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

	Androgenic Transactivation Activity in MDA-kb2 Reporter Cells Final Report						
AUTHOR(S)	:						
STUDY CON	MPLETION DATE:	27Jan2012					
TEST FACI	LITY:	CeeTox, Inc. 4717 Campus Drive Kalamazoo, MI 49008 USA					
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SPONSOR(S	<sup>(</sup> ):	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: 9070-100107ARTA

Study Title: Androgenic Transactivation Activity in MDA-kb2 Reporter Cells

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.

Associate Study Director

27 Jan 2012 Date

## FLAGGING STATEMENT

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#### QUALITY ASSURANCE STATEMENT

Study Title: Androgenic Transactivation Activity in MDA-kb2 Reporter Cells

Study Number: 9070-100107ARTA

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
27Jun2011	Draft Protocol Audit	27Jun2011	27Jun2011
20Oct2011 and 21Oct2011	In-Process Audit	21Oct2011	21Oct2011
22Jan2012	Data Binder	22Jan2012	22Jan2012
23Jan2012	Draft Report	24Jan2012	24Jan2012

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			
	Associate Study Director			
	Director of Laboratory Operations			
	Senior Scientist/Endocrine Group Leader			
	Scientist			
	Scientist			
	Scientist			
	Director of Project Management			

#### **Study Dates**

Study initiation date: June 24, 2011 Experimental start date: October 13, 2011 Experimental termination date: November 4, 2011 Study termination date: January 27, 2012

#### **Deviations from the Protocol**

See Appendix 2. There were seven 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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#### **EXECUTIVE SUMMARY**

#### 1.1 Study Design

The objective of this study was to analyze the test substances for androgenic transactivation activity (agonism and antagonism) using the MDA-kb2 reporter cell line. The MDA-kb2 cell line was derived from a human breast cancer line transfected with an androgen receptor promoter linked to a luciferase gene. Consequently, the MDA-kb2 cell line can measure the ability of a test substance to induce (agonism) or antagonize Androgen Receptor (AR) mediated transactivation via luciferase gene expression. Cell viability was monitored by a two-read propidium iodide (PI) uptake assay.

Final concentration ranges of subsequent run(s) were adjusted based on assessments of precipitation observed in the first run. Solubility was visually observed for runs 1 and 2, and was read on the nepheloskan for run 3.

Two runs were conducted on octylmethoxycinnamate. The final concentrations were:  $10^{-6.5}$ ,  $10^{-6}$ ,  $10^{-5.5}$ ,  $10^{-5}$ ,  $10^{-4.5}$ ,  $10^{-4.5}$ ,  $10^{-3.5}$  and  $10^{-3}$  M for run 1 (13-October-2011) and  $10^{-7.5}$ ,  $10^{-7}$ ,  $10^{-6.5}$ ,  $10^{-6}$ ,  $10^{-5.5}$ ,  $10^{-5}$ ,  $10^{-5}$ ,  $10^{-4.5}$  and  $10^{-4}$  M for run 2 (20-October-2011). Three runs were conducted on oxybenzone, octylsalate and octocrylene. The final concentrations were:  $10^{-6.5}$ ,  $10^{-6}$ ,  $10^{-5.5}$ ,  $10^{-5}$ ,  $10^{-4.5}$ ,  $10^{-4.5}$  and  $10^{-3.5}$  and  $10^{-3}$  M for run 1 (13-October-2011) and  $10^{-7.5}$ ,  $10^{-7}$ ,  $10^{-6.5}$ ,  $10^{-5.5}$ ,  $10^{-5}$ ,  $10^{-4.5}$ ,  $10^{-4.5}$  and  $10^{-4}$  M for runs 2 and 3 (20-October-2011) and  $10^{-7.5}$ ,  $10^{-7}$ ,  $10^{-6.5}$ ,  $10^{-6.5}$ ,  $10^{-5.5}$ ,  $10^{-5}$ ,  $10^{-4.5}$  and  $10^{-4}$  M for runs 2 and 3 (20-October-2011) and 3-November-2011). The third run was used to clarify results of the first two runs for oxybenzone, octylsalate and octocrylene. Every run contained one agonism plate, one antagonism plate, and one cytotoxicity plate for each substance tested.

Solubility was recorded visually on runs 1 and 2 (13-October-2011 and 20-October-2011; (see deviation 2, Appendix 2). The Nepheloskan was used for run 3 (3-November-2011) at the concentrations of  $10^{-7.5}$ ,  $10^{-7}$ ,  $10^{-6.5}$ ,  $10^{-6}$ ,  $10^{-5.5}$ ,  $10^{-5}$ ,  $10^{-4.5}$ ,  $10^{-4}$ ,  $10^{-3.5}$  and  $10^{-3}$  for oxybenzone, octylsalate and octocrylene. The Nepheloskan was used (3-November-2011) at the concentrations of  $10^{-7.5}$ ,  $10^{-7}$ ,  $10^{-6.5}$ ,  $10^{-6}$ ,  $10^{-5.5}$ ,  $10^{-5}$ ,  $10^{-4.5}$ , and  $10^{-4}$  for octylmethoxycinnamate due to the limited availability of test substance.

For agonist plates, all concentrations were tested in replicates of 6/plate, with the addition of 2 replicates/plate that incorporated the antagonist nilutamide which is used as a CeeTox internal control. Replicates incorporating the nilutamide allow for the identification of non-specific (i.e., non-androgen receptor mediated) induction of the luciferase gene.

For antagonist plates, all test substance concentrations included four replicates with 1 nM DHT and four replicates with 1000 nM DHT. Replicates incorporating 1000 nM DHT allowed for the identification of assay interference.

For cytotoxicity plates, all concentrations were tested in replicates of 6/plate, with the addition of 2 replicates/plate that incorporated digitonin. Replicates incorporating digitonin allow for the identification of assay interference.

The duration of exposure was 24 hours. A complete concentration response curve for each of 3 reference compounds (dihydrotestosterone (DHT), nilutamide (NIL) and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (p,p'-DDE)) was run each time the transcriptional activation assay was performed.

#### 1.2 Results

Solubility was visually observed for run 1 and run 2. Solubility was run on the Nepheloskan for run 3. The top concentration for all test substances in run 1 (13-October-2011) was  $10^{-3}$ M. Precipitation was observed at  $10^{-4}$ ,  $10^{-3.5}$  and  $10^{-3}$  M in octylmethoxycinnamate. Precipitation was observed at  $10^{-3.5}$  and  $10^{-3}$  M in oxybenzone. Precipitation was observed at  $10^{-4}$ ,  $10^{-3.5}$  and  $10^{-3}$  M in octylsalate. Precipitation was observed at  $10^{-4}$ ,  $10^{-3.5}$  and  $10^{-3}$  M in octocrylene. The suitable top concentration of each test substance for use in later runs was 10<sup>-4</sup> M, based on these observations. In run two (20-October-2011), slight precipitation was observed at 10<sup>-4</sup> M in octylmethoxycinnamate and octylsalate. There was no evidence of precipitation in oxybenzone and octocrylene. In run 3, solubility was run on the Nepheloskan. With the criteria of  $\geq 3$  times the vehicle control octylsalate had a solubility limit of 10<sup>-4.5</sup> M, oxybenzone had no solubility limit (soluble at all concentrations tested), octylmethoxycinnamate had a solubility limit of  $10^{-4.5}$  M, and octocrylene had a solubility limit of  $10^{-5}$  M. Cytotoxicity ( $\geq 20\%$  reduction in cell viability) was observed in oxybenzone and octylsalate at  $10^{-3.5}$  and  $10^{-3}$  M in the first run (13-October-2011). Cytotoxicity was noted in oxybenzone at  $10^{-4}$  in the second run (20-October-2011). Cytotoxicity was observed in octocrylene at  $10^{-4.5}$ ,  $10^{-4}$ ,  $10^{-3.5}$  and  $10^{-3}$  M in the first run (13-October-2011) and at the top two doses,  $10^{-4.5}$  and  $10^{-4}$  M, in the second run (20-October-2011) and at  $10^{-4}$  M in the third run (3-November-2011).

In all independent runs of the agonist transcriptional activation assay, these test substances (octylmethoxycinnamate, oxybenzone, octylsalate and octocrylene) did not result in an increase in luciferase activity at any of the viable soluble concentrations tested (RPC<sub>max</sub><20%).

In two of two independent runs of the antagonist transcriptional activation assay, octylmethoxycinnamate did not result in a differential between the high antagonism and the low antagonism of greater than 50% at more than one viable soluble concentration.

In two of three independent runs of the antagonist transcriptional activation assay, oxybenzone did result in a differential between the high antagonism and the low antagonism of greater than 50% at more than one viable soluble concentration  $(10^{-4.5} \text{ and } 10^{-4})$ .

In three of three independent runs of the antagonist transcriptional activation assay, octylsalate did not result in a differential between the high antagonism and the low antagonism of greater than 50% at more than one viable soluble concentration.

In three of three independent runs of the antagonist transcriptional activation assay, octocrylene did not result in a differential between the high antagonism and the low antagonism of greater than 50% at more than one viable soluble concentration.

#### 1.3 Conclusion

Octylmethoxycinnamate, octylsalate and octocrylene do not demonstrate agonism or antagonism of AR mediated transactivation when tested in the MDA-kb2 cell model system. Oxybenzone does not demonstrate agonism, however there was a exposure dependent antagonism of AR-mediated transactivation when tested in the MDA-kb2 cell model system.

## 2.0 INTRODUCTION

#### 2.1 Purpose

The objective of this study was to analyze the test substances for androgenic transactivation activity using the MDA-kb2 reporter cell line. The MDA-kb2 cell line is derived from a human breast cancer line transfected with an androgen receptor promoter linked to a luciferase gene. Consequently, the MDA-kb2 cell line can measure the ability of a test substance to induce (agonism) or antagonize AR mediated transactivation via luciferase gene expression.

The MDA-kb2 cell line is derived from human breast cancer cells. These cells were transformed with an androgen responsive luciferase reporter plasmid driven by the mouse mammary tumor virus promoter (MMTV). The MMTV promoter was chosen for transformation because it is a robust viral promoter and is well characterized as being androgen responsive. Consequently, the MDA-kb2 cell line can measure the ability of a test substance to induce AR-mediated transactivation of luciferase gene expression, i.e., the cell line can be used to assess the ability of a test substance to act as an agonist of AR. Antagonism can be distinguished by the differential ellicited from the co-administration of the test article and AR agonist DHT at a high concentration (1000 nM) versus the co-administration of the test article and the AR agonist DHT at a low concentration (1 nM).

#### 2.2 Regulatory Citations

Currently this assay has not been validated as part of the EDSP Tier 1 testing program and is not mandated.

#### 3.0 MATERIALS AND METHODS

#### 3.1 Test Substance

#### 3.1.1 Test substance details

Test substance name:2-Hydroxy-4-methoxybenzophenone

	(Oxybenzone)
Test substance manufacturer:	Ivy Fine Chemicals Corporation
CAS number:	131-57-7
Description:	Light yellow powder
Solvent used:	DMSO
Batch identification:	20100801
Expiry date:	August 1, 2012
Purity:	99.92%
Molecular formula:	$C_{14}H_{12}O_3$
Molecular weight:	228.25
Storage conditions:	Room Temperature
Test substance name:	2-Ethylhexyl p-methoxycinnamate
	(Octylmethoxycinnamate); Octyl 4-
	methoxycinnamate
Test substance manufacturer:	Acros Organics
CAS number:	5466-77-3
Description:	Clear colorless liquid
Solvent used:	DMSO
Batch identification:	A0293319
Recertification date:	Not Provided
Purity:	99.8%
Molecular formula:	$C_{18}H_{26}O_3$
Molecular weight:	290.39
Storage conditions:	Room Temperature
Test substance name:	Octyl Salicylate (Octylsalate); 2-
	Ethylhexyl salicylate
Test substance manufacturer:	Sigma Aldrich
CAS number:	118-60-5
Description:	Colorless liquid
Solvent used:	DMSO
Batch identification:	44698PJ
Recertification date:	Not Provided
Purity:	99.6%
Molecular formula:	$C_{15}H_{22}O_3$
Molecular weight:	250.33
Storage conditions:	Room Temperature
Test substance name:	2-Ethylhexyl 2-cyano-3,3-

diphenylacrylate (Octocrylene)Test substance manufacturer:Sigma AldrichCAS number:6197-30-4Description:Yellow viscous liquid	Test substance name:	2-Ethylhexyl 2-cyano-3,3-				
Test substance manufacturer:Sigma AldrichCAS number:6197-30-4Description:Yellow viscous liquid		diphenylacrylate (Octocrylene)				
CAS number:6197-30-4Description:Yellow viscous liquid	Test substance manufacturer:	Sigma Aldrich				
Description: Yellow viscous liquid	CAS number:	6197-30-4				
	Description:	Yellow viscous liquid				
Solvent used: DMSO	Solvent used:	DMSO				

Batch identification:	01697MJ
Recertification date:	Not Provided
Purity:	99.2%
Molecular formula:	$C_{24}H_{27}NO_2$
Molecular weight:	361.48
Storage conditions:	Room Temperature

Certificates of analysis for the test substances are presented in Appendix 3.

#### 3.1.2 Vehicle selection

Dimethyl sulfoxide (DMSO) was selected as a suitable vehicle for test substances. Therefore, solutions with a test substance concentration of up to  $10^{-3}$  M (the highest concentration tested) can be prepared while limiting the final concentration of DMSO in the assay medium to 0.5% (v/v). Dihydrotestosterone, nilutamide, p,p'-DDE and test substances were all prepared on the day of dosing (13-October-2011 for run 1, 20-October-2011 for run 2, and 3-November-2011 for run 3).

#### 3.2 Cell Line

#### 3.2.1 Source

The stably transfected MDA-kb2 cell line was used in this study. The cell line was obtained from ATCC (Appendix 3). The cells were certified as Mycoplasma Free (Appendix 4).

#### 3.2.2 Stability of the cell line

The stability of the cell line was monitored by the use of the following reference chemicals: dihydrotestosterone (DHT), nilutamide (Nil) and p,p'-DDE. A complete concentration response curve for each reference compound was run each time the transcriptional activation assay was performed.

#### 3.2.3 Cell culture and plating conditions

Cells were maintained in Leibovitz's L-15 culture medium containing 10% fetal bovine serum, in an incubator at ~37°C without CO<sub>2</sub>. The MDA-kb2 cell line is not contact inhibited and can be grown to confluence. Cells were subcultivated at a 1:2 to 1:8 subcultivation ratio. The cells were suspended with complete medium and plated into wells of a 96-well cell culture plate at a density of ~1 X 10<sup>4</sup> cells/100  $\mu$ L/well. The cells were then placed into an incubator without CO<sub>2</sub> at ~37°C overnight prior to chemical exposure.

#### 3.3 Chemical Exposure and Assay Plate Organization

Each test substance was prepared for addition to the cell system by making a 400 mM stock. Dilutions were prepared in DMSO to 400x final target concentration. Ten microliter aliquots of the substance dilutions were added to 2 mL media in deep well plates and mixed to yield concentrations of test material 2-fold greater than the desired final concentration.

After the overnight post-seeding incubation, the plates were removed from the incubator and the media was aspirated. Fifty microliters of media and appropriate controls were added to the seeded plates. To achieve the final exposure concentrations each 2X solution was diluted 2-fold in the 96-well plate containing the cells and media and controls.

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	1	2	3	4	5	6	7	8	9	10	11	12
А	Blank*	DHT (10 nM)	VC**	VC	Conc. 1	Conc. 2	Conc. 3	Conc. 4	Conc. 5	Conc. 6	Conc. 7	Conc. 8
В	↓***	$\rightarrow$	$\downarrow$	$\downarrow$	$\downarrow$	$\rightarrow$	$\downarrow$	$\downarrow$	$\downarrow$	$\downarrow$	$\downarrow$	$\downarrow$
С	$\rightarrow$	$\rightarrow$	$\downarrow$	$\downarrow$	$\downarrow$	$\downarrow$	$\downarrow$	$\downarrow$	↓	$\downarrow$	$\downarrow$	$\downarrow$
D	$\downarrow$	$\downarrow$	$\downarrow$	↓	$\downarrow$	$\downarrow$	$\downarrow$	↓	↓	↓	$\downarrow$	↓
Е	$\downarrow$	$\downarrow$	↓	$\downarrow$	$\downarrow$	$\downarrow$	$\downarrow$	$\downarrow$	↓	$\downarrow$	$\downarrow$	↓
F	$\downarrow$	$\downarrow$	$\downarrow$	↓	$\downarrow$	$\downarrow$	$\downarrow$	↓	↓	↓	$\downarrow$	↓
G	As above + antagonist (10 µM nilutamide)											
Н	$\downarrow$	$\downarrow$	$\downarrow$	$\downarrow$	$\downarrow$	$\downarrow$	$\downarrow$	$\downarrow$	↓	$\downarrow$	$\downarrow$	↓
DIT	1.1 1											

DHT = dihydrotestosterone

\*Blank wells contain media only (no cells)

\*\*Vehicle control (VC) wells contain cells and media + 0.5% (v/v) DMSO

\*\*\*↓ Indicates the composition of the well is identical to the well directly above it

A .	•
Antao	onism
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	1	2	3	4	5	6	7	8	9	10	11	12
А	Blank*	*** (nM)	VC**	VC	Conc. 1	Conc. 2	Conc. 3	Conc. 4	Conc. 5	Conc. 6	Conc. 7	Conc. 8
В	↓***	↓	$\downarrow$	↓	$\downarrow$	↓	↓	$\downarrow$	$\downarrow$	$\downarrow$	$\downarrow$	$\downarrow$
С	$\downarrow$	$\rightarrow$	$\downarrow$	$\rightarrow$	$\downarrow$	$\rightarrow$	$\rightarrow$	$\downarrow$	$\downarrow$	$\rightarrow$	$\downarrow$	$\downarrow$
D	$\downarrow$	$\rightarrow$	$\downarrow$	$\rightarrow$	$\downarrow$	$\rightarrow$	$\rightarrow$	$\downarrow$	$\downarrow$	$\rightarrow$	$\downarrow$	$\downarrow$
Е				As abov	e + (1000)	) nM DH	T instead	l of 1 nM	DHT)			-
F	$\downarrow$	$\rightarrow$	$\downarrow$	↓	$\downarrow$	↓	↓	$\downarrow$	↓	$\rightarrow$	↓	$\downarrow$
G	$\downarrow$	$\rightarrow$	$\downarrow$	→	↓	→	→	$\downarrow$	↓	$\downarrow$	↓	$\downarrow$
Н	$\downarrow$	$\rightarrow$	$\downarrow$	$\rightarrow$	$\downarrow$	$\rightarrow$	$\rightarrow$	$\downarrow$	$\downarrow$	$\downarrow$	$\downarrow$	$\downarrow$

\*\*\*10 nM dihydrotestosterone (DHT) Maximal induction control wells

\*Blank wells contain media only (no cells)

\*\*Vehicle control (VC) wells contain cells and media + 0.5% (v/v) DMSO

\*\*\*\* $\downarrow$  Indicates the composition of the well is identical to the well directly above it

Rows A-D are low agonist (1 nM DHT)

Rows E-H are high agonist (1000 nM DHT)

After adding the reference chemicals/test substances, the plates were incubated in an incubator at  $\sim$ 37°C without CO<sub>2</sub> for  $\sim$ 24 hours.

For the agonism plates, all concentrations were tested in replicates of 6/plate. In addition, for each concentration, 2 replicates/plate were prepared that incorporated the AR antagonist nilutamide. Replicates incorporating an AR antagonist allow for the identification of non-specific (i.e., non-AR-mediated) induction of the luciferase gene as true AR-mediated induction is inhibited by addition of an antagonist whereas non-specific induction is not.

For the antagonism plates, all concentrations were tested in replicates of 4/plate. Four replicates were co-administered 1 nM DHT and test article at each concentration. Four replicates were co-administered 1000 nM DHT and test article at each concentration. Replicates incorporating 1000 nM DHT allowed for the identification of assay interference.

In view of the short-term nature of studies of this type, no analyses of stability, homogeneity or achieved concentration(s) were carried out on preparations of the test substance or positive control chemicals, either before or after the treatment phase. This was not considered to have affected the integrity of the study. For the reference control compounds, stability was demonstrated by an appropriate response in the assay system.

#### 3.4 Assays

#### **3.4.1** Cytotoxicity assay

Cell viability was monitored by a two-read propidium iodide (PI) uptake assay. PI was a light sensitive dye and all procedures were conducted under low light conditions. PI could not cross the plasma membrane of intact and viable cells. Cells that were dead or dying had weakened plasma membranes which allowed PI to enter the cytosol of the damaged cells. Once inside the cell, PI intercalated into DNA/RNA and yielded a fluorescent signal. In the two-read procedure, the first read was taken immediately after full exposure to controls and test articles. This measured "background" fluorescence. The cells were then lysed and a second read was taken. This read indicated cell death. The first read was then subtracted from the second read. The results of the subtracted reads were directly proportional to the viability of the cells. The control and test substance data were normalized to vehicle control to generate percent cell viability.

Cells were seeded as described in Section 3.2.3, with the exception that a black-walled 96well cell culture plate was used. The cells were exposed to the test chemicals in replicates of 6 (rows A-F) while the last 2 rows (G and H) received 125  $\mu$ M digitonin as a positive control for cell death. Following chemical exposure, the growth medium was removed and 50  $\mu$ L of a PI working solution (44  $\mu$ M in phosphate buffered saline) was added to each well. Background fluorescence was evaluated by measuring fluorescence immediately on a Packard Fusion fluorescence plate reader at an excitation wavelength of 544 nm and an emission wavelength of 612 nm. Following this determination, 50  $\mu$ L of a 2% (v/v) Triton X-100 solution was added to each well and the plate was incubated at room temperature for  $\sim$ 15 minutes to fully lyse all cells in the wells before measuring fluorescence at the same wavelengths.

The background-corrected fluorescence was calculated for each well by subtracting the results of the first read from the results of the second read. The change in cell viability was determined by comparing treated wells to the vehicle control wells. A  $\geq$ 20% reduction in cell viability was considered evidence of cytotoxicity.

#### 3.4.2 Precipitation assessment

Solubility limits for runs 1 and 2 (13-October-2011 and 20-October-2011) were determined by visual observation. For run 3, the limit of solubility was determined by Nephelometry. A 96-well clear bottom plate containing 200  $\mu$ L of every test concentration in cell culture media was evaluated using the Nepheloskan. Nephelometry measured the particulate light scattering.

#### 3.4.3 Transcriptional activation assay

A luciferase assay was performed as described in CeeTox Standard Operating Protocol (SOP) 2041 using the reagents listed below. Luciferase assay reagent was prepared as described in CeeTox SOP 2041 (proprietary information).

Reagent	Supplier	Catalog #
Trisma Base	Sigma	T6066
Magnesium Chloride	Sigma	M2393
EDTA	Sigma	E5134
Dithiothreitol	Sigma	D9779
ATP	Sigma	A2383
Coenzyme A	Sigma	C3019
AMP	Sigma	A1752
Luciferin	Promega	E160E
Glycerol	Sigma	G5516
Triton-X100	Sigma	T8787
Bovine Serum Albumin	Sigma	A9418
CDTA	Sigma	D0922

## **3.5** Agonist Transcriptional Activation Assay Data Analysis and Interpretation

In order to determine the relative transcriptional activity as compared to the positive control (PC), 10 nM DHT, the luminescence data from each plate were analyzed according to the steps outlined below. Wells incorporating nilutamide were analyzed in an identical fashion to wells not incorporating nilutamide, except that the data were normalized by subtracting the mean value for the nilutamide-containing vehicle control (VC) wells.

- 1. Any cytotoxic concentrations (as defined in Section 3.4.1) were excluded from data analysis.
- 2. The mean value for the VC wells was calculated.
- 3. The mean value for the VC wells was subtracted from each well to normalize the data.
- 4. The mean value for the normalized PC wells was calculated.
- 5. The normalized value for each well was divided by the mean value of the normalized PC wells (with the normalized mean of the PC wells being defined as 100% relative transcriptional activity). The final value for each well is the relative transcriptional activity for that well compared to the mean normalized PC response.

The data were then interpreted according to the following steps:

- 1. Where appropriate, LogPC<sub>50</sub>, LogPC<sub>10</sub>, LogEC<sub>50</sub> and Hill slope values were calculated.
- 2. For the test substance, the maximum response relative to the positive control  $(RPC_{Max})$  was determined. In each individual run of the transcriptional activation assay, if  $RPC_{max}$  was less than 20%, the test substance was considered to have given a negative response for AR agonism.
- 3. For each individual run of the transcriptional activation assay, the acceptability of the data was evaluated using the following criteria:
  - The mean normalized luciferase signal of the PC (10 nM DHT) should be at least 4-fold that of the mean VC on each plate.
  - The results of the reference compounds, nilutamide and DHT, should be within the acceptable ranges.
- 4. If the acceptability criteria outlined above were met, that run of the transcriptional activation assay was considered to be definitive
- 5. The test substance was considered negative if  $\text{RPC}_{\text{Max}}$  was <20% in at least 2 definitive runs of the transcriptional activation assay. The test substance was considered positive if  $\text{RPC}_{\text{Max}}$  was  $\geq$ 20% in at least 2 definitive runs of the transcriptional activation assay.

## **3.6** Antagonist Transcriptional Activation Assay Data Analysis and Interpretation

In order to determine the relative transcriptional activity as compared to the positive control (PC), 10 nM DHT, the luminescence data from each plate were analyzed according to the steps outlined below. Wells incorporating 1 nM DHT were analyzed in an identical fashion to wells incorporating 1000 nM DHT, except that the data was normalized to the induced control with 1 nM DHT or 1000 nM DHT, respectively.

- 1. Any cytotoxic concentrations (as defined in Section 3.4.1) were excluded from data analysis.
- 2. The mean value for the VC wells was calculated.

- 3. The mean value for the VC wells was subtracted from each well to normalize the data.
- 4. The mean value for the induced control with 1 nM DHT was calculated.
- 5. The mean value for the induced control with 1000 nM DHT was calculated.
- 6. The wells dosed with test or control substance and 1 nM DHT were normalized to the mean value for the induced control with 1 nM DHT.
- 7. The wells dosed with test or control substance and 1000 nM DHT were normalized to the mean value for the induced control with 1000 nM DHT.
- 8. Averages of antagonist % maximal induction control were calculated (test or control substance a with 1 nM DHT).
- 9. Averages of high agonist control % maximal induction control were calculated (test or control substance a with 1000 nM DHT).
- 10. Differentials were calculated (averages of high agonist control % maximal induction control minus averages of antagonist % maximal induction control).

The data were then interpreted according to the following steps:

- 1. Where appropriate, RICMax, Differential  $IC_{50}$ , Differential  $IC_{30}$ , LogEC<sub>50</sub> and Hill slope values were calculated.
- 2. If the differential between the high antagonism and the low antagonism was greater then 50% and had a dose response (more than one data point) in two of two runs, than the test substance was considered positive.
- 3. If the differential between the high antagonism and the low antagonism was less then 50% and did not have a dose response (more than one data point) in two of two runs, than the test substance was considered negative.
- 4. For each individual run of the transcriptional activation assay, the acceptability of the data was evaluated using the following criteria:
  - The mean normalized luciferase signal of the PC (10 nM DHT) should have been at least 4-fold that of the negative control on each plate.
- 5. If the acceptability criteria outlined above were met, that run of the transcriptional activation assay was considered to be definitive.

## 4.0 **RESULTS AND DISCUSSION**

#### 4.1 Concentration Range for the Test Substance

The final concentrations were:  $10^{-6.5}$ ,  $10^{-6}$ ,  $10^{-5.5}$ ,  $10^{-5}$ ,  $10^{-4.5}$ ,  $10^{-4}$ ,  $10^{-3.5}$  and  $10^{-3}$  M for run 1 (13-October-2011) and  $10^{-7.5}$ ,  $10^{-7}$ ,  $10^{-6.5}$ ,  $10^{-6}$ ,  $10^{-5.5}$ ,  $10^{-5}$ ,  $10^{-4.5}$  and  $10^{-4}$  M for runs 2 and 3 (20-October-2011) and 3-November-2011). Test concentrations were reduced after the first run due to observed precipitation.

## 4.2 Transcriptional Activation Assay Acceptance Criteria

In all valid independent runs of the assay, the mean luciferase activity of the PC (10 nM DHT) was greater than 4-fold that of the mean luciferase activity of the VC on each plate.

Test article data and data from the 3 reference compounds were excluded from evaluation and interpretation in instances of excessive cytotoxicity or precipitation observed in the valid independent runs.

## 4.3 Transcriptional Activation Assay Results

Two runs were conducted on octylmethoxycinnamate and three runs were conducted on octylsalate, oxybenzone and octocrylene. The third run was to clarify antagonism classification on borderline substances (substances whose results suggested antagonism just before cytotoxicity).

In all independent runs of the agonist transcriptional activation assay, test substances (octylmethoxycinnamate, oxybenzone, octylsalate and octocrylene) did not result in an increase in luciferase activity at any of the viable soluble concentrations tested ( $RPC_{max} < 20\%$ ).

In two of two independent runs of the antagonist transcriptional activation assay, octylmethoxycinnamate did not result in a differential between the high antagonism and the low antagonism of greater than 50% at more than one viable soluble concentration.

In two of three independent runs of the antagonist transcriptional activation assay, oxybenzone did result in a differential between the high antagonism and the low antagonism of greater than 50% at more than one viable soluble concentration  $(10^{-4.5} \text{ and } 10^{-4})$ .

In three of three independent runs of the antagonist transcriptional activation assay, octylsalate did not result in a differential between the high antagonism and the low antagonism of greater than 50% at more than one viable soluble concentration.

In three of three independent runs of the antagonist transcriptional activation assay, octocrylene did not result in a differential between the high antagonism and the low antagonism of greater than 50% at more than one viable soluble concentration.

#### 4.4 Discussion

The suitable top concentration of test substances for use in the transcriptional activation assays was  $10^{-4}$  M, based on precipitation observed at concentrations  $\ge 10^{-4}$  M. Cytotoxicity ( $\ge 20\%$  reduction in cell viability) was observed in oxybenzone and octylsalate at  $10^{-3.5}$  and  $10^{-3}$  M in the first run (13-October-2011). Cytotoxicity was noted in oxybenzone at  $10^{-4.5}$ ,  $10^{-4}$ , the second run (20-October-2011). Cytotoxicity was observed in octocrylene at  $10^{-4.5}$ ,  $10^{-4}$ ,

 $10^{-3.5}$  and  $10^{-3}$  M in the first run (13-October-2011) and at the top two doses,  $10^{-4.5}$  and  $10^{-4}$  M, in the second run (20-October-2011) and  $10^{-4}$  M in the third run (3-November-2011).

In all independent runs of the transcriptional activation assay, test substances did not result in an increase in luciferase activity in agonism plates at any of the viable soluble concentrations tested ( $RPC_{max} < 20\%$ ).

In all independent runs of the transcriptional activation assay, octylmethoxycinnamate, octylsalate and octocrylene did not result in a differential on the antagonism plates greater than 50% for two or more viable soluble doses.

In two of three independent runs of the transcriptional activation assay, oxybenzone did result in a differential on the antagonism plates greater than 50% for two viable soluble doses.

## 5.0 CONCLUSIONS

Octylmethoxycinnamate, octylsalate and octocrylene did not demonstrate agonism or antagonism of AR-mediated transactivation when tested in the MDA-kb2 cell model system. Oxybenzone did not demonstrate agonism, however there was an exposure dependent antagonism of AR-mediated transactivation when tested in the MDA-kb2 cell model system.

## 6.0 **REFERENCES**

Wilson, VS., Bobseine, K., Lambright, CR., and Gray, LE., Jr. (2002). A novel cell line, MDA-kb2, which stably expresses an androgen and glucocorticoid-responsive reporter for detection of hormone receptor agonists and antagonists. *Toxicol. Sci.* **66**, 69-81.

## **TABLES SECTION**

Chemical	Concentration	RT	A PC)	RTA v	with Nil of PC)	Cell Vi	iability f VC)	Precipitation
Chemical	( <b>M</b> )	Mean	SD	Value 1	Value 2	Mean	SD	Value
	-6.5	0.4	0.2	0.7	0.3	105	6	-
	-6	1.6	0.5	-0.1	0.3	110	9	_
	-5.5	0.9	0.3	0.9	-0.5	107	6	-
	-5	1.7	0.3	-0.3	-0.5	102	9	-
Octylmethoxycinnamate	-4.5	0.9	0.3	-0.8	-1.0	100	2	-
	-4	-0.2	0.2	-1.1	-1.6	89	6	+
	-3.5	0.0	0.4	-1.1	-1.8	90	5	+
	-3	-0.1	0.3	-1.4	-1.7	91	4	+
	-6.5	0.5	0.6	-0.6	-1.5	96	5	-
Octylsalate	-6	1.0	0.5	3.8	-0.5	104	9	-
	-5.5	0.5	0.4	3.3	-0.7	95	2	-
	-5	-0.2	0.1	-0.6	-1.2	97	5	-
	-4.5	-0.4	0.1	0.0	-0.6	97	5	-
	-4	-0.6	0.1	-0.8	-1.2	88	7	+
	-3.5	*	*	*	*	**70	**5	+
	-3	*	*	*	*	**66	**5	+
	-6.5	0.5	0.2	7.0	0.1	105	5	-
	-6	1.0	0.6	4.3	0.1	106	4	-
	-5.5	-0.5	0.3	0.2	-0.3	102	6	-
Ostosmulana	-5	-1.2	0.2	1.0	-0.8	95	5	-
Octocrylene	-4.5	*	*	*	*	**74	**2	-
	-4	*	*	*	*	**59	**4	+
	-3.5	*	*	*	*	**49	**6	+
	-3	*	*	*	*	**60	**6	+
	-6.5	0.4	0.2	1.3	-2.9	104	6	-
	-6	0.8	0.4	0.9	-2.9	106	5	-
	-5.5	0.0	0.2	4.4	-3.3	102	4	-
Owybonzono	-5	-0.2	0.3	-1.5	-2.9	101	4	-
Oxybelizolie	-4.5	0.0	0.2	23.8	-1.8	99	6	-
	-4	-0.2	0.2	12.0	-2.8	94	4	-
	-3.5	*	*	*	*	**66	**5	+
	-3	*	*	*	*	**63	**6	+

## TABLE 1Results of 1st Valid Transcriptional Activation Assay Agonist

RTA = Relative Transcriptional Activation

PC = Positive Control (10 nM DHT)

VC = Vehicle Control

SD = Standard Deviation

+ = Precipitation observed

- = No precipitation observed

\* = data not evaluated due to observed Cytotoxicity

\*\* = Cytotoxicity observed

Chemical	Concentration	RT (% of	A PC)	RTA v (% c	vith Nil of PC)	Cell Vi	iability f VC)	Precipitation
	(M)	Mean	SD	Value 1	Value 2	Mean	SD	Value
	-11.5	1.9	0.3	-0.2	-1.1	100	3	
-	-11	2.7	0.5	1.5	-0.7	101	3	
	-10.5	9.6	0.8	5.8	-0.5	102	5	
ршт	-10	26.9	2.6	5.6	-0.3	104	6	
DHI	-9.5	79.4	8.6	7.3	1.2	101	5	
	-9	95.6	5.2	13.5	3.6	101	3	
	-8.5	111.7	9.4	17.3	7.1	101	5	
	-8	87.1	7.0	41.1	26.2	96	5	
	-7.5	0.9	0.1	2.1	-0.9	103	7	
	-7.0	-0.1	0.4	1.8	-0.9	104	10	
	-6.5	-0.5	0.2	0.8	-1.0	102	5	
NU	-6.0	-0.8	0.1	-0.6	-0.9	102	7	
1111	-5.5	-0.2	0.1	1.6	0.1	98	3	
	-5.0	0.5	0.2	0.8	0.6	97	6	
	-4.5	2.1	0.3	-0.6	0.1	84	5	
	-4.0	*	*	*	*	**61	**1	
	-7.5	3.8	0.8	4.2	-0.5	106	5	-
	-7.0	3.2	0.4	2.5	-0.7	107	6	-
	-6.5	3.5	0.5	1.9	-0.6	105	5	-
mDDE	-6.0	1.7	0.5	5.6	-0.7	101	7	-
ppDE	-5.5	1.2	0.3	4.0	0.3	98	3	-
	-5.0	0.8	0.3	1.9	0.4	97	4	-
	-4.5	1.9	0.5	2.9	1.3	95	2	-
	-4.0	*	*	*	*	**68	**4	-

 TABLE 1
 Results of 1<sup>st</sup> Valid Transcriptional Activation Assay Agonist (Continued)

RTA = Relative Transcriptional Activation

PC = Positive Control (10 nM DHT)

VC = Vehicle Control

SD = Standard Deviation

+ = Precipitation observed

- = No precipitation observed

\* = data not evaluated due to observed Cytotoxicity

\*\* = Cytotoxicity observed

shaded areas not evaluated

Chemical	Concentration (LogM)	Low Agonis Induc Antagoni	t Maximal ction (%)	High Agoni Induction (100	st Maximum 0 nM DHT) (%)	Differential
		Mean	SD	Mean	SD	
10 nM DHT		110.0	8.3	104.9	7.5	
VC		0.0	0.5	100.5	11.9	
Induced Controls (1nM DHT)		100.0	9.0	99.5	11.4	
	-6.5	113.6	11.8	108.9	5.6	-4.7
	-6.0	104.7	9.0	106.6	14.3	1.9
	-5.5	128.8	5.2	115.8	14.3	-13.0
Octvlmethoxycinnamate	-5.0	102.8	13.8	99.2	11.9	-3.7
	-4.5	98.5	15.9	94.5	9.7	-4.0
	-4.0	64.4	13.0	67.9	6.4	3.4
	-3.5	69.2	10.9	66.4	5.0	-2.8
	-3.0	//./	2.5	/8.9	6.6	1.2
10 nM DHT		109.9	10.2	94.9	15.8	
VC VC		0.0	0.3	92.3	6.5	
Induced Controls (1nM DHT)		100.0	7.7	107.7	9.2	
	-6.5	119.9	12.2	116.0	8.3	-3.9
	-6.0	114.0	3.9	115.2	6.2	1.2
Octylsalate	-5.5	125.1	5.5	127.9	5.7	2.7
	-5.0	101.6	3.2	135.1	5.4	33.5
	-4.5	104.9	6.4	139.2	15.9	34.3
	-4.0	66.9	6.7	160.5	6.7	#93.7
	-3.5	*	*	*	*	*
	-3.0	*	*	*	*	*
10 nM DHT		126.3	7.2	100.9	12.5	
VC		0.0	0.3	92.8	7.1	
Induced Controls (1nM DHT)		100.0	3.9	107.2	10.9	
	-6.5	130.4	5.6	111.2	15.1	-19.1
	-6.0	111.6	9.9	120.8	9.8	9.2
	-5.5	109.2	14.9	132.1	12.1	22.9
Octocrylene	-5.0	61.3	7.3	118.9	7.7	#57.6
o cover yrene	-4.5	*	*	*	*	*
	-4.0	*	*	*	*	*
	-3.5	*	*	*	*	*
	-3.0	*	*	*	*	*
10 nM DHT		98.9	6.1	98.1	12.1	
	_	0.0	0.3	95.8	9.2	-
induced Controls (InM DHT)	65	100.0	0./	104.2	9.1	12.2
	-0.3	105.0	10.0	11/.8	4.5	12.2
	-0.0	121.4	4.5	137.4	64	16.0
	-5.0	102.4	4.0	142.6	3.7	40.2
Oxybenzone	-4.5	72.1	9.1	181.7	1.2	#109.5
	-4.0	21.1	2.2	174.8	11.8	#153.8
	-3.5	*	*	*	*	*
	-3.0	*	*	*	*	*

## TABLE 2Results of 1st Valid Transcriptional Activation Assay Antagonist

VC = Vehicle Control

SD = Standard Deviation

\* = data not evaluated due to observed Cytotoxicity, Cytotoxicity values shown on corresponding agonist table shaded areas = does not apply

# = differential > 50

Chemical	Concentration (LogM)	Low A Maximal I Antagoni	gonist Induction Ism (%)	High Agon Induction (100	ist Maximum 00 nM DHT) (%)	Differential
		Mean	SD	Mean	SD	
10 nM DHT		117.1	9.0	104.5	9.2	
VC		0.0	0.3	98.9	3.0	
Induced Controls (1nM DHT)		100.0	12.4	101.1	2.8	
	-11.5	142.5	15.4	124.4	9.5	-18.1
	-11	108.1	8.5	114.5	5.2	6.3
	-10.5	140.3	13.9	124.2	13.8	-16.1
рнт	-10	115.4	13.3	112.4	3.3	-3.0
DIII	-9.5	149.0	20.1	134.2	7.8	-14.8
	-9	163.3	27.2	132.0	4.0	-31.2
	-8.5	159.7	12.5	134.1	8.7	-25.6
	-8	152.4	7.1	121.6	13.4	-30.8
10 nM DHT		112.6	10.4	103.5	5.8	
VC		0.0	0.3	99.5	5.8	
Induced Controls (1nM DHT)		100.0	10.1	100.5	6.5	
	-7.5	96.8	6.4	100.6	9.6	3.8
	-7.0	89.0	9.3	109.5	11.7	20.5
	-6.5	62.1	4.1	124.1	11.6	#62.0
Nil	-6.0	20.3	2.4	106.4	6.0	#86.1
1411	-5.5	6.7	0.9	124.2	8.5	#117.5
	-5.0	3.5	0.7	121.7	18.2	#118.2
	-4.5	4.4	0.6	84.9	5.4	#80.6
	-4.0	*	*	*	*	*
10 nM DHT		118.8	15.9	102.6	9.0	
VC		0.0	0.2	101.0	4.3	
Induced Controls (1nM DHT)		100.0	2.6	99.0	13.4	
	-7.5	112.3	9.4	111.6	7.2	-0.7
	-7.0	111.2	2.3	103.3	5.2	-7.9
	-6.5	133.5	10.9	118.0	10.3	-15.5
nnDDF	-6.0	108.6	9.3	100.8	6.3	-7.9
рров	-5.5	101.7	3.4	117.0	6.9	15.3
	-5.0	62.9	7.4	109.2	12.9	46.3
	-4.5	25.3	2.9	111.4	11.5	#86.2
	-4.0	*	*	*	*	*

# TABLE 2Results of 1st Valid Transcriptional Activation Assay Antagonist<br/>(Continued)

VC = Vehicle Control

SD = Standard Deviation

\* = data not evaluated due to observed Cytotoxicity, Cytotoxicity values shown on corresponding agonist table shaded areas = does not apply

# = differential > 50

	Concentration	RT	A	RTA v	with Nil	Cell Vi	iability	Precipitation
Chemical	(M)	(% of	PC)	(% 0	of PC)	(% 0	f VC)	Treeprention
	(112)	Mean	SD	Value 1	Value 2	Mean	SD	Value
	-7.5	0.2	0.2	0.0	0.5	104	6	-
Octylmethoxycinnamate	-7.0	0.1	0.2	-0.3	0.3	107	6	-
	-6.5	0.2	0.3	-1.4	0.8	99	6	-
Octulmethoxycinnemate	-6.0	0.2	0.3	-2.1	0.2	102	12	-
Octymieutoxyciinianiate	-5.5	1.0	0.1	1.6	1.2	99	5	-
	-5.0	0.5	0.2	-0.8	0.5	103	9	-
	-4.5	-0.2	0.2	-0.5	-1.0	100	11	-
	-4.0	-0.7	0.2	-2.7	-2.4	'80	3	+
	-7.5	0.3	0.3	10.6	0.4	99	7	-
	-7.0	0.0	0.3	6.1	0.2	97	9	-
	-6.5	0.2	0.2	0.9	0.3	98	8	-
	-6.0	0.1	0.4	4.2	-0.3	97	5	-
Octylsalate	-5.5	0.4	0.2	2.1	0.8	92	6	-
	-5.0	-0.2	0.2	4.8	1.4	88	6	-
	-4.5	-0.5	0.2	0.4	-0.5	87	9	-
	-4.0	-0.7	0.1	-0.7	-1.2	84	4	+
	-7.5	0.0	0.2	7.0	-5.6	104	3	-
	-7.0	0.0	0.3	2.7	-5.3	104	5	-
	-6.5	0.3	0.3	8.4	-5.5	103	6	-
	-6.0	-0.3	0.1	8.1	-4.7	101	6	-
Octocrylene	-5.5	-0.4	0.2	9.6	-5.1	97	3	-
	-5.0	-1.1	0.1	-1.0	-5.9	94	4	-
	-4.5	*	*	*	*	**67	**3	-
	-4.0	*	*	*	*	**55	**2	-
	-7.5	0.1	0.4	3.4	-4.8	99	3	-
	-7.0	0.0	0.1	1.1	-4.6	101	4	-
	-6.5	0.2	0.4	2.1	-4.8	97	3	-
	-6.0	-0.3	0.2	-4.8	-5.3	97	4	-
Oxybenzone	-5.5	0.0	0.1	0.9	-3.7	97	5	-
	-5.0	-0.2	0.2	0.7	-4.8	96	5	-
	-4.5	-0.3	0.2	-1.8	-5.3	92	4	-
	-4.0	*	*	*	*	**#80	**4	-

## TABLE 3Results of 2nd Valid Transcriptional Activation Assay Agonist

RTA = Relative Transcriptional Activation

PC = Positive Control (10 nM DHT)

VC = Vehicle Control

SD = Standard Deviation

+ = Precipitation observed

- = No precipitation observed

\* = data not evaluated due to observed Cytotoxicity

\*\* = Cytotoxicity observed

'= true value 80.38

#= true value 79.96

	Generation	RT	Α	RTA v	with Nil	Cell Viability		
Chemical	Concentration	(% of	PC)	(% c	of PC)	(% 0	f VC)	
	(11)	Mean	SD	Value 1	Value 2	Mean	SD	
	-11.5	0.8	0.2	6.3	-1.2	102	5	
	-11	1.3	0.3	-2.0	-1.9	101	7	
	-10.5	6.6	1.1	4.6	-1.2	98	5	
DUT	-10	29.4	3.6	4.7	-0.9	104	6	
DEI	-9.5	81.8	7.0	-0.4	0.0	103	7	
	-9	107.3	11.9	10.9	2.2	102	6	
	-8.5	115.6	10.4	20.7	7.0	103	6	
	-8	113.8	6.5	58.4	33.9	98	4	
	-7.5	-0.3	0.1	0.3	-0.1	102	2	
	-7.0	-0.7	0.1	0.1	-0.3	103	7	
	-6.5	-0.6	0.1	0.6	0.1	92	4	
NI:1	-6.0	-0.8	0.2	0.9	-0.2	99	5	
INII	-5.5	-0.2	0.2	1.7	1.5	96	6	
	-5.0	0.5	0.1	4.7	1.3	99	5	
	-4.5	2.4	0.4	1.4	0.5	83	4	
	-4.0	*	*	*	*	**66	**3	
	-7.5	-0.1	0.3	10.2	-9.2	101	8	
	-7.0	-0.2	0.3	-11.4	-11.2	101	8	
	-6.5	0.2	0.2	5.2	-10.8	101	13	
mDDE	-6.0	-0.2	0.2	-11.2	-10.2	102	11	
PPDDE	-5.5	0.4	0.2	2.8	-7.8	93	8	
	-5.0	0.8	0.5	11.3	-8.3	92	8	
	-4.5	1.5	0.2	4.8	-9.1	96	6	
	-4.0	*	*	*	*	**66	**3	

TABLE 3Results of 2nd Valid Transcriptional Activation Assay Agonist<br/>(Continued)

RTA = Relative Transcriptional Activation

PC = Positive Control (10 nM DHT)

VC = Vehicle Control

SD = Standard Deviation

+ = Precipitation observed

- = No precipitation observed

\* = data not evaluated due to observed Cytotoxicity

\*\* = Cytotoxicity observed

Chemical	Concentration (LogM)	Low Agonis Induc Antagoni	t Maximal ction ism (%)	High Agoni Induction (100	st Maximum 0 nM DHT) (%)	Differential
	_	Mean	SD	Mean	SD	
10 nM DHT		110.0	6.2	105.7	8.7	
VC		0.0	0.2	95.2	15.4	
Induced Controls (1nM DHT)		100.0	11.5	104.8	4.1	
	-7.5	94.1	13.6	98.2	9.2	4.1
	-7.0	97.8	11.1	104.4	2.7	6.6
	-6.5	97.3	15.6	102.3	11.8	5.0
Octylmethoxycinnamate	-6.0	83.2	15.5	90.3	17.5	7.1
	-5.5	99.9	4.8	92.1	10.1	-7.7
	-5.0	90.8	13.2	91.5	21.3	0.8
	-4.5	83.3	10.4	85.2	15.6	1.9
	-4.0	44.3	4.7	45.2	10.6	0.9
10 nM DHT		119.7	10.2	118.4	5.9	
VC VC		0.0	0.5	108.3	9.5	
Induced Controls (1nM DHT)		100.0	11.5	91.7	6.2	
	-7.5	107.7	16.0	97.6	4.8	-10.1
	-7.0	98.5	15.5	83.4	8.6	-15.1
Octylsalate	-6.5	115.2	17.9	98.0	5.5	-17.2
	-6.0	97.6	10.6	95.2	3.9	-2.3
	-5.5	132.7	18.3	108.3	9.8	-24.3
	-5.0	117.4	13.8	118.5	5.2	1.2
	-4.5	124.6	12.5	135.6	13.8	11.0
	-4.0	76.6	48.2	128.1	27.0	#51.6
10 nM DHT		90.6	14.1	109.8	7.8	
VC		0.0	0.1	102.2	9.1	
Induced Controls (1nM DHT)		100.0	12.9	97.8	6.3	
	-7.5	98.5	6.1	103.1	12.6	4.6
	-7.0	100.2	6.3	99.8	8.4	-0.3
	-6.5	108.1	12.3	115.1	11.1	7.0
Octocrylene	-6.0	94.6	6.8	106.1	8.2	11.5
0.000	-5.5	102.1	11.8	125.3	10.7	23.2
	-5.0	62.6	3.9	112.7	14.6	#50.1
	-4.5	*	*	*	*	*
10 M DUE	-4.0	*	*	*	*	*
10 nM DHT		111.3	20.2	116.9	22.9	
VU Induced Controls (1nM DUT)		0.0	0.5	99.2	14.0	
Induced Controls (IIIWI DH1)	75	112.0	19.2	100.8	12.5	22.2
	-7.5	108.9	22.0	87.3	4.2	-23.2
	-6.5	108.7	13.3	95.8	4.3	-12.9
	-6.0	109.5	10.2	107.8	9.7	-1.7
Oxybenzone	-5.5	123.1	18.1	122.1	7.4	-1.0
	-5.0	123.5	17.6	157.6	11.1	34.1
	-4.5	89.0	5.7	169.2	16.2	#80.3
	-4.0	*	*	*	*	*

## TABLE 4Results of 2nd Valid Transcriptional Activation Assay Antagonist

VC = Vehicle Control

SD = Standard Deviation

\* = data not evaluated due to observed Cytotoxicity, Cytotoxicity values shown on corresponding agonist table shaded areas = does not apply

# = differential > 50

Chemical	Concentration (LogM)	Low Ag Maximal I Antagoni	gonist nduction sm (%)	High Agon Induction (100	ist Maximum 0 nM DHT) (%)	Differential
	_	Mean	SD	Mean	SD	
10 nM DHT		103.9	8.5	87.7	4.1	
VC		0.0	0.2	97.3	3.8	
Induced Controls (1nM DHT)		100.0	17.4	102.7	6.2	
	-11.5	102.7	7.2	100.8	6.6	-1.9
	-11	97.0	7.1	94.7	8.2	-2.3
	-10.5	112.9	9.4	107.2	9.4	-5.7
рит	-10	97.9	16.8	97.1	5.7	-0.8
DIII	-9.5	125.9	10.3	115.3	3.7	-10.6
	-9	121.3	6.1	119.1	16.8	-2.2
	-8.5	137.3	26.7	118.5	3.7	-18.9
	-8	124.4	18.0	120.4	8.7	-4.1
10 nM DHT		119.4	15.5	106.2	12.5	
VC		0.0	0.4	104.8	4.5	
Induced Controls (1nM DHT)		100.0	11.0	95.2	10.8	
	-7.5	102.8	13.8	108.8	16.1	6.0
	-7.0	90.0	9.5	102.5	10.8	12.5
	-6.5	67.2	14.8	102.9	5.5	35.7
Nil	-6.0	19.6	2.5	106.8	4.3	#87.2
1 MI	-5.5	6.5	0.7	119.6	6.0	#113.1
	-5.0	3.6	0.2	121.6	10.4	#118.0
	-4.5	3.7	0.9	78.2	3.4	#74.5
	-4.0	*	*	*	*	*
10 nM DHT		114.2	10.7	129.3	20.6	
VC		0.0	0.2	99.8	10.7	
Induced Controls (1nM DHT)		100.0	19.4	100.2	7.9	
	-7.5	90.4	11.2	101.1	4.3	10.7
	-7.0	87.2	12.5	91.6	8.2	4.4
	-6.5	101.3	7.9	103.8	9.5	2.5
nnDDE	-6.0	95.3	13.7	101.5	9.7	6.2
рров	-5.5	94.4	5.5	110.7	18.4	16.3
	-5.0	58.7	8.2	119.3	6.0	#60.6
	-4.5	27.4	2.4	133.2	9.9	#105.9
	-4.0	*	*	*	*	*

# TABLE 4Results of 2nd Valid Transcriptional Activation Assay Antagonist<br/>(Continued)

VC = Vehicle Control

SD = Standard Deviation

\* = data not evaluated due to observed Cytotoxicity, Cytotoxicity values shown on corresponding agonist table shaded areas = does not apply

# = differential > 50

Chemical	Concentration	RT (% of	A PC)	RTA v (% c	with Nil of PC)	Cell Vi (% of	iability f VC)	Precipitation	
	(M)	Mean	SD	Value 1	Value 2	Mean	SD	Value 1	Value 2
	-7.5							0.3	0.3
	-7.0							0.2	0.3
	-6.5							0.2	0.3
	-6.0							0.3	0.4
Octylmetnoxycinnamate	-5.5							0.4	0.3
	-5.0							0.3	0.3
	-4.5							0.6	0.7
	-4.0							+1.9	+2.1
	-7.5	-0.1	0.3	8.4	-0.3	109	7	0.2	0.3
	-7.0	0.0	0.3	4.3	-1.4	109	4	0.2	0.2
	-6.5	0.3	0.4	0.6	-0.8	113	3	0.2	0.3
	-6.0	-0.1	0.4	1.2	-1.0	112	6	0.2	0.3
Octylealate	-5.5	0.5	0.3	0.6	0.1	104	6	0.3	0.4
Octyfsalate	-5.0	0.3	0.2	39.0	-0.3	110	7	0.3	0.2
	-4.5	0.1	0.3	11.0	0.1	112	7	0.5	0.6
	-4.0	0.0	0.2	0.1	-1.0	88	9	+0.9	+1.2
	-3.5							+1.7	+1.6
	-3.0							+0.7	+1.1
	-7.5	0.3	0.4	6.5	-2.2	111	3	0.4	0.2
	-7.0	0.3	0.4	7.8	-1.4	108	6	0.3	0.2
	-6.5	0.3	0.3	1.6	-2.6	116	7	0.2	0.3
	-6.0	0.1	0.1	-0.4	-5.7	115	4	0.3	0.2
Octocrylene	-5.5	0.5	0.2	1.4	-1.0	110	6	0.2	0.2
Octoerytene	-5.0	0.1	0.2	7.1	-1.6	107	7	0.3	0.3
	-4.5	-0.6	0.1	-0.9	-4.1	90	9	+4.1	+4.4
	-4.0	*	*	*	*	**66	**3	+28.0	+29.1
	-3.5							+23.9	+25.5
	-3.0							+19.2	+20.4
	-7.5	0.1	0.2	5.7	-2.4	116	3	0.3	0.3
	-7.0	0.1	0.3	5.7	-2.2	107	29	0.6	0.3
	-6.5	0.6	0.4	-0.7	-2.7	120	12	0.3	0.3
	-6.0	0.1	0.3	-1.9	-2.5	121	8	0.2	0.3
Oxybenzone	-5.5	0.8	0.5	0.3	-1.6	116	12	0.3	0.4
ONYDERLORE	-5.0	0.5	0.3	-0.2	-1.0	121	9	0.3	0.3
	-4.5	0.2	0.2	5.5	-2.3	124	7	0.3	0.3
	-4.0	0.2	0.2	-2.0	-3.2	97	18	0.3	0.3
	-3.5							0.4	0.5
	-3.0							0.3	0.5

#### **Results of 3<sup>rd</sup> Valid Transcriptional Activation Assay Agonist** TABLE 5

RTA = Relative Transcriptional Activation PC = Positive Control (10 nM DHT)

VC = Vehicle Control

SD = Standard Deviation

+ = Precipitation  $\ge 3$  times vehicle control

\* = data not evaluated due to observed Cytotoxicity

\*\* = Cytotoxicity observed

shaded areas not evaluated

		RT	Ά	RTA w	ith Nil	Cell Viability		
Chamical	Concentration	(% of	PC)	(% of	PC)	(% of	VC)	
Chemicai	(M)	Mean	SD	Value 1	Value 2	Mean	SD	
	-11.5	0.4	0.3	0.5	-0.8	100	9	
	-11	1.6	0.4	1.3	-0.5	106	11	
	-10.5	7.2	2.0	6.2	0.1	108	14	
рит	-10	28.2	5.9	4.6	-0.6	106	12	
DIII	-9.5	81.2	6.7	9.7	0.9	103	14	
	-9	118.0	12.3	4.3	3.2	107	6	
	-8.5	144.3	22.2	14.5	5.8	117	7	
	-8	140.0	28.8	38.3	27.1	98	7	
	-7.5	-0.4	0.2	2.7	-1.3	98	10	
	-7.0	-0.3	0.3	2.2	-0.5	101	13	
	-6.5	-0.2	0.2	-0.3	-1.2	101	10	
NU	-6.0	-0.3	0.4	-0.5	-1.7	92	7	
INII	-5.5	0.4	0.4	2.0	-0.6	98	10	
	-5.0	1.9	0.5	2.9	1.6	97	11	
	-4.5	2.9	0.5	-0.1	-0.8	95	3	
	-4.0	*	*	*	*	**56	**4	
	-7.5	0.0	0.3	-2.0	-3.1	106	13	
	-7.0	0.3	0.2	1.0	-2.8	108	9	
	-6.5	0.4	0.4	-1.4	-2.1	113	15	
m DDE	-6.0	0.1	0.4	-1.3	-2.5	117	16	
рроос	-5.5	1.2	0.5	0.9	-0.9	110	16	
	-5.0	1.9	0.8	2.9	-0.9	103	16	
	-4.5	2.2	0.5	0.6	-0.8	118	11	
	-4.0	*	*	*	*	**68	**5	

TABLE 5Results of 3rd Valid Transcriptional Activation Assay Agonist<br/>(Continued)

RTA = Relative Transcriptional Activation

PC = Positive Control (10 nM DHT)

VC = Vehicle Control

SD = Standard Deviation

+ = Precipitation observed

- = No precipitation observed

\* = data not evaluated due to observed Cytotoxicity

\*\* = Cytotoxicity observed

Chemical	Concentration (LogM)	Low A Maximal I Antagon	gonist Induction ism (%)	High Agon Induction (100	Differential	
	_	Mean	SD	Mean	SD	
10 nM DHT		110.8	75.8	119.5	8.0	
VC		0.0	0.3	101.9	11.8	
Induced Controls (1nM DHT)		100.0	18.1	98.1	15.5	
	-7.5	108.7	11.0	105.2	13.7	-3.5
	-7.0	111.3	21.0	104.3	22.8	-7.0
	-6.5	115.0	29.5	112.1	24.8	-2.9
Ostylsələtə	-6.0	119.0	36.7	107.9	17.4	-11.1
Octylsalate	-5.5	140.3	24.2	133.1	31.4	-7.2
	-5.0	132.1	15.3	118.9	20.6	-13.2
	-4.5	122.7	24.4	146.4	38.6	23.7
	-4.0	91.0	23.9	157.6	32.2	#66.6
10 nM DHT						
VC		0.0	0.2	95.9	10.8	
Induced Controls (1nM DHT)		100.0	22.9	104.1	15.0	
	-7.5	96.8	23.7	107.4	13.5	10.5
	-7.0	109.7	20.0	101.7	18.4	-8.0
	-6.5	117.6	29.7	123.8	32.3	6.2
Ostsomulans	-6.0	111.4	27.3	94.2	14.5	-17.2
Octocrytene	-5.5	105.0	26.2	125.4	23.6	20.4
	-5.0	73.5	16.0	133.4	8.9	#59.9
	-4.5	21.6	6.8	91.4	20.1	#69.8
	-4.0	*	*	*	*	*
10 nM DHT						
VC		0.0	0.3	103.7	9.5	
Induced Controls (1nM DHT)		100.0	21.0	96.3	27.5	
	-7.5	97.2	20.4	101.2	19.9	4.0
	-7.0	96.7	20.4	96.2	40.1	-0.5
	-6.5	119.8	31.5	103.9	40.7	-15.9
Ovybenzone	-6.0	101.9	25.6	111.0	58.7	9.1
OxyDenZOIIC	-5.5	134.8	28.6	133.8	62.8	-1.1
	-5.0	124.0	18.5	152.0	71.6	28.0
	-4.5	75.6	10.1	176.5	24.7	#100.9
	-4.0	29.5	7.0	198.5	40.7	#169.0

## TABLE 6Results of 3<sup>rd</sup> Valid Transcriptional Activation Assay Antagonist

VC = Vehicle Control

SD = Standard Deviation

\* = data not evaluated due to observed Cytotoxicity, Cytotoxicity values shown on corresponding agonist table shaded areas = does not apply

# = differential > 50

Chemical	Concentration (LogM)	Low A Maximal I Antagoni	gonist nduction sm (%)	High Agon Induction (100	Differential	
		Mean	SD	Mean	SD	
10 nM DHT		115.8	24.0	118.0	12.7	
VC		0.0	0.1	104.3	22.5	
Induced Controls (1nM DHT)		100.0	17.0	95.7	19.2	
	-11.5	117.0	18.4	97.7	19.3	-19.2
	-11	109.1	9.9	119.5	17.4	10.4
	-10.5	130.6	33.6	115.7	32.0	-15.0
рит	-10	113.1	18.3	117.1	32.9	3.9
DIII	-9.5	128.4	34.6	119.0	24.8	-9.4
	-9	155.4	18.0	122.9	11.3	-32.5
	-8.5	161.9	13.4	127.6	26.1	-34.3
	-8	140.0	25.7	118.6	16.2	-21.4
10 nM DHT		122.9	15.1	110.2	12.3	
VC		0.0	0.5	102.7	10.2	
Induced Controls (1nM DHT)		100.0	16.9	97.3	16.2	
	-7.5	105.3	4.3	107.9	6.1	2.7
	-7.0	77.3	8.8	113.8	6.0	36.5
	-6.5	56.2	6.1	116.4	17.6	#60.3
Nil	-6.0	16.7	3.6	108.2	13.0	#91.6
1411	-5.5	8.8	2.3	123.6	14.9	#114.8
	-5.0	6.1	1.3	115.0	9.9	#108.8
	-4.5	6.9	1.2	74.8	19.7	#68.0
	-4.0	*	*	*	*	*
10 nM DHT		122.7	19.8	110.2	15.6	
VC		0.0	0.1	103.6	25.6	
Induced Controls (1nM DHT)		100.0	23.0	96.4	23.6	
	-7.5	110.5	15.5	95.8	19.7	-14.7
	-7.0	106.0	10.8	87.5	16.9	-18.6
	-6.5	120.1	24.1	105.6	25.9	-14.5
<b>PPDDE</b>	-6.0	100.2	18.8	98.5	28.0	-1.6
рров	-5.5	102.1	23.2	114.0	35.1	12.0
	-5.0	62.6	13.0	118.3	26.0	#55.7
-	-4.5	31.0	3.1	112.5	30.1	#81.5
	-4.0	*	*	*	*	*

# TABLE 6Results of 3<sup>rd</sup> Valid Transcriptional Activation Assay Antagonist<br/>(Continued)

VC = Vehicle Control

SD = Standard Deviation

\* = data not evaluated due to observed Cytotoxicity, Cytotoxicity values shown on corresponding agonist table shaded areas = does not apply

# = differential > 50

## TABLE 7LogPC50, LogPC10, LogEC50 and Hill Slope Values for the Reference<br/>Chemicals

Agoni	ist											
	LogPC <sub>50</sub>			LogPC <sub>10</sub>			LogEC <sub>50</sub>			Hill Slope		
Name	1 <sup>st</sup> Valid Assay	2 <sup>nd</sup> Valid Assay	3 <sup>rd</sup> Valid Assay	1 <sup>st</sup> Valid Assay	2 <sup>nd</sup> Valid Assay	3 <sup>rd</sup> Valid Assay	1 <sup>st</sup> Valid Assay	2 <sup>nd</sup> Valid Assay	3 <sup>rd</sup> Valid Assay	1 <sup>st</sup> Valid Assay	2 <sup>nd</sup> Valid Assay	3 <sup>rd</sup> Valid Assay
DHT	-9.8	-9.8	-9.8	-10.5	-10.4	-10.4	-9.8	-9.7	-9.6	1.9	1.7	1.3
Nil	-	-	-	-	-	-	-	-	-	-	-	-
ppDDE	-	-	-	-	-	-	-	-	-	-	-	-

PC = Positive Control (10 nM DHT)

#### Antagonist

	Dif	ferential l	C <sub>50</sub>	Dif	fferential	IC <sub>30</sub>	LogEC <sub>50</sub>		Hill Slope			
Name	1 <sup>st</sup> Valid Assay	2 <sup>nd</sup> Valid Assay	3 <sup>rd</sup> Valid Assay	1 <sup>st</sup> Valid Assay	2 <sup>nd</sup> Valid Assay	3 <sup>rd</sup> Valid Assay	1 <sup>st</sup> Valid Assay	2 <sup>nd</sup> Valid Assay	3 <sup>rd</sup> Valid Assay	1 <sup>st</sup> Valid Assay	2 <sup>nd</sup> Valid Assay	3 <sup>rd</sup> Valid Assay
DHT	-	-	-	-	-	-	-	-	-	-	-	-
Nil	-6.6	-6.4	-6.7	-6.9	-6.6	-7.1	-6.4	-6.4	-6.7	-1.5	-1.6	-0.9
ppDDE	-5.0	-5.1	-5.1	-5.3	-5.3	-5.3	-4.9	-4.8	-4.9	-1.3	-1.5	-1.3

Differential = High agonist minus low agonist

Differential  $IC_{50}$  = concentration at which the high agonist minus low agonist is 50%

	Relative Inhibitory Concentration Max (RICMax)							
Name	1 <sup>st</sup> Valid Assay	2 <sup>nd</sup> Valid Assay	3 <sup>rd</sup> Valid Assay					
DHT	-	-	-					
Nil	96.5	96.4	93.9					
ppDDE	74.7	72.6	69.0					

## **FIGURES SECTION**
## FIGURE 1 Octylmethoxycinnamate – Agonist

#### 13Oct2011







The two separate graphs represent the data (Means $\pm$ Standard Deviation) from the two different independent runs of the assay in the absence of antagonist (n =6/concentration).

FIGURE 2 Octylmethoxycinnamate – Antagonist



The top graph represents the cells co-dosed with 1 nM DHT. The bottom graph represents the cells co-dosed with 1000 nM DHT.



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The top graph represents the cells co-dosed with 1 nM DHT. The bottom graph represents the cells co-dosed with 1000 nM DHT.



#### Octy Isalicy lateag 120 r 110 100 % of Maximal Induction Control 90 80 70 60 50 40 30 20 10 0 -6 -5 -4 -3 -7 Concentration [LogM]

### 13Oct2011





The two separate graphs represent the data (Means $\pm$ Standard Deviation) from the two different independent runs of the assay in the absence of antagonist (n =6/concentration). The limit of cyotoxicity was -4.0 logM in run one.





The graph represents the data (Means $\pm$ Standard Deviation) from an independent runs of the assay in the absence of antagonist (n =6/concentration).





<sup>13</sup>Oct2011

The top graph represents the cells co-dosed with 1 nM DHT. The bottom graph represents the cells co-dosed with 1000 nM DHT. The limit of cyotoxicity was -4.0 logM.





The top graph represents the cells co-dosed with 1 nM DHT. The bottom graph represents the cells co-dosed with 1000 nM DHT.





The top graph represents the cells co-dosed with 1 nM DHT. The bottom graph represents the cells co-dosed with 1000 nM DHT.





The two separate graphs represent the data (Means $\pm$ Standard Deviation) from the two different independent runs of the assay in the absence of antagonist (n =6/concentration). The limit of cytotoxicity was -5.0 logM for runs one and two.

## FIGURE 5 Octocrylene – Agonist (Continued)



The graph represents the data (Means $\pm$ Standard Deviation) from an independent runs of the assay in the absence of antagonist (n =6/concentration). The limit of cytotoxicity was -4.5 logM for run three.





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The top graph represents the cells co-dosed with 1 nM DHT. The bottom graph represents the cells co-dosed with 1000 nM DHT. The limit of cytotoxicity was -5.0 logM for run one.





The top graph represents the cells co-dosed with 1 nM DHT. The bottom graph represents the cells co-dosed with 1000 nM DHT. The limit of cytotoxicity was -5.0 logM for run two.

FIGURE 6 Octocrylene – Antagonist (Continued)



The top graph represents the cells co-dosed with 1 nM DHT. The bottom graph represents the cells co-dosed with 1000 nM DHT. The limit of cytotoxicity was -4.5 logM for run three.











The graph represents the data (Means $\pm$ Standard Deviation) from an independent runs of the assay in the absence of antagonist (n =6/concentration).





The top graph represents the cells co-dosed with 1 nM DHT. The bottom graph represents the cells co-dosed with 1000 nM DHT. The cytotoxicity limit for run one was -4.0 logM.

### FIGURE 8 Oxybenzone – Antagonist (Continued)



The top graph represents the cells co-dosed with 1 nM DHT. The bottom graph represents the cells co-dosed with 1000 nM DHT. The cytotoxicity limit for run two was -4.5 logM.

FIGURE 8 Oxybenzone – Antagonist (Continued)









The two separate graphs represent the data (Means $\pm$ Standard Deviation) from the two different independent runs of the assay in the absence of antagonist (n =6/concentration).



FIGURE 9 DHT – Agonist (Continued)

The graph represents the data (Means $\pm$ Standard Deviation) from an independent runs of the assay in the absence of antagonist (n =6/concentration).





The top graph represents the cells co-dosed with 1 nM DHT. The bottom graph represents the cells co-dosed with 1000 nM DHT.





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The top graph represents the cells co-dosed with 1 nM DHT. The bottom graph represents the cells co-dosed with 1000 nM DHT.

















The two separate graphs represent the data (Means $\pm$ Standard Deviation) from the two different independent runs of the assay in the absence of antagonist (n =6/concentration). The cytotoxicity limit is -4.5 logM.



# FIGURE 11 Nilutamide – Agonist (Continued)

The graph represents the data (Means $\pm$ Standard Deviation) from an independent runs of the assay in the absence of antagonist (n =6/concentration). The cytotoxicity limit is -4.5 logM.





13Oct2011

The top graph represents the cells co-dosed with 1 nM DHT. The bottom graph represents the cells co-dosed with 1000 nM DHT. The cytotoxicity limit is -4.5 logM.







The top graph represents the cells co-dosed with 1 nM DHT. The bottom graph represents the cells co-dosed with 1000 nM DHT. The cytotoxicity limit is -4.5 logM.







The top graph represents the cells co-dosed with 1 nM DHT. The bottom graph represents the cells co-dosed with 1000 nM DHT. The cytotoxicity limit is -4.5 logM.







The two separate graphs represent the data (Means $\pm$ Standard Deviation) from the two different independent runs of the assay in the absence of antagonist (n =6/concentration). The cytotoxicity limit is -4.5 logM.



#### 3Nov2011



The graph represents the data (Means $\pm$ Standard Deviation) from an independent runs of the assay in the absence of antagonist (n =6/concentration). The cytotoxicity limit is -4.5 logM.





The top graph represents the cells co-dosed with 1 nM DHT. The bottom graph represents the cells co-dosed with 1000 nM DHT. The cytotoxicity limit is -4.5 logM.





The top graph represents the cells co-dosed with 1 nM DHT. The bottom graph represents the cells co-dosed with 1000 nM DHT. The cytotoxicity limit is -4.5 logM.

FIGURE 14 ppDDE – Antagonist (Continued)



The top graph represents the cells co-dosed with 1 nM DHT. The bottom graph represents the cells co-dosed with 1000 nM DHT. The cytotoxicity limit is -4.5 logM.

# **APPENDICES SECTION**

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APPENDIX 1 Data Spreadsheets

### **Data Spreadsheets**






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Experiment date:	13oct2011			1	blank - no	cells, veh	icle control			Spreadsh	eet locked	on: 11/10/2011		
TopCount Model B9912V, S	Serial# 408672		1	1	neq. contro	ol = cells +	vehicle.	07		Green sha	aded areas	unlocked cells for data	a entry	
Assay Conducted by:		-			Compoun	d	Octylsalic	ylate			-			
A REAL PROPERTY OF A REAL PROPER	26		2						5 6		-		FOLD INDU	ICTION
Octylsalicylate ag	blank 10nMDHT	neg. control	neg. control	-6.5	-6.0	-5.5	-5.0	-4.5	-4.0	-3.5	-3.0		-	10nMDH neg. control neg. control -6.5 -6.0 -5.5 -5.0 -4.5 -4.0 -3.5 -3.0
Ê	100 1403550	25700	25900	33400	34250	26700	22950	20050	18250	17150	11250		1	53.5 1.0 1.0 1.3 1.3 1.0 0.9 0.8 0.7 0.4 0.5
C	50 1286600	27550	28450	36450	40050	32450	24750	23650	19850	11800	14850			49.1 1.1 1.1 1.4 1.5 1.2 0.9 0.9 0.8 0.5 0.6
D E	0 1118550	20650	32950	30800	35500	35450	23900	19250	16800	14150	14500		-	42.7 0.8 1.3 1.2 1.4 1.4 0.9 0.7 0.6 0.5 0.6
Ē	50 1318250	27550	27400	30800	44750	36100	23850	22050	20100	11800	10750			50.3 1.1 1.0 1.2 1.7 1.4 0.9 0.8 0.8 0.5 0.4
G	0 435700	81100	21700	28800	84900	79150	28350	30000	26200	15400	16300	with 10µM Nilutamide	Mean	<b>49.9</b> 1.0 1.3 1.5 1.2 0.9 0.8 0.7 0.5 0.5
н	0 232500	17400	25400	17050	30150	27000	21450	28950	21500	18300	15100	with 10µM Nilutamide	Std Dev	
Mean	50 1307325	26217	-	33042	38975	32600	24067	20667	18433	13267	12975		CV%	7.7 13.7 23.5 17.5 13.0 5.2 8.6 7.0 18.7 13.6
Std Dev	32 100723	3594		7753	6809	4523	1244	1785	1288	2476	1763		Relative Tra	100 2.0 2.5 3.0 2.5 1.8 1.6 1.4 1.0 1.0
SEM	13 41120	1037	5 <u>8</u>	3165	2780	1847	508	729	526	1011	720			
CV% Relative Transcriptional Act	03.2 7.7	13.71	8 8	23.5	17.5	13.9	0.0	0.0	0.0	18.7	13.0			60.0 Ctylsalicylate ag
Rows G&H														
Mean	0 334100	36400	5 S	23225	57525	53075	24900	32475	23850	16850	15700			50.0
Std Dev	0 143684	29979	5	7884	38714	36876	4879	4985	3323	2051	849			
Mean VC	26217	Mean Nilutar	mide Control	36400					-		<u> </u>			<u>640.0</u>
														9300
Subtraction of VC from w	Vella		non control		6.0		6.0	4.5	4.0	2.5	20			
A	50 1336983	-2517	-6167	-4617	4433	1383	-3267	-6667	-9.0	-15967	-12067			£ 20.0 +
в	100 1377333	-1217	-317	7183	8033	483	-3417	-6167	-7967	-9067	-14967			
C	50 1260383	1333	2233	10233	13833	6233	-1467	-2567	-6367	-14417	-11367		-	10.0 +
E	50 1327583	3133	-167	18983	22433	11083	-2317	-6767	-8417	-11767	-13867			
F	50 1292033	1333	1183	4583	18533	9883	-2367	-4167	-6117	-14417	-15467			
G	0 399300	44700	-14700	-7600	48500	42750	-8050	-400	-10200	-21000	-20100	with 10µM Nilutamide	-	NON 2010 5" 5" 5" 5" 4" 3" 3" 3"
Corrected Data Means	0 196100	-19000	-11000	-16/50	-6250	-9400	-14950	-/450	-14900	-10100	-21300	with TOPM Nilutamide	-	Concentration (logM)
Octylsalicylate ag	blank 10nM DHT	neg. control	8 8	-6.5	-6.0	-5.5	-5.0	-4.5	-4.0	-3.5	-3.0			
Mean	50 1281108	0	5	6825	12758	6383	-2150	-5550	-7783	-12950	-13242			
Std Dev SEM	32 100723	3594		7753	6809	4523	1244	1785	1288	2476	1763			Octylsalicylate ag
CV%	03.2 7.9			113.0	53.4	70.9	-57.8	-32.2	-16.5	-19.1	-13.3			20
Relative Transcriptional Act	tivity 1.0	0.0	<u>i</u> (1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		1	\$100
Aconist: % of Maximal In	duction Control										-			
Octylsalicylate ag	blank 10nMDHT	neq. control	neq. control	-6.5	-6.0	-5.5	-5.0	-4.5	-4.0	-3.5	-3.0			<b>80</b>
Α	0.0 104.4	-0.2	-0.5	-0.4	0.3	0.1	-0.3	-0.5	-0.7	-1.2	-0.9			
B	0.0 107.5	-0.1	0.0	0.6	0.6	0.0	-0.3	-0.5	-0.6	-0.7	-1.2			
D	0.0 98.4	-0.4	0.2	0.4	0.7	0.5	-0.1	-0.2	-0.5	-0.9	-0.9			
E	0.0 103.6	0.2	0.0	1.5	1.8	0.9	0.0	-0.5	-0.7	-0.9	-1.1			8
F	0.0 100.9	0.1	0.1	0.4	1.4	0.8	-0.2	-0.3	-0.5	-1.1	-1.2		<u>1</u>	20
G H	0.0 31.2	3.5	-1.1	-0.6	-0.5	-0.7	-0.6	-0.6	-0.8	-1.6	-1.6	with 10µM Nilutamide	-	
% of Maximal Induction (	Control							0.0				inter repartition inter	-	
Octylsalicylate ag	blank 10nM DHT	neg. control	2	6.5	-6.0	-5.5	-5.0	4.5	4.0	-3.5	-3.0			Dr 10 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
Viean Std Dev	0.0 100.0	0.0	3	0.5	1.0	0.5	-0.2	-0.4	-0.6				-	State and Concentration (logit)
SEM	0.0 3.2	0.1	ų į	0.2	0.2	0.1	0.0	0.1	0.0		Q		-	Concentration (roger)
00070			1 1			-			. <u> </u>		1			
		-	-								-			Viability (% Control)
PC10		1	PC50								-		-	Octvisalicviate ag 100M DHT neg. confineg. conf -6.51 -6.0 -5.51 -5.0 -4.5 -4.0 -3.51
					§ 24									Mean 103 100 97 96 104 95 97 97 88 70
							1		1 8		-			StdDev 3 4 6 5 9 2 5 5 7 5
	arrest of						-		-		-			SEM 1 2 3 2 4 1 2 2 3 2 %CV 2 9 39 54 51 89 21 51 51 83 70
	PC10			H			1	PC60			_	-		
1.2				12	2									
													1	
1.0				1.0	•								FIt	rance be
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0.8				8.0										
														120m
0.6				0.6	6	_			_	_				U Bottom
				140										5.5 EC50
0.4														-16.9 Hill slope
0.2				- 34										
100 A														ž 60-
0.0														
0.0 0.2	0.4 0.6	0.8 1.0	1.2		0.0	0.2	0.4	0.6	0.8	1.0	1.2		14	8 40
6					66.6	SIN!	10.20	1738 ·	1983	200	1926		-	
			5 2			-			i		s - 1			* *
							1							-7 -6 -5 -4 -3

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Count Model B9912V, S	Serlal# 40867	2				neq. contro	I = cells +	vehicle.			Green sha	ded areas	unlocked cells for data	entry	
1/11 13:23 ay Conducted by:						Study Num Compound	iber: 9	9070-10010 Octylsalicyl	7ARTA ate						
Isalicylate Ag	blank	10nMDHT n	neg. control	neg. control	-7.5	-7.0	-6.5	-6.0	-5.5	-5.0	-4.5	4.0		FOLD IND	UCTION 10nMDH neg.control neg.control <u>7.5</u> <u>7.0</u> <u>-5.5</u> <u>-5.0</u> <u>-5.5</u> <u>-5.0</u> <u>-4.5</u> <u>-4.0</u>
AB	0	199250	4450	2500	3150	4300	4650	3450 3300	5100 5250	5800 4550	5350 3350	3600			49.9         1.1         0.6         0.8         1.1         1.2         0.9         1.3         1.5         1.3         0.9           62.7         1.1         1.0         0.7         0.7         0.9         0.8         1.3         1.1         0.8         0.9
c	0	292900	4400	3900	3550	4300	6050	4400	5100	4350	3350	3800			73.3 1.1 1.0 0.9 1.1 1.5 1.1 1.3 1.1 0.8 1.0
E	50	250750	4700	4600	4550	4500	5600	4600	6700	5500	4400	4750			60.3         1.2         1.3         1.3         1.3         1.4         1.2         1.3         0.3           62.8         1.2         1.2         1.1         1.4         1.2         1.4         1.1         1.4           71.8         1.2         1.1         1.4         1.2         1.4         1.1         1.4
G	100	112150	3600 11500	10750	31300	20150	4300	11950	4550 10200	4500 113350	4300 38100	4550 8950	with 10µM Nilutamide	Mean	71.8         0.9         0.7         0.8         0.9         1.1         0.6         1.1         1.1         1.1         1.1           68.1         1.0         0.9         1.0         1.2         1.0         1.3         1.2         1.1         1.0
н	50	67950	7450	5150	7900	4850	6650	5900	8950	7900	8850	6050	with 10µM Nilutamide	Std Dev SEM	13.0         0.2         0.2         0.2         0.2         0.2         0.2         0.2         0.1         0.2         0.2           5.3         0.1
ev.	25	272300	3996		3725	4092	4850	3808	5392 732	4908	4300	3908		CV% Relative Tra	10.1 17.4 24.9 19.9 19.6 25.9 13.6 12.1 19.4 15.5 ar 100 1.5 1.4 1.5 1.8 1.4 2.0 1.8 1.5 1.4
-	17	21202	201		379	332	388	403	299	243	341	247			
e Transcriptional Act	Mty	1.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			80.0 Octylsalicylate Ag
G&H	25	90050	8713		19600	12500	8475	8925	9575	60625	23475	7500		-	70.0
2V	35	31254	2956		16546	10819	2581	4278	884	74564	20683	2051		-	60.0
vc	3996		Vean Nilutar	mide Control	8713										§ 50.0 -
action of VC from w	ella		Interest	non control	76	7.0		6.0		6.0	4.6	4.0			240.0
A	0	195254	454	-1496	-846	304	654	-546	1104	1804	1354	-396			230.0
C	0	288904	404	-96	-1246	-1246	2054	404	1254	354	-646	-496			100
E	0 50	349454 246754	654 704	4 604	1154	1104	1104	854	1654 2704	754	1054 404	-746 754		-	
F	100	282754	-396 2788	-1096 2038	-796 22588	-396 11438	304 1588	-1746 3238	554 1488	504 104638	304 29388	554 238	with 10µM Nilutamide	-	and 10 10 10 10 10 10 10 10 10
H cted Data Meana	50	59238	-1263	-3563	-813	-3863	-2063	-2813	238	-813	138	-2663	with 10µM Nilutamide		State Concentration (logM)
alicylate Aq	blank 1	OnM DHT n	eq. control		-7.5	-7.0	-6.5	-6.0	-5.5	-5.0	4.5	-4.0			
24	42	51934	696	d.	-2/1	814	950	988	732	596	834	-00			Ontvisalinviate An
	17 167.3	21202	201		-342.4	332 849.3	388	403	299 52.4	243 05.3	341 274.3	-690.9			120 - Occysancyraic Ag
e Transcriptional Act	ivity	1.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			5100
ist: % of Maximal Inc	duction Con	trol	intron per	neg control	-75	-7.0	-6.5	-6.0	-5.5	-5.0	-4.5	-4.0			2 80 -
A	0.0	72.8	0.2	-0.6	-0.3	0.1	0.2	-0.2	0.4	0.7	0.5	-0.1			1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
č	0.0	107.7	0.2	0.0	-0.5	0.1	0.2	0.2	0.5	0.2	-0.2	-0.2			
E	0.0	92.0	0.2	0.0	0.4	0.4	0.4	0.3	1.0	0.3	0.4	-0.3			2 1 40 T
F G	0.0	105.4 38.6	-0.1	-0.4	-0.3	-0.1	0.1	-0.7	0.2	0.2	0.1	0.2	with 10µM Nilutamide	-	
H Maximal Induction C	0.0 Control	22.1	-0.5	-1.3	-0.3	-1.4	-0.8	-1.0	0.1	-0.3	0.1	-1.0	with 10µM Nilutamide	-	* 0 +
alicylate Aq	blank 1	OnM DHT n	eq. control	Sł. – Z	-7.5	-7.0	-6.5	-6.0	-5.5	-5.0	-4.5	-4.0			NOT control 15 10 25 20 55 50 25 20
ev.	0.0	19.4	0.3		0.3	0.3	0.4	0.4	0.3	0.2	0.3	0.2			NOT REAL Concentration (logM)
	0.0		u. 1		9.1	0.1		0.2		0.1	0.1	0.1			Vieblike (V. Control)
															Viability (% control)
PC10				PC50											Octylsallcylate Ag         10nM DHT         neg. contineg. cont         -7.5         -7.0         -5.5         -5.0         -5.5         -5.0         -4.5           Mean         90         100         101         109         113         112         104         110         1
					1.										StdDev         4         4         3         7         4         3         6         6         7           SEM         2         2         1         3         2         1         2         3         3
	PC1	0						P	C60						%CV 4.3 4.4 3.4 6.6 3.6 2.9 5.1 6.1 6.1 (
					1	2									
2					1.									2920	#interse*
														Chart	#OK
															Octyfeallcy/ate Ag
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.0 0.2 (			-				-		_			_			5 20-
1.0 0.2 1														1	76 20- #

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Experiment date: 13Oct2011 TopCount Model B9912V. Serial# 408672 11/11/11 13:23 Assay Conducted by: blank = no cells. vehicle control neg. control = cells + vehicle. Study Number: 9070-100107ARTA Compound Octocrylene Spreadsheet locked on: 11/10/2011 Green shaded areas unlocked cells for data entr FOLD IND ICTION black HOcMD 10nM DHineg, control induced co Ith 1nM DHT Ith 1nM DHT Ith 1nM DHT Ith 1nM DHT Ith 1000nM DH 93415 89225 86120 92930 0.3 0.3 0.4 1.9 50.8 38. 48.7 36.4 38.4 113130 107945 105420 1792 1245 44. 43. 38. 22.3 48.3 47.0 1.0 51.4 39.1 49.8 40.9 8.9 h 1000nM DH 38.2 39.1 49. 1.9 DOODM DH old Induction (1nM 2.1 1.0 4.2 103.2 48.1 Mean Std Dev SEM 113571 6335 3167 1171625 10066 49561 875 24780 4370 04225 34248 98512 876. td Dev EM 2.6 1.3 5.6 0 5.4 2.7 13.3 1. 0.1 873 16.1 13.5 0.8 CV% Relative Transcription 100 43.8 49.6 04 nduction (100 Std Dev SEM CV% 5.3 2.7 12.1 3.0 4.6 6.4 3.2 13.3 4.1 5.1 2.6 9.0 3.3 0. 0.3 4.5 1.5 2.1 0.1 0.1 1240325 579413 78673 109434 39336 54717 39675 5280 2640 13.3 2.3 105568 972913 1120488 1161588 1259625 1375150 73091 111226 154512 100046 123687 18963 2329 5036 525 2.3 Std Dev SEM CV% 06.1 110.0 119.3 130.3 117.5 54.9 3.8 100 Mean VC 24125 Mean DHT 1000nM Control 972913 Fold Induction (1nM DHT) 60.0 Subtraction of VC from w InMOHT neg. control 
 uced cont
 \_6.5

 910025
 1150275

 868125
 1107175

 837075
 1116125
 <u>-5.5</u> 903475 865475 1155125 <u>-5.0</u> 592925 460525 591675 <u>-4.0</u> -17175 -15625 -15675 50.0 -3.5 -18575 -19575 -19025 4.5 -24075 1200275 -24125 1079825 -23975 1055325 854275 1045525 1030075 120775 134775 100375 -15975 -16975 -18025 4025 -2225 -1475 th 1nM DHT 40.0 -24075 1110925 -325 905175 1216425 1000125 919925 513225 161275 12975 -19575 -152 th 1nM DHT 30.0 -2407 257 11768 963025 919025 1198879 133037 19822 139591 131322 5472 7030 1317 -732 20.0 10.0 VC Corrected Data Means AVG cntrl Octocrylene ant blank 10nM DH neg. contro nduced con 4.0 -19188 484 1111586 63351 -24063 0 880100 1147500 982500 795 34248 49561 87536 961000 539588 64560 129300 -15363 -16550 0.0 Std Dev SEM 92 th 92 th 25 2 2 20 4376 87: -11.4 19.0 - CAR ... Concentration (logM) Antagonist: % of 1 nM DHT (Normalized value of each weil/mean value of normalized mean (induced control) Octoorviene ant Iblank 110nM DHT inea control linduced cont <u>-6.5</u> .6.0 .5.5 .3 Fold Induction (1000nMDHT) -6.5 130.7 125.8 126.8 138.2 60.0 97. 103.4 136. 57.4 1nM DL 118.8 117.0 113.6 98.3 131.2 104.5 52.3 67.2 58.3 15. 11. 18. th 1nM DHT th 1nM DHT th 1nM DHT 50.0 122. 102. 40.0 117.2 115. 94.2 117.2 130.1 136.5 128.4 53. h 1000nM DH 30.0 87 oldind 20.0 ntagonist % of Max -6.0 10.0 -5.5 -5.0 4.5 4.0 -3.0 -6.5 -3.5 0.0 100.0 Mean Std Dev SEM -2.7 126. 0.0 2000 25 25 25 25 25 25 25 25 25 130.4 109.2 STATE COMPANY 100MB CBAT 0.0 20 92.8 7.1 High Agoni Maximal 100. 107.2 111.2 132.1 Mean Std Dev SEM -2.4 18.9 9.6 Concentration (logM) 3.6 0.0 6. Octocrylene ant Differential -6.5 -6.0 -5.0 -4.0 -3.5 -3.0 -5.5 -4.5 0.4 -25.4 92.8 Viability (% Control -5.5 -5.0 22.9 57.6 -5.1 Relative inhibitory Con 100.0 61.3 -5.5 -5.0 ntration Max (RICMax) 38.7 DIffe Differential IC 22.9 neg. cont in 100 Mean 100 49 StdDev SEM y = 69.395x + 404.6 R<sup>2</sup> = 1 Differential IC30 y = 69.395x + 404.6 Offerential IC60 70.0 70.0 60.0 60.0 Fit >-5 #Ok 50.0 50.0 Antagonist % of Maximal Induction Control (Average) 40.0 40.0 0 Bottom 124.6 Top -5.0 ECS0 -1.5 Hill slope ist % of Maximal Inducion Control (Average) 120 30.0 30.0 100 80 60 20.0 20.0 10.0 10.0 0.0 0.0 40 -5.5 -5.4 -5.3 -5.2 -5.0 -5.4 -5.6 -5.1 -4.9 -5.6 -5.5 -5.3 -5.2 -5.1 -5.0 -4.9 20 05 -5.5 oncentration (LogM) <-6.5 #Ok Fit Chart High Agonist control. % of Maximal Induction Control (Average) 0 Bottom 124.9 Top -7.0 EC50 1.7 Hill slope . 120 100 80 60 40 20 Vaximal 1 Average) Ontrol 0.5 -5.5 Concentration [LogM] 4

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Experiment date: 13oct2011 Spreadsheet locked on: 11/10/2011 blank - no cells, vehicle control TopCount Model B9912V, Serial# 408672 neg. control - cells + vehicle. Study Number: 9070-100 Green shaded areas unlocked cells for data entry 11/11/11 13:23 9070-100107 Assay Conducted by Oxybenzone Compound FOLD INDUCTION blank 10nMDHT neq. control neq. control -5.5 -5.0 -4.5 -4.0 10nM DH neq. control neq. control -6.5 -6.0 -5.5 -5.0 -4.5 -4.0 -3.5 -3.0 50 1291050 100 1415950 28650 21400 31400 25450 23850 24400 3435 2965 28100 24050 3200 100 41.3 0.8 0.8 11 0.9 0.9 0.7 0.9 0.8 0 0.0 25400 3650 1300 29850 30600 3560 4585 27800 45.3 1.0 1.0 1.1 1.5 10 0.8 0.9 0.8 0.1 0.0 46100 31200 155 315 56.5 48.7 1.5 1.0 50 176530 100 152255 32100 33900 33100 3660 33300 29650 31250 2550 3950 4050 1.0 1.0 0. 0. 50 164865 31700 31150 4145 4825 35600 31750 33250 27400 3450 420 52.8 1.0 1 1 0 176950 40250 34350 43200 3300 56.6 0.1 31550 28550 29150 33400 31800 1.3 1.1 1.4 0.9 0.9 1.1 10 0 0 00740 345 300 9670 3845 15045 595 448300 26720 3800 20350 1020 Ith 10µM Nilutamide Std Dev 6.2 0.1 0. 0.2 0.1 0.1 0.1 0.1 0.0 0.0 SEM 2.5 0.0 0.0 0,1 0.0 0.1 0.0 0.0 0.0 0.0 Mean Std Dev SEM 58 1568833 43292 31192 27992 CV% 31238 36842 30767 27567 3600 2533 12.4 13.0 7.6 15.0 9.0 14.0 7.9 11.8 9.0 56.2 38 193864 15 79145 4241 2782 2819 1151 3916 1599 2419 987 346 142 100 2.0 2.3 2.8 2.0 1.8 2.0 1.8 0.2 0.2 6881 3251 Relative Tra 1224 280 1327 141 58 56.2 CV% 64.5 12.4 13.58 7.6 15.0 9.0 14.0 7.9 11.8 9.6 0.0 Oxybenzone ag 60.0 Relative Transcriptional Activity Rows G&H 50.0 82413 
 69850
 67575
 91150
 48550
 251850
 152900

 45538
 41189
 83863
 15486
 277822
 161645
 Mean 0 552475 3525 3225 Std Dev 0 162528 63025 389 \$40.0 Mean VC 31238 Mean Nilutamide Control 82413 30.0 Subtraction of VC from wells blank 10nMDHT neg. control neg. control -51 -4.0 2 20.0 50 1259813 100 1384713 50 1734063 100 1491313 50 1617413 0 1738263 3113 4363 5363 7463 10213 3113 -7188 -5838 -5738 13 -3838 563 -3138 -3438 13 -438 -6838 -638 -2588 163 -30238 -7388 -158 -983/ -28038 14613 14863 15463 -9838 -5788 -38 -2238 513 -2088 -1388 -27588 -29938 -638 2663 1863 -88 313 863 1163 2063 -27288 -29688 10.0 -27188 -28088 -1588 17013 4363 2013 2163 463 0.0 173826 9013 -2688 -27938 -27238 tonth OHT 5° 5° 5° 4° 4° so 30 00 78388 1963 -22913 -78613 -79163 5849 2363 1428 18478 -78963 with 10µM Nilutamide -79413 with 10µM Nilutamide 189.001 35513 -43813 -27013 -50563 Concentration (logM) Corrected Data Means blank 10nM DHT neg. control <u>-5.0</u> <u>-4.5</u> <u>-4.0</u> <u>-3.5</u> <u>-3.0</u> -3246 -471 -3671 -27638 -2870 -6.5 -6.0 -5.5 Oxybe 58 1537596 38 193864 15 79145 Mean Std Dev 5604 12054 -2870 -46 4241 2819 3916 1151 1599 2419 3251 987 1327 346 1425 278 6881 Oxybenzone ag 1136 SEM 280 141 120 04.5 12.0 57.1 -0150.8 -120.7 -513.7 -88.0 -1.3 -5.0 49.0 CV% AN 100 Relative Transcriptional Activity 1.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 Agonist: % of Maximal Induction Control 80 blank 10nM DHT neg. control neg. control -6.5 -6.0 -5.5 -5.0 4.5 -4.0 -3.5 benzone ad -0, -0.2 81. -0.5 60 0.0 90.1 -0.1 0.0 -0.4 -0.2 -1.8 0.0 0.0 112.8 0.1 0.2 10 0.1 0.0 0.0 -0.4 -1.8 97.0 0.1 -0.1 0.0 -1.8 2 40 0.0 105.2 0.0 0.0 0 0.3 0.0 0.1 -1.8 -1 % Relativ 0.0 113.1 0.6 0.0 -0.2 -0.1 0.1 0.0 -1.8 -1.8 0.3 0.8 20 0.0 1.5 -1.5 23.8 12.0 -5.1 -5.1 with 10µM Nilutamide 5.1 G 38.0 4.4 -5.2 with 10µM Nilutamide 0.0 23.1 -3.5 -3.2 -3.3 -2.9 -1.8 -2.8 -5.1 0 % of Maximal Induct 10th DH \$ \$° \$° \$° \$° \$° \$° 00 Oxybenzone ag blank 10nM DHTne control <u>-6.5 -6.0 -5.5 -5.0 -4.5 -4.0 -3.5</u> -3.0 Mean 0.0 100.0 12.6 5.1 0.0 -0.2 -0.2 100 Std Dev Concentration (logM) 0.0 0.3 0.4 0.2 0.3 0.2 0.2 SEM Viability (% Control) PC10 PC50 Mean 106 101 10 StdDev SEM %CV 4.4 PC10 PC60 1.2 1. 1.0 #Interse 1.0 FIt Chart #Ok 0.8 0.8 Oxybenzone ag 120 0.6 0.6 0 Bottom 0.4 Top -5.7 EC50 100 0.4 0.4 -71.9 Hill slope 80 0.2 0.2 60 0.0 -0.0 -40 0.0 0.2 0.4 0.6 0.8 1.0 1.2 0.0 0.2 0.4 0.6 0.8 1.0 1.2 20 맞 -5 Concentration [LogM] -3

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Experiment date: 20Oct2011 TopCount Model B9912V, Serial# 408672 Spreadsheet locked on: 11/10/2011 Green shaded areas unlocked cells for data entry blank - no cells, vehicle control neg. control - cells + vehicle. 11/11/11 13:23 Study Number: 9070-100107ARTA Assay Conducted by Compound Oxybenzone FOLD INDUCTION -7.0 blank 10nM DHT neg. control neg. control -4.5 -4.1 10nMDH neg. control neg. control -7.5 -6.5 59.4 59.7 64.2 725 0.9 1.0 0.9 1 54900 55145 8400 8700 0.9 1.0 870 945 880 6900 890 0. 0.9 1.0 0.8 0.4 9700 7200 800 750 750 655 59370 47810 9450 9250 12200 9250 9300 770 725 1000 78 51.7 D 8600 1180 795 990 795 1.0 1.2 0.9 1.0 1.3 0.9 0.9 0.8 0.8 9000 8350 11650 9800 7900 54.0 65.5 49945 920 895 1290 990 9000 9550 1.0 1.3 1.1 1.4 1.0 0.8 895 1.0 1.0 E 1.0 0. 790 0.0 10 10 11 11 0.9 1.0 0 0.1 64700 14450 G 3646 62900 493 481 ith 10µM Nilutan 59 with 10µM Nilutamide Std Dev 5.4 0.2 0.1 0.2 0.1 0.1 0.1 0.1 0.1 0. SEM 2.2 0.0 0.1 0.0 0.1 0.0 0.0 0.0 Mean Std Dev SEM CV% Relative Transcr Rows G&H 20.2 
 0.8
 18.4
 11.6
 5.1
 9.9
 10.3

 1.7
 1.9
 1.4
 1.7
 1.5
 1.4
 33 54618 9242 9708 9233 10300 7625 9442 8200 7675 735 CV% 9.2 9.2 10.5 6.4 41 50238 854 881 807 100 1961 629 1896 479 811 47 Relative Tran 17 13 330 10.5 247 9.24 774 360 11.0 17 2051 801 257 196 331 19 122.5 20.2 18.4 0.4 9.2 Oxybenzone 70.0 0.0 0.0 10 0.0 0.0 0.0 0.0 0.0 0.0 0.0 60.0 Mean Std Dev 44525 32969 25 323200 35 58548 40700 31396 35200 37350 21779 26304 17525 37075 33325 25450 1803 17359 20966 13506 982 46 50.0 Mean VC 9242 Mean Nilutamide Control 44525 \$40.0 Subtraction of VC from wells B 30.0 -4.0 -1992 -1942 -1242 -1242 -1742 -1742 -2692 10nMDHT neg. control neg. control blank -7.0 -6.5 -50 -4.5 -742 -1042 458 1708 -42 -292 35775 -842 -542 -2042 -1942 -1342 -2692 1358 -342 -1542 2558 3658 658 -542 1408 208 -2342 -1842 -2942 408 -392 758 658 -242 -442 -1792 -1392 539758 -542 -892 20.0 54220 208 50 58445 2958 -1992 10.0 -1292 -292 -1342 -1292 308 -1642 46885 -642 58 2408 208 49020 -242 558 758 0.0 596158 10mm DHT o. o. o. o. o. 16 10 50 with 10µM Nilutamide with 10µM Nilutamide 2017 18375 60 1142 482 -9525 -3437 G 36 3007 do 258 247 -257 -350 .00 Concentration (logM) Corrected Data Means Oxybenzo -6.0 -5.5 -5.0 blank 10nM DHT -7.5 -6.5 -4.5 -4.0 ea, control -7.0 33 536942 467 -1617 -1042 -1567 -189 Mean 105 200 Std Dev SEM CV% 41 5023 854 1961 1896 881 479 811 807 47 Oxybenzone 801 420.2 257 -7548.2 774 360 -54.5 196 331 -77.9 330 -51.5 17 20510 247 193 120 122.5 9.4 179.2 239.5 AND 100 1.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 Relative Transcriptional Activity 0.0 Agonist: % of Maximal Induction Control 2 80 blank 10nM DHT neg. control neg. control -6.5 -4.0 -4.5 100.5 0.1 -0.2 -0.4 0.0 -0.1 0.0 -0. 0.3 -0.3 -0.1 -0 F 60 -0.4 -0.1 0.0 0.3 -0. T 108 0 0.6 0 -0.3 -0.4 0 1 -0 -0.4 -0.3 E 40 0.0 87.3 0.0 0 -0.1 0. 0. -0.: 0.1 -0.4 -0. 0.0 91.3 111.0 -0.3 0.0 0.4 0.7 0.0 -0.2 0.0 0.1 -0.1 0.1 20 % Kelativ 0.0 -0.2 -0. 0.0 0.1 0. -0.2 0.0 -0.3 -0.5 0.0 59.6 3.8 3.4 1.1 -4.8 0.9 0.7 -1.8 -6.4 V with 10µM Nilutamide G 6 2 0.0 44.2 -5.6 -4.8 -4.8 -4.6 -4.8 -5.3 -3.7 -4.8 -5.3 -6.5 with 10uM Nilutamide 0 H % of Maximal Induc 10mm OH res.control A.O 15 10 05 00 55 50 25 Oxybenzone -7.0 -6.5 -6.0 -5.5 -5.0 -4.5 -4.0 blank 10nM DHT eg. control 7.5 Mean Std Dev SEM 0.0 100.0 0.0 0.1 0.0 0.2 -0.3 0.0 -0.2 -0.3 0.0 9.4 3.8 0.2 0.4 0.1 0.4 0.2 0.2 0.2 Concentration (logM) 0.0 Viability (% Control) PC10 PC50 M DHT neg. con neg. con -7.5 Mean 97 8 99 103 QC 10 StdDev SEM %CV 5.1 2.8 PC60 PC10 1.2 1.2 #Interse 1. 1.0 Fit ct #Ok Chart 0.8 0.8 Oxybenzone 120 0 Bottom 06 0.6 0.1 Top 100 80 60 40 -7.3 EC50 -1.2 Hill slope 0.4 0.4 0.2 0.2 0.0 0.0 0.0 0.2 0.4 0.6 0.8 1.0 0.2 0.6 1.2 0.0 0.4 0.8 1.0 1.2 20 -8 -4 -6 Concentration [LogM] -5

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Concentration [LogM]

Experiment date: 3Nov2011 TopCount Model B9912V, Serial# 408672 Spreadsheet locked on: 11/10/2011 Green shaded areas unlocked cells for data entry blank - no cells, vehicle control neg. control - cells + vehicle. 11/11/11 13:23 Study Number: 9070-100107ARTA Assay Conducted by Compound Oxybenzone FOLD INDUCTION -6.5 -4.0 -40 10nMDHT neg. control neg. control -6.0 55 45 10nMDH neg. control neg. contro -70 -6.5 -60 -55 45 4750 4400 4100 5100 5250 1.0 0.9 1.0 1.1 1.0 3750 4800 3450 3400 47.6 0.8 1.0 204950 2800 450 380 425 650 0.6 5100 5450 375 455 3800 5700 500 580 62.6 1.1 0.9 1.5 1.3 1.2 26975 5150 6650 6350 515 0.9 690 65.6 1.2 1.3 1.3 1.3 1.5 1.3 3500 4600 4250 4750 5200 4050 4650 4700 5600 445 460 5100 4350 4650 4550 5400 64.9 27960 570 4800 4250 3850 8350 555 555 390 465 705 5350 62.1 1.1 1.1 1.0 1.2 1.9 0.9 1.0 26775 695 4500 6750 65.6 1.0 1.3 0.9 1.6 1.0 1.6 1.1 1.1 G 24250 101 89 140 1340 th 10uM Nilutam e Std Dev 6.9 0.2 0.2 0.3 0.1 0.1 0.2 0.1 0.2 0.2 0.0 2.8 0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1 Mean Std Dev 4467 730 6375 1242 CV% 11.3 Relative Trar 100 18.9 
 13.1
 16.3
 18.4
 19.2
 19.5
 15.7
 13.5

 1.7
 1.7
 2.2
 1.7
 2.4
 2.1
 1.8
 9.8 264508 29867 4308 814 4592 883 5683 893 4725 639 4758 4592 1.6 1.8 SEM CV% Relative Transcriptional Activity 190 12193 235 245 298 10.3 437 361 507 365 261 13.5 11.3 18.4 19.2 19.5 15.7 9.8 Oxybenzone Ag 70.0 1.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 Rows G& 60.0 Mean 89225 13825 18175 14885 18350 14496 9325 3712 8100 12150 3536 12325 18000 7000 Std Dev 30724 8076 50.0 Mean VC 4308 Mean Nilutamide Control 13825 \$40.0 Subtraction of VC from wells 30.0 blan 10nMDHT -5.5 control -6.5 -6.0 -5.0 -4.5 -4.0 -208 792 942 792 42 342 -508 242 1492 242 -58 -458 442 2342 200642 -150 -58 842 -908 -508 2192 842 -558 492 -858 442 892 -258 192 -558 42 342 392 1292 20.0 265442 792 692 278192 1142 1442 1392 2042 2592 1492 10.0 1392 2742 2642 492 1042 192 -808 292 -58 1092 4042 292 -408 342 275292 142 1242 263442 0.0 -278192 2442 10mh DHT res.control 70 8.9 8.9 5° 5° 2° 2° 19 1042 232 1487 1477 -4875 825 -425 1437 -5275 ith 10µM Nilutamide 97125 th 10uM Nilutamide Concentration (logM) **Corrected Data Means** blank 10nM DHTneg control -7.5 -7.0 -50 -45 Oxybenzone Aq Mean -6.5 -6.0 -5.5 -4.0 450 466 190 103.0 2067 260200 283 158 150 283 1375 417 Std Dev 29867 814 601 730 883 1242 893 639 10 Oxybenzone Ag SEM CV% 12193 11.5 245 298 461.0 361 507 365 261 153.3 235 437 120 71.3 AUNTON 100 1.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 Relative Transcriptional Activity 0.0 Agonist: % of Maximal Induction Control 2 80 Oxybenzone Ag blank 10nMDHT neg. control neg. control -6.0 -5.5 -5.0 -4.0 -7.5 -7.0 -6.5 -4.5 0.2 -0.3 0.8 0.0 77.1 0.1 -0.2 0.0 0.0 -0.1 -0.6 -0.2 0.3 60 0.2 -0.2 0.3 0.0 102.0 0.3 -0.2 0.3 0.3 0.0 106.9 04 -0.3 0.0 0.6 0.6 0.5 10 0.6 E 40 0.0 105.8 -0.3 0. 0.1 0.1 0.5 0.2 0.4 0.1 0. 101.2 0.0 1.6 0.0 0.0 0.1 0.3 0.2 0.4 0.5 -0.2 20 1.1 0.0 0.0 -0.1 0.5 1.0 0.1 0.5 0.1 0. Γ 0.0 37.3 4.0 0.9 5.7 -0.7 -1.9 0.3 -0.2 5.5 10µM Nilutamide 5 -21 0.0 -2.5 2 20.6 -2.3 -2.6 -2.4 -2.2 -27 -1.6 -1.0 -2.3 -3.2 ith 10µM Nilutamide 0 % of Maximal Indu on Control NORTH OFF 189. Control 10 10 00 00 00 00 00 00 00 00 blank 10nM DHT neg. control -7.5 -7.0 -6.5 -6.0 -5.5 -5.0 -4.5 -4.0 Oxybe 0.8 0.3 0.0 0. 0. 0.1 0.5 0.2 Std Dev Concentration (logM) 0 0: 0.; 04 0.1 SEM 0.0 0.1 0.1 0.1 0.2 0.1 0.2 0 1 0 1 Viability (% Control) PC10 PC50 ne Ag 10nM DHT neg contineg cont -6.5 -5.5 -5.0 121 Mean 116 116 113 107 121 124 StdDev 12 9 SEM %CV 20 PC10 PC60 1.2 1.2 #Interse 1.0 1.0 FIt ct Chart #Ok 0.8 0.8 Oxybenzone Ag 120 0.6 0.6 0 Bottom 100 0.4 0.4 80 0.2 0.2 60 0.0 0.0 40 0.0 0.2 0.4 0.6 0.8 1.0 1.2 0.0 0.2 0.4 0.6 0.8 1.0 1.2 5 20 -8 --7 -6 -5 Concentration [LogM

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Spreadsheet locked on: 11/10/2011 Green shaded areas unlocked cells for data entry





Experiment date: 20Oct2011 blank = no cells vehicle control Spreadsheet locked on: 11/10/2011 TopCount Model B9912V, Serial# 408672 neq. control - cells + vehicle. Green shaded areas unlocked cells for data entry 9070-100107ARTA 11/11/11 15:00 Study Number: Assay Conducted by Comp une DHT FOLD INDUCTION 
 10nMDHT neg. control
 neg. control

 0
 755950
 11550
 12450
 10nM DH neq. control neq. control -9.5 blank -11.5 -11.0 -9.0 -11.5 -11.0-10.5 -10.0 -8.5 -8.0 62.5 16.1 67.9 59.3 15900 22050 48900 19435 821000 67890 717450 1.3 1.8 4.0 56.1 53360 1.0 44.1 66260 12550 105 1855 20750 49400 21315 56730 821200 88525 74045 54.8 1.5 1.7 4.1 17.6 46.9 67.9 73.2 61.2 66.9 62.2 64.3 24100 18110 743850 80985 622000 75245 15.0 52.8 18.5 41.3 61.5 51.4 66.3 62.5 68.3 66840 12250 1280 1220 165 6755 5330 6388 80275 75635 55.2 54.4 1 0 1.4 20 5.6 50020 1740 4.4 1.4 13500 11450 19000 19600 59150 24840 549700 680400 77750 82650 52.5 1.6 4.9 20.5 56.3 6356 45.4 71695 13200 9500 18250 21800 58750 19610 57535 702300 821300 810400 59.3 15 4.9 16.2 47.5 58. 67.9 67.0 1.8 vith 10µM Nilutamide Mean vith 10µM Nilutamide Std Dev 17.3 64.1 3.6 1410 504 248 4192 56 46. 60 65. 16950 1663 4.6 0.2 0.6 5.8 3. 3.9 6.6 0 0.1 SEM 1.5 0.0 0.0 0.1 0.2 0.8 1.6 2.4 
 0.0
 0.4
 12.6
 11.6
 8.3
 10.0
 8.0
 5.6

 2.6
 3.1
 8.2
 30.7
 82.1
 107.1
 115.3
 113.6
 6.5 33 683042 12100 17608 21125 56175 209500 560833 731792 787542 775650 CV% 9.7 Mean Std Dev 61 4458 1172 1210 1989 7099 24288 46650 79577 69742 43650 Relative Tra 100 1.8 SEM 1820 494 812 2898 9916 19045 32487 2847 1782 181.7 9.09 9.4 12.0 11.0 8.3 0.8 10.9 8.9 DHT CV% 0.5 0.9 5.0 1.0 0.0 0.1 0.3 1.1 80.0 Relative Trans Rows G&H 70.0 Mean 14450 38950 40350 26125 71700 120375 336975 100 292725 27625 44700 Std Dev 71 48826 19725 35921 495 27719 26941 1874 41366 64948 116284 60.0 50.0 Mean VC 12100 Mean Nilutamide Control 27625 240.0 Subtraction of VC from wells 8 30.0 blank 10nMDHT -11.5 eq. control n control -11.0 9 74385 -550 350 -1550 3800 9950 36800 182250 521500 808900 666800 705350 450 6450 8650 37300 873150 20.0 -20105 555200 809100 728350 65050 700 100 -650 65630 150 1100 4450 5300 12000 55450 41200 169000 626750 731750 797750 790650 10.0 64665 6350 488100 609900 740350 1400 7500 47050 236300 537600 668300 765400 814400 62350 6900 0.0 70485 1100 6150 4665 18400 56325 690200 80920 798300 10th OH 0.1 10. 10.5 100 95 -2600 9700 control 90 85 00 -282 -10675 4247 -13525 3092 3173 73325 13867 391575 with 10µM Nilutamide 227125 with 10µM Nilutamide ..... Concentration (logM) Corrected Data Means DHT blank 10nM DHT -11.5 <u>-11.0</u> <u>-10.5</u> <u>-10.0</u> <u>-9.5</u> <u>-9.0</u> <u>-8.5</u> <u>-8.0</u> eq. control 9025 44075 197400 548733 719692 775442 763550 1989 7099 24288 46650 79577 69742 43650 33 670942 Mean Std Dev 0 1172 338 5508 61 4458 1210 DHT SEM 25 18201 494 812 2898 9916 19045 32487 28472 17820 140 
 22.0
 10.1
 12.3
 8.5
 11.1
 9.0
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 0.0
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 1.1
 1.2
 1.1
 22.0 CV% 181.7 0.0 CINIDA 120 Relative Transcriptional Activity 1.0 0.0 Agonist: % of Maximal Induction Control 100 blank 10nM DHT neg. control neg. control -115 -11.0 -10.5 -10.0 -9.5 -9.0 -8.5 -8.0 80 1.5 5.5 27.2 30.0 77.7 120.6 82.7 120.6 0.0 110.9 -0.1 0.1 0.6 99.4 105.1 97.0 -0.2 130.1 108.6 60 97.8 96.4 92.9 25.2 31.6 35.2 93.4 72.7 80.1 109.1 90.9 99.6 118.9 117.8 110.3 110.9 0.0 0.1 0.7 1.8 8.3 2 0.2 0.0 0.9 6.1 40 -0.1 1.0 114.1 121.4 0.0 0. 105. 0.2 -0.4 0.9 1.4 7.0 27. 83.9 102.9 120.6 119.0 10 20 44.1 -1.6 4.4 6.3 4.6 4. -0.4 10.9 20.7 58.4 v 33.9 v th 10µM Nilutamide -2.0 34.4 -0.9 H 0.0 th 10uM Nilutamide \* 0 % of Maximal Induction Control 10res OFT control 115 110 105 100 9° 9° 8° 20 blank 10nM DHT control -11.5 -11.0 -10.5 -10.0 -9.5 -9.0 -8.5 -8.0 DHT 100.0 Mean 0.0 0.0 0.8 1.3 6.6 107.3 , and 10.4 Std Dev 6. 7 6.5 Concentration (logM) SEM 0.0 27 0 1 01 0 1 04 1.5 2.8 4.8 42 27 Viability (% Control) -10.0 -10.5 -10.0 -9.5 PC10 29.4 PC50 29.4 81.8 IOnM DHT neg. conineg. con -11.5 -11.0 -10.5 -10.0 -9.5 6.6 -10 -0.8 Mean 100 104 102 101 98 104 103 103 SEM 5 y = 45.704x + 486.47 y = 104.73x + 1076.7 %CV PC60 PC10 R2= 1  $R^{2} = 1$ 35.0 90.0 80.0 30.0 -9.8 FIt 70.0 Chart #Ok 25.0 60.0 DH 120 20.0 50.0 0 Bottom 114.9 Top -9.7 EC50 100 40.0 15.0 30.0 80 1.7 Hill slope 10.0 20.0 60 5.0 10.0 40 0.0 0.0 -10.5 -10.4 -10.3 -10.2 -10.1 -10.0 -9.9 -10.6 -9.9 -10.1 -10.0 -9.8 -9.7 -9.6 -9.5 -9.4 20 -11.6 -10.6 -9.6 -8.6 Concentration [LogM

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Experiment date: 13oct2011 blank - no cells, vehicle control Spreadsheet locked on: 11/10/2011 TopCount Model B9912V, Senal# 408672 neq. control - cells + vehicle. Green shaded areas unlocked cells for data entry 11/11/11 15:00 Study Number: 9070-100107 Nilutamide (Nil) Assay Conducted by: Compound FOLD INDUCTION 10nM DH neg. control neg. control 10nMDHT neg. control neg. control -6.5 -6.0 -5.5 -5.0 -4.5 -4.0 -7.5 -7.0 -6.5 -6.0 -5.5 -5.0 -4.5 -4.0 1302000 22300 2585 38000 20300 16100 15600 23250 32400 57850 800 49.8 0.9 1.5 0.8 0.6 0.6 0.0 12 23 0.0 1212000 24350 2120 3760 3645 1805 1570 2250 12000 24550 23000 29100 32250 47750 1900 46.4 0.9 0.8 1.4 0.7 0.6 0.5 0.9 1.1 1.8 0.1 48.0 0.1 1.4 0. 0.9 1. 136615 29150 2735 3950 2945 2260 15450 21250 33900 51900 2950 52.3 15 0.6 0 33200 25550 36850 38750 2240 1860 15450 18050 24950 21400 55800 59600 2150 1544450 27700 2860 3335 59.1 11 1.4 11 0.9 0.6 10 1.3 21 0.1 57.5 0.1 1501000 24000 28000 35400 0.9 1.5 1.1 0.7 0.7 0.8 1.4 2.3 33400 700 7145 535 6415 th 10µM Nilutamide 0.6 6780 3550 5350 3500 12 tean 52.2 ith 10µM Nilutamide Std Dev 5.1 0.1 0.0 0.2 0.1 0.1 0.1 0.1 0. 0.0 SEM 2.1 0.0 0.0 0.1 0.1 0.0 0.0 0.0 0.1 0.0 58 1363242 74 134472 26121 3183 37858 24233 19650 15258 4993 3277 1932 23067 1540 32733 54100 2117 4430 2158 959 CV% Relative Tra 9.9 100 12.2 
 3.0
 20.0
 16.7
 12.7
 6.7
 6.5
 8.2
 44.4

 2.8
 1.8
 1.4
 1.1
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 2.4
 4.0
 0.2
 Mean Std Dev SEM CV% 2038 1338 789 16.7 12.7 54898 919 629 864 1809 391 468 126. 12.18 44.4 9.9 3.0 6.7 0.5 8.2 Nilutamide ag 60.0 Relative Transcriptional Activity 10 0.0 00 00 00 00 00 00 00 Rows G&H 51200 49250 41925 33300 54625 52350 28638 26234 16370 3111 13470 1626 50.0 Mean Std Dev 43213 24001 40000 1325 50 404125 71 54058 540.0 Mean VC 26121 Mean Nilutamide Control 43213 2 30.0 subtraction of VC from wells -4.5 -4.0 2 20.0 -2871 -1571 -3121 31729 21629 25579 25779 1275879 -382 -1002 10521 6279 -25321 -27 11879 -582 -1771 11479 -8071 -10421 -3621 -3521 -14121 2979 6129 7779 7229 9279 -24221 118587 -4921 1227729 10.0 1340029 3029 1229 13379 3329 -10671 -487 -23171 1579 7079 2479 -3721 -1171 29679 33479 1518329 10729 -10671 -23971 0.0 1474879 1262 -8071 -2262 10mm DHT 15 10 00 00 00 50 AS 40 3583 -42013 322688 399138 -9813 245 102 -7713 -8213 1788 th 10µM Nilutamide 282 20938 10288 -1226 -1251 -1286 -41763 th 10uM Nilutamid н Concentration (logM) 100 Corrected Data Means Nilutamide aq blank 10nM DHT ne control -5.5 4.5 -4.0 27979 -23963 4430 959 -1888 -6471 -10863 -3054 Mean 58 1337121 1173 6613 74 134472 1147 4993 3277 1932 1540 2117 959 391 Std Dev 3183 Nilutamide a SEM 133 789 629 864 54898 91 203 180 120 46 CV% 120.2 10.1 9.8 -204.5 -50.0 -17.8 -50.4 32.0 15.8 -4.0 AVI 100 Relative Transcriptional Activity 1.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 Agonist % of Maximal Induction Control 80 blank 10nMDHT neg. control neg. control -7.5 -7.0 -6.5 -6.0 -5.5 -5.0 4.5 4. 95.4 -0.3 0.0 0.9 -0.4 -0.7 -0.8 -0.3 0.5 2.4 -1.0 0.0 60 0 88.7 91.8 -0.1 -0.4 0.9 -0.6 -0.8 -11 -0.1 0.2 1.6 -1.8 0.0 0.0 0.8 -0.4 -0.8 -0.2 1.9 -1.8 -0.3 100.2 -0.8 E 40 0.0 0.2 1.0 0.2 -0.3 -0.4 0.6 0.1 1.9 -1.7 0.0 113.6 0.1 0.5 0.8 02 -0 : -0.8 -0. 0.5 -1.8 Relative 2.5 0.0 110.3 -0.2 0.0 0.9 0.1 -0.6 -0.6 -0.4 0.7 -1.7 20 . -0.6 rith 10µM Nilutamide G 0.0 24.1 -0.7 2.7 2.1 1.8 0.8 -0.6 1.6 0.8 -3.1 0.0 29.9 -0.9 0.6 0.1 th 10uM Niluta \* ۰ ۵ % of Maximal In 10ma OHT res.control 1.5 1.0 as a o a b a o Nilutamide aq blank 10nM DHT ne control -7.0 -6.5 -6.0 -5.5 -5.0 4.5 -4.0 -7.5 Mean 0.0 100.0 0.0 0.9 -0.1 -0.5 -0.8 -0.2 0.5 2.1 Std Dev Concentration (logM) 0.0 10.1 0.2 0 1 0.4 0 1 0.1 0.1 0.: 0.3 0.1 0.0 0.2 0.1 0.0 0.1 SEM 0.0 4.1 0.1 0.1 Viability (% Control) PC10 PC50 Ide ac 10nM DHT neg. conineg. con -6.5 -5.1 104 103 97 Mean 10 102 84 100 98 StdDev SEM %CV PC60 PC10 1.2 1.2 1.0 1.0 #Interse Fit ct Chart #Ok 0.8 0.8 Nilutamide ag 120 0 Bottom 0.6 0.6 2.1 Top -4.9 EC50 Induction Control 100 0.4 0. 7.5 Hill slope 80 0.2 0.2 60 % of Maximal 0.0 0.0 40 0.0 0.2 0.4 0.6 0.8 1.0 1.2 0.0 0.2 0.4 0.6 0.8 1.0 12 20 -8 -4 -7 -6 Concentration [LogM] -5

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Experiment date: 13Oct2011 TopCount Model 89912V. 8eria# 408672 11/11/11 15:00 Assay Conducted by: blank = no cells, vehicle control neg. control = cells + vehicle. Study Number: 9070-100107ARTA Spreadsheet locked on: 11/10/2011 Green shaded areas unlocked cells fr cked cells for data entry 10nM DH neg. FOLD IN with 1nM DHT with 1nM DHT with 1nM DHT with 1nM DHT 774150 716400 766800 673400 17200 663050 6715 7035 6695 545 4375 5295 4440 4880 59.1 8996 801750 723250 667950 47370 51065 48065 16415 16500 14450 443 405 343 59.3 651400 8689 7360 13000 13850 57.6 48.8 52.3 58.8 58.2 33. 31.5 67.8 58.0 70.5 50.9 44.7 48.7 48.0 43.2 64.3 65.4 64.5 44.4 57.7 51.9 53.3 56.2 61.1 64.3 61.8 57.9 60.0 53.6 54.7 51.0 54.7 71. 73.7 94957 727699 674617 121 the Des 7320-47128 73564 56363 74767 37383 697 348 Std Dev 1.6 2.3 692 346 3036 1518 384 0.1 0.6 0.2 0.0 29.2 0.2 0.6 <u>10.0</u> 9.0 13.1 9.9 6.4 10.3 6.4 9.0 29.2 elative Tr 100 89.0 86.2 79.4 54.4 54.5 59.2 56.0 66.9 19.5 7.6 CV% Relative Transcriptional Ac 1.0 0.8 57.5 65 
 Mican
 56.0
 53.6

 Std Dev
 3.1
 3.1

 SEM
 1.5
 1.5

 CV%
 5.5
 5.0

 Relative Tran
 100
 96.2

 94.4
 94.3
 35.4
 60.5
 37.3
 67.4
 95.7
 46.1

 3.4
 6.1
 6.2
 6.2
 3.2
 4.5
 9.7
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 1.7
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 6.3
 0.4
 10.5
 0.2
 5.5
 0.7
 14.7
 6.2

 97.2
 97.3
 105.7
 119.6
 102.8
 119.6
 117.3
 82.4
 821125 891713 100910 Mean Std Dev SEM 46400 46721 23361 51791 77173 94024 25896 38586 47012 93210 46605 48018 67692 145853 24009 33846 72926 43072 21536 856 0.9 200.0 CV9 5.5 9.4 9.2 Mean VC 15075 Mean DHT 1000nM Control 811725 Fold Induction (1nM DHT 60.0 Subtraction of VC from well: 50.0 blank 10nM DHT neg. control In -15075 -15025 -15025 884575 878375 853875 2125 1175 -2075 817425 786675 708175 759075 647975 701325 757825 751725 636325 421675 458625 495575 173425 149075 149925 52075 55275 51875 31025 29275 25425 28675 37875 29325 -1417 -1397 -1357 tth 1nM DHT 40.0 with 1nM DHT with 1nM DHT 51875 39475 95447 97077 95727 33725 742625 654525 675175 122 658325 596025 465575 12942 19275 133 7209 30.0 845129 854179 766979 754279 773029 871929 861779 849875 736575 793625 719825 788775 906675 765625 831625 954625 1006875 858725 1048325 80232 91602 85747 89032 88962 92722 Ith 1000nM 0 -150 -150 -150 -527 -667 -472 h 1000nM ð 20.0 th 1000nM DHT C Corrected Data Mea /G cntrl 10.0 717613 659538 47128 69200 23600 <u>-5.5</u> 49675 6976 0nM DH1 834450 -6.5 0.0 1 -15038 741288 74767 460363 15046 2625 3240 428 -1376 a a a a a a a a Std Dev 7680 nes com 20 .0 38401 37383 1518 Concentration (logM) 9.2 10.1 6.6 10.5 6.6 12.0 14.0 19.0 13.2 02 0.1 0.0 tagonist: % of 1 nM DHT (Norm Fold Induction (1000nMDH) 10nM DHT neg. control induced con -7.5 -6.5 80.0 102.4 94.6 101.4 88.8 89.9 110.3 106.1 95.5 88.1 105.5 87.4 102.2 85.8 90.4 95.6 119.3 0.3 56. h 1nM DH 70.0 61.9 66.9 62.8 125.7 th 1nM DHT th 1nM DHT th 1nM DHT 115.2 97.3 60.0 4.0 60.0 50.0 40.0 20.0 10.0 40.0 -Η 106.7 95.8 81.7 108.9 98.5 113.2 92.0 99.1 107.2 114.4 121.2 111.1 1 Ξ 01.0 94.2 132 81.0 intagonist % of Maximal rol (A) 3 M DHT ne -7.0 4.0 blank -6.5 Mean Std Dev SEM 0.0 10.4 10.1 6.4 9.3 4.1 2.4 0.7 0.6 0.0 0.9 PRO COMPO ور ور ور ور ور ور ور ور 20 High Agonist of Mean Std Dev SEM rol (Averag 103.5 100.5 Conc tion (logM Nilutamide ant Differential -7.5 -7.0 -5.0 -6.5 -6.0 -5.5 -4.5 -4.0 0.1 -9.1 99.5 0.5 Viability (% Control -7.0 -7.0 -6.5 ential IC60 20.5 62.0 Relative inh Differential 10 Diffe InM DHT neg. con induced -7.0 -6.6 -6. Mean 100 101 103 10 StdDev SEM y = 83.052x + 601.85 R<sup>2</sup> = 1 3.6 y = 83.052x + 601.85 Differential 1030 70.0 70.0 60.0 60.0 Fit Chart -6.4 #Ok 1 50.0 50.0 ist % of Maximal Induction Control (Average 40.0 40.0 0 Bottom 98.7 Top -6.4 EC50 -1.5 Hill slope 120 30.0 30.0 100 20.0 20.0 ist % of Maximal Control (Average) 80 10.0 10.0 60 0.0 0.0 40 -7.0 -6.9 -6.8 -6.7 -6.6 -6.5 -7.1 -6.4 -7.0 -6.9 -6.8 -6.7 -6.6 -6.5 -7.1 -6.4 -6.5 Concentration [LogM] Fit Chart >-4.5 #Ok High Agonist control. % of Maximal Induction Control (Average 0 Bottom 114.4 Top -4.4 EC50 -8.6 Hill slop . . 120 . . 100 80 60 Control 40 20 0 -6.5 -5 tration (LogM

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Experiment date: 20Oct2011 blank - no cells, vehicle control Spreadsheet locked on: 11/10/2011 TopCount Model B9912V, Serial# 408672 neq. control - cells + vehicle. Green shaded areas unlocked cells for data entry 11/11/11 15:26 Study Number: 9070-100107ARTA Assay Conducted by Compound Nilutamide (Nil) FOLD INDUCTION IOnM DHT neg. control neg. control -6.0 -5 -4.5 10nMDH n -5.0 4.5 -4.0 -5.0 -4.0 ontrol neg. control -7.0 -6.5 -6.0 594800 825 995 855 455 3200 780 1165 2325 64.7 0.9 0.8 0.5 0.6 0. 1.3 2.5 0.1 625900 540850 9850 9400 1135 2395 2315 0.1 765 4600 520 470 3650 4050 700 68.1 11 0.8 0.5 0.6 0.4 58.8 0.9 0.8 0.6 0.5 0.4 2.5 1.1 1.3 506100 8950 9400 7250 8050 535 570 5050 8150 12150 2360 55.1 1.0 0.6 0.5 1.3 765 0.6 D 0.8 0.9 600950 11100 8150 5550 555 5650 8550 1205 2460 65.4 0.9 0.6 0.6 0 27 0.1 592200 9550 58 5000 690 5000 775 1310 188 64.4 0.9 0.6 0.5 0.8 0.5 0.8 14 2.1 0.1 5 with 10uM Nilutamide G 23070 13800 1585 1870 20300 251 4210 3/ lean 62.8 0.6 0. 2.5 0. Std Dev 4.8 0.0 0.1 0.0 h 10µM Niluta 0.1 0.1 0.1 0.1 0.2 SEM 0.0 2.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.1 Mean Std Dev 33 576800 41 44376 4433 945 11942 661 CV% Relative Tran 7.7 11.3 
 11.0
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 1.3
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 1.4
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 0.1
 9192 1042 5092 444 8158 913 22900 2053 7250 5550 746 592 183 1.6 SEM CV% Relative Transcriptional Activ 17 18116 301 326 181 304 386 373 270 838 122.5 7.7 11.34 21.3 11.2 5.5 11.0 8.7 13.4 9.0 30.9 Nilutamide 70.0 1.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 Rows G&H 60.0 Mean 0 228200 15363 15800 14875 17175 17325 24400 32450 20775 Std Dev 0 12021 133/ 1768 1379 2157 4207 1061 13647 3571 50.0 Mean VC 9192 Mean Nilutamide Control 15363 2400 Subtraction of VC from wells 30.0 blank 10nMDHT neg. control neg. control -4. intemide -6. -5.5 -942 -2242 -394: -5992 245 -8392 -4643 139 585608 80 1405 20.0 -2192 508 -1042 -642 -1442 658 208 -242 758 -642 208 -1542 -4592 -3692 -3992 -4492 -5542 -5142 2158 2158 50 616708 14758 -8442 531658 13958 -8892 10.0 -3492 -1542 -3842 -4142 2958 2858 14408 -8692 496908 591758 1908 -1942 -1042 -3642 -4192 -3542 15408 -8542 0.0 583008 -1142 -3342 229 -4192 3908 965 -8642 10mm OH 50 control 0.5 ×0 00 50 25 15 10 221338 488 4938 26738 -15063 -1563 1638 168 333 9788 793 th 10uM Nilutamide th 10µM Nilutam C89. Concentration (logM) Corrected Data Means Nilutamide blank 10nM DHT neg. contro -7.5 -7.0 -6.5 -6.0 -5.5 -5.0 -4.5 -4.0 Mean 567608 -1041 -4100 -364 -4758 945 -103 2750 661 -860 91 183 Std Dev 41 44376 1042 797 444 74 205 Nilutamide 270 SEM 18116 181 30 386 37 30 326 83 120 7.8 -41.1 -10.8 -20.5 -19.9 -88.3 24.0 15.0 CV% 122.5 -2.1 AU 100 Relative Transcriptional Activity 1.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 Agonist % of Maximal Induction Control 2 80 blank 10nMDHT neg. control neg. control iutamide -7.5 -7.0 -6.5 -6.0 -5.5 -5.0 -4.5 -4.1 -1.1 -0.2 0.4 0.0 103.2 0.1 -0.2 -0.4 -0.8 -0.7 -1.3 60 0.0 108.7 -0.3 -0.8 -0.1 -1.0 -0.4 0.4 0.1 2.6 -11 0.0 037 0.0 -0.1 -0.3 -0.7 J 8 -0.9 0.1 0.4 2.5 -11 0.0 87.5 0.0 0.0 -0.3 -0.6 -0.7 -0.2 2.5 £ 40 -1.5 104.3 -0.3 -0.2 -0.6 -0.0 -0.6 -0.1 0.5 0.0 2. % Relative 102.7 -0 -0 -0.7 -0 0 • -0 20 with 10µM Nilutamide 39.0 36.0 4.7 1.4 0.3 0.3 0.3 0.1 0.9 -2.7 0. % of Maximal Induction C 10mm OHT control 15 10 50 50 55 50 A. A. Nilutamide blank 10nM DHT neq. control -7.5 -7.0 -6.5 -6.0 -5.5 -5.0 -4.5 -4.0 Mean 100.0 -0.3 -0.7 -0.6 -0.8 -0.2 0.0 0.0 0.5 2.4 ..... Std Dev 0. 7.8 0.2 0.1 0.1 0.1 0.2 0: Concentration (logM) 3.2 0 1 SEM 0 1 0.1 0.0 0 1 0.1 0 1 0.0 Viability (% Control) PC10 PC50 -7.0 OnM DHT neg. conineg. con -75 -6.0 -5.5 -5.0 4.5 4.0 Mean 100 100 105 102 92 99 96 99 83 66 StdDev SEM %CV PC10 PC60 1.2 1.2 #Interse 1.0 1.0 FIL ct #Ok Chart 0.8 0.8 Nilutamide 120 0 Bottom 0.6 0.6 2.4 Top 100 -4.9 EC50 0.4 0.4 80 6.7 HIII slope 0.2 0.2 60 of Maxmail I 0.0 0.0 40 0.0 0.2 0.4 0.6 0.8 1.0 1.2 0.0 0.2 0.4 0.6 0.8 1.0 1.2 20 -8 -5 -4 Concentration [LogM]

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Experiment date:	13oct2011		blank - no cells, veh	Icle control	Spreadsheet locked on:	11/10/2011	
TopCount Model B9	912V, Serlal# 408672		neq. control = cells -	vehicle.	Green shaded areas uni	locked cells for data entry	
Assay Conducted by			Compound	PPDDE			
						FOLD INDUCTION	DN
ppDDE ag	DIANK 100MDH	neg. control neg. control 31850 2680	-7.5 -7.0 -5.5 68400 72100 74850	49700 47150	-5.0 -4.5 -4.0 30150 52050 46750	100	MDHineg.control neg.control -7.5 -7.0 -5.5 -5.0 -5.5 -5.0 -4.5 -4.0
8	100 1541100	36200 2840	0 97800 77600 86500	50650 45950	38950 58650 44000		46.5 1.1 0.9 3.0 2.3 2.6 1.5 1.4 1.2 1.8 1.3
C	0 1525350	36100 2940	0 93600 84300 89450	61550 55400	48600 71100 54500		46.1 1.1 0.9 2.8 2.5 2.7 1.9 1.7 1.5 2.1 1.6
E	0 1697650	30650 3660	0 97850 82900 95500	65400 51150	45900 60700 53300		51.3 0.9 1.1 3.0 2.5 2.9 2.0 1.5 1.4 1.8 1.6
F	100 1512000	34200 3865	0 102500 81700 90400	60300 55450	47150 70250 52900	4	45.7 1.0 1.2 3.1 2.5 2.7 1.8 1.7 1.4 2.1 1.6
GH	0 450400	28200 25750	0 100250 80450 70800 0 33950 31800 33050	120900 102000 A	70950 80150 40350 With 48300 01850 51450 With	h 10µM Nilutamide Mean 4 h 10µM Nilutamide Std Dev	46.8         1.0         2.5         2.5         2.6         1.8         1.6         1.4         1.9         1.6           2.3         0.1         0.4         0.2         0.2         0.1         0.2         0.2         0.1
						SEM	0.9 0.0 0.2 0.1 0.1 0.1 0.1 0.1 0.1 0.1
Mean Std Day	50 1549525	33108	91283 81183 86050	58775 51942	45158 61383 51342	CV%	5.0 11.1 13.5 7.0 8.8 11.8 8.8 11.2 12.6 9.5
SEM	18 31441	1064	5014 2322 3093	2838 1871	2062 3149 1991		
CV%	89.4 5.0	11.13	13.5 7.0 8.8	11.8 8.8	11.2 12.0 9.5		60.0 ppDDE ag
Rows G&H	nal Activity 1.0	0.0	0.1 0.1 0.1	0.0 0.0 0	0.0 0.0 0.0		
Mean	0 395525	41975	70100 56125 51925	79175 74675	59625 74000 45900		50.0
Std Dev	0 86090	27909	51124 34401 26693	67493 39492	16016 17183 7849		Ean
Mean VC	33108	Mean Nilutamide Contro	A 41975				9 <sup>40,0</sup>
Subtraction of VC	from wells						2 30.0 +
ppDDE ag	blank 10nMDH	neg. control neg. control	-7.5 -7.0 -6.5	-6.0 -5.5	-5.0 -4.5 -4.0		8
A	50 1441292	-1258 -630	8 35292 38992 41742	16592 14042	6042 19842 13642		· 20.0
C	0 1492242	2992 -370	8 60492 44492 53392 8 60492 51192 56342	28442 22292	5842 25542 10892 15492 37992 21392		10.0
D	50 1513542	392 184	2 54442 55392 46492	31942 23442	18092 21542 23492		
F	100 1478892	-2458 -349	2 64742 49792 62392 2 69392 48592 57292	27192 22342	12/92 2/592 20192 14042 37142 19792		0.0
G	0 414425	41775 -1177	5 64275 38475 28825	84925 60625	28975 44175 -1625 with	h 10µM Nilutamide	Joh other 1, 1, 2, 8, 8, 5, 5, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8,
Corrected Data Me	0 292675	-13775 -1622	5 -8025 -10175 -8925	-10525 4775	6325 19875 9475 with	h 10µM Nilutamide	Concentration (logM)
ppDDE ag	blank 10nM DH	neg. control	-7.5 -7.0 -6.5	-6.0 -5.5 -	-5.0 -4.5 -4.0		
Mean Std Dev	50 1516417	0	58175 48075 52942	25667 18833	12050 28275 18233		
SEM	18 31441	1064	5014 2322 3093	2838 1871	2062 3149 1991		120 - ppDDE ag
CV%	89.4 5.1		21.1 11.8 14.3	27.1 24.3	41.0 27.3 20.7		2
Relative transcriptio	nar Activity 1.0	0.0	0.0 0.0 0.0	0.0 0.0 1	0.0 0.0 0.0		§100 -
Agonist: % of Maxi	mai Induction Control						
ppDDE ag	0.0 95.0	-0.1 -0.4	<u>-7.5</u> -7.0 -6.5 4 2.3 2.6 2.8	<u>-6.0</u> -5.5 1.1 0.9	<u>-5.0</u> <u>-4.5</u> <u>-4.0</u> 0.4 1.3 0.9		8
В	0.0 99.4	0.2 -0.3	3 4.3 2.9 3.5	1.2 0.8	0.4 1.7 0.7		5 60 t
C	0.0 98.4	0.2 -0.	2 4.0 3.4 3.7 1 3.6 3.7 3.1	1.9 1.5	1.0 2.5 1.4		<u></u> <u> </u>
E	0.0 109.8	-0.2 0.3	2 4.3 3.3 4.1	2.1 1.2	0.8 1.8 1.3		2
F	0.0 97.5	0.1 0.4	4 4.6 3.2 3.8 8 4.2 2.5 1.9	1.8 1.5 5.6 4.0	0.9 2.4 1.3 1.9 2.9 -0.1 with	h 10uM Nilutamide	1 20
н	0.0 19.3	-0.9 -1.	1 -0.5 -0.7 -0.6	-0.7 0.3	0.4 1.3 0.6 with	h 10µM Nilutamide	
% of Maximal Indu	ction Control	neg control	-75 -70 -65	-60 -55 -	50 45 40		
Mean	0.0 100.0	0.0	3.8 3.2 3.5	1.7 1.2	0.8 1.9		man C soon is a second s
Std Dev	0.0 5.1	0.2	0.8 0.4 0.5	0.5 0.3	0.3 0.5		Concentration (logM)
SEM	0.0 2.1	0.1	0.3 0.2 0.2	0.2 0.1	0.1 0.2		
				<u> </u>		Viab	ibility (% Control)
	PC10	PC50				ppDi	DDE ag 10nM DHT neg. con neg. con -7.5 -7.0 -6.5 -6.0 -5.5 -5.0 -4.5 -4.0
							Mean 110 100 105 106 107 105 101 98 97 95 68
							StoDev 6 1 6 5 6 5 7 3 4 2 4
	PC10			PC60			%CV 5.3 1.3 6.1 4.9 5.2 4.5 6.6 2.8 4.5 2.5 5.9
1.2			12	a control			
1.0			1.0			Fit et	lerse 1
						Chart #Ok	
0.8			0.8				120 - ppDDE ag
0.6			0.6				0 Bottom
5						8	100 - 12.8 Top
0.4			0.4			ŏ	80 2. Hill slope
02						Prote Land	
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0.0			0.0				40-
0.0 0.2	0.4 0.6	0.8 1.0 1.2	0.0 0.2	0.4 0.6 0	0.8 1.0 1.2	ž	
						0 *	
				118			Concentration [LogM]

Study Number: 9070-100107ARTA

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th 1nM DH

with 1nM DH with 1nM DH with 1nM DH

FOLD INDUCTION 10nM DH 55.7

41. 43. 48.

ed con 38.8

40.8 40.7 39.4 50.0 42.1 42.6 44.3 43.8 45.6

44.0

47.6 64 0 45.8

57.3 52.3

5.

Spreadsheet locked on: 11/10/2011 Green shaded areas unlocked cells for data entry

blank - no cells, vehicle control blank = no cells, venicle control neg. control = cells + vehicle. Study Number: 970-100107ARTA Compound ppDDE

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1033250

665350 554050 523650

264800 237900

95430 95195

24750

9749

10196



Experiment date: 13Oct2011 TopCount Model B9912V. Serial# 408672 11/11/11 15:26 Assay Conducted by:



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FOLD INDUCTION

66

Spreadsheet locked on: 11/10/2011 Green shaded areas unlocked cells for data entry

blank - no cells, vehicle control neg. control - cells + vehicle.

Compound

Study Number: 9070-100107ARTA

DDDDE



Experiment date: 20Oct2011 TopCount Model B9912V, Serial# 408672

11/11/11 15:26

Assay Conducted by


# **Data Spreadsheets**



Study Number: 9070-100107ARTA

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# **Data Spreadsheets**

Experiment date: 3Nov2011 TopCount Model B9912V, Serial# 408672 Spreadsheet locked on: 11/10/2011 Green shaded areas unlocked cells for data entry blank - no cells, vehicle control neg. control - cells + vehicle. 11/11/11 15:26 Study Number: 9070-100107ARTA Assay Conducted by Compound DODDE FOLD INDUCTION -6.5 -4.5 -7.5 -6.5 -6.0 -5.5 10nMDH1r eg. control n control -7 -7.0 -4. 10nMDH -4.5 control 22845 4050 400 460 6450 620 8400 13400 1800 1255 52.5 1.4 2.7 2.9 3900 4650 5200 3700 9200 9450 11100 2994 5150 3050 4750 5000 520 405 7150 1065 1315 68.8 0.7 1.3 0.9 2.4 3.0 2569 4100 4850 505 5300 600 1075 1800 59 1.1 2.5 1430 1.2 32545 4250 4600 470 4900 7100 5300 9800 825 74.7 12 1.1 1.6 23 1.9 3.3 8550 7200 10500 12150 72.8 2.4 31710 510 5100 5700 4300 1.2 1.0 3.4 1.3 2.0 380 435 4400 1740 2915 4850 3600 3450 7250 1 1321 735 15700 9000 015 600 2775 I0uM N 65. 1.1 Std Dev 8.5 0.5 0.3 0.3 0.1 0.2 0.2 0.3 0.5 SEM 3.5 0.0 0.1 0.1 0.1 0.1 0.2 0.1 0.2 0.1 Mean Std Dev SEM 4354 520 21.1 21.2 16.0 22.1 12.8 14.8 1.9 1.7 2.7 3.4 3.7 5.3 286475 4425 5117 5342 4767 7667 9817 10683 15042 CV% 13.0 11.9 10.2 13.6 14.8 1368 37125 716 696 1128 1009 1294 2166 222 Relative Tran 100 15 15 18 292 16.2 528 10.9 884 559 12.8 1515 150 284 461 412 90 244.9 11.95 13.0 21.1 22.1 14.8 CV% 13.0 21.2 ppDDE Ag 80.0 Relative \* 1.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.1 Rows G&H 70.0 12938 7981 5825 2157 10350 7566 8025 7575 12975 15650 7566 12650 19250 2899 12021 105050 38254 60.0 Std Dev \$ 50.0 4354 Mean VC Mean Nilutamide Control 12938 240.0 Subtraction of VC from wells 30.0 10nMDHT neg. control neg. control -4.0 blank -7 : 9046 4846 5096 6746 4196 2846 1046 846 696 2746 1346 -754 1846 -304 946 946 -54 -904 8196 22409 -304 -354 4046 7446 24 2096 646 454 296 846 -654 -554 -1304 2796 1646 5446 3046 2896 20.0 295046 796 6296 8796 396 346 746 496 546 746 46 6396 3896 6146 7796 252546 -254 13646 10.0 321096 -104 246 496 9946 10496 312746 0.0 287196 10mm OHT 5° 5° 5° 04 15 10 50 00 14813 with 10µM Nilutamide -2188 with 10µM Nilutamide 11916 391 -668 936 -393 8063 256 or 650 -658 -863 -248 -2638 -233 -793 . An Concentration (logM) Corrected Data Means PPDDE AC blank 10nM DHT neg. control -5.0 -4.5 -4.0 -7.5 -7.0 -6.5 -6.0 -5.5 Mean Std Dev SEM 282121 3313 5463 6329 1068 763 988 413 1009 37125 716 696 284 1128 1294 2166 884 1368 559 2222 ppDDE Ag 29 120 CV% 13.2 1010.9 91.3 114.2 244.0 30.1 39.0 21.0 20.8 244.9 AW 100 Relative Transcriptional Activity 1.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 Agonist: % of Maximal Induction Control
ppDDE Ag
blank
10nMDH1neg.control
neg.control 2 80 -7.5 -7.0 -6.5 -5.0 45 -4.0 79.4 0.7 0.4 0.7 2.6 -0. -0.5 1.4 3.2 2.9 -0.1 60 2.2 П 89 0.2 0.3 0.6 18 4 -0.1 0 40 1.0 113.8 0.0 0. 0.1 0.2 0.3 1.9 2.4 1.4 3.5 110.9 101.8 -0.2 0.3 0.3 0.5 0.0 3.7 0. 0.1 1.1 2.2 0.2 -0.2 0.0 0.0 -0.3 -0.3 1.0 1.0 2.8 4.6 0 20 -42.2 3.3 -2.0 1.0 -14 -1.2 0.9 2.9 0.6 5.3 with 10uM Nilutamide 0 14 \* 0.0 23.1 -2.4 -2.3 -3.1 -2.8 -2.1 -2.5 -0.9 -0.9 -0.8 -0.8 with 10uM Nilutamide H 0 + % of Maximal Induct 10mm DHT nea.control og og og og 15 10 15 20 ppDDE Ag OnM DH1 neg. -7.0 -6.5 -6.0 -5.5 -5.0 -4.5 -4.0 blank control -7.5 100.0 0.0 0.0 0.4 Mean Std Dev 0.0 0.3 0.1 1.2 1.9 2.2 0.0 13.2 0.2 0.3 0.2 04 0.5 0.8 0.5 Concentration (logM) SEM 0.0 5.4 0.1 0.1 0.1 0.2 0.1 0.2 0.3 0.2 Viability (% Control IOnM DHT neg. con neg. con PC10 PC50 DDE Ag -7.5 -7.0 -6.5 -6.0 -5.5 -5.0 -4.5 Mean 106 108 113 117 100 10 StdDev SEM %CV 4 12 5 8.3 PC60 PC10 12 1.7 #Interse 1.0 1.0 ct #Ok Fit Chart 0.8 0.8 PPDDE Ag 120 0.6 0.6 0 Bottom 2.3 Top -5.5 EC50 100 0.4 0.4 1.5 Hill slope 80 0.2 . 0.2 60 0.0 -0.0 40 0.0 0.2 0.4 0.6 0.8 1.0 12 0.2 0.4 1.0 0.0 0.6 0.8 1.2 20 -8 -5 -4 Concentration [LogM

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# **Data Spreadsheets**



APPENDIX 2 Deviation Forms

Deviation & Investigation         Study Number (if applicable):       9070-100107 ARTA         Date of Reporting:       11Nov2011         Date of Reporting:       13Oct2011.20Oct2011         Date of Occurrence:       3Nov2011         Associate Involved:       Image: Study Number (if applicable):         Date of Occurrence:       3Nov2011         Associate Involved:       Image: Study Number (if applicable):         Signature       Image: Study Director/Principal Investigator):         Image: Study Numary of Deviation       Image: Study Director/Principal Investigator):         Compound lot supplied by sponsor differs from the lot indicated in the protocol.         Action Taken and Determination of Impact on Study Data and/or Facility Compliance: (Image: Study Pack		-			Form #:	SOP-1003-
Study Number (if applicable): 9070-100107 ARTA Date of Reporting: 11Nov2011 Reporting Associate: 13Oct2011.20Oct2011 Date of Occurrence: 3Nov2011 Associate Involved: Description of Deviation: Oxybenzone (2-hydroxy-4-methoxybenzone) Lot number in the protocol was 20080801. Lot Supplied by sponsor was 20100801. (Reporting Associate) Type of Deviation (determined by Study Director/Principal Investigator): SOP Deviation (Deviation by SD/PI/Test Facility Management/Designee: Compound lot supplied by sponsor differs from the lot indicated in the protocol. Action Taken and Determination of Impact on Study Data and/or Facility Compliance:!\Mark	In vitro models to predict tox	idty De	eviation & Investig	ation		
Date of Reporting:       11Nov2011       Reporting Associate:         Date of Occurrence:       13Oct2011.20Oct2011         Description of Deviation:       Oxybenzone (2-hydroxy-4-methoxybenzone) lot number in the protocol was 20080801. Lot         Supplied by sponsor was 20100801.       Image: Control of the protocol was 20080801. Lot         Signature       Image: Control of Deviation         Signature       Date:         Deviation (determined by Study Director/Principal Investigator):         SOP Deviation (meeting associate)         Type of Deviation Investigation by SD/PI/Test Facility Management/Designee:         Compound lot supplied by sponsor differs from the lot indicated in the protocol.         Action Taken and Determination of Impact on Study Data and/or Facility Compliance:         No impact on study         Signature:         Date:         D	Study Number (if a	pplicable}:	9070-100107AR	ſA		
Date of Occurrence:       3Nov2011       Associate Involved:         Description of Deviation:	Date of Reporting:	11Nov2011	Repo	rting Associate:		
Description of Deviation:          Oxybenzone (2-hydroxy-4-methoxybenzone) lot number in the protocol was 20080801. Lot         supplied by sponsor was 20100801.         Signature         Date:         (Reporting Associate)         Type of Deviation (determined by Study Director/Principal Investigator):         SOP Deviation         SoP Deviation         Optication         Optication         Optication         Optication         SoP Deviation         Optication         Optication         SoP Deviation         Action Taken and Determination of Impact on Study Data and/or Facility Compliance:         No impact on study         Junct         Junct         Signature:         Date:         Date: <t< td=""><td>Date of Occurrenc</td><td>13Oct201 e: <u>3Nov2011</u></td><td>1,200ct2011 Assoc</td><td>ciate Involved:</td><td></td><td></td></t<>	Date of Occurrenc	13Oct201 e: <u>3Nov2011</u>	1,200ct2011 Assoc	ciate Involved:		
Oxybenzone (2-hydroxy-4-methoxybenzone) lot number in the protocol was 20080801. Lot         supplied by sponsor was 20100801.         Signature         Date:         Image: I	Description of Devi	ation:				
supplied by sponsor was 20100801.       It is the difference of the sponsor was 20100801.         Signature       Date: 10 How 2 Ol (         Reporting Associate)       Date: 10 How 2 Ol (         Type of Deviation (determined by Study Director/Principal Investigator):       No Deviation         SOP Deviation       Protocol Deviation       GLP Deviation         Summary of Deviation Investigation by SD/PI/Test Facility Management/Designee:       Compound lot supplied by sponsor differs from the lot indicated in the protocol.         Action Taken and Determination of Impact on Study Data and/or Facility Compliance:       Mode Date         No impact on study       July Data and/or Facility Compliance:         Signature:       Date: 10 How 2 Ol (         Signature:       Date: 10 How 2 Ol (         Signature:       Date: 10 How 2 Ol (	Oxybenzone (2-hyc	droxy-4-metho	xybenzone] lot nur	nber in the prote	ocol was 200	)80801.Lot 📿
Signature Date: 10 Kov 201( (Reporting Associate) Type of Deviation (determined by Study Director/Principal Investigator): SOP Deviation (Deviation Deviation CLP Deviation No Deviation Summary of Deviation Investigation by SD/PI/Test Facility Management/Designee: Compound lot supplied by sponsor differs from the lot indicated in the protocol. Action Taken and Determination of Impact on Study Data and/or Facility Compliance: No impact on study Signature: Date: 10 How 201( SD/PI/Test Facility Management	supplied by sponso	r was 2010080	1.		1.0	wild be INNOV 6
Signature   Signature     Date:     Image: I					1 the se	2011
Signature       Date: 10 Hov 201(         (Reporting Associate)       Investigator):         SOP Deviation (determined by Study Director/Principal Investigator):       No Deviation         SOP Deviation       Protocol Deviation       Investigator):         SOP Deviation       Protocol Deviation       Investigator):         Summary of Deviation Investigation by SD/PI/Test Facility Management/Designee:       Compound lot supplied by sponsor differs from the lot indicated in the protocol.         Action Taken and Determination of Impact on Study Data and/or Facility Compliance:       Market Automatication of Impact on Study Data and/or Facility Compliance:         No impact on study       Market Automatication       Market Automatication of Impact on Study Data and/or Facility Compliance:         Signature:       Date: 10 Hold 201(         SD/PI/Test Facility Management       Date: 10 Hold 201(				~to	2	
(Reporting Associate)         Type of Deviation (determined by Study Director/Principal Investigator):         SOP Deviation       Protocol Deviation         SOP Deviation       Protocol Deviation         Summary of Deviation Investigation by SD/PI/Test Facility Management/Designee:         Compound lot supplied by sponsor differs from the lot indicated in the protocol.         Action Taken and Determination of Impact on Study Data and/or Facility Compliance:         No impact on study         Signature:         Signature:         Signature:         Date:       Date:         SD/PI/Test Facility Management	Signature			Date: 11	May 2	211
Type of Deviation (determined by Study Director/Principal Investigator):         SOP Deviation       Protocol Deviation       BLP Deviation       No Deviation         Summary of Deviation       Investigation by SD/PI/Test Facility Management/Designee:       Compound lot supplied by sponsor differs from the lot indicated in the protocol.         Action Taken and Determination of Impact on Study Data and/or Facility Compliance:       No         No impact on study       Investigation       Investigation         Signature:       Date:       IOHay 2D(/         SUPPI/Test Facility Management       Date:       IOHay 2D(/		(Reporting			10000	
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SOP Deviation       Protocol Deviation       Investigation       Investigation         Summary of Deviation       Investigation       by SD/PI/Test Facility Management/Designee:         Compound lot supplied by sponsor differs from the lot indicated in the protocol.         Action Taken and Determination of Impact on Study Data and/or Facility Compliance:         No impact on study       Investigation         Signature:       Date:       IOHOU 2DI/         Signature:       SD/PI/Test Facility Management       Date:       IOHOU 2DI/	Type of Deviation (	determined by	/Study Director/Pri	ncipal Investigat	for):	
Summary of Deviation Investigation by SD/PI/Test Facility Management/Designee: Compound lot supplied by sponsor differs from the lot indicated in the protocol.  Action Taken and Determination of Impact on Study Data and/or Facility Compliance:	SOP Deviatio	n 🛛 🏾 🖾 Proto	ocol Deviation	GLP Deviatio	n 🗆	No Deviation
Compound lot supplied by sponsor differs from the lot indicated in the protocol.  Action Taken and Determination of Impact on Study Data and/or Facility Compliance: II Merid No impact on study  Signature: Date: IO Hou 20((	Summary of Deviati	on Investigatio	on by SD/PI/Test Fa	cility Manageme	ent/Designe	e:
Action Taken and Determination of Impact on Study Data and/or Facility Compliance: II Mark No impact on study	Compound lot supr		or differs from the l	at indicated in t		
Action Taken and Determination of Impact on Study Data and/or Facility Compliance: II Ment No impact on study Signature: SD/PI/Test Facility Management Action Taken and Determination of Impact on Study Data and/or Facility Compliance: II Ment Date: 10 Hou 20((	<u>composition supp</u>			or malcaled in h		denim tatal moduli di kaina
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Action Taken and Determination of Impact on Study Data and/or Facility Compliance: II Nor No impact on study Signature: SD/PI/Test Facility Management Action Taken and Determination of Impact on Study Data and/or Facility Compliance: II Nor Date: 10 Hou 20((						
No impact on study	Action Taken and D	etermination	of Impact on Study	Data and/or Fo	acility Comp	liance:11 No
Signature: Date: 10 Hou 20(/			or impact on blog			16-11
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Signature: Date: 10, Nov 20(/ SD/PI/Test Facility Management	No impact on study			ß	Joter Show	d vot
SD/PI/Test Facility Management	No impact on study			مى مىرى	July 1	or your
	No impact on study			می ری Date: ۱	July 2	
	No impact on study Signature:	/PI/Test Facility		می ری Date: <u> </u> C	Abou 2	
	No impact on study Signature:	'PI/Test Facility	/ Management	می Date: IC	they 2	2 2 2 1

Study Number: 9070-100107ARTA

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# **Deviation Forms (Continued)**

	Form #: SOP-1003-F-1.
)	CECION Deviation & Investigation
	In vitro models to predict toxicity
	Study Number (if applicable): 9070-100107ARTA
	Date of Reporting: <u>11Nov2011</u> Reporting Associate
	Date of Occurrence: 200ct2011, Associate Involved:
	Description of Deviation:
	Solubility was observed visually rather than read on the nepheloskan.
	the set
	and the
	Signature Date: () Date: ()
	(Reporting Associate)
	Type of Deviation (determined by Study Director/Principal Investigator):
	Type of Deviation (determined by Study Director/Principal Investigator):
	Type of Deviation (determined by Study Director/Principal Investigator): SOP Deviation Protocol Deviation GLP Deviation No Deviation Summary of Deviation Investigation by SD/PI/Test Facility Management/Designee:
	Type of Deviation (determined by Study Director/Principal Investigator): SOP Deviation Protocol Deviation GLP Deviation No Deviation Summary of Deviation Investigation by SD/PI/Test Facility Management/Designee: Solubility was observed visually rather than read on the nepholoskan.
	Type of Deviation (determined by Study Director/Principal Investigator):         SOP Deviation       Protocol Deviation         GLP Deviation       No Deviation         Summary of Deviation Investigation by SD/PI/Test Facility Management/Designee:         Solubility was observed visually rather than read on the nepheloskan.
	Type of Deviation (determined by Study Director/Principal Investigator):         SOP Deviation       Protocol Deviation         SUmmary of Deviation Investigation by SD/PI/Test Facility Management/Designee:         Solubility was observed visually rather than read on the nepheloskan.
	Type of Deviation (determined by Study Director/Principal Investigator): SOP Deviation Protocol Deviation GLP Deviation No Deviation Summary of Deviation Investigation by SD/PI/Test Facility Management/Designee: Solubility was observed visually rather than read on the nepheloskan.
	Type of Deviation (determined by Study Director/Principal Investigator):         SOP Deviation       Protocol Deviation       Deviation         Summary of Deviation Investigation by SD/PI/Test Facility Management/Designee:         Solubility was observed visually rather than read on the nepheloskan.         Action Taken and Determination of Impact on Study Data and/or Facility Compliance:
	Type of Deviation (determined by Study Director/Principal Investigator):         SOP Deviation       Protocol Deviation         Summary of Deviation Investigation by SD/PI/Test Facility Management/Designee:         Solubility was observed visually rather than read on the nepheloskan.         Action Taken and Determination of Impact on Study Data and/or Facility Compliance:         Solubility was run on the nepheloskan for run 3 with all test articles.
	Type of Deviation (determined by Study Director/Principal Investigator):         SOP Deviation       Protocol Deviation       INo Deviation         Summary of Deviation Investigation by SD/PI/Test Facility Management/Designee:       Solubility was observed visually rather than read on the nepheloskan.         Action Taken and Determination of Impact on Study Data and/or Facility Compliance:       INo         Solubility was run on the nepheloskan for run 3 with all test articles.       Investigation
	Type of Deviation (determined by Study Director/Principal Investigator):         SOP Deviation       Protocol Deviation         Summary of Deviation Investigation by SD/PI/Test Facility Management/Designee:         Solubility was observed visually rather than read on the nepheloskan.         Action Taken and Determination of Impact on Study Data and/or Facility Compliance:         Solubility was run on the nepheloskan for run 3 with all test articles.         Mark
	Type of Deviation (determined by Study Director/Principal Investigator):         SOP Deviation       Protocol Deviation       INo Deviation         Summary of Deviation Investigation by SD/PI/Test Facility Management/Designee:       Solubility was observed visually rather than read on the nepheloskan.         Action Taken and Determination of Impact on Study Data and/or Facility Compliance:       INo Deviation         Solubility was run on the nepheloskan for run 3 with all test articles.       Investigation
	Type of Deviation (determined by Study Director/Principal Investigator):         SOP Deviation       Protocol Deviation         Summary of Deviation Investigation by SD/PI/Test Facility Management/Designee:         Solubility was observed visually rather than read on the nepheloskan.         Action Taken and Determination of Impact on Study Data and/or Facility Compliance:         Solubility was run on the nepheloskan for run 3 with all test articles.         Job Training         Job Training         Date:         Image:
	Type of Deviation (determined by Study Director/Principal Investigator):         SOP Deviation       Protocol Deviation       Iso Deviation         Summary of Deviation Investigation by SD/PI/Test Facility Management/Designee:       Solubility was observed visually rather than read on the nepheloskan.         Action Taken and Determination of Impact on Study Data and/or Facility Compliance:       Solubility was run on the nepheloskan for run 3 with all test articles.         Solubility was run on the nepheloskan for run 3 with all test articles.       Solubility was run on the nepheloskan for run 3 with all test articles.         Signature:       Solubility Management

Study Number: 9070-100107ARTA

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# APPENDIX 2 Deviation Forms (Continued)

Contours			Form #:	SOP-1003-F-
In vitro models to predict toxicity	Deviation &	Investigation		
Study Number (if applie	:able): <u>9070-1</u>	00107ARTA		
Date of Reporting: 11	Nov2011	Reporting Associate	э:	
Date of Occurrence:	130ct2011,200ct20 3Nov2011	11 Associate Involved	:	
Description of Deviation	1:			
According to the proto	col, the final percer	ntage of DMSO in dosir	ng solution sho	uld be 0.1% (v/v
The actual final DMSO	percentage is 0.5%.			
-				
Signature		Date:	11 Nov 2011	
(F	eporting Associate			
Transformer				
rype of Deviation (defe	rmined by Study Di	ector/Principal Investig	gator): 	500 O O O
USOP Deviation	⊠Protocol Devic	tion UGLP Devia	tion	No Deviation
Summary of Deviation I	nvestigation by SD/	PI/Test Facility Manage	ment/Designe	e:
The final percertage of	DMSO in dosing so	utions was 0.5%.		
(				
Action Taken and Dete	mination of Impact	on Study Data and/or	Facility Comp	liance:
No impact on study. Th	e vehicle controls ir	icluded in this study on	d used for cor	mariton also
had 0.5% DMSO		cicaed in this study of		
100 0.3% DM30.			******	
Cimenting			111	
signature:		Date: /	1 Nov 2011	
SD/PI/T	est Facility Manage	ment		
Standard Operating Procedu	e			Page 1 of

Study Number: 9070-100107ARTA

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# **Deviation Forms (Continued)**

		Form #:	SOP-1003-F-
In vitro models to predict toxicity	& Investigation		
Study Number (if applicable): 9070	-100107ARTA		
Date of Reporting: <u>8Dec2011</u>	Reporting Associate:		
13Oct2011,20Oct Date of Occurrence: <u>3Nov2011</u>	2011, Associate Involved:		
Description of Deviation:			
The purity used for methoxycinnamate w	as taken from the MSDS ra	ther than th	ne C of A (98% vs
99.8% respectively).			
Signature	Date:	8 Dec	2011
(Reporting Associa	te)	0 000	
inches and second			
Type of Deviation (determined by Study)	Director/Principal Investigo	tor):	
Type of Deviation (determined by Study)	Director/Principal Investigo	tor):	No Deviation
Type of Deviation (determined by Study SOP Deviation Protocol Dev Summary of Deviation Investigation by SE	Director/Principal Investigo viation ØGLP Deviatio D/PI/Test Facility Managem	tor): on E ent/Desian	]No Deviation ee:
Type of Deviation (determined by Study SOP Deviation Protocol Dev Summary of Deviation Investigation by SE The wrong purity was used to proport the	Director/Principal Investigo viation ØGLP Deviatio D/PI/Test Facility Managem	tor): on E ent/Design	]No Deviation ee:
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Type of Deviation (determined by Study SOP Deviation Protocol Deviation Investigation by SL Summary of Deviation Investigation by SL The wrong purity was used to prepare the Action Taken and Determination of Impa No impact on study as the difference be the difference was paglicible	Director/Principal Investigo viation ØGLP Deviatio D/PI/Test Facility Managem e initial stock. act on Study Data and/or F tween the actual purity an	tor): ent/Design acility Com d the purity	No Deviation ee: pliance: used was 1.8%,
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Type of Deviation (determined by Study SOP Deviation Protocol Deviation Investigation by SL Summary of Deviation Investigation by SL The wrong purity was used to prepare the Action Taken and Determination of Impa No impact on study as the difference be the difference was negligible.	Director/Principal Investigo viation ØGLP Deviatio D/PI/Test Facility Managem e initial stock.	tor): ent/Design acility Com d the purity	No Deviation ee: pliance: rused was 1.8%,
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# **Deviation Forms (Continued)**

Form #: SOP-1003-F-1.0
In vitro models to predict toxicity
Study Number (if applicable): 9070-100107ARTA
Date of Reporting: <u>8Dec2011</u> Reporting Associate:
Date of Occurrence: 60ct2011, 310ct2011 Associate Involved:
Description of Deviation:
According to the protocol, the study director, study monitor, and sponsor will sign any protocol
amendments. One protocol amendment signed on 6Oct2011, and two protocol amendments
signed 31Oct2011 did not have study monitor or sponsor signature. They did receive copies of the protocol amendments.
Signature Date: 8 Bec 2011
Type of Deviation (determined by Study Director/Principal Investigator):         SOP Deviation       Protocol Deviation         GLP Deviation       No Deviation         Summary of Deviation Investigation by SD/PI/Test Facility Management/Designee:         Study monitor and sponsor signatures were not obtained for protocol amendments
Action Taken and Determination of Impact on Study Data and/or Facility Compliance:
Protocol amendments after October for this study included study monitor signature.
Signature: Date: 8 Dec 2011
SD/PI/Test Facility Management

Study Number: 9070-100107ARTA

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# **Deviation Forms (Continued)**

In vitro models to predict toxicity         Study Number [if applicable]:       9070-100107ARTA         Date of Reporting:       24 Jan 2012         Date of Reporting:       24 Jan 2012         Date of Occurrence:       8Dec 2012         Date of Occurrence:       8Dec 2012         Date of Deviation:       Associate Involved:         Description of Deviation:       Date:         The hard copies of the two deviations signed and scanned on 8Dec 2012 were misplaced         Signature       Date:         Reporting Associate)         Type of Deviation (determined by Study Director/Principal Investigator):	applicable):       9070-100107ARTA         ::       24 Jan 2012         ::	In vitro models to predict toxicity         Study Number (if applicable):       9070-10         Date of Reporting:       24Jan2012         Date of Occurrence:       8Dec2012         Date of Occurrence:       8Dec2012         Description of Deviation:         The hard copies of the two deviations signed         Signature         Reporting Associate)         Type of Deviation (determined by Study Direct         SOP Deviation	Date: 24Jan 2012
Study Number (if applicable):       9070-100107ARTA         Date of Reporting:       24Jan2012         Date of Reporting:       24Jan2012         Date of Occurrence:       8Dec2012         Description of Deviation:       Associate Involved:         The hard copies of the two deviations signed and scanned on 8Dec2012 were misplaced         Signature       Date:         Image: 24Jan2012       Date:         Description of Deviation:       Date:         The hard copies of the two deviations signed and scanned on 8Dec2012 were misplaced         Image: Reporting Associate)       Date:         Type of Deviation (determined by Study Director/Principal Investigator):	applicable]:       9070-100107ARTA         ::       24Jan2012         ::	Study Number (if applicable):       9070-10         Date of Reporting:       24Jan2012         Date of Occurrence:       8Dec2012         Description of Deviation:         The hard copies of the two deviations signe         Signature         [Reporting Associate]         Type of Deviation         [SOP Deviation]	Date: 24Jan 2012
Date of Reporting:       24Jan2012       Reporting Associate:         Date of Occurrence:       8Dec2012       Associate Involved:         Description of Deviation:       Associate on 8Dec2012 were misplaced         The hard copies of the two deviations signed and scanned on 8Dec2012 were misplaced         Signature       Date:       245an 20(2         Reporting Associate)       Date:       245an 20(2	: 24Jan2012       : 1/3       Reporting Associate:         :: 24Jan2012       : 1/3       Reporting Associate:         :: 24Jan2012       : 1/3       : 1/3         :: : : : : : : : : : : : : : : : : : :	Date of Reporting: 24Jan2012	Associate Involved:
Date of Occurrence:       8Dec2012       3       4       Associate Involved:       1         Description of Deviation:       The hard copies of the two deviations signed and scanned on 8Dec2012 were misplaced         Signature       Date:       24Jan 20(2         Reporting Associate)       Type of Deviation (determined by Study Director/Principal Investigator):	ce:       8Dec2012       3 3 3       Associate Involved:	Date of Occurrence: <u>BDec2012</u>	Associate Involved: d and scanned on 8Dec2012 were misplaced.  Date: 24Jan 20(2
Description of Deviation: <u>The hard copies of the two deviations signed and scanned on 8Dec2012 were misplaced</u> Signature Date: <u>24Jan 20(2</u> [Reporting Associate] Type of Deviation (determined by Study Director/Principal Investigator):	<i>i</i> ation: <u>f the two deviations signed and scanned on 8Dec2012 were misplaced.</u> Date:       24Jan 20(2         (Reporting Associate)         (determined by Study Director/Principal Investigator):         On       □Protocol Deviation         Image: Deviation       □No Deviation         Image: Deviation       □No Deviation	Description of Deviation: <u>The hard copies of the two deviations signe</u> Signature [Reporting Associate] Type of Deviation (determined by Study Direction [SOP Deviation] Protocol Deviat	Date: 24Jan 2012
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	on Protocol Deviation IGLP Deviation INo Deviation	SOP Deviation Protocol Deviat	ctor/Principal Investigator):
SOP Deviation Protocol Deviation SGLP Deviation No Devia	tion Investigation by SD/PI/Test Facility Management/Designee:		on
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	510 JUL 28 10	Action Taken and Determination of Impact	on Study Data and/or Facility Compliance:
Action Taken and Determination of Impact on Study Data and/or Facility Compliance:	Determination of Impact on Study Data and/or Facility Compliance:	No impact on the study as the electronic co	ny was retained
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			and the south
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Action Taken and Determination of Impact on Study Data and/or Facility Compliance:	Determination of Impact on Study Data and/or Facility Compliance:	No impact on the study as the electronic co	ny was retained
Action Taken and Determination of Impact on Study Data and/or Facility Compliance:	Determination of Impact on Study Data and/or Facility Compliance:	the imposer of the stody as the electronic co	oy was refained.

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# **Deviation Forms (Continued)**

flaaTeur=	Form #:	SOP-100
In vitro models to predic taxidity		
Study Number (if applicable):9070-100107ARTA		
Date of Reporting: 27Jan2012 Reporting Assoc	ciate	
19Oct2011 and         Date of Occurrence:       02Nov2011         Associate Involv	ved:	
Description of Deviation:		~ ~
The time of seeding was verbally communicated but not reco	orded.	
		1999a - <b>1</b> 90 <b>2</b> 3554
Signature Dat	e: 27 Ja	~2012
(Reporting According)		
(Reporting Associate)		
Type of Deviation (determined by Study Director/Principal Inv	estigator):	
Type of Deviation (determined by Study Director/Principal Inv	estigator):	
Type of Deviation (determined by Study Director/Principal Inv	estigator): eviation	□No Deviatio
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Type of Deviation (determined by Study Director/Principal Inv SOP Deviation Protocol Deviation &GLP De Summary of Deviation Investigation by SD/PI/Test Facility Man The time of seeding was verbally communicated but not reco Action Taken and Determination of Impact on Study Data an No impact on study.	estigator): eviation agement/Desi orded. d/or Facility Co	□No Deviatio gnee: ompliance:
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Type of Deviation (determined by Study Director/Principal Inv         SOP Deviation       Protocol Deviation         Summary of Deviation Investigation by SD/PI/Test Facility Man         The time of seeding was verbally communicated but not record         Action Taken and Determination of Impact on Study Data an         No impact on study.         Signature         SD/PI/Test Facility Management	estigator): eviation agement/Desi orded. d/or Facility Co	□No Deviatio gnee: ompliance: 20 / Z_
Type of Deviation (determined by Study Director/Principal Inv SOP Deviation Protocol Deviation SD/PI/Test Facility Man The time of seeding was verbally communicated but not reco Action Taken and Determination of Impact on Study Data an No impact on study. Signature Date SD/PI/Test Facility Management	estigator): eviation agement/Desi arded. d/or Facility Co	□No Deviatio gnee: ompliance: 20 / Z_

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# APPENDIX 3 Certificate of Analysis – Oxybenzone

		IVYC IVY FINE C http://www.i	HEMICALS Wychem.com	S	
	Product Name	2-HYDROX	ХҮ-4-МЕТНОХҮР	BENZ	ZOPHENONE
	Synonym	Oxybenzone			
	Catalog Number	HH13-026	ř.		
	CAS Number	131-57-7			-
	Batch Number	20100801	Quantity		200 KG
	Manu. Date	August 2, 2010	Expiry Date		August 1, 2012
	Date of Report	August 2, 2010	Package		
	Quality Specifications		Specifications ( In l	house	9.)
	a.				£
	Test	Standa	rd		Results
	Appearance	Light yellow to gre	en crystalline er	Light yellow crystalline powder 99.92% 63.8 °C to 64.8 °C	
	Assay (HPLC)	98% mi	in		
	Melting Point	62 °C to 6	5 °C		
11 1 Mail 100 March 100 March	Loss on Drying	0.5% m	ax	0.07%	
	Heavy Metals	<= 5 pp	m		2.9 ppm
	Conclusion: Conf	form		1	
					č

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# APPENDIX 3 Certificate of Analysis – Octocrylene



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Certificate of Analysis - Octylmethoxycinnamate



# ATCC

### **Certificate of Analysis**

ATCC<sup>®</sup> Number: Lot Number:

Name: Description: Species: Expiration Date:

MDA-kb2 **Breast Carcinoma** Human (Homo sapiens) Not applicable

CRL-2713\*\*\*

3984776

Test	Specifications	Results
Total cells/mL	Report results	5.7 x 10 <sup>6</sup>
Ampule passage number	Report results	37
Post-freeze viability	≥ 50.0%	89.8%
Growth properties	Adherent	Adherent
Morphology	Epithelial-like* and/or rounded	Epithelial-like and rounded
Test for mycoplasma contamination Hoechst DNA stain (indirect) Agar culture (direct)	None detected None detected	None detected None detected
Species determination: Isoenzyme assay (interspecies)	Human B (G6PD variant)	Human B (G6PD variant)
Species determination: STR analysis (intraspecies)	Human (Unique DNA Profile) D5S818: 11 D13S317: 12 D7S820: 10 D16S539: 9 VWA: 17, 18 THO1: 6 Amelogenin: X TPOX: 10 CSF1PO: 10, 12	Human (Unique DNA Profile) D5S818: 11 D13S317: 12 D7S820: 10 D165539: 9 vWA: 17, 18 THO1: 6 Ameiogenin: X TPOX: 10 CSFIPO: 10, 12
Sterility test (BacT/ALERT 3D) iAST bottle (aerobic) at 32°C iNST bottle (anaerobio) at 32°C	No growth No growth	No growth No growth

Epithelial-like: Any adherent cells of a polygonal shape with clear, sharp boundaries between them.

Quality Control Manager; Quality, Compliance and Biosafety

ATCC hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and correct to the best of the company's knowledge and belief.

This product is intended to be used for laboratory research use only. It is not intended for use in humans, animals, or for diagnostics.

ATCC products may not be resold, modified for resale, used to provide commercial services, or to manufacture commercial products without prior written agreement from ATCC.

ATCC (American Type Culture Collection) P.O. Box 1549 Manassas, VA 20108 USA www.atcc.org

800-638-6597 or 703-365-2700 Fax: 703-365-2750 E-mail: tech@atcc.org or contact your local distributor

13 April 2009 Date

- Page 1 of 2 -

CONFIDENTIAL AND PROPRIETARY
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Template Doc ID: 7075 Template Effective Date: 12/31/2008

Study Number: 9070-100107ARTA

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**Mycoplasma Free** 

Mycoplasma Testing Services 156 Fay Brook Drive • Saranac Lake   Phone: 518-891-2356 • Fax: 518-891	VY 12983 Please end -5753 each slide	close this completed for to avoid delays in pro	rm with cessing.
Dare Sent: 31 Aug 20(1	Sample Designa	ntion or #: MDIA-KD2 LC	1:3984776
Name: Bionique will submit results only to the p	erson named above)		
Company/University <u>CooToX</u>	Cell Type:	Adhcrent	Nosadherent
Complete Mailing Address: (Results are 717 Campus Dr.	mailed let class USPS) NormalT	ransfect Monoclonal	
alamazon MI 4900	B Flask VT150	Roller Bottle \$\$2 lite	r suspension
	Bioreactor	Other	
Optional: FAX #:			
Date received at Bionique Te	www.bionique.com	For Research Use Only 11 Code #: 476	0236 60
Date received at Bionique Te <u>M-100 CELLSHIPPER DN</u> <u>NEGATIVE:</u>	www.bionique.com sting Labs: $4/1/11$ C A FLUOROCHROME ASSAY A reaction with staining lim	For Research Use Only 11 Code #: <u>476</u> <u>RESULTS:</u> ited to the nuclear reg	0236 60
Date received at Bionique Te. <u>M-100 CELLSHIPPER DN</u> <u>K</u> NEGATIVE:	www.bionique.com sting Labs: <u>4/1/11</u> A FLUOROCHROME ASSAY : A reaction with staining lim no mycoplasmal contaminat	For Research Use Only 11 Code #: <u>476</u> RESULTS: ited to the nuclear reg	0236 60
Date received at Bionique Te. <u>M-100 CELLSHIPPER DN</u> <u>X</u> NEGATIVE: POSITIVE:	www.bionique.com sting Labs: <u>4/1/11</u> A FLUOROCHROME ASSAY A reaction with staining lim no mycoplasmal contaminat A significant amount of extra mycoplasmal contamination	For Research Use Only 11 Code #: <u>476</u> RESULTS: itted to the nuclear reg ion. anuclear staining which	y 0236 60 tion, which indica
Date received at Bionique Ter <u>M-100 CELLSHIPPER DN</u> <u>X</u> NEGATIVE: POSITIVE: INCONCLUS	www.bionique.com sting Labs: <u>4/1/11</u> A FLUOROCHROME ASSAY A reaction with staining lim no mycoplasmal contaminat A significant amount of extra mycoplasmal contamination IVE:	For Research Use Only 11 Code #: <u>476</u> RESULTS: ited to the nuclear region. anuclear staining which	y 0236 60 rion, which indica
Date received at Bionique Ter <u>M-100 CELLSHIPPER DN</u> <u>NEGATIVE:</u> POSITIVE: INCONCLUS	www.bionique.com	For Research Use Only 11 Code #: <u>476</u> RESULTS: ited to the nuclear region. anuclear staining which muclear staining consi or nuclear degenerat	y 0236 60 tion, which indica ch strongly sugge stent with low - le
Date received at Bionique Ter M-100 CELLSHIPPER DN NEGATIVE: INEGATIVE: INCONCLUS	www.bionique.com	For Research Use Only 11 Code #: <u>476</u> RESULTS: ited to the nuclear region. anuclear staining which muclear staining consi or nuclear degeneration internation or viral CIE contaminant or viral CIE contamination.	y 0236 60 tion, which indica th strongly sugge stent with low - le ion. istent with bacter PE. Morphology

Thank you for allowing us to assist you, and for using the CELLshipper. (dc: 3003 att # 2; 10/9/2003)

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	TEST PROTOCOL
TO BE COMPLETED	BY THE STUDY SPONSOR:
Study Sponsor:	NIEHS/NTP Chief Toxicology Branch)
Address:	P.O. Box 12233
	Phone: (
Study Monitor:	Kesculti mongle rolk, rec
	E-mail:
NIEHS/NTP In Telephone No.: Facsimile No.: E-mail:	vestigator
(Contract No. HF Study Monitor Telephone No.:	ISN273200900005C; NIEHS Control No. N01-ES-00005) (ILS, Inc, Durham, NC)
Facsimile No.: E•mail:	

# Geeton MANDROGENIC TRANSACTIVATION ACTIVITY IN MDA-KB2 REPORTER CELLS Study #:9070-100107ARTA Table of Contents Solubility/Precipitation Assay Page 3 of 13

# APPENDIX 5 Protocol and Protocol Amendments

#### **Protocol and Protocol Amendments APPENDIX 5**

	Study #:9070-100107ARTA
Study Records to be maintained:	

## Protocol and Protocol Amendments



Study Number: 9070-100107ARTA

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CONTINUE ANDROGENIC TRANSACTIVATION ACTIVITY IN MDA-KB2 REPORTER CELLS

Study #:9070-100107ARTA

### **Title of Study**

Androgenic Transactivation Activity in MDA-kb2 Reporter Cells

### **Purpose of Study**

The purpose of this study is to analyze test substances for androgenic transactivation activity using the MDA-kb2 reporter cell line. The MDA-kb2 cell line is derived from a human breast cancer line transfected with an androgen receptor promoter linked to a luciferase gene. Consequently, the MDA-kb2 cell line can measure the ability of a test substance to induce or antagonize AR-mediated transactivation via luciferase gene expression.

### **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.

### **Quality Assurance**

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

### **Test Facility**

CeeTox, Inc. 4717 Campus Drive Kalamazoo, MI 49009 USA

### **Materials and Methods**

### **Test Substance**

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

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

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	Cooton ANDROGENIC TRANSACTIVATION AC	ANDROGENIC TRANSACTIVATION ACTIVITY IN MDA-KB2 REPORTER CELLS		
	Storage	Room Temperature		
	Test Substance: 2-Ethylhexyl p-methoxycinnamate (Octylmethoxycinnamate) CAS No. 5466-77-3			
Source:Acros OrganicsLot/Batch No.:A0293319ILS Repository No.:11-32				
	Formula:	C <sub>18</sub> H <sub>26</sub> O <sub>3</sub>		
	Description:	Clear colorless liquid		
	Storage	Room Temperature		
	Test Substance: Octyl Salicyl	ate (Octylsalate)		
	CAS No.	118-60-5		
Source: Sigma-Aldrich		Sigma-Aldrich		
	Lot/Batch No.:	44698PJ		
	ILS Repository No.:	11-30		
	Formula:	C <sub>15</sub> H <sub>22</sub> O <sub>3</sub>		
	Description:	Colorless liquid		
	Storage	Room Temperature		
Test Substance: 2-Ethylhexyl 2-Cyano-3,3-Diphenylacrylate (Octocrylene)				
	CAS No.	6197-30-4		
	Source:	Sigma-Aldrich		
	Lot/Batch No.:	01697MJ		
		Page 7 of 13		

Cooton Androgenic Transactivat	ON ACTIVITY IN MDA-KB2 REPORTER CELLS	Study #:9070-100107ARTA	
ILS Repository No.:	11-31		
Formula:	C <sub>24</sub> H <sub>27</sub> NO <sub>2</sub>		
Description:	Yellow viscous liquid		
Storage	Room Temperature		
Preparation of Test Su	Preparation of Test Substance		
Test substances will b vehicle and serially d (to a final concentrati prepared on the day substances will not be	Test substances will be prepared as a stock in DMSO (Dimethylsulfoxide), or appropriate vehicle and serially diluted in the same solvent to prepare solutions for dilutions with media (to a final concentration of ≤ 0.1% (v/v)). Fresh dilutions of the stock solutions will be prepared on the day of use in the assay. Dose concentrations of test and control substances will not be verified using analytical methods.		
Positive and Negative	e Reference Substances		
0.5 DMSO: negative control group DHT (Dihydrotestosterone): CAS No: 521-18-6 (strong agonist) Nilutamide: CAS No. 63612-50-0 (strong antagonist, no agonism) p,p'-DDE (1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene) CAS No 82413-20-5: (weak antagonist)			
Reference substa media of 0.5% ( to prepare solution be prepared on the second	nces will be prepared as a sto v/v)), or appropriate vehicle and ons for dilutions with media. Fre he day of use in the assay.	ck in DMSO (final concentration in d serially diluted in the same solvent sh dilutions of the stock solutions will	
Certificates of a and appended t (Table 1).	nalysis will be provided by the v to the study report for the positive	vendor and stored in the study data e and negative reference substances	
Note: A certificate of analysis will report. Confirmation of the identit positive, negative reference substar data and appended to the study re of the study report.	be provided by the sponsor and will be sto y of the test substance, characterization ar ices certificates of analysis will be obtained port. Test substance will be either returned t	red in the study data and appended to the study ad stability will be verified by the sponsor. For from the vendor and will be stored in the study o the Sponsor or destroyed following finalization	
Transactivation Assays			
	Page 8 of 13		

Study Number: 9070-100107ARTA

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#### CONTON - ANDROGENIC TRANSACTIVATION ACTIVITY IN MDA-KB2 REPORTER CELLS

Study #:9070-100107ARTA

This test system was designed to identify substances capable of inducing androgen and glucocorticoid-receptor (GR) mediated gene expression (transactivation). Anti-androgen activities will also be evaluated in this model.

The transactivation model utilizes MDA-kb2 cells (derived from human breast cancer cell line, MDA-MB-453) transfected with an AR promoter linked to a luciferase gene (Wilson, *et al.* 2002). The cell line was obtained from ATCC. Cell culture medium and cell culture reagents are purchased from Hyclone and Gibco (Invitrogen).

### Androgen Transactivation Assay Test System

The ability of unknown substances to induce an AR-dependent response in a cell system will be determined using an MDA-kb2 human breast carcinoma cell line constructed with a luciferase reporter gene for the androgen response element (ARE). The MDA-kb2 cell line was derived from the breast cancer cell line, MDA-MB-453 by stable transfection with a mouse mammary tumor virus (MMTV) luciferase-neo reporter gene construct (Wilson *et al.*, 2002). The MDA-MB-453 parent cell line has been shown to express high levels of functional, endogenous androgen receptor (AR). However estrogen receptor alpha, and progesterone receptor are not detectable at the mRNA level and estrogen receptor beta is expressed only at very low levels. This cell line does contain glucocorticoid receptor (GR).

MDA-kb2 cell line will be tested and determined to be mycoplasm free prior to testing sponsor's substances in the transactivation assays. Cells will be initially grown in Leibovitz's L-15 medium containing 10% fetal bovine serum without antibiotics at approximately  $37^{\circ}$ C and without CO<sub>2</sub>. The doubling time for these cells is approximately 40-48 hr.

MDA-kb2 cells will be seeded into opaque sided 96-well cell culture plates at a density of approximately 10,000 cells/well in the medium described above. The cells will be then grown for approximately 24 hrs prior to the addition of the test substances.

Each test substance will be prepared for addition to the cell system by making a 400 mM stock. Dilutions will be prepared in DMSO to 400X final target concentration.  $10 \ \mu$ l aliquots of the substance dilutions will be added to 2 mL media in deep well plates and mixed to yield concentrations of test material 2-fold greater than the desired final concentration. To achieve the final exposure concentrations each 2X solution will be diluted 2-fold in another 96-well plate containing the cells and 50 mL media and appropriate controls.

Once completed each plate will be returned to the incubator and incubated for approximately 24 hours at approximately  $37^{\circ}$ C without CO<sub>2</sub>. Each test substance exposure concentration will be in replicates of six. Several control groups will be included o in each set of plates (agonist or antagonist) as follows: vehicle control (0.5% DMSO), maximal response agonist control (dihydrotestosterone; DHT, CAS No 521-18-6), antagonist (nilutamide, CAS No

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#### CONTONIN ANDROGENIC TRANSACTIVATION ACTIVITY IN MDA-KB2 REPORTER CELLS

Study #:9070-100107ARTA

63612-50-0) only control, and antagonist (nilutamide) or agonist (DHT) at each exposure concentration. In all situations the amount of stock solution solvent (DMSO) will be held constant at 0.5%.

Antagonism experiment plates will be co-exposed to a near maximal induction producing concentration of agonist, dihydrotestosterone (1 nM DHT) with vehicle or test substance. In the presence of an antagonist, the luciferase activity induced by DHT would be reduced proportionally to the concentration of the antagonist. The well-characterized antagonist, nilutamide will be run as a positive control. Concentrations of test substances with cytotoxicity below 80% will be eliminated from the analysis.

### Reference

Wilson, VS., Bobseine, K., Lambright, CR., and Gray, LE., Jr. (2002). A novel cell line, MDA-kb2, which stably expresses an androgen and glucocorticoid-responsive reporter for detection of hormone receptor agonists and antagonists. *Toxicol. Sci.* **66**, 69-81.

### **Calculations for Transactivation Data**

Luminescence will be measured with a luminescence counter. These data will be transferred to Microsoft Excel® worksheets for determination of standard statistical parameters such as the Mean, Standard Deviation, Standard Error of the Mean, and Coefficient of Variation. At this point the mean values of response will be reviewed for outlier values. All processed data will be examined to determine if negative and positive induction controls within each plate are within acceptable limits. The acceptance criteria used will be as follows: Background value ratio of vehicle control to antagonist control should be less than 10-fold, and the ratio of positive control to vehicle control should be greater than 3-fold. Each data point will be normalized to the average of the vehicle-only treated control (fold induction). The final Fold Induction results will be transferred into GraphPad Prism version 5.01 or xlfit as individual data points in plate block format.

Test substances will be considered positive for agonism and or antagonism based upon two (or three) independent runs. If two runs give comparable and therefore reproducible results, it will not be necessary to conduct a third run. Data interpretation criteria are shown in the table below for agonism.

Agonism	Positive	and	Negative	Decision	Criteria
---------	----------	-----	----------	----------	----------

Positive	If the RPC <sub>Max</sub> obtained is equal to or exceeds 20% of the
	positive control (DHT) in at least two of two runs
Negative	If the RPC <sub>Max</sub> fails to achieve at least 20% of the response
	of the positive control in two of two or two of three runs

Antagonism Positive and Negative Decision Criteria

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CROTON AND AND A CONTRACTIVATION ACTIVITY IN MDA-KB2 REPORTER CELLS		Study #:9070-100107ARTA	
Positive	If the differential between the high antagonism are greater than 509 response (more than one data point	antagonism and low % and have a dose ) in two of two runs.	
Negative	If the differential fails to achieve at	least 50% difference	
	and does not have a dose response	, in two of two runs.	

### Cytotoxicity Assay

Cell viability will be monitored by Propidium Iodide (PI) uptake. PI is a dye that cannot cross the plasma membrane of intact and viable cells. Cells that are dead or dying have weakened plasma membrane which allows PI to enter the cytosol of the damaged cells. Once inside the cell the PI intercalates into DNA/RNA and yields a fluorescent signal. Fluorescence is directly proportional to cell viability. PI is a light sensitive substance; therefore all procedures will be conducted under low light conditions.

Cells will be seeded into a 96-well black sided culture plate at the same time cells are seeded for the AR transactivation assays as described above (i.e. vehicle control (0.5% DMSO), maximal response agonist control (dihydrotestosterone; DHT, CAS No 521-18-6), nilutamide, CAS No 63612-50-0) only control, and antagonist (nilutamide) or agonist (DHT) at each exposure concentration. PI assays will be performed on the test substance alone and additionally on the test substance in the presence of DHT for all concentrations examined in the transactivation assays (see above). In all situations the amount of stock solution solvent (DMSO) will be held constant at 0.5%. The PI working solution will be prepared by adding PI powder to phosphate buffered saline (PBS) for a final concentration of 4.4  $\mu$ M. Following approximately 24 hr incubation with the test substances the growth medium will be removed from the plate and 50  $\mu$ l of the PBS/PI solution will be added. The plate will be maintained under low light conditions. Background fluorescence will be evaluated by reading fluorescence following a minimum of 5 minutes on a fluorescent plate reader at an excitation wavelength of 544 nm and an emission wavelength of 612 nm. Following this determination, 50  $\mu$ l of a 2% triton 100X solution prepared in water will be added and the plate will be incubated at room temperature for a minimum of 15 minutes and read at the same wavelengths. The total amount of fluorescence or cells present on the plate will be determined by subtracting the first read from the second read. The change in cell viability will be determined by comparing treated wells to the untreated or control wells. A 20% drop below vehicle treated controls will be considered cytotoxic.

### Solubility/Precipitation Assay

The limit of solubility will be determined by a light scattering procedure that uses Nephelometry (Nepheloskan Ascent by Labsytems). A plate (without cells) will be prepared that contains 200  $\mu$ l of the growth medium and the test substance at all exposure concentrations being evaluated. Nephelometry measures particulate light scattering. If a substance produces a consistent signal  $\geq$  3 times the vehicle control signal, that concentration will be considered to have precipitation.

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CONTINUE ANDROGENIC TRANSACTIVATION ACTIVITY IN MDA-KB2 REPORTER CELLS

Study #:9070-100107ARTA

This technique is an effective means of determining changes in the cell culture and dosing matrices. However, it should be noted that changes in fluid turbidity can be affected by the test substance reaching saturation and precipitating out of the solution or by the substance causing the precipitation of components in the culture and dosing media such as protein or salts. In situations where the transactivation response continues to increase with increasing exposure concentrations or receptor binding curves respond as expected beyond the apparent solubility, it is likely that the reason for the change in apparent solubility was due to matrix component precipitation and not test substance precipitation.

### Acceptance Criteria

Stability of the cell line will be monitored by using DHT as the agonist reference control and Nilutamide as the strong positive antagonist control. Nonylphenol will be used as a mild reference control for antagonism. A complete concentration range for each reference control will be run every time the AR transactivation assay is performed.

Background Criteria: The mean of the vehicle control wells (VC) divided by average background wells must be less than 20.

Fold induction: The target mean luciferase activity of the positive control (10 nM DHT) will be at least 3 fold that of the mean vehicle control on each plate.

## Study Reports

The data to be reported in the draft final report and final report will include (but will not be limited to) the following information: assay date and run number, laboratory personnel involved in the study, chemical/test substance information (including but not limited to chemical name, code, molecular weight, concentrations tested, notes regarding solubility).

### **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 this protocol and all amendments will be issued to the Sponsor as well as CeeTox and placed into the study binder.

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CONTINUE ANDROGENIC TRANSACTIVATION ACTIVITY IN MDA-KB2 REPORTER CELLS

Study #:9070-100107ARTA

### **Data Retention and Archiving**

All raw data, documentation, records, protocol, and the final report generated as a result of this study will be retained at CeeTox for 15 years. Retention of the materials after 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

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Study Number: 9070-100107ARTA

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#### Protocol Amendment

Study Number: 9070-100107ARTA

<u>Title of Study to be Amended:</u> Androgenic Transactivation Activity in MDA-kb2 Reporter Cells

<u>Reason for Amendment to Protocol:</u> Current Study Director's work load and number of studies directing has reached a maximal capacity deemed acceptable by test facility management.

**Change:** will be designated the Study Director for this study.

### **Signature**

CeeTox, Inc.



06 OCt 2011 Date

06 017 2011 Date

President

CeeTox Study # 9070-100107ARTA

6-Oct-11

Study Number: 9070-100107ARTA

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**Protocol and Protocol Amendments** 



**Protocol Amendment** 

Study Number: 9070-100107ARTA

<u>**Title of Study to be Amended:**</u> Androgenic Transactivation Activity in MDA-kb2 Reporter Cells

**<u>Reason for Amendment to Protocol:</u>** A typograpical error was found in the protocol.

#### Change:

The CAS number for p,p'DDE was listed in the protocol as 82413-20-5.

The CAS number for p,p'DDE will now be listed in the protocol as 72-55-9.

Signature

CeeTox, Inc.



<u>31 Oct zoll</u> Date

Study Director (Project Manager)

CeeTox Study # 9070-100107ARTA

31-Oct-11

Study Number: 9070-100107ARTA

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Protocol and Protocol Amendments



**Protocol Amendment** 

Study Number: 9070-100107ARTA

Title of Study to be Amended: Androgenic Transactivation Activity in MDA-kb2 **Reporter Cells** 

Reason for Amendment to Protocol: The Two-Read Propidium lodide SOP has been revised and the protocol is being amended to reflect these changes. A typo was identified in both CeeTox SOP and the protocol which stated the final concentration of propidium iodide was 4.4 µM. The correct concentration, and the concentration prepared and used in the study, was 44 µM.

Change:

The section titled Cytotoxicity Assay, Paragraph 2, the sentence 4 stated:

"The PI working solution will be prepared by adding PI powder to phosphate buffered saline (PBS) for a final concentration of 4.4 µM."

The section titled Cytotoxicity Assay, Paragraph 2, the sentence 4 will now state:

"The PI working solution will be prepared by adding PI powder to phosphate buffered saline (PBS) for a final concentration of 44 µM."

### Signature

CeeTox, Inc.



<u>31 - Oct - 201(</u> Date

CeeTox Study # 9070-100107ARTA

31-Oct-11

Study Number: 9070-100107ARTA

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### **Protocol and Protocol Amendments**



#### **Protocol Amendment**

Study Number: 9070-100107ARTA

<u>Title of Study to be Amended:</u> Androgenic Transactivation Activity in MDA-kb2 Reporter Cells

Reason for Amendment to Protocol: Client requested amendment

Change:

Section Data Retention and Archiving will now state:

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

NTP Archives 615 Davis Drive, Suite 300 Durham, NC 27713

#### Signature

CeeTox, Inc.

Study Monitor



CeeTox Study # 9070-100107ARTA

Study Director (Project Manager)

12-	6	-	11
Date			

6 Dec 2011 Date

6-Dec-11

Study Number: 9070-100107ARTA

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### **Protocol and Protocol Amendments**

# CeeTox≥

**Protocol Amendment** 

Study Number: 9070-100107ARTA

Title of Study to be Amended: Androgenic Transactivation Activity in MDA-kb2 Reporter Cells

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

Ch	nn	101	a	,
0.11			6	1

The Table of Contents will now read:
Signatures
Tille of Sludy
Purpose of Study
Compliance Statement
Quality Assurance
Test Facility
Materials and Methods
Yesi Substance
Tesi Substance: 2-Hydroxy-4-Methoxybenzophenone (Oxybenzone)
Test Substance: 2-Ethylhexyl p-methoxycinnamate (Octylmethoxycinnamate)
Tesi Substance: Octyl Salicylate (Octylsalate)
Test Substance: 2-Ethylhexyl 2-Cyano-3,3-Diphenylacrylate (Octocrylene)
Transactivation Asays
Androgen Transactivation Assay Test System
Reference
Calculations for Transactivation Data
Cytotoxicity Assay
Solubility/Precipitation Assay
Acceptance Criteria
Study Reports
Alterations of the Study Design
Data Relention and Archiving
Study Records to be maintained;

Signature



Study Director (Project Manager)

CeeTox Study # 9070-100107ARTA

1/24/17 Date

25 Jan 2012 Date

24-Jan-11

# Study Number: 9070-100107ARTA

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