conductory Impartment Second	—	A	—		D	E	T E	G	T					—		ī.		
Provinte Provinte Provinte Pr		Experiment Date:		c			TARB	6	Н		Assays Con	K nducted by:		M	N			
	3 1	10/27/2011 11:33																
Idea Server Type UPU (tot) Server Type	4	1		ug protein/assay tube =														
TO Tod Achiv/Maer Mil) SO/F - SUB10 F1015 1 Tod Achiv/Maer Mil) SO/F - SO/F SO/F - SO/F			Tube	Sample Type	DPM (1mL)	Binding DPM (100	Specific Binding	Mean	Ĩ									
10 10 200 1002 000 10 200 1002 000 1000	8 9 10		3 4 5	Total Activity (Master Mix)	65781 58416 64532 59920	Ē	63276.0 65781.0 58416.0 64532.0 59920.0	61919.5										
Diff Table Table Sample Type Constraints Bineth Left Direct (be) Restar	13 14 15 16 17 18		7 8 9 10 11	Total Binding (Solvent Control)	2485 2328 2363 2084 2325	2033.2 2068.2 1789.2 2030.2	6571 6100 6205 5368 6091	5982.5										
Description Table Sample Type Consistion (beff) Description (beff) Restar Restar Number (beff) State (beff) State (beff)	19 20 21		12		2149	1854.2	5563		1									
232 2540 31 R188 (MSB) 40 -125 2.0 2.3 8.1 0.0 2.3 0.0 2.26 (17) 1.2 270 2800 14 40 -125 2.2 2.4 8.1 0.0 2.3 0.0 2.36 0.0 2.36 0.0 2.37 0.0 2.3 0.0 2.3 0.0 2.3 0.0 2.3 0.0 2.3 0.0 2.3 0.0 2.3 0.0 2.3 0.0 2.3 0.0 2.3 0.0 2.3 0.0 2.3 0.0<		DPM	Tube	Sample Type		Binding DPM (1mL)	Specific Binding	Binding	Residual		Specific	Deviation	SEM	% CV	Bound vs. Total			
20 2000 10 0 10 0 10 0 10 <th10< th=""> 10 10 1</th10<>		254.0 257.0		R1881 (NSB)							0.0	2.3	0.9	2.5E+17		1		
20 20 <th20< th=""> 20 20 <th2< td=""><td>26</td><td>262.0</td><td>15</td><td> /</td><td>-8</td><td>-32.8</td><td>-98.5</td><td>-1.6</td><td>-2.4</td><td>6.0</td><td></td><td>1 1</td><td>1</td><td></td><td>1.3</td><td>\vdash</td><td>г</td><td></td></th2<></th20<>	26	262.0	15	/	-8	-32.8	-98.5	-1.6	-2.4	6.0		1 1	1		1.3	\vdash	г	
32 330 19 Ft81 -7 32 114.5 1.3 0.9 0.8 1.8 1.0 0.9 0.1 1.1 0.0 0.1 0.0 0.	28	344.0 293.0	17	1 1	-6	49.2	147.5	2.5	1.7	2.8		/	1		1.7		100-5	
33 350 92 R181 4 0.2 184.0 3.2 0.8 0.3 3.8 0.5 0.4 10.5 17.0 0.5 350 350.0 25 R181 4 0.2 20.6 0.3 3.3 0.5 0.4 10.5 17.3 27.5 17.0 13.0 14.5 12.2 0.8 0.5 7.3 27.7 13.0 20.7 77.7 13.0 20.7 77.7 13.0 20.7 77.7 13.0 20.7 77.7 13.0 0.0 0.5 0.1 13.0 0.2 7.3 2.7 0.4 0.5 0.1 13.0 0.2 0.7 7.3 2.7 0.4 0.5 0.1 0.0 0.5 0.1 0.1 0.1 0.0 0.1 0.0 0.1	30 31	333.0 361.0	19 20	R1881	-7 -7	38.2 66.2	114.5 198.5	1.9 3.3	0.9	0.8 5.3	1.8	1.6	0.9	88.1	1.6 1.7	(%) Bupu	-	\backslash
30 State 22 R181 40 222 765 113 1.5 22 123 0.9 0.5 7.3 2.7 2.7 2.7 30 M000 20 R181 40 2022 706.5 124 0.01 0.00 0.05 7.3 2.7 2.7 30 M000 20 R1801 100.02 223.6 54.4 1.3 1.7 53.2 1.8 1.0 3.3 67.7 3.4 4.7 4.8 4.2 4.8 4.8 4.8 4.8 4.8 4.8 4.8 4.8 4.8 4.8 4.8 4.8 4.8 4.8 4.8 4.8 4.8 <td>33</td> <td>358.0</td> <td>22</td> <td>R1881</td> <td>-8</td> <td>63.2</td> <td>189.5</td> <td>3.2</td> <td>0.6</td> <td>0.3</td> <td>3.8</td> <td>0.6</td> <td>0.4</td> <td>16.5</td> <td>1.7</td> <td>alc Br</td> <td></td> <td>-</td>	33	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	alc Br		-
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$		383.0	24	D1001	-8	88.2	264.5	4.4	1.8	3.3	123		0.5	73	1.9	in Sper	- 1	
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	37 38	547.0	26	Kiooi	-0	252.2	756.5	12.6	-0.1	0.0	12.0	0.6	0.5	1.0	2.7	Mea	1	
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	39 40	1316.0 1380.0	28 29	R1881	-10 -10	1021.2 1085.2	3063.5 3255.5	51.2 54.4	-1.9 1.3	3.7 1.7	53.2	1.8	1.0	3.3	6.4 6.7	1_		-9.9 0
48 391.0 37 Desamehasone -3 46.2 288.5 4.8 4.2 0.1 5.1 2.0 1.2 88.0 1.3 47 38.0 30 -3 61.2 1.38 -3.3 0.1 5.1 2.0 1.2 88.0 1.3 4.1 47 38.0 -40 3.8 -3 61.2 1.3 -40 0.3 1.3 4.1 -4.4 606.2 1.00 0.0 0.1 0.0 1.2 0.1 6.1 0.0 0.0 1.2 0.1 0.0 1.2 0.1 0.0 1.2 0.1 0.0 1.2 0.1 0.0 0.1 0.0 0.1 0.0 0.1 0.0 0.0 0.1 0.0 0.0 0.1 0.0 0.	41 42 43	2320.0 2234.0	31	R1881	-11 -11	2025.2 1939.2	6075.5 5817.5	101.6 97.2	3.1	9.5 1.5	98.4	2.7	1.6	2.8	11.2 10.8	ſ		
47 38.0 39 30 33 92 188.5 32 188.5 32 188.5 33 0.5 0.5 0.3 33 0.4 0.2 1.7 1.7 46 000.0 41 000.0 41 000.0 0.4 0.3 33.0 0.5 0.5 0.5 0.0 0.0 1.3 4.7 47 000.0 44 0 0 177 177 178 177 177 178 178 177 178 178 178 178 178 178 179 0.5 0.5 0.0 0.1 1.2 0.1 0.5 0.1 0.5 0.1 0.5	45	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	1		
40 960.0 41 4 665.2 1266.5 32.9 4.1 0.0 0.0 4.8 0 61 1988.0 43 Dexamethasone -5 1991.2 477.5 78.8 -1.2 0.1 0.0 0.6 1.2 0.1 0.0 61 1988.0 43 Dexamethasone -6 1991.2 487.5 78.8 -1.2 0.1 0.0 0.6 1.2 0.1 0.0	47 48	358.0 962.0	39 40	Dexamethasone	-3	63.2 667.2	189.5 2001.5	3.2 33.5	-1.9 0.5	3.6 0.3	33.0	0.4	0.3	1.3	1.7 4.7	┨		De
61 1988.0 43 Decamefusione -5 1012 477.5 79.8 -1.2 1.5 80.9 1.0 0.6 1.2 6.1 <th6.1< th=""> <th6.1< th=""> <th6.1< td="" th<=""><td>49 50</td><td>950.0 945.0</td><td>41 42</td><td></td><td>4 4</td><td>655.2 650.2</td><td>1965.5 1950.5</td><td>32.9 32.6</td><td>-0.1 -0.3</td><td>0.0</td><td></td><td></td><td></td><td></td><td>4.6 4.6</td><td>F</td><td>90-</td><td>•</td></th6.1<></th6.1<></th6.1<>	49 50	950.0 945.0	41 42		4 4	655.2 650.2	1965.5 1950.5	32.9 32.6	-0.1 -0.3	0.0					4.6 4.6	F	90-	•
164 2282.0 46 Dexamethasone -0 1907.2 6001.5 06.8 2.2 5.0 07.1 2.4 1.4 2.5 11.0 10.5	51 52 53	1886.0 1914.0 1924.0	44 45	Dexamethasone	-5 -5	1619.2 1629.2	4857.5 4887.5	81.2 81.7	0.2 0.7	0.0	executions and	1.0	0.6		9.3 9.3	guque (70-	
100 2420.0 49 Dexamethasone -7 1802.2 565.8 94.5 -3.3 15.4 96.4 2.7 1.5 2.8 10.9	54 55	2262.0 2175.0	46 47	Dexamethasone	-6 -6	1967.2 1880.2	5901.5 5640.5	98.6 94.3	2.2 -2.1	5.0 4.5	97.1	2.4	1.4	2.5	11.0 10.5	Specific	50 40	
60 2200.0 52 Dexamethasone 8-8 1066.2 568.5 10.1 1.4 1.8 99.5 1.2 0.7 1.2 1.1 10.9 61 2251.0 53 Peramethasone 9-8 2002.2 600.4 1.7 2.7 1.2 0.7 1.2 1.1 10.9 63 2271.0 56 Dexamethasone 9 104.4 682.5 104.1 1.6 4.2 11.1 10.4 1.1 3.3 1.9 3.3 11.2 1.1 1.1 1.0 1.1 1.1 1.0 1.1 1.1 1.0 1.1 1.1 1.0 1.1 <th< td=""><td>57 58</td><td>2180.0 2192.0</td><td>49 50</td><td>Dexamethasone</td><td>-7 -7</td><td>1885.2 1897.2</td><td>5655.5 5691.5</td><td>94.5 95.1</td><td>-3.9 -3.3</td><td>15.4 11.1</td><td>96.4</td><td>27</td><td>1.5</td><td>2.8</td><td>10.6 10.6</td><td>Mean</td><td>30 - 20 -</td><td></td></th<>	57 58	2180.0 2192.0	49 50	Dexamethasone	-7 -7	1885.2 1897.2	5655.5 5691.5	94.5 95.1	-3.9 -3.3	15.4 11.1	96.4	27	1.5	2.8	10.6 10.6	Mean	30 - 20 -	
122 2280.0 34 1.0 -3 2001.2 000.3 10.4 1.1 2.7 1 1 1.1 <t< td=""><td>60 61</td><td>2290.0 2251.0</td><td>52 53</td><td>Dexamethasone</td><td>-8 -8</td><td>1995.2 1956.2</td><td>5985.5 5868.5</td><td>100.1 98.1</td><td>1.4 -0.6</td><td>1.8 0.4</td><td>99.5</td><td>1.2</td><td>0.7</td><td>1.2</td><td>11.1 10.9</td><td>1</td><td></td><td></td></t<>	60 61	2290.0 2251.0	52 53	Dexamethasone	-8 -8	1995.2 1956.2	5985.5 5868.5	100.1 98.1	1.4 -0.6	1.8 0.4	99.5	1.2	0.7	1.2	11.1 10.9	1		
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	62 63	2296.0 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			
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	65 66	2320.0 2247.0	57 58	Dexamethasone	- 0 -10	2025.2 1952.2	6075.5 5856.5	101.6 97.9	2.8 -0.8	8.0 0.7	97.1	0.9	0.5	0.9	11.2 10.9	-		
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	68	2233.0	60	Octocrylene	-10 -4	1938.2 1173.2	5814.5 3519.5	97.2 58.8	-1.5 8.5	2.3 72.0	50.4	11.7	6.7	23.1	10.8 7.1	4		
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	71	1395.0	63	1.73	4	1100.2	3300.5	55.2	4.8	23.3	74.6	22		2.9	6.8	┢		0
76 2146.0 68 -6 1851.2 563.5 92.8 1.8 3.4 10.4 <t< td=""><td>73 74</td><td>1833.0 1761.0</td><td>65 66</td><td></td><td>-5 -5</td><td>1538.2 1466.2</td><td>4614.5 4398.5</td><td>77.1 73.5</td><td>2.4</td><td>6.0 1.3</td><td>-</td><td></td><td>bibela bibela</td><td>erossi terest</td><td>8.9 8.5</td><td>9</td><td>- * 83</td><td>++</td></t<>	73 74	1833.0 1761.0	65 66		-5 -5	1538.2 1466.2	4614.5 4398.5	77.1 73.5	2.4	6.0 1.3	-		bibela bibela	erossi terest	8.9 8.5	9	- * 83	++
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	76	2146.0 2089.0	68	Octocrylene	-6	1851.2	5553.5	92.8	1.8 -1.0	3.4	91.2	1.5	0.9	1.6	10.4) Gupup		
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	78 79	2259.0 2258.0	70 71	Octocrylene	-7 -7	1964.2 1963.2	5892.5 5889.5	98.5 98.4	2.5 2.4	6.1 5.8	96.5	3.5	2.0	3.6	10.9 10.9	pedic F		
D4 2270.0 76 Octoorylene -0 1984.2 5652.5 99.5 2.1 4.4 99.5 4.0 2.3 4.0 11.0 85 2200.0 77 -9 1905.2 5715.5 95.5 -1.9 3.5 -1.9 3.5 -1.9 3.5 -1.9 3.5 -1.1 -11 -	81 82	2150.0 2156.0	73 74	Octocrylene	-8 -8	1855.2 1861.2	5565.5 5583.5	93.0 93.3	-4.1 -3.8	17.1 14.7	94.2	1.9	1.1	2.0	10.4 10.4	Mean S	-	
86 2358.0 78 -9 2083.2 6189.5 103.5 6.1 38.6 11.4 87 2191.0 79 Octoorylene -10 1896.2 6588.5 95.1 -2.4 5.6 97.1 3.4 2.0 3.5 10.6 88 2309.0 80 -10 2014.2 6042.5 101.0 3.5 12.8 10.8 10.8 90 2337.0 82 Octoorylene -11 2042.2 6128.5 102.4 4.9 24.4 98.1 4.4 2.8 4.5 110.6 90 2337.0 82 Octoorylene -11 2042.2 6128.5 102.4 4.9 24.4 98.1 4.4 2.8 4.5 11.3 91 2161.0 83 -11 1806.2 5598.5 93.6 -3.9 15.1 10.5	83 84	2217.0 2279.0	75 76	Octocrylene	-8 -9	1922.2 1984.2	5766.5 5952.5	96.4 99.5	-0.8 2.1	0.6	99.5	4.0	2.3	4.0	10.7 11.0	1	الم	
88 2309.0 80 -10 2014.2 6042.5 101.0 3.5 12.6 11.2 89 2191.0 81 -10 1866.2 5688.5 95.1 -2.4 5.6 10.8 90 2337.0 82 Octoorylene -11 2042.2 6128.5 102.4 4.9 24.4 98.1 4.4 2.6 4.5 11.3 91 2161.0 83 -11 1866.2 5598.5 93.6 -3.9 15.1 10.5	86 87	2358.0 2191.0	78 79	Octocrylene	- 0 -10	2063.2 1896.2	6189.5 5688.5	103.5 95.1	6.1 -2.4	36.6 5.6	97.1	3.4	2.0	3.5	11.4 10.6	┢	11.11	
<u>91</u> 2161.0 83 -11 1866.2 5598.5 93.6 -3.9 15.1 10.5	88 89	2309.0 2191.0	80 81		-10 -10	2014.2 1896.2	6042.5 5688.5	101.0 95.1	3.5 -2.4	12.6 5.6	etoreset in		100290302		11.2 10.6			
	91	2161.0	83	OCLOCIVIENE	-11	1866.2	5598.5	93.6	-3.9	15.1	90.1	-	2.0	4.5	10.5			

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

xperiment Date:			Study Number:	9070-100107	7ARB				Assays Cor	ducted by:					
t substance: 7/2012 13:57	Oxybenzone														
		ug protein/assay tube =							-				-		
				1					_						
	Tube	Sample Type	DPM (1mL)	Specific Binding DPM (100 uL) - NSB	Total Specific Binding (300 uL)	Mean									
1	1 2		59644 57430		59644.0 57430.0										
	3 4 5	Total Activity (Master Mix)	55671 58179 58770		55671.0 58179.0 58770.0	57405.7									
	6 7 8 9		54740 2161 2188 2221		54740.0 5742 5823 5922										
	10 11 12	Total Binding (Solvent Control)	2197 2248 2240	1950.0 2001.0 1993.0	5850 6003 5979	5886.5									
DPM			Concentration	Specific Binding	Total Specific	Specific		Squared	Mean Specific	Standard			% Ligand Bound vs.	5	
1mL) from LSC	Tube	Sample Type	log[M]	DPM (1mL) - NSB	Binding (3mL)	Binding (%)	Residual	Residual	Binding (%)	Deviation	SEM	% CV	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		
271.0 218.0	14		-6 -8	24.0	72.0	1.2	0.9	0.8					1.4		_
246.0 272.0	16 17		-6 -6	-1.0 25.0	-3.0 75.0	-0.1 1.3	-0.4	0.1					1.3		100
215.0	18		-8	-32.0	-96.0	-1.6	-1.9	3.8					1.1	8	
306.0 308.0	19 20	R1881	-7 -7	59.0 61.0	177.0 183.0	3.0 3.1	1.9	3.4	2.8	0.4	0.2	14.7	1.6	Guip	80
293.0 381.0	21 22	R1881	-7 -8	46.0 134.0	138.0 402.0	2.3 6.8	1.2	1.4	7.5	1.8	1.0	24.0	1.5	Bin	60
367.0	23	Kitooi	-8	120.0	360.0	6.1	-2.8	7.9	1.5	1.0	1.0	24.0	1.9	Specific	40
434.0 1197.0	24 25	R1881	-8 -9	187.0 950.0	561.0 2850.0	9.5 48.4	0.6	0.4	49.3	0.8	0.5	1.7	2.3 6.3	-	20
1216.0 1230.0	26 27		-9 -9	969.0 983.0	2907.0 2949.0	49.4 50.1	0.9	0.8					6.4 6.4	2	-
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		0
1999.0 1950.0	29 30	2 4 1	-10 -10	1752.0 1703.0	5256.0 5109.0	89.3 86.8	1.8 -0.6	3.4 0.4	1 1				10.4		
2273.0 2057.0	31 32	R1881	-11 -11	2026.0 1810.0	6078.0 5430.0	103.3 92.2	8.3	69.3 7.2	95.8	6.4	3.7	6.7	11.9 10.7		
2053.0	33		-11	1806.0	5418.0	92.0	-2.9	8.3					10.7		
281.0 236.0	37 38	Dexamethasone	-3 -3	34.0 -11.0	102.0	1.7	1.5 -0.8	2.2 0.6	0.5	1.1	0.7	211.4	1.5		
256.0 625.0	39 40	Dexamethasone	-3 -4	9.0 378.0	27.0 1134.0	0.5	0.2	0.0	19.2	0.4	0.2	2.0	1.3		
632.0	41	Dexametriasone	-4	385.0	1155.0	19.6	-0.2	0.0	18.2	0.4	0.2	2.0	3.3	1	100
617.0 1710.0	42 43	Dexamethasone	-4	370.0 1463.0	1110.0 4389.0	18.9 74.6	-1.0 4.7	0.9	70.8	3.3	1.9	4.6	3.2	×.	80
1592.0 1606.0	44 45		-5 -5	1345.0 1359.0	4035.0 4077.0	68.5 69.3	-1.3 -0.6	1.6 0.3		1			8.3 8.4	Binding	60
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 2031.0	47 48		-6 -6	1774.0 1784.0	5322.0 5352.0	90.4 90.9	-0.5	0.2					10.6 10.6	Spedic	40
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	lean	20
2059.0 2136.0	50 51		-7 -7	1812.0	5436.0 5667.0	92.3 96.3	-1.4 2.6	1.9					10.8	2	F
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		۰Ŀ
2091.0 2185.0	53 54	9 - S	-8 -8	1844.0 1938.0	5532.0 5814.0	94.0 98.8	0.0 4.8	0.0 22.6	3 3				10.9 11.4	8	10
2114.0 2114.0	55 56	Dexamethasone	-9 -9	1867.0 1867.0	5601.0 5601.0	95.1 95.1	1.1	1.2	92.9	3.9	2.3	4.2	11.0		
1980.0	57		-9	1733.0	5199.0	88.3	-5.7	32.7					10.3		
2147.0 2132.0	58 59	Dexamethasone	-10 -10	1900.0 1885.0	5700.0 5655.0	96.8 96.1	2.8	7.8	95.6	1.6	0.9	1.7	11.2		
2087.0 1354.0	60 61	Oxyberrene	-10	1840.0	5520.0 3321.0	93.8 56.4	-0.3	0.1 25.0	61.2	6.8	3.9	11.2	10.9		
1602.0	62	Oxybenzone	4	1355.0	4065.0	69.1	7.6	58.3	01.2	0.8	3.8	11.2	8.4		
1390.0 2150.0	63 64	Oxybenzone	-4 -5	1143.0 1903.0	3429.0 5709.0	58.3 97.0	-3.2	10.0 39.3	92.0	5.2	3.0	5.7	7.3		Г
2061.0	65	SATURATION	-5	1814.0	5442.0	92.4	1.7	3.0	02.0			v.1	10.8		100
1945.0 2131.0	66 67	Oxybenzone	-5 -6	1698.0 1884.0	5094.0 5652.0	86.5 96.0	-4.2	17.5 4.6	96.2	0.9	0.5	1.0	10.2	8	80
2155.0 2119.0	68 69		-6 -6	1908.0 1872.0	5724.0 5616.0	97.2 95.4	-0.9	0.9 7.6					11.3 11.1	Binding	ł
2109.0	70	Oxybenzone	-7	1862.0	5586.0	90.4	-2.8	26.5	97.5	3.8	2.2	3.9	11.1		60
2125.0 2246.0	71 72		-7 -7	1878.0 1999.0	5634.0 5997.0	95.7 101.9	-4.3 1.8	18.8 3.4		e states -	Jellin .	0.0713	11.1	Spedito	40
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	Mean	20
2195.0 2313.0	74		-8 -8	1948.0 2066.0	5844.0 6198.0	99.3 105.3	-1.2 4.8	1.5					11.5	-	~
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		ol
2156.0 2241.0	77 78		-9 -9	1909.0 1994.0	5727.0 5982.0	97.3 101.6	-3.3	11.2					11.3	8	-
2210.0 2222.0	79 80	Oxybenzone	-10 -10	1963.0 1975.0	5889.0 5925.0	100.0	-0.6 0.0	0.4	100.8	0.9	0.5	0.9	11.5 11.6	l.	
2244.0	81		-10	1997.0	5991.0	100.7 101.8	1.1	1.2					11.7		
2126.0 2303.0	82 83	Oxybenzone	-11 -11	1879.0 2056.0	5637.0 6168.0	95.8 104.8	-4.9 4.1	24.1 16.9	101.2	4.8	2.8	4.7	11.1 12.0		
2270.0	84		-11	2030.0	6069.0	104.8	2.4	5.9	2	5 C		1	11.9	6	

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

Experiment Date:			Study Number:	9070-100107	TARB				Assays Con	inducted by:					
						-								4	
		ug protein/assay tube =						/						4	
1				- Caraifie	Tabl								-	-	
	Tube	Sample Type	DPM (1mL)	Specific Binding DPM (100 uL) - NSB	(300 uL)	Mean									
	1 2 3 4 5	Total Activity (Master Mix)	59644 57430 55671 58179 58770	1111	59644.0 57430.0 55671.0 58179.0 58770.0	57405.7									
	5 6 7 8 9		54740 2161 2188 2221		58770.0 54740.0 5742 5823 5922										
	10 11 12	Total Binding (Solvent Control)) 2197 2248 2240	1974.0 1950.0 2001.0 1993.0	5850 6003 5979	5886.5									
DPM (1mL) from LSC	Tube	Sample Type	Concentration log[M]	Specific Binding DPM (1mL) - NSB	Total Specific Binding (3mL)	Specific Binding (%)		Squared Residual		Standard Deviation	SEM	% CV	% Ligand Bound vs. Total Activity	5.	
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	1	
271.0 218.0	14 15		-6 -6	24.0 -29.0	72.0 -87.0	1.2	0.9	0.8					1.4	+	~~~~
246.0 272.0	16 17	1	-8 -6	-1.0 25.0	-3.0 75.0	-0.1 1.3	-0.4 1.0	0.1		4		4	1.3 1.4	-	100-T
215.0	18	R1881	-0 -8 -7	-32.0	-96.0	-1.6	-1.9	3.8	2.8	0.4	0.2	14.7	1.1	8	-1
308.0	20	K1881	-7	61.0	183.0	3.1	1.9	3.4 3.8	2.8	0.4	0.2	14.7	1.6	Binding (-
293.0 381.0	21 22	R1881	-7 -8	46.0 134.0	138.0 402.0	2.3 6.8	1.2	1.4 4.4	7.5	1.8	1.0	24.0	1.5	C Bu	60-
367.0 434.0	23 24		-8 -8	120.0	360.0	6.1 9.5	-2.8	7.9					1.9	Specific	40-
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	Wean S	20-
1216.0 1230.0	26 27		-9 -9	969.0 983.0	2907.0 2949.0	49.4 50.1	0.9	0.8			(6.4 6.4	2	F
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	1	아는 -11
1999.0 1950.0	29 30	/	-10 -10	1752.0 1703.0	5256.0 5109.0	89.3 86.8	1.8 -0.6	3.4 0.4			I		10.4	1	354
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	1	
2057.0 2053.0	32 33		-11 -11	1810.0 1806.0	5430.0 5418.0	92.2 92.0	-2.7 -2.9	7.2 8.3					10.7 10.7		
281.0 236.0	37 38	Dexamethasone	-3 -3	34.0 -11.0	102.0 -33.0	1.7 -0.6	1.5 -0.8	2.2 0.6	0.5	1.1	0.7	211.4	1.5	ŧ	
256.0	39	4 5 J	-3	9.0	27.0	0.5	0.2	0.0					1.3		
625.0 632.0	40	Dexamethasone	4	378.0 385.0	1134.0 1155.0	19.3 19.6	-0.6 -0.2	0.3	19.2	0.4	0.2	2.0	3.3 3.3	-	100-
617.0	42		-4	370.0	1110.0	18.9	-1.0	0.9	70.8		10	18	3.2	(%)	80-
1710.0 1592.0	43	Dexamethasone	-5 -5	1463.0 1345.0	4389.0 4035.0	74.6 68.5	4.7	22.5 1.6	70.8	3.3	1.9	4.6	8.9 8.3	2	-
1606.0	45 46	Dexamethasone	-5 -8	1359.0	4077.0	69.3 83.0	-0.6	0.3 62.6	88.1	4.5	2.6	5.1	8.4	c Bud	60 -
2021.0	47	Dexamenason	-8	1774.0	5322.0	90.4	-0.5	0.2	00.1	9.0	2.0	6. s	10.6	pecific	40-
2031.0 2061.0	48	Dexamethasone	-6 -7	1784.0 1814.0	5352.0 5442.0	90.9 92.4	0.0 -1.3	0.0	93.7	2.2	1.3	2.4	10.6	S UB	F
2059.0	50		-7	1812.0	5436.0	92.3	-1.4	1.9				-	10.8	No.	20-
2136.0 2103.0	51 52	Dexamethasone	-7 -8	1889.0 1856.0	5667.0 5568.0	96.3 94.6	2.6 0.6	6.5 0.3	95.8	2.6	1.5	2.7	11.2 11.0	1	•
2091.0 2185.0	53 54	1	-8 -8	1844.0 1938.0	5532.0 5814.0	94.0 98.8	0.0	0.0					10.9 11.4	L	-10
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	1	
2114.0 1980.0	56 57		-9 -9	1867.0 1733.0	5601.0 5199.0	95.1 88.3	1.1 -5.7	1.2 32.7	$\mathbf{I}_{\mathbf{I}}$		1		11.0 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	1	
2132.0 2087.0	59 60		-10 -10	1885.0 1840.0	5655.0 5520.0	96.1 93.8	2.0 -0.3	4.1 0.1		<u> </u> '			11.1 10.9	1	
1947.0 2015.0	61 62	Methoxycinnamate	4	1700.0 1768.0	5100.0 5304.0	86.6 90.1	-10.9	118.1 54.8	89.3	2.4	1.4	2.6	10.2 10.5	Ŀ	
2035.0	63	Methoxycinnamate	-4	1788.0	5364.0	91.1	-6.4	40.7				28	10.6	-	F
2148.0 2066.0	64 65	Methoxycinhamave	-5 -5	1901.0 1819.0	5703.0 5457.0	96.9 92.7	-0.6 -4.8	0.4 23.1	96.4	3.5	2.0	3.6	11.2 10.8	1	100-
2201.0 2194.0	66 67	Methoxycinnamate	-5 -6	1954.0 1947.0	5862.0 5841.0	99.6 99.2	2.1	4.3	99.0	0.3	0.2	0.3	11.5	E	80-
2191.0	68	Methoxyonnamate	-6	1944.0	5832.0	99.1	1.6	2.5	60.0	0.0	U.2	0.0	11.5	Binding	-
2182.0 2184.0	69 70	Methoxycinnamate	-6 -7	1935.0 1937.0	5805.0 5811.0	98.6 98.7	1.1	1.2	95.7	8.5	4.9	8.9			60
2254.0	71		-7	2007.0	6021.0	102.3	4.8	22.8				-	11.8	Specific	40
1935.0 2265.0	72 73	Methoxycinnamate	-7 -8	1688.0 2018.0	5064.0 6054.0	86.0 102.8	-11.5 5.3	131.8 28.5	100.3	2.6	1.5	2.6	10.1	Mean	-
2164.0	74	-	-8	1917.0	5751.0	97.7	0.2	0.0				-	11.3	3	20 -
2216.0 2196.0	75	Methoxycinnamate	-8 -9	1969.0 1949.0	5907.0 5847.0	100.3 99.3	2.8	8.1 3.3	97.7	1.6	0.9	1.6	11.6 11.5	1	o[
2134.0 2160.0	77 78	1	-9 -9	1887.0 1913.0	5661.0 5739.0	96.2 97.5	-1.3	1.8				4	11.2 11.3	\vdash	Mart
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	1	
2292.0 2212.0	80 81		-10 -10	2045.0 1965.0	6135.0 5895.0	104.2 100.1	6.7 2.6	45.1 7.0					12.0 11.6	-	
2126.0	82	Methoxycinnamate	-10	1879.0	5637.0	95.8	-1.7	3.0	100.9	5.1	2.9	5.0	11.1	1	
2120.0	83		-11	1983.0	5949.0	101.1	3.6	12.6			4		11.7		

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

Experiment Date:			Study Number:	9070-10010	TARB				Assays Con	nducted by:					
est substance: 027/2012 14:00	Octyl Salicyla	te	<u> </u>			('	'	'					4	-	
2112012						4							4		
		ug protein/assay tube =				$\underline{-}$	<u></u>								
	Tube	Sample Type	DPM (1mL)	Specific Binding DPM (100 uL) - NSB	(300 uL)	Mean									
	1 2 3 4 5 6	Total Activity (Master Mix)	59644 57430 55671 58179 58770 54740		59644.0 57430.0 55671.0 58179.0 58770.0 54740.0	57405.7									
	6 7 8 9 10 11 12	Total Binding (Solvent Control)	2161 2188 2221	1914.0 1941.0 1974.0 1950.0 2001.0 1993.0	54740.0 5742 5823 5922 5850 6003 5979	5886.5									
DPM (1mL) from LSC	Tuba	Sample Type	Concentration log[M]	Specific	Total Specific	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	1 10 10 10 10 10 10 10 10 10 10 10 10 10	-6 -6	24.0 -29.0	72.0	1.2	0.9	0.8					1.4	<u> </u>	
218.0 246.0	15		-6	-1.0	-87.0 -3.0	-1.5 -0.1	-1.8 -0.4	3.2 0.1			()		1.1		Б
272.0	17		-6	25.0	75.0	1.3	1.0	0.9			()		1.4		100
215.0 306.0	18	R1881	-6 -7	-32.0	-96.0 177.0	-1.6 3.0	-1.9	3.8	2.8	0.4	0.2	14.7	1.1	×.	80-
308.0	20		-7	61.0	183.0	3.1	2.0	3.8					1.8	Binding	-
293.0 381.0	21 22	R1881	-7 -8	46.0 134.0	138.0 402.0	2.3 6.8	-2.1	1.4	7.5	1.8	1.0	24.0	1.5		60-
367.0	23	TTTOOT	-8	120.0	360.0	6.1	-2.8	7.9	1.0	1.0	1	27.0	1.9	Specific	40-
434.0 1197.0	24 25	R1881	-8 -9	187.0 950.0	561.0 2850.0	9.5 48.4	0.6	0.4	49.3	0.8	0.5	1.7	2.3 6.3	8	E.
1216.0	26	R1001	-9	969.0	2907.0	49.4	0.9	0.8	98.0	0.0	0.0	15a	6.4	Mea	20-
1230.0	27	04004	-9	983.0	2949.0	50.1	1.6	2.5					6.4		0
1862.0 1999.0	28	R1881	-10 -10	1615.0 1752.0	4845.0 5256.0	82.3 89.3	-5.1	26.4 3.4	86.1	3.5	2.0	4.1	9.7 10.4		-11
1950.0	30		-10	1703.0	5109.0	86.8	-0.6	0.4					10.2	-	
2273.0	31 32	R1881	-11 -11	2026.0 1810.0	6078.0 5430.0	103.3 92.2	8.3	69.3 7.2	95.8	6.4	3.7	6.7	11.9 10.7		
2057.0 2053.0	33		-11	1806.0	5418.0	92.2 92.0	-2.7 -2.9	7.2 8.3			()	<u> </u>	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		
236.0 256.0	38		-3 -3	-11.0	-33.0 27.0	-0.6 0.5	-0.8 0.2	0.6		+ +	(1.2	<u> </u>	125
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	1	100-
632.0 617.0	41		4	385.0	1155.0	19.6	-0.2	0.0		-	· · · · · · · · · · · · · · · · · · ·		3.3	1	100
617.0 1710.0	42	Dexamethasone	-4 -5	370.0 1463.0	1110.0 4389.0	18.9 74.6	-1.0 4.7	0.9 22.5	70.8	3.3	1.9	4.6	3.2 8.9	8	80-
1592.0 1606.0	44 45		-5 -5	1345.0	4035.0 4077.0	68.5 69.3	-1.3 -0.6	1.6	an Chaptara				8.3 8.4	Bulpu	
1606.0	45	Dexamethasone	-5 -6	1359.0	4077.0	69.3 83.0	-0.6	0.3 62.6	88.1	4.5	2.6	5.1	8.4 9.8		60-
2021.0	47	Deatherster	-6	1774.0	5322.0	90.4	-0.5	0.2					10.6	pecific	40-
2031.0 2061.0	48	Dexamethasone	-6 -7	1784.0 1814.0	5352.0 5442.0	90.9 92.4	0.0	0.0	93.7	2.2	1.3	2.4	10.6	u S	-
2059.0	50	Dexamenations	-7	1812.0	5436.0	92.3	-1.4	1.9	80.1		1	4.7	10.8	Mea	20-
2136.0	51	A 10 10 10 10 10 10 10 10 10 10 10 10 10	-7	1889.0	5667.0	96.3	2.6	6.5	05.0	~~		27	11.2	1	•
2103.0 2091.0	52 53	Dexamethasone	-8 -8	1856.0 1844.0	5568.0 5532.0	94.6 94.0	0.6	0.3	95.8	2.6	1.5	2.7	11.0	1	-10
2185.0	54		-8	1938.0	5814.0	98.8	4.8	22.6					11.4	<u> </u>	
2114.0	55 56	Dexamethasone	-9 -9	1867.0 1867.0	5601.0 5601.0	95.1 95.1	1.1	1.2	92.9	3.9	2.3	4.2	11.0 11.0		
2114.0 1980.0	57		-9	1733.0	5199.0	88.3	1.1 -5.7	1.2 32.7	ti nanga s	the second	(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	1	
2132.0 2087.0	59	1	-10 -10	1885.0 1840.0	5655.0 5520.0	96.1 93.8	2.0 -0.3	4.1	÷ 7	1	('		11.1		
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 1223.0	62		4	270.0 976.0	810.0 2928.0	13.8	-24.7	607.8		-			2.7	4	
1836.0	63 64	Octyl Salicylate	-5	1589.0	4767.0	49.7 81.0	11.3 -3.0	128.3 8.9	84.0	5.5	3.2	6.6	6.4 9.6	1	Г
1828.0	65		-5	1581.0	4743.0	80.6	-3.4	11.5	1. A A				9.6		100 8
2020.0 2221.0	66 67	Octyl Salicylate	-5 -6	1773.0 1974.0	5319.0 5922.0	90.4 100.6	6.4 10.3	40.9 106.2	97.3	3.2	1.8	3.3	10.6 11.6	8	80-
2097.0	68	own own part	-6	1850.0	5550.0	94.3	4.0	15.9	With a	-	(11.0	uding	
2148.0 2100.0	69 70	Octyl Salicylate	-6	1901.0 1853.0	5703.0 5559.0	96.9 94.4	6.6 4.1	43.4	91.6	3.3	1.9	3.6	11.2	c Buc	60-
2100.0 2060.0	70	Octyl Salicylate	-7 -7	1853.0 1813.0	5559.0 5439.0	94.4 92.4	4.1	17.1	91.0	3.3	1.8	3.0	11.0	Spedic	
1974.0	72		-7	1727.0	5181.0	88.0	-2.3	5.2	1						40
1407.0	73	Octyl Salicylate	-8	1160.0 1358.0	3480.0 4074.0	59.1	-31.2	972.2 444.8	73.1	16.3	9.4	22.2	7.4	Mean	20
1605.0 2031.0	74 75		-8 -8	1358.0	4074.0 5352.0	69.2 90.9	-21.1 0.6	444.8 0.4			()		8.4	1	-
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	1	-11
2119.0 1951.0	77 78		-9 -9	1872.0	5616.0 5112.0	95.4 86.8	5.1 -3.5	26.1	<u> </u>		·'		11.1	<u> </u>	2221
2016.0	79	Octyl Salicylate	-10	1769.0	5307.0	90.2	-0.1	0.0	93.0	2.6	1.5	2.8	10.2	1	
2088.0 2114.0	80		-10	1841.0	5523.0	93.8	3.5	12.4	104074	1 11111		1220	10.9	ŧ.	
2114.0	81	<u></u> ′	-10	1867.0	5601.0	95.1 98.0	4.9	23.5 59.4	97.4	2.6	1.5	2.7	11.0	1	
2114.0	82	Octyl Salicylate	-11	1923.0	5769.0		7.7								

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

Experiment Date:	6-Oct-11		Study Number:	9070-100107	TARB		T	T	Assays Con	nducted by:					
														4	
2/12012 14.00													-	-	
/		ug protein/assay tube =												-	
	Tube	Sample Type	DPM (1mL)	Specific Binding DPM (100 uL) - NSB		Mean									
	1 2 3 4 5 6	Total Activity (Master Mix)	59644 57430 55671 58179 58770 54740		59644.0 57430.0 55671.0 58179.0 58770.0 54740.0	57405.7									
	6 7 8 9 10 11 11	Total Binding (Solvent Control)	2161 2188 2221		54740.0 5742 5823 5922 5850 6003 5979	5886.5									
DPM	Tuba		Concentration	Specific Binding	Total Specific	Specific		Squared	Mean Specific	Standard			% Ligand Bound vs.		
(1mL) from LSC	Tube	Sample Type	log[M]	DPM (1mL) - NSB		Binding (%)	Residual	Residual	Binding (%)	Deviation	SEM	% CV	Total Activity		
260.0 271.0	13 14	R1881 (NSB)	-6 -6	13.0 24.0	39.0 72.0	0.7	0.3	0.1	0.0	1.3	0.5	#DIV/0!	1.4	1	
218.0	15		-6	-29.0	-87.0	-1.5	-1.8	3.2					1.1	-	-
246.0 272.0	16 17	/	-6 -6	-1.0 25.0	-3.0 75.0	-0.1 1.3	-0.4	0.1		1		-	1.3	-	100-T
215.0	18		-6	-32.0	-96.0	-1.6	-1.9	3.8	1 1	1			1.1	8	- 1
306.0 308.0	19 20	R1881	-7 -7	59.0 61.0	177.0 183.0	3.0 3.1	1.9	3.4 3.8	2.8	0.4	0.2	14.7	1.6		80-
293.0	21	//	-7	46.0	138.0	2.3	1.2	1.4			[]		1.5	Binding	60-
381.0 367.0	22 23	R1881	-8 -8	134.0 120.0	402.0 360.0	6.8 6.1	-2.1	4.4 7.9	7.5	1.8	1.0	24.0	2.0	Cilic	100 C
434.0	24	<u> </u>	-8	187.0	561.0	9.5	0.6	0.4	3 3	1000			2.3	Se	40-
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	Mean	20-
1216.0 1230.0	26 27	//	-9 -9	969.0 983.0	2907.0 2949.0	49.4 50.1	0.9	0.8 2.5				<u> </u>	6.4 6.4		, L
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	1	-11
1999.0 1950.0	29 30		-10 -10	1752.0 1703.0	5256.0 5109.0	89.3 86.8	1.8 -0.6	3.4 0.4					10.4	-	1000
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	1	
2057.0 2053.0	32 33	/	-11 -11	1810.0 1806.0	5430.0 5418.0	92.2 92.0	-2.7	7.2 8.3				-	10.7 10.7	4	
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	1	
236.0 256.0	38	/	-3 -3	-11.0	-33.0	-0.6	-0.8	0.6			1		1.2	1	
256.0 625.0	39 40	Dexamethasone	-3 -4	9.0 378.0	27.0 1134.0	0.5	0.2	0.0	19.2	0.4	0.2	2.0	1.3 3.3	1	Г
632.0	41	/	-4	385.0	1155.0	19.6	-0.2	0.0					3.3	1	100
617.0 1710.0	42 43	Dexamethasone	-4 -5	370.0 1463.0	1110.0 4389.0	18.9 74.6	-1.0 4.7	0.9	70.8	3.3	1.9	4.6	3.2	S	80
1592.0	44	Deadhround	-5	1345.0	4035.0	68.5	-1.3	1.6	1			1.11	8.3	uding	
1606.0 1875.0	45 46	Dexamethasone	-5 -6	1359.0 1628.0	4077.0 4884.0	69.3 83.0	-0.6	0.3 62.6	88.1	4.5	2.6	5.1	8.4 9.8	c Bindir	60-
2021.0	47	Deadhreansanna	-6	1774.0	5322.0	90.4	-0.5	0.2	00		4.0	v	10.6	pechic	40-
2031.0 2061.0	48 49	Dexamethasone	-6 -7	1784.0 1814.0	5352.0 5442.0	90.9 92.4	0.0	0.0	93.7	2.2	1.3	2.4	10.6 10.8	L S	
2059.0	50	Dexamentasone	-7	1812.0	5436.0	92.3	-1.4	1.9	80.1	2.2	1.8	2.4	10.8	Mea	20
2136.0	51	/	-7	1889.0	5667.0	96.3	2.6	6.5					11.2	1	•
2103.0 2091.0	52 53	Dexamethasone	-8 -8	1856.0	5568.0 5532.0	94.6 94.0	0.6	0.3	95.8	2.6	1.5	2.7	11.0	-	-10
2185.0	54	· · · · · · · · · · · · · · · · · · ·	-8	1938.0	5814.0	98.8	4.8	22.6			L		11.4	<u> </u>	
2114.0 2114.0	55 56	Dexamethasone	-9 -9	1867.0 1867.0	5601.0 5601.0	95.1 95.1	1.1	1.2	92.9	3.9	2.3	4.2	11.0 11.0	-	
1980.0	57	d/	-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	1	
2132.0 2087.0	59 60	'	-10 -10	1885.0 1840.0	5655.0 5520.0	96.1 93.8	2.0 -0.3	4.1 0.1				<u> </u>	11.1	-	
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	1	
1285.0 1225.0	62 63	· · · · · · · · · · · · · · · · · · ·	4	1038.0 978.0	3114.0 2934.0	52.9 49.8	1.6	2.6		++			6.7 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	1	100
1905.0 1955.0	65 66	/	-5 -5	1658.0 1708.0	4974.0 5124.0	84.5 87.0	-0.3 2.3	0.1				-	10.0 10.2	×.	100 -
2319.0	67	Octocrylene	-8	2072.0	6216.0	105.6	7.5	56.0	98.6	6.9	4.0	7.0	12.1		80-
2177.0 2049.0	68 69	/	-6 -6	1930.0 1802.0	5790.0 5406.0	98.4 91.8	0.2 -6.3	0.1 39.4			1		11.4	Binding	
2259.0	70	Octocrylene	-7	2012.0	6036.0	102.5	-0.3	39.4	97.3	7.2	4.1	7.4	10.7	_	60-
2213.0	71	/	-7	1966.0	5898.0	100.2	2.1	4.3			1		11.6	Specific	40-
1996.0 2246.0	72 73	Octocrylene	-7 -8	1749.0	5247.0 5997.0	89.1 101.9	-9.0 3.8	80.7 14.1	100.0	1.8	1.0	1.8	10.4	Mean	F
2176.0	74		-8	1929.0	5787.0	98.3	0.2	0.0	Tere	1 1 1	,		11.4	1	20 -
2206.0 2254.0	75 76	Ortoonlana	-8 -9	1959.0	5877.0	99.8	1.7	3.0	00.0	2.5	20	2.5	11.5	-	
2196.0	77	Octocrylene	-9	2007.0 1949.0	6021.0 5847.0	102.3 99.3	1.2	17.3	99.0	3.5	2.0	3.5	11.8	-	-11
2119.0	78	/	-9	1872.0	5616.0	95.4	-2.7	7.4					11.1	1	
2159.0 2153.0	79	Octocrylene	-10 -10	1912.0	5736.0 5718.0	97.4 97.1	-0.7	0.5	97.4	0.3	0.2	0.3	11.3	-	
2164.0	81	//	-10	1917.0	5751.0	97.7	-0.4	0.2	Course of	a survey a		-	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	1	
1965.0	83	1	-11	1718.0	5154.0	87.6	-10.6	111.6			· · · · · · · · · · · · · · · · · · ·		10.3	•	

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
r or r o	J · ·

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

Charles River Laboratories
Sprague-Dawley
90 days
< 1
8.8 mg/mL
Bradford Method
Bio-Rad Dye Reagent Concentrate
500-0006
210007463
FedEx – priority overnight
Dry Ice

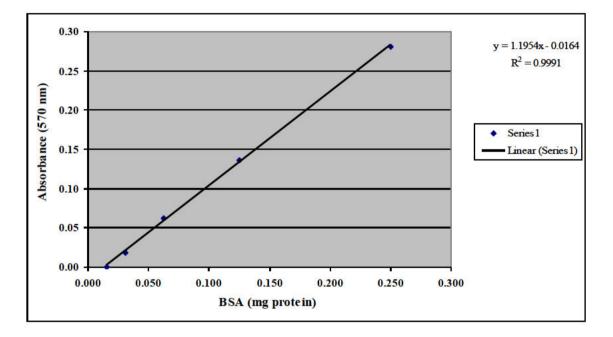
Third Run – October 06, 2011

Isolation Procedure

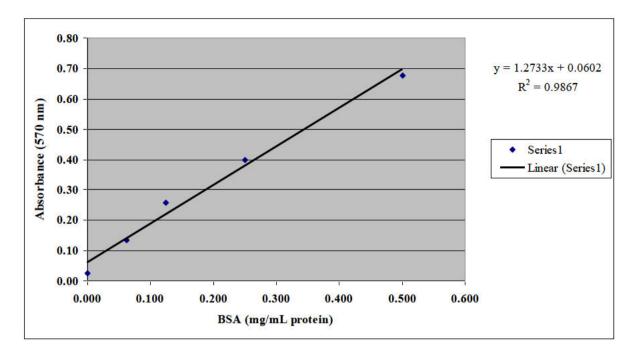
- □ Make sure the homogenizer probe is pre-chilled before use, by placing it in a beaker of low-salt TEDG buffer on ice.
- □ Inspected prostate tissue for healthy appearance (no fibrous, inflamed, edematous or infected appearance) and discard any tissues that appear compromised; trim excess fascia if 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.
- \Box 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).

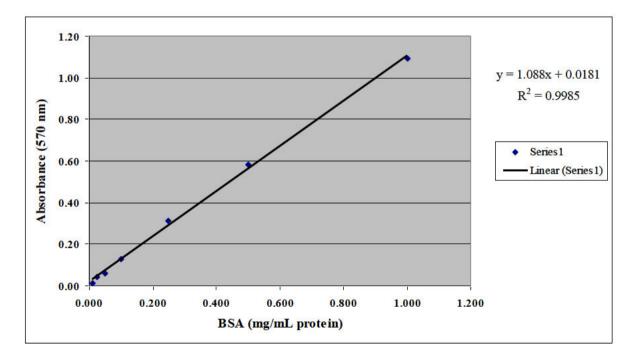


Calibration Curve - First Run - September 20, 2011



Calibration Curve - Second Run - September 22, 2011

Calibration Curve - Third Run - October 06, 2011



-	Itun D	utu i iutt it	Inp Inpu	itum Depte	111001 2 09 /								
		1	2	3	4	5	6	7	8	9	10	11	12
	А	buffer bkg	buffer blank	water blank	0.5	0.5	0.5	3x cyto	3x cyto	3x cyto	buffer bkg	buffer blank	water blank
_	В	buffer bkg	buffer blank	water blank	0.25	0.25	0.25	5x cyto	5x cyto	5x cyto	buffer bkg	buffer blank	water blank
5	С	buffer bkg	buffer blank	water blank	0.125	0.125	0.125	10x cyto	10x cyto	10x cyto	buffer bkg	buffer blank	water blank
010	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
100	Е	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
	Н	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 Plate Map – First Run – September 20, 2011

Raw Data– First Run – September 20, 2011 Plate Seq#: 8072

Comment:	Acq	uired: Thurs	day, June 03	3, 2010 4:11	PM Tempe	erature Min/	Max: 0.0/0.0)°C				
Absorbance-A	F	ile Report: C	:\Fusion dat	a files\MTT	_(null)_06-	03-10_1142	.TXT					
	1	2	3	4	5	6	7	8	9	10	11	12
А	0.056	0.412	0.414	0.839	0.922	0.943	1.544	1.711	1.369	0.044	0.042	0.045
В	0.050	0.416	0.414	0.629	0.695	0.707	1.154	1.226	1.194	0.048	0.042	0.047
С	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
Е	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
Н	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 D	ata Plate M	1ap- second	l Run – Sep	tember 22	2, 2011							
	1	2	3	4	5	6	7	8	9	10	11	12
А	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty	empty
В	empty	empty	empty	3x cyto	3x cyto	3x cyto	empty	empty	2	2	2	empty
С	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
Н	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:	Acqui	red: Wednes	day, March	30, 2011 5:2	25 PM Tem	perature Mi	n/Max: 0.0/0).0−C				
Absorbance-A	F	ile Report: C	:\Fusion dat	a files\MTT	_(null)_03-3	30-11_1551	TXT					
	1	2	3	4	5	6	7	8	9	10	11	12
А	0.04	0.039	0.04	0.038	0.039	0.038	0.037	0.038	0.038	0.04	0.04	0.039
В	0.402	0.388	0.394	1.116	1.089	1.09	0.399	0.407	1.336	1.355	1.302	0.41
С	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
Н	0.039	0.039	0.043	0.038	0.038	0.04	0.043	0.037	0.039	0.039	0.039	0.039

	1	2	3	4	5	6	7	8	9	10	11	12
А	neat cyto	neat cyto	neat cyto	water	empty	empty	empty	empty	empty	2	2	2
В	2X cyto	2X cyto	2X cyto	water	empty	empty	empty	empty	empty	1	1	1
С	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
Н	water	water	water	water	empty	empty	empty	empty	empty	0.01	0.01	0.01

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

Raw Data – Third Run – October 06, 2011 Plate Seq#: 9198

Comment:	Acqui	red: Sunday,	, September	25, 2011 5:2	28 PM Tem	perature Mi	n/Max: 0.0/0).0°C				
Absorbance-A	File	Report: C:\l	Fusion data	files\MTT_((null)_09-25	-11_1732.T	XT					
	1	2	3	4	5	6	7	8	9	10	11	12
А	3.310	3.310	3.310	0.140	0.044	0.041	0.040	0.041	0.042	2.065	2.125	2.071
В	2.708	2.611	2.590	0.142	0.041	0.041	0.042	0.049	0.043	1.300	1.177	1.230
С	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
Н	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

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.



27007 2011 Date

CeeTox, Inc.

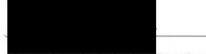


Director of Project Management CeeTox, Inc.

2700+2011 Date



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.



270CT 2011 Date

Senior Scientist/Endocrine Group Leader CeeTox, Inc.



Director of Project Management CeeTox, Inc.

27 Oct 2011 Date



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.



270672011 Date

Senior Scientist/Endocrine Group Leader CeeTox, Inc.



Director of Project Management CeeTox, Inc.

J7042011 Date

Study Number: 9070-100107ARB

APPENDIX 3 Deviation Forms

In vitro models to predict toxic	âły				
Study Number (if app	licable):	Batch ARB002			
Date of Reporting:	26-Sep-11	Reporting	Associate:		
Date of Occurrence:	20-Sep-11, 2 and 06-Oct-1		nvolved: <u>El</u>	DSP Lab	
Description of Deviation	on:				
All centrifuge spins we	ere performed at 2	700 x g, not 600 x g d	is stated in the j	protocol. Th	is is necess
To prevent loss of the	HAP pellet.				
Signature		26 Oct2011	Date:	26-0	ct-11
	(Reporting As	sociate)	0		
Type of Deviation (det	termined by Study	Director/Principal Inv	estigator):		
SOP Deviation			GLP Deviation		No Deviati
Summary of Deviation	Investigation by	SD/PI/Test Facility Ma	nagement/Desi	ignee:	
Increased centrifugation	on speed to preve	ent loss of the HAP pell	ət.		
protocol.					
	the second s	act on Study Data and	/or Facility Con	mpliance:	
Action Taken and Det	ermination of imp				
Action Taken and Deta	10				
	10				
	10				

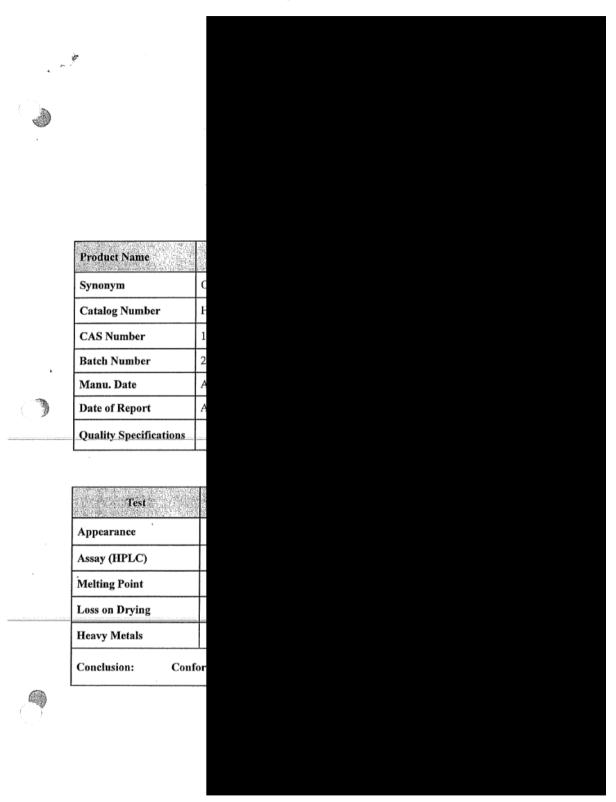
100 011 80			Form #:	SOP-1
In vitro models to predict toxicity	Deviation &	Investigation		
Study Number (if applica	ble): Batch A	RBOO2		
Date of Reporting: _2	0-Sep-11	Reporting Associate:		
Date of Occurrence:	20-Sep-11, 22-Sep-11 and 06-Oct-11	_ Associate Involved:	EDSP Lab	
Description of Deviation:				
150 mL of 60% HAP was	measured and washe	d three times at 2 hour int	ervals with 50 r	mM Tris. Th
was allowed to settle ove	rnight at 4°C because	it settles much better and	the results are n	nore accura
Signature	01.	School Date:	26-0	Dct-11
	(Reporting Associate)			
Type of Deviation (determ			_	
SOP Deviation		est Facility Management/	Designee:	
Summary of Deviation Inv Washed 3x then let HAP	restigation by SD/PI/T	est Facility Management/	Designee:	
Summary of Deviation Inv	restigation by SD/PI/T	est Facility Management/	Designee:	
Summary of Deviation Inv Washed 3x then let HAP day.	restigation by SD/PI/Ti settle overnight instead	est Facility Management/	Designee: might, was twic	No Deviati
Summary of Deviation Inv Washed 3x then let HAP	restigation by SD/PI/Ti settle overnight instead	est Facility Management/ l of wash once, settle ove	Designee: might, was twic	
Summary of Deviation Inv Washed 3x then let HAP day. Action Taken and Determ	restigation by SD/PI/Ti settle overnight instead	est Facility Management/ l of wash once, settle ove	Designee: might, was twic	
Summary of Deviation Inv Washed 3x then let HAP day. Action Taken and Determ	restigation by SD/PI/Ti settle overnight instead	est Facility Management/ l of wash once, settle ove	Designee: might, was twic	
Summary of Deviation Inv Washed 3x then let HAP day. Action Taken and Determ None. Deviation results i	restigation by SD/PI/Ti settle overnight instead	est Facility Management/ l of wash once, settle ove udy Data and/or Facility	Designee: might, was twic Compliance:	e and use t
Summary of Deviation Inv Washed 3x then let HAP day. Action Taken and Determ None. Deviation results i	restigation by SD/PI/Ti settle overnight instead	est Facility Management/ l of wash once, settle ove udy Data and/or Facility 	Designee: might, was twic Compliance:	
Summary of Deviation Inv Washed 3x then let HAP day. Action Taken and Determ None. Deviation results i	restigation by SD/PI/Ti settle overnight instead	est Facility Management/ l of wash once, settle ove udy Data and/or Facility 	Designee: might, was twic Compliance:	e and use t

In vitro models to predict toxicity
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 19 th 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 20 th missed temperature documentation. It was determined upon investigation that there was no impact on study number 9070-100107ARB due to the missed temperature documentation or 20September2011.
Type of Deviation (determined by Study Director/Principal Investigator): Facility Deviation from SOP-4007
Summary of Deviation Investigation by SD/Pt/Test Facility Management/Designee:
The records of the temperatures of the listed refrigerators and freezers were examined. All contents a 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 20 th time period.
Signature: Date: <u></u> Date:DCGL671

In vitro models to predict toxicity	Deviation 8	& Investigation	orm #:	SOP-1003-F
Study Number (if appli	icable): _9070-	100107ARB		
Date of Reporting:	27-Jan-12	Reporting Associate:		
Date of Occurrence:	06-Jul-11	Associate Involved:		
Description of Deviation	on:			
The lot number for oxy	benzone in the prot	ocol (20080801) was not the	lot provid	ed (20100801)
SOP Deviation	Protocol Devi Investigation by SD.	irector/Principal Investigato ation GLP Deviation /PI/Test Facility Managemer	Designe	
Action Taken and Dete	ermination of Impac	ct on Study Data and/or Fac	illity Comp	illance:
None.	ermination of Impac	Date:		an-12

In vitro models to predict toxicit		Investigation		
Study Number (if ap	plicable): 9070-1	00107ARB		
Date of Reporting:	04-Jan-12	_ Reporting Associate: _		
Date of Occurrence	20-Sep-11, 22-Sep-1 and 06-Oct-11	1 Associate Involved:		
Description of Devia	tion:	_		
Wrong purity was use	ed for methoxycinnam	ate. Used 98% instead of	99.8%.	
<u></u>				
Signature		Date:	04-Jc	an_12
	(Reporting Associate)		04-30	211-12
	(hepoling Associate)	1		
1				
Type of Deviation (de	etermined by Study Dir	ector/Principal Investigato	or):	
Type of Deviation (de				No Deviation
SOP Deviation	Protocol Devia			
SOP Deviation	Protocol Devia	tion GLP Deviation		
SOP Deviation	Protocol Devia	tion GLP Deviation		
SOP Deviation	Protocol Devia	tion GLP Deviation		
SOP Deviation Summary of Deviation Wrong purity was use	Protocol Devia n Investigation by SD/F ed for methoxycinname	tion GLP Deviation	∏t nt/Designee	9:
SOP Deviation Summary of Deviatio Wrong purity was use Action Taken and De	Protocol Devia n Investigation by SD/F ed for methoxycinname	tion GLP Deviation PI/Test Facility Manageme ate.	∏t nt/Designee	9:
SOP Deviation Summary of Deviatio Wrong purity was use Action Taken and De	Protocol Devia n Investigation by SD/f ed for methoxycinnami termination of Impact	tion GLP Deviation PI/Test Facility Manageme ate.	∏t nt/Designee	9:
SOP Deviation Summary of Deviatio Wrong purity was use Action Taken and De	Protocol Devia n Investigation by SD/f ed for methoxycinnami termination of Impact	tion GLP Deviation PI/Test Facility Manageme ate.	∏t nt/Designee	9:
SOP Deviation Summary of Deviatio Wrong purity was use Action Taken and De	Protocol Devia n Investigation by SD/f ed for methoxycinnami termination of Impact	tion GLP Deviation PI/Test Facility Manageme ate.	∏t nt/Designee	9:
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SOP Deviation Summary of Deviation Wrong purity was use Action Taken and De None. After dilutions. Signature:	Protocol Devia n Investigation by SD/f ed for methoxycinnami termination of Impact	tion GLP Deviation PI/Test Facility Manageme ate. on Study Data and/or Fac gible. Date:	nt/Designed	ə: liance:
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Ueeiox 🛚	Deviation &	Investigation	Form #:	SOP-1003-F-
In vitre models to predict toxicity Study Number (if applica	ble): _9070-10	00107ARB		
Date of Reporting:	04-Jan-12	_ Reporting Associate: _		
Date of Occurrence: 21	-Sep-11	Associate Involved:		
Description of Deviation:				
Sponsor was not asked to	sign amendment	s according to the protoco	ol.	
Signature		Date:	04-Jc	10
	porting Associate)		04-50	11-12
/ (//6				
Type of Deviation (detern	nined by Study Dir	ector/Principal Investigato	r):	
SOP Deviation	Protocol Devia	tion GLP Deviation		lo Deviation
Summary of Deviation Inv	restigation by SD/F	l/Test Facility Managemer	nt/Designee	
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Sponsor was notified of postated in the protocol. Action Taken and Determ None. Sponsor signature study. Signature:	ending Amendme	on Study Data and/or Fac	sign the an	iance:



CERTIFICATE OF ANALYSIS Product 29116

Specifications

Appearance Infrared spectrometry Separat. techn. GC Axid value Specific abs. A. (15v1cm) Specific abs. A. (15v1cm) Specific arxity Befractive index Stabilizer

CLEAR COLDURLESS TO YELLOW LIQUID AUTHENTIC 977.5 % <1 mg KOH9g 9880 (al 307 to 308 mm in methanol) (2505 CC) 1 607 to 1.012 1.5441 to 1.3474 (2476, 589 mm) 0.05 to 0.1 % BHT

General Product Data

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



Lot Specific Data for Lot No.: A0293319

Appearance
nfrared spectrometry
Separat. techn. GC
Acid value
Specific abs. A (1%/1cm)
Specific gravity
Refractive index
Stabilizer

CLEAR COLOURLESS LIQUID CLEAR COLOURLESS LIQUID AUTHENT(99.8 % 0.1 mg KOH/g 865 (at 307 to 308 nm in methanol) (25/25*C) 1.0396 1.5453 (20*C, 589 nm) 0.09 % BHT



Quality Assurance Manager

Acros Organics

Geel/West Zone 2, Janssen Pharmacoutication 38, 8-2440 Geel, Belgium. Tel +32 14/57.52.11 - Fax +32 14/59.34.34 internet: http://www.acros.com I Reagent Lane, Fair Lanen, NJ 07410,USA. Fax 201-796-1329

MRI-N TP/Tesk 1492

A-1

Page 1 of 1

Certificate of Analysis

≥99.0 %

≤100 APHA

≤3.0 ppm

<1.0 ppm

<1.0 ppm

≤10.0 ppm

Product Name

Product Number Product Brand CAS Number Molecular Formula Molecular Weight 2-Ethylhexyl salisylate, 299% W614600 ALDRICH 118-60-5 (HO)C₈H₄CO₂CH₂CH(C₂H₆)(CH₂)₃CH₅ 250.33

2

TEST Appearance (Color) Appearance (Form) Retractive Intex at 20 ° C Infrared spectrum Purity (6 C) Color Test Arsenic (As) Cadmium (Co) Mercury (Hg) Lead (Pb) Specification Date: Date of QC Release: Print Date:

SPECIFICATION Colorless Liquid 1.600 - 1.504 Conformato Structure

Caloriess Liquid 1.602 Conforms 99.6 % 10 AFHA < 1.0 ppm < 1.0 ppm < 1.0 ppm < 1.0 ppm DEC 2006 DEC 2006 DEC 2008

LOT 44698PJ RESULTS



Quality Centrol Milwaukes, Wisconsin USA

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Certificate of Analysis

SPECIFICATION

Viscous Liquid

Conforms to Structure

Yellow

≥96.5 %

Product Name

Product Number Product Brand CAS Number Nolecular Formula Nolecular Weight 2-Ethy hexyl 2-cyano-3,3-diphenylaciylate, 97% 415929 ALDRICH 6197-30-4 $(C_6H_2)_2C=C(CN(CO_2CH_2CH(C_2H_3)(CH_2)_3CH_3)$ 361,48

TEST

Appearance (Color) Appearance (Form) Infrared spectrum Purity (GC) Specification Date: Date of QC Release:





Quality Control Milwaukee, Wisconsin USA

LOT 01697MJ RESULTS

Yellow Viscous Liquid Conforms 99.2 % OCT 2008 OCT 2008 OCT 22 2008

APPENDIX 5 Protocol and Protocol Amendments

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



FINAL PROTOCOL

Androgen Receptor Binding (Rat Prostate Cytosol)

Data Requirements: OPPTS 890.1150



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

Study #: 9070-100107ARB

CeeToxim PROTOCOL - IN VITRO ANDROGEN RECEPTOR BINDING (OPPTS 890.1150)

TEST PROTOCOL

Study Sponsor:	NIEHS/NTP	Chief Toxicology Branch)
Address:	P.O. Box 12233	
		Phone:
	Research Triangle Park, NC	
Study Monitor:		
		E-mail:
Sponsor Protocol/	Project No.:	

NIEHS/NTP Investigator

Telephone No.:	
Facsimile No.:	
E-mail:	
Contract Office	Technical Representative

(Contract No. HHSN273200900005C; NIEHS Control No. N01-ES-00005)

Study Monitor

	(ILS, Inc, Durham, NC)
Telephone No.:	
Facsimile No.:	
E-mail:	

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CeeTox PROTOCOL - IN VITRO ANDROGEN RECEPTOR BINDING (OPPTS 890.1150)

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Study #: 9070-100107ARB

	Ham PROTOCOL – IN VITRO ANDROGEN RECEPTOR BINDING (OPPTS	Study #: 9070-100107ARB
16.	Alterations of the Study Design	
17.	Data Retention and Archiving	

Roge 4 of 1.6

Coolorin PROTOCOL - IN VITRO ANDROGEN RECEPTOR BINDING (OPPTS 890.1150)

Study #: 9070-100107AR8

Signatures

	.1 1
	26/11
Study Sponsor	Date

Study Monitor

7/6/11 Date

Study Director

06 July 2011 Date

CeeTonia PROTOCOL - IN VITRO ANDROGEN RECEPTOR BINDING (OPPTS 890.1150)

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 [³H] 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.

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890.1	150)	
Т	est 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-Ethylhex	yl 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 Sali	cylate (Octylsalate)
	CAS No.	118-60-5
	Source:	Sigma-Aldrich
	Lot/Batch No.:	44698PJ
	ILS Repository No.:	11-30

CeeTox an PROTOCOL - IN VITRO ANDROGEN RECEPTOR BINDING (OPPTS 890.1150)

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Study #: 9070-100107ARB

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:	C24H27NO2
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.

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Continue PROTOCOL - IN VITRO ANDROGEN RECEPTOR BINDING (OPPTS 890.1150)

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 H₂O. 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

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CONTRACTOR PROTOCOL - IN VITRO ANDROGEN RECEPTOR BINDING (OPPTS 890.1150)

- 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 \sim 7.4 at approximately 4°C (the solution will be adjusted with HCl (\sim 1M) or NaOH (\sim 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 [3H]-R1881 Stock Solutions:

- For example the original stock of [³H]–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 [³H]–R1881 stock solution and dilutions at approximately -20°C.
- A 1 x 10⁸ M stock of [³H]–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 [³H]–R1881 will be maintained with the study records.

Preparation of 100X Radioinert R1881 Solutions:

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Ceeton PROTOCOL - IN VITRO ANDROGEN RECEPTOR BINDING (OPPTS 890.1150)

- 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 μ M radioinert R1881 stock will be prepared by pipetting the 1 μ M stock 1:10 in an amber vial. This will be the 0.1 μ M 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 μ M triamcinolone acetonide working solution will be prepared for the assay by making a 1:10 dilution of the 600 μ M 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.

Standard	Initial R1881 Concentration (M)	Final R1881 Concentration (M) in AR Assay Tube			
Negative Control	0	0			
0	O (Ethanol)	0			
NSB	1 × 10 ⁵	1 x 10°			
S1	3 x 10 ⁻⁶	1 x 10 ⁷			
\$2	3 × 10 ⁻⁷	1 × 10 ⁸			
\$3	3 x 10 ⁻⁸	1 x 10°			
S4	3 x 10°	1 x 10 ⁻¹⁰			
S5	3 x 10 ¹⁰	1 x 10 ¹¹			

Table 1. Standard Curve – Recommended Standard Curve Concentrations

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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. $30 \ \mu$ l of 0.01 μ M [³H]-R1881 (1 x 10⁸ M) and 50 μ l triamcinolone acetonide (60 μ M 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 μ l of 1 μ M) 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 μ l of test substance stocks or radioinert R1881 standards will be added. 300 μ l 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.

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)	ΗΑΡ (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

Table 2. Competitive Assay Tube Layout

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Study #: 9070-100107ARB

	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					

CeeToxes PROTOCOL – IN VITRO ANDROGEN RECEPTOR BINDING (OPPTS 890.1150)

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 prelabeled, pre-cooled 12x75 mm test tubes. One HAP tube will be prepared for each incubation tube. 100 μ l 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 $4^{\circ}C$ and $600 \times g$. 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.

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12. Competitive Binding Analyses

Estimating the IC50

Plot the data for the standard curve and each test substance as the percent [3 H]-R1881 bound versus the molar concentration. IC₅₀ 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:

 $Y = Bottom + \frac{(Top - Bottom)}{1 + 10^{(logiC50-X) Hill Slope + log ((top-bottom)/(50-bottom)-1)}}$

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_{so} for R1881 by the IC_{so} of the competitor and expressing as a percent.

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

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 [³H]-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.

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Ceetoxam PROTOCOL – IN VITRO ANDROGEN RECEPTOR BINDING (OPPTS 890.1150)

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

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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

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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.

<u>Change:</u> 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.

21 Sept 2011 Date

Study Director (Project Manager)

CeeTox Study # 9070-100107ARB

21-Sep-11

Protocol Amendment

Study Number: 9070-100107 ARB

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

Reason for Amendment to Protocol: Client requested amendment

<u>Change:</u> 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

615 Davis Drive, Suite 300 Durham, NC 27713

Signature

CeeTox, Inc.





12-6-11 Date

<u>06 Dec 11</u> Date

CeeTox Study # 9070-100107ARB

6-Dec-11