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

CeeTox
*In vitro* models to predict toxicity

# H295R Steroidogenesis Assay

## Final Report

**DATA REQUIREMENT(S):** OPPTS 890.1550 (2009)

**AUTHOR(S):** [redacted]

**STUDY COMPLETION DATE:** 21 February 2012

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**LABORATORY PROJECT ID:** Study Number: 9070-100107STER
Sponsor Contract No. HHSN273200900005C
NIEHS Control No. N01-ES-00005

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## STATEMENT OF DATA CONFIDENTIALITY CLAIMS

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## GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

**Study Number:** 9070-100107STER

**Study Title:** H295R Steroidogenesis Assay

I, the undersigned, hereby declare that this study was performed in accordance with EPA GLP regulations Title 40 CFR 160 with the exception of section 160.113. Dose concentration of test and control substances were not verified using analytical methods.

*1.) There were four protocol deviations and one facility deviation; however, the deviations did not impact the integrity of the data in this report (Appendix 12).*

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

[redacted]
Study Director

21 Feb 2012
Date

## FLAGGING STATEMENT

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# QUALITY ASSURANCE STATEMENT

**Study Title:** H295R Steroidogenesis Assay

**Study Number:** 9070-100107STER

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

| Date(s) of Inspection/Audit | Inspection/Audit | Date(s) reported to Study Director | Date(s) reported to Management |
|---|---|---|---|
| 27 Jun 2011 | Draft Protocol | 27 Jun 2011 | 27 Jun 2011 |
| 20 Jul 2011 and 22 Jul 2011 | In-Process | 22 Jul 2011 | 22 Jul 2011 |
| 03 Feb 2012 | Data Binder | 03 Feb 2012 | 03 Feb 2012 |
| 06 Feb 2012 | Draft Report | 06 Feb 2012 | 06 Feb 2012 |

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

[redacted] 21 Feb 2012

Date

Quality Assurance Auditor
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# GENERAL INFORMATION

## Contributors

The following contributed to this report in the capacities indicated:

| Name | Title |
|---|---|
| [redacted] | Study Director |
| | Director of Project Management |
| | Director of Laboratory Operations |
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| | Lead Cell Culture Scientist |
| | Scientist |
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| | Scientist |
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| | Associate Scientist |

## Study Dates

Study initiation date: 12 July 2011
Experimental start date: 19 July 2011
Experimental termination date: 29 July 2011

## Deviations from the Protocol

See Appendix 12. No deviation impacted the integrity of the data in this report.

## Other

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

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

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# 1.0 EXECUTIVE SUMMARY

## 1.1 Study Design

The objective of this study was to evaluate the ability of four test chemicals to affect the steroidogenic pathway beginning with the sequence of reactions occurring after the gonadotropin hormone receptors through the production of testosterone and estradiol/estrone. The assay used the H295R human adrenocarcinoma cell line.

The four chemicals tested in the assay were 2-Hydroxy-4-Methoxybenzophenone (Oxybenzone), 2-Ethylhexyl p-methoxycinnamate (Octylmethoxycinnamate), Octyl Salicylate (Octylsalate), and 2-Ethylhexyl 2-Cyano-3,3-Diphenylacrylate (Octocrylene). The final concentrations of each compound tested in the steroidogenesis assay were: 0.0001, 0.001, 0.01, 0.1, 1, 10, and 100 µM.

Four independent runs of the steroidogenesis assay were conducted. Three of the four assays were analyzed for each compound. All test chemicals, reference chemicals, and solvent controls were tested in replicates of 3/plate, with the exception of the solvent controls on the quality control (QC) plate. Six solvent control wells were analyzed on the QC plate. The H295R supplemented medium used in the assay at the time of plating, dosing, and harvest contained 10 µM 22R-hydroxycholesterol. The duration of exposure was 48 hours. A QC plate containing two doses of reference chemicals forskolin and prochloraz was run each time the assay was performed. Cell viability was assessed after the 48 hour exposure using the MTT assay. Testosterone and estradiol levels were measured using HPLC/MS-MS by OpAns, LLC (Durham, NC). All concentrations that exhibited greater than 20% cytotoxicity in the MTT cell viability assay were excluded from the statistical analysis of testosterone and estradiol levels.

## 1.2 Results

For octyl salicylate, the highest concentration that could be tested in the assays was 100 µM based on solubility and cytotoxicity results. Induction or inhibition of testosterone was not observed in Runs 3 or 4 after octyl salicylate exposure. Statistically significant induction of testosterone was observed at 100 µM octyl salicylate in Run 2. Statistically significant induction of estradiol was observed at 10 and 100 µM octyl salicylate in Run 2 and at 100 µM in Run 3. Inhibition of estradiol was observed at 0.0001, 0.001, 0.01, and 1 µM octyl salicylate in Run 3. Although statistically significant results were identified at the 100 µM octyl salicylate exposure concentration, precipitation was noted under the microscope after the 48 hour exposure period at this concentration in all three runs of the assay. When the octyl salicylate results for Run 3 were normalized to cell viability, the increases and decreases in estradiol noted above were no longer statistically significant. Induction or inhibition of estradiol was not observed at any octyl salicylate exposure concentrations in Run 4.

The highest concentration of oxybenzone that could be tested in the assays was 100 µM based on solubility and cytotoxicity results. Statistically significant induction of testosterone was observed at the 100 µM concentration for oxybenzone in all three runs of the assay. Statistically significant estradiol induction was observed at 10 and 100 µM in Runs 2 and 3 and at 0.1, 1, 10, and 100 µM in Run 4. Although statistically significant results were identified at the 100 µM oxybenzone concentration in all three runs, precipitation was noted under the microscope after the 48 hour exposure period at this concentration in Runs 3 and 4.

The highest concentration of octocrylene that could be analyzed was 1 µM in Runs 2 and 4, and 100 µM in Run 3 based on cytotoxicity results. Cytotoxicity greater than 20% was observed at 10 and 100 µM in Runs 2 and 4. No precipitation was observed at time of exposure. No statistically significant effects on estradiol or testosterone were observed in Run 2 at any of the concentrations that were analyzed. In Run 3, statistically significant inhibition of testosterone and estradiol were observed at the 10 and 100 µM octocrylene doses. Although statistically significant results were identified at the 100 µM octocrylene concentration in Run 3, precipitation was noted under the microscope after the 48 hour exposure period at this concentration in this run. When the Run 3 results were normalized to cell viability, statistically significant inhibition of testosterone was observed at 100 µM and statistically significant inhibition of estradiol was observed at 100 µM. After normalization, a statistically significant increase in estradiol was observed at 1 µM. In Run 4, statistically significant induction of estradiol was observed at the 1 µM concentration.

The highest concentration of octylmethoxycinnamate that could be tested in the assays was 0.1 µM in Run 1 and 100 µM in all Runs 2 and 3 based on solubility results. Precipitation was observed prior to exposure in Run 1 at the 1, 10, and 100 µM concentrations. Cytotoxicity greater than 20% was not observed in any of the three runs at any of the concentrations tested. No statistically significant effects were observed on testosterone or estradiol production at any of the concentrations that were analyzed in any of the three runs.

## 1.3 Conclusion

Octyl salicylate treatment resulted in variable effects on testosterone and estradiol production. Statistically significant effects on hormone production were observed; however, the results were not consistent across the three independent runs of the assay. Based on the data interpretation procedure outlined in the OECD test guideline (Test Guideline 456), octyl salicylate is negative for effects on testosterone, as statistically significant effects were observed at one concentration in one run of the assay. The statistically significant effect was not confirmed in subsequent runs. Treatment with octyl salicylate showed a statistically significant increase in estradiol in two of the three runs of the assay (10 and 100 µM in Run 2; 100 µM in Run 3). When the results for Run 3 were normalized to cell viability, the noted changes in estradiol were no longer statistically significant.

The effect on estradiol was not consistent across the three runs; however, a trend was observed where increased estradiol concentrations coincided with increasing exposure concentration of octyl salicylate.

Oxybenzone exposure resulted in statistically significant induction of both testosterone and estradiol in all three runs of the assay. For testosterone, statistically significant induction was observed at the highest concentration tested (100 μM) in all three runs. Based on the criteria outlined in OECD Test Guideline 456, the effects of oxybenzone on testosterone in each run would be classified as equivocal. The equivocal results could be clarified with additional runs of the steroidogenesis assay with a narrower concentration range around the 100 μM oxybenzone dose. For estradiol, statistically significant induction was observed at the two highest concentrations (10 and 100 μM) tested in Runs 2 and 3 and at the four highest concentrations tested in Run 4 (0.1, 1, 10, and 100 μM). Based on the data interpretation criteria outlined in the OECD test guideline for the assay, oxybenzone would be classified as positive in the steroidogenesis assay for effects on estradiol.

Octocrylene exposure resulted in variable effects on testosterone and estradiol. No statistically significant effects were observed in one run of the assay. Inhibition of testosterone and estradiol was observed at two exposures in one run, while induction of estradiol was observed at one exposure concentration in the remaining run. Because of cytotoxicity, the two highest exposure concentrations of octocrylene were analyzed in only one of the three runs of the assay. No statistically significant effects on testosterone were identified at any of the concentrations that could be analyzed in all three runs of the assay (0.0001 – 1 μM). Statistically significant reductions in testosterone were observed at the two highest octocrylene concentrations tested (10 and 100 μM) in one run; however, theses concentrations also showed reduced viability to 81 and 89% of vehicle controls, respectively. For estradiol, statistically significant reductions were also observed at the two highest octocrylene concentrations tested in one run. As with the testosterone results these effects were observed at concentrations where the viability was reduced to 81 and 89% of vehicle controls, respectively. A statistically significant increase in estradiol was observed at the 1 μM concentration in one run of the assay (Run 4). After normalization to cell viability, a statistically significant increase in estradiol was observed at the 1 μM octocrylene concentration in Run 3 as well.

No statistically significant effects on testosterone or estradiol production were observed at any of the concentration that could be analyzed after octylmethoxycinnamate exposure. Based on the data interpretation criteria outlined in the OECD test guideline, octylmethoxycinnamate is negative in the steroidogenesis assay.

# 2.0 INTRODUCTION

## 2.1 Purpose

The objective of this study was to evaluate the ability of four test chemicals to affect the steroidogenic pathway beginning with the sequence of reactions occurring after the gonadotropin hormone receptors through the production of testosterone and estradiol/estrone using the H295R cell line.

The human H295R cell line is a human adrenocarcinoma cell line that expresses genes that encode for all the key enzymes for steroidogenesis.

## 2.2 Regulatory Citations

OPPTS 890.1550: Steroidogenesis (Human Cell Line – H295R). 2009.

# 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, Octyl 4-methoxycinnamate (Octylmethoxycinnamate) |
| 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, 2-Ethylhexyl Salicylate (Octylsalate) |
| 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 Aldrich |
| CAS number: | 6197-30-4 |
| Description: | Yellow viscous liquid |
| 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 13.

### 3.1.2 Vehicle Selection

Dimethyl sulfoxide (DMSO; Sigma Aldrich, lot number RNBB7617) was selected as a suitable vehicle for all compounds. Forskolin and prochloraz were prepared on July 19, 2011, July 20, 2011, July 26, 2011, and July 27, 2011 for use in this study. Test chemicals were prepared in DMSO on July 19, 2011, July 20, 2011, July 26, 2011, and July 27, 2011 for use in this study.

The 22R-hydroxycholesterol was prepared in ethanol on July 18, 2011, July 19, 2011, July 20, 2011, July 25, 2011, July 26, 2011, and July 27, 2011.

## 3.2 Control Substances

### 3.2.1 Forskolin

| | |
|---|---|
| Source: | Sigma Aldrich (St. Louis, MO) |
| CAS number: | 66575-29-9 |
| Description: | White powder |
| Solvent used: | DMSO |
| Lot number: | 097K50653V, 109K50571V |
| Expiration date: | May 2016, July 2016 |
| Purity: | 99%, 98% |
| Molecular formula: | $C_{22}H_{34}O_7$ |
| Molecular weight: | 410.50 |
| Storage conditions: | Room Temperature |

### 3.2.2 Prochloraz

| | |
|---|---|
| Source: | Sigma Aldrich (St. Louis, MO) |
| CAS number: | 67747-09-5 |
| Description: | White powder |
| Solvent used: | DMSO |
| Lot number: | SZE6220X |
| Expiration date: | 08 Aug 2012 |
| Purity: | 99.1% |
| Molecular formula: | $C_{15}H_{16}C_{13}N_3O_2$ |
| Molecular weight: | 376.67 |
| Storage conditions: | Room Temperature |

### 3.2.3 Other Materials

#### 3.2.3.1 22R-Hydroxycholesterol

| | |
|---|---|
| Source: | Sigma Aldrich (St. Louis, MO) |
| CAS number: | 17954-98-2 |
| Description: | White powder |
| Solvent used: | Ethanol |
| Lot number: | 089K4132, 060M4098 |
| Retest Date: | July 2012, June 2013 |
| Purity: | 99.0% |
| Molecular weight: | 402.65 |

| | |
|---|---|
| Storage conditions: | Room Temperature |

## 3.3 Cell Line

### 3.3.1 Source

The H295R cell line was used in this study. The cell line was obtained from the American Type Culture Collection (ATCC CLR-2128; Lot #7635054), Manassas, VA.

### 3.3.2 Stability of the Cell Line

The stability of the cell line was monitored by the use of the following reference chemicals: forskolin and prochloraz. Two concentrations for each reference chemical were included on a QC plate each time the assay was performed and the fold change values for testosterone and estradiol were compared to the acceptable values summarized below (values taken from the cited guideline). Additionally, basal production of testosterone and estradiol on the QC plate were compared to the acceptable values below (from the cited guideline).

| | **Testosterone** | **Estradiol** |
|---|---|---|
| Basal Production | ≥5 times method detection limit | ≥2.5 times method detection limit |
| Induction (10 µM Forskolin) | ≥2 times solvent control | ≥7.5 times solvent control |
| Inhibition (1 µM Prochloraz) | ≤0.5 times solvent control | ≤0.5 times solvent control |

### 3.3.3 Cell Culture and Plating Conditions

Cells were maintained in Dulbecco's modified Eagle's medium/nutrient mixture F-12 Ham with 15 mM HEPES (Sigma, Lot#021M8304), sodium bicarbonate (Sigma, Lot#068K0105), ITS+Premix (Becton Dickinson, Catalog #354352, Lot #05245, 09233), and 2.5% Nu-Serum (Becton Dickinson, Catalog #355500, Lot #81515, hormone concentrations in undiluted Nu-Serum: testosterone: 3754 pg/mL, estradiol: 3846 pg/mL) in a 5% $CO_2$ incubator at approximately 37°C. H295R cells were grown for five passages, frozen in liquid nitrogen, then thawed and cultured for seven or eight additional passages prior to use in the assay. The culture medium was supplemented with 10 µM 22R-hydroxycholesterol at the time of plating, dosing, and harvest. The concentration of 22R-hydroxycholesterol was chosen based on laboratory proficiency experiments previously conducted at CeeTox. The cells were plated into wells of a 24-well cell culture plate at a density of 300,000 cells/mL. The cells were then placed into a 5% $CO_2$ incubator at approximately 37°C for approximately 24 hours prior to chemical exposure.

## 3.4 Chemical Exposure and Assay Plate Organization

The test chemicals were dissolved in DMSO to make 200 mM stocks and then serially diluted 1:10 in DMSO. 22R-hydroxycholesterol was dissolved in ethanol to make a 40 mM stock and then diluted in supplemented medium to a final concentration of 10 µM. The test chemicals were then diluted 1:2000 in supplemented medium containing 10 µM 22R-hydroxycholesterol to prepare mastermix solutions. Forskolin and prochloraz were dissolved in DMSO to make 100 mM solutions and then serially diluted in DMSO. Forskolin and prochloraz were then diluted 1:2000 in supplemented medium containing 10 µM 22R-hydroxycholesterol. When added to the cell culture plates, these dilutions yielded final concentrations of 1 µM and 10 µM for forskolin, 0.1 µM and 1 µM for prochloraz and 0.0001, 0.001, 0.01, 0.1, 1, 10, and 100 µM for the test chemicals, with the final concentration of DMSO in the medium being held constant at 0.05% (v/v). The final ethanol concentration in the supplemented medium was 0.025% (v/v). In Run 1, a protocol deviation resulted in final forskolin concentrations of 3.33 µM and 10 µM (see Appendix 12).

For octylmethoxycinnamate, the concentrations of all stock solutions prepared differed by approximately 2% from the indicated concentrations as the purity used when preparing the compound was lower than the purity listed on the certificate of analysis (99.8% purity listed on certificate of analysis; 98% purity used in calculation for compound preparation). This difference was noted, but was determined not to have an impact on the integrity of the data.

The cells were checked microscopically for good attachment and proper morphology prior to dosing. The medium was removed from the cells and replaced with 1 mL of medium containing 10 µM 22R-hydroxycholesterol and the concentrations of test chemicals indicated in the table below. All concentrations were tested in replicates of 3/plate. Assay plates were organized as detailed below:

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| **A** | Solvent Control | Solvent Control | Solvent Control | 0.1 µM | 0.1 µM | 0.1 µM |
| **B** | 100 µM | 100 µM | 100 µM | 0.01 µM | 0.01 µM | 0.01 µM |
| **C** | 10 µM | 10 µM | 10 µM | 0.001 µM | 0.001 µM | 0.001 µM |
| **D** | 1 µM | 1 µM | 1 µM | 0.0001 µM | 0.0001 µM | 0.0001 µM |

A concurrent quality control plate was included with each of the independent runs of the test chemical plates. The QC plate was prepared and dosed in the same manner as the test chemicals with either forskolin or prochloraz according to the following plate map.

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| **A** | Blank | Blank | Blank | Background | Background | Background |
| **B** | DMSO | DMSO | DMSO | DMSO + MeOH | DMSO + MeOH | DMSO + MeOH |
| **C** | Forskolin 1 µM | Forskolin 1 µM | Forskolin 1 µM | Prochloraz 0.1µM | Prochloraz 0.1µM | Prochloraz 0.1µM |
| **D** | Forskolin 10 µM | Forskolin 10 µM | Forskolin 10 µM | Prochloraz 1µM | Prochloraz 1µM | Prochloraz 1µM |

Note: In Run 1, the wells labeled as forskolin 1 µM (C1, C2, C3) were dosed with 3.33 µM forskolin. The wells were dosed with 1 µM forskolin in the other two runs of the assay.

After adding the reference chemical/test chemical dosing solutions, the plates were incubated in a 5% $CO_2$ incubator at approximately 37°C for approximately 48 hours. After the 48 hour exposure, each well was examined under the microscope and images were taken of the vehicle control wells as well as the two greatest non-cytotoxic concentrations (based on observation under the microscope). The media was collected from all wells in two equal aliquots and stored at approximately -80°C until shipment to the analytical laboratory for hormone measurement. After media removal, cell viability measured by MTT assay, described in section 3.5.1 below.

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 is not considered to have affected the integrity of the study. For the positive control compounds, stability is demonstrated by an appropriate response in the assay system.

## 3.5 Assays

### 3.5.1 Cytotoxicity Assay

Cell viability was monitored by MTT assay after the 48 hour exposure. On the QC plates, wells designated to receive methanol (control wells for cell death measurements) were rinsed twice with PBS (Gibco, Cat#10010, Lot#979431), then incubated in methanol(Sigma, Cat#494437, Lot#SHBB0973V) for 30 minutes at room temperature. After the methanol incubation, the methanol-treated wells were rinsed again with PBS three times. Following media removal and/or methanol treatment, 0.5 mL of 0.5 mg/mL MTT (Sigma, Cat#M5655, Lot# MKBD8254V, MKBG1611V) solution in supplemented medium containing 10 µM 22R-hydroxycholesterol was added to each well of the test chemical and QC plates. The plates were incubated at approximately 37°C in a 5% $CO_2$ incubator for 3 hours. Following the 3 hour incubation, the MTT solution was removed from each well and 0.5 mL of isopropanol (Sigma, Cat#278475, Lot#77996MMV; Cat#I9516, Lot#13696CPV) was added to each well. Plates were incubated at room temperature for 20 minutes with shaking. Following this incubation, absorbance at 570 nm and 650 nm were measured on a Packard Fusion plate reader. The absorbance at 650 nm was subtracted from the absorbance at 570 nm to calculate the MTT value for each well.

The change in cell viability was determined by comparing treated wells to the solvent control wells. A greater than 20% reduction in cell viability was considered evidence of cytotoxicity.

### 3.5.2 Precipitation Assay

Final dilutions of test chemical in supplemented media were observed visually for evidence of precipitation. If precipitation was observed, the concentration was considered insoluble and was excluded from further analysis.

### 3.5.3 Hormone Measurement System

Testosterone and estradiol levels were measured using HPLC/MS-MS at OpAns, LLC (Durham, NC). The method detection limit is 100 pg/mL for testosterone and 10 pg/mL for estradiol. The resulting minimum basal production levels based on the specifications in the test guideline (OPPTS 890.1550) are 500 pg/mL for testosterone and 25 pg/mL for estradiol. The report from this test site is provided as Appendix 14 in this report.

## 3.6 Data Analysis and Interpretation

### 3.6.1 QC Plates

Mean values (pg/mL) and standard deviations for testosterone and estradiol were calculated for each concentration of the reference chemicals and the solvent controls, as well as for the blank and background wells. Relative changes in hormone production were calculated using the following equation:

Relative Change = [Hormone] in each well ÷ [Hormone] of mean solvent (vehicle) control

For forskolin induction of testosterone, the background hormone production was subtracted from the forskolin-treated wells and blank and solvent control wells before calculating the relative change. Background hormone production was calculated from three wells with cells on the QC plate that received no 22R-hydroxycholesterol at the time of exposure.

### 3.6.2 Test Chemical Plates

Mean values (pg/mL) and standard deviations for testosterone and estradiol were calculated for each concentration of the test chemical, reference chemicals, and the solvent controls. Relative changes in testosterone and estradiol production were calculated using the equation below:

Relative Change = [Hormone] in each well ÷ [Hormone] of mean solvent (vehicle) control

All concentrations that exhibited greater than 20% cytotoxicity in the MTT cell viability assay were excluded from further analysis. Concentrations where precipitation was observed were also excluded from further analysis.

Normality of the data was evaluated using Shapiro-Wilk's test. Homogeneity of the variances between the treatment groups was evaluated using Levene's test. If the p-values were greater than 0.05 in both tests, statistical significance between each treatment group and the control group was evaluated using Dunnett's test. If the p-values were less than or equal to 0.05 in either the normality or the homogeneity test, a log transformation was performed on the data to attempt to approximate a normal distribution. If, following the transformation, p-values were greater than 0.05 in both the normality and homogeneity tests, Dunnett's test was performed on the transformed data to evaluate statistical significance between each treatment group and the control group. If, following the log transformation, p-values were less than or equal to 0.05 in either the normality or the homogeneity test, the non-transformed data set was analyzed using the nonparametric Kruskal-Wallis test followed by Dunn's test to evaluate statistical significance between each treatment group and the control group.

# 4.0 RESULTS AND DISCUSSION

## 4.1 Concentration Range for the Test Substance

All four chemicals were tested at the following concentrations: 0.0001, 0.001, 0.01, 0.1, 1, 10, and 100 μM. The concentrations of all solutions prepared for octylmethoxycinnamate differed by approximately 2% from the indicated concentrations as the purity used when preparing the compound was lower than the purity listed on the certificate of analysis (99.8% purity listed on certificate of analysis; 98% purity used in calculation for compound preparation). Precipitation was not observed for any of the compounds at any of the concentrations tested prior to exposure of the cells, with the exception of octylmethoxycinnamate in Run 1. Precipitation of octylmethoxycinnamate was observed at the 1, 10, and 100 μM concentrations in Run 1 prior to dosing. These concentrations were excluded from the statistical analysis. Precipitation was not observed at any concentrations of octylmethoxycinnamate in the other two runs of the assay.

## 4.2 Assay Acceptance Criteria

In all four independent runs of the assay, the basal production of testosterone and estradiol on the quality control plates were above the required levels specified in section 3.3.2. In addition, the fold change required after induction with 10 μM forskolin and inhibition with 1 μM prochloraz on the quality control plates met the requirements specified in section 3.3.2 for both the testosterone and estradiol analyses.

The coefficients of variation for solvent control replicate wells for testosterone and estradiol within a plate based on absolute concentrations were less than 30% as specified in the test guideline (OPPTS 890.1550) for all test chemical plates. The between plate coefficient of variation for solvent controls based on fold change was 2.73% for testosterone and 2.61% for estradiol for octyl salicylate. The between plate coefficient of variation for solvent controls for

oxybenzone based on fold change was 4.84% for testosterone and 3.45% for estradiol. For octocrylene, the between plate coefficient of variation for solvent controls based on fold change was 4.98% for testosterone and 4.14% for estradiol. For octylmethoxycinnamate, the between plate coefficient of variation for solvent controls based on fold change was 8.35% for testosterone and 6.83% for estradiol. The between plate coefficients of variation fall within the specifications outlined in the test guideline for all compounds tested.

## 4.3 Assay Results and Discussion

For octyl salicylate, the highest concentration that could be tested in the assays was 100 µM. No precipitation was observed at time of exposure. Cytotoxicity greater than 20% was not observed in any of the three runs at any of the concentrations tested. Precipitation was observed in the wells at the 100 µM exposure concentration following the 48 hour exposure period in all three runs. Induction or inhibition of testosterone was not observed in Runs 3 or 4 after octyl salicylate exposure. Statistically significant induction of testosterone was observed at 100 µM octyl salicylate in Run 2. Statistically significant induction of estradiol was observed at 10 and 100 µM octyl salicylate in Run 2 and at 100 µM in Run 3. Inhibition of estradiol was observed at 0.0001, 0.001, 0.01, and 1 µM octyl salicylate in Run 3. When the octyl salicylate results for Run 3 were normalized to cell viability, the noted increases and decreases in estradiol were no longer statistically significant. Induction or inhibition of estradiol was not observed at any concentrations in Run 4.

The highest concentration of oxybenzone that could be tested in the assays was 100 µM. No precipitation of the compound was observed at time of exposure. Cytotoxicity greater than 20% was not observed in any of the three runs at any of the concentrations tested. Precipitation was observed in the wells at 100 µM following the 48 hour exposure period in two of the three runs (Runs 3 and 4). Statistically significant induction of testosterone was observed at the 100 µM concentration for oxybenzone in all three runs of the assay. Statistically significant estradiol induction was observed at 10 and 100 µM in Runs 2 and 3 and at 0.1, 1, 10, and 100 µM in Run 4.

For octocrylene, the highest concentration that could be tested in the assays was 1 µM in Runs 2 and 4, and 100 µM in Run 3. Cytotoxicity greater than 20% was observed at 10 and 100 µM in Runs 2 and 4. The cytotoxic concentrations were omitted from the statistical analysis. No precipitation was observed at time of exposure at any of the concentrations tested. Precipitation was observed in the wells at 100 µM following the 48 hour exposure period in all three runs. No statistically significant effects on estradiol or testosterone were observed in Run 2 at any of the concentrations tested. In Run 3, statistically significant inhibition of testosterone and estradiol were observed at the 10 and 100 µM octocrylene doses. When the Run 3 results were normalized to cell viability, statistically significant inhibition of testosterone was observed at 100 µM and statistically significant inhibition of estradiol was observed at 100 µM. After

normalization, a statistically significant increase in estradiol was observed at 1 µM. In Run 4, statistically significant induction of estradiol was observed at the 1 µM concentration.

For octylmethoxycinnamate, the highest concentration that could be tested in the assays was 0.1 µM in Run 1 and 100 µM in all Runs 2 and 3. Precipitation was observed prior to dosing in Run 1 at the 1, 10, and 100 µM concentrations. These concentrations were omitted from the statistical analysis. Cytotoxicity greater than 20% was not observed in any of the three runs at any of the concentrations tested. No statistically significant effects were observed on testosterone or estradiol production at any of the concentrations that could be analyzed in any of the three runs.

## 5.0 CONCLUSIONS

Octyl salicylate treatment resulted in variable effects on testosterone and estradiol production. Statistically significant effects on hormone production were observed; however, the results were not consistent across the three independent runs of the assay. Based on the data interpretation procedure outlined in the OECD test guideline (Test Guideline 456), octyl salicylate is negative for effects on testosterone as statistically significant effects were observed at one concentration in one run of the assay. The statistically significant effects were not confirmed in subsequent runs. Octyl salicylate treatment was associated with statistically significant increases in estradiol in two of the three runs of the assay (10 and 100 µM in Run 2; 100 µM in Run 3). When the results for Run 3 were normalized to cell viability, the noted changes in estradiol were no longer statistically significant. The statistically significant effects were not consistent across the three runs; however, a trend was observed where increased estradiol concentrations coincided with increasing exposure concentration of octyl salicylate.

For oxybenzone, statistically significant induction of testosterone and estradiol was observed in all three runs of the assay. For testosterone, statistically significant induction was observed at the highest concentration tested (100 µM) in all three runs. Based on the criteria outlined in OECD Test Guideline 456, the effects of oxybenzone on testosterone in each run would be classified as equivocal. The equivocal results could be clarified with additional runs of the steroidogenesis assay with a narrower concentration range around the 100 µM oxybenzone dose. For estradiol, statistically significant induction was observed at the two highest concentrations (10 and 100 µM) tested in Runs 2 and 3 and at the four highest concentrations tested in Run 4 (0.1, 1, 10, and 100 µM). Based on the data interpretation criteria outlined in the OECD test guideline for the assay, oxybenzone would be classified as positive in the steroidogenesis assay for effects on estradiol.

Octocrylene exposure resulted in variable effects on testosterone and estradiol. No statistically significant effects on testosterone or estradiol were observed in one run of the assay (Run 2). Inhibition of testosterone and estradiol was observed at two exposures in one run (Run 3), while induction of estradiol was observed at one exposure concentration in the remaining run (Run 4).

Because of cytotoxicity the two highest exposure concentrations of octocrylene could be analyzed in only one of the three runs of the assay. No statistically significant effects on testosterone were identified at any of the concentrations that could be analyzed in all three runs of the assay (0.0001 – 1 μM). Statistically significant reductions in testosterone were observed at the two highest octocrylene concentrations tested (10 and 100 μM) in one run; however, theses concentrations also showed reduced viability to 81 and 89% of vehicle controls, respectively. For estradiol, statistically significant reductions were also observed at the two highest octocrylene concentrations tested in one run. As with the testosterone results these effects were observed at concentrations where the viability was reduced to 81 and 89% of vehicle controls, respectively. A statistically significant increase in estradiol was observed at the 1 μM concentration in one run of the assay (Run 4). After normalization to cell viability, a statistically significant increase in estradiol was observed in a second run as well (Run 3).

No statistically significant effects on testosterone or estradiol production were observed at any of the concentrations that could be analyzed after octylmethoxycinnamate exposure. Based on the data interpretation criteria outlined in the OECD test guideline, octylmethoxycinnamate is negative in the steroidogenesis assay.

## 6.0 REFERENCES

Endocrine Disruptor Screening Program Test Guidelines. *OPPTS 890.1550: Steroidogenesis (Human Cell Line – H295R).* EPA 640-C-09-003. October 2009.

OECD Guideline for the Test of Chemicals. *H295R Steroidogenesis Assay.* Test Guideline 456. 28 July 2011.

# TABLES SECTION

**TABLE 1 Results of MTT Cell Viability Assay – QC Plate**

| Condition | Cell Viability – Run 1 (% of SC) | | Cell Viability - Run 2 (% of SC) | | Cell Viability – Run 3 (% of SC) | | Cell Viability – Run 4 (% of SC) | |
|---|---|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** |
| Blank | 100.4 | 4.97 | 104.3 | 2.69 | 104.3 | 2.81 | 100.7 | 3.98 |
| Background | 94.7 | 2.09 | 95.4 | 4.14 | 102.5 | 1.79 | 98.9 | 0.94 |
| SC + Methanol | 9.1 | 1.98 | 5.6 | 2.51 | 11.4 | 1.99 | 5.1 | 0.14 |
| Forskolin 1 µM * | 101.9 | 1.19 | 107.8 | 1.52 | 109.3 | 1.17 | 105.0 | 1.55 |
| Forskolin 10 µM | 97.5 | 4.35 | 107.0 | 2.43 | 108.5 | 4.17 | 102.4 | 2.58 |
| Prochloraz 0.1 µM | 93.5 | 1.49 | 98.3 | 2.20 | 101.2 | 0.47 | 100.7 | 0.86 |
| Prochloraz 1 µM | 94.0 | 3.07 | 99.4 | 1.19 | 101.5 | 0.78 | 99.5 | 2.37 |

SC = Solvent Control
SD = Standard Deviation
*Forskolin concentration in Run 1 was 3.33 µM. Protocol deviation is attached in Appendix 12.

**TABLE 2 Results of MTT Cell Viability Assay – Octyl Salicylate**

| Concentration (µM) | Cell Viability – Run 2 (% of SC) | | Cell Viability – Run 3 (% of SC) | | Cell Viability – Run 4 (% of SC) | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| 0.0001 | 102.8 | 1.51 | 92.7 | 2.05 | 96.5 | 2.90 |
| 0.001 | 101.0 | 0.78 | 92.3 | 0.72 | 98.5 | 0.23 |
| 0.01 | 101.7 | 0.68 | 95.1 | 1.19 | 99.2 | 1.01 |
| 0.1 | 99.2 | 1.05 | 97.2 | 2.31 | 99.2 | 2.40 |
| 1 | 102.1 | 2.89 | 94.3 | 2.45 | 92.9 | 2.20 |
| 10 | 101.5 | 3.54 | 92.0 | 2.27 | 97.5 | 1.40 |
| 100 | 99.8 | 1.11 | 92.6 | 1.30 | 93.6 | 2.01 |

SD = Standard Deviation

**TABLE 3 Results of MTT Cell Viability Assay - Oxybenzone**

| Concentration (µM) | Cell Viability – Run 2 (% of SC) | | Cell Viability – Run 3 (% of SC) | | Cell Viability – Run 4 (% of SC) | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| 0.0001 | 98.0 | 0.53 | 96.2 | 0.85 | 95.2 | 1.51 |
| 0.001 | 95.1 | 0.53 | 92.6 | 1.22 | 96.8 | 1.28 |
| 0.01 | 95.7 | 1.42 | 94.8 | 1.59 | 96.3 | 1.05 |
| 0.1 | 97.8 | 1.03 | 95.6 | 2.58 | 98.7 | 0.96 |
| 1 | 97.6 | 0.32 | 93.6 | 3.88 | 94.3 | 2.48 |
| 10 | 95.5 | 2.09 | 93.2 | 2.33 | 95.4 | 1.00 |
| 100 | 91.2 | 2.09 | 88.6 | 5.84 | 92.6 | 2.17 |

SD = Standard Deviation

**TABLE 4 Results of MTT Cell Viability Assay - Octocrylene**

| Concentration (µM) | Cell Viability – Run 2 (% of SC) | | Cell Viability – Run 3 (% of SC) | | Cell Viability – Run 4 (% of SC) | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| 0.0001 | 100.3 | 1.92 | 91.8 | 3.01 | 96.4 | 3.78 |
| 0.001 | 98.8 | 1.43 | 94.2 | 1.46 | 96.8 | 1.54 |
| 0.01 | 98.1 | 1.41 | 97.3 | 2.94 | 98.8 | 2.51 |
| 0.1 | 101.1 | 1.19 | 99.8 | 0.95 | 99.1 | 1.10 |
| 1 | 97.9 | 0.54 | 93.9 | 3.31 | 95.8 | 3.33 |
| 10 | 73.7* | 1.31 | 81.0 | 3.10 | 78.4* | 1.23 |
| 100 | 66.0* | 0.87 | 89.0 | 5.55 | 77.5* | 3.70 |

SD = Standard Deviation

*Concentrations omitted from statistical analysis because of cytotoxicity.

**TABLE 5 Results of MTT Cell Viability Assay - Octylmethoxycinnamate**

| Concentration (µM) | Cell Viability – Run 1 (% of SC) | | Cell Viability – Run 2 (% of SC) | | Cell Viability – Run 3 (% of SC) | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| 0.0001 | 94.8 | 2.94 | 97.9 | 1.83 | 91.3 | 3.00 |
| 0.001 | 95.7 | 0.61 | 98.6 | 0.75 | 93.9 | 2.10 |
| 0.01 | 98.2 | 1.51 | 100.1 | 0.87 | 96.7 | 0.79 |
| 0.1 | 98.5 | 3.89 | 101.1 | 0.57 | 98.4 | 0.66 |
| 1 | 90.7 | 4.20 | 98.7 | 1.74 | 94.3 | 5.82 |
| 10 | 94.1 | 4.67 | 96.9 | 1.74 | 95.0 | 1.19 |
| 100 | 99.8 | 2.27 | 100.9 | 3.97 | 107.6 | 6.84 |

SD = Standard Deviation

## TABLE 6 QC Plate Raw Data and Fold Change Results – Testosterone

| | Average Testosterone (pg/mL) | | | | Testosterone Fold Change over SC | | | | Testosterone Fold Change over SC – Background Subtracted | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Concentration** | **Run 1** | **Run 2** | **Run 3** | **Run 4** | **Run 1** | **Run 2** | **Run 3** | **Run 4** | **Run 1** | **Run 2** | **Run 3** | **Run 4** |
| Background | 1056 | 1135 | 1073 | 1262 | 0.49 | 0.45 | 0.57 | 0.54 | N/A* | N/A* | N/A* | N/A* |
| Blank | 2174 | 2581 | 1873 | 2304 | 1.01 | 1.03 | 1.00 | 0.99 | 1.03 | 1.05 | 1.00 | 0.97 |
| DMSO | 2143 | 2511 | 1874 | 2339 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 1 µM Forskolin** | 2930 | 3764 | 2595 | 3120 | 1.37 | 1.50 | 1.38 | 1.33 | 1.73 | 1.91 | 1.90 | 1.73 |
| 10 µM Forskolin | 3349 | 4751 | 3019 | 3780 | 1.56 | 1.89 | 1.61 | 1.62 | 2.11 | 2.63 | 2.43 | 2.34 |
| 0.1 µM Prochloraz | 1914 | 1816 | 1149 | 1818 | 0.89 | 0.72 | 0.61 | 0.78 | N/A* | N/A* | N/A* | N/A* |
| 1 µM Prochloraz | 1009 | 1188 | 501 | 1153 | 0.47 | 0.47 | 0.27 | 0.49 | N/A* | N/A* | N/A* | N/A* |

For forskolin induction of testosterone, background hormone concentration is subtracted from all other concentrations prior to calculating fold change values.

*N/A = not applicable

**Forskolin concentration in Run 1 was 3.33 µM. Protocol deviation is attached in Appendix 12.

## TABLE 7 QC Plate Raw Data and Fold Change Results – Estradiol

| | Average Estradiol (pg/mL) | | | | Estradiol Fold Change over SC | | | |
|---|---|---|---|---|---|---|---|---|
| **Concentration** | **Run 1** | **Run 2** | **Run 3** | **Run 4** | **Run 1** | **Run 2** | **Run 3** | **Run 4** |
| Background | 72 | 41 | 56 | 77 | 0.47 | 0.21 | 0.31 | 0.35 |
| Blank | 158 | 208 | 182 | 228 | 1.04 | 1.04 | 1.01 | 1.02 |
| DMSO | 153 | 200 | 179 | 223 | 1.00 | 1.00 | 1.00 | 1.00 |
| 1 µM Forskolin* | 1787 | 1343 | 1254 | 1538 | 11.69 | 6.72 | 7.01 | 6.91 |
| 10 µM Forskolin | 2002 | 2116 | 1661 | 2477 | 13.10 | 10.60 | 9.28 | 11.13 |
| 0.1 µM Prochloraz | 148 | 133 | 104 | 171 | 0.97 | 0.67 | 0.58 | 0.77 |
| 1 µM Prochloraz | 68 | 57 | 52 | 83 | 0.45 | 0.29 | 0.29 | 0.37 |

*Forskolin concentration in Run 1 was 3.33 µM. Protocol deviation is attached in Appendix 12.

## TABLE 8 Quality Control Plate Results for Testosterone

| | Run 1 | Run 2 | Run 3 | Run 4 |
|---|---|---|---|---|
| Basal Production – Blank Wells | 2174 pg/mL | 2581 pg/mL | 1873 pg/mL | 2304 pg/mL |
| Basal Production – Solvent Control Wells | 2143 pg/mL | 2511 pg/mL | 1874 pg/mL | 2339 pg/mL |
| Induction (10 µM Forskolin) | 2 | 3 | 2 | 2 |
| Inhibition (1 µM Prochloraz) | 0.5 | 0.5 | 0.3 | 0.5 |

For forskolin induction of testosterone, background hormone concentration is subtracted from all other concentrations prior to calculating fold change values.

## TABLE 9 Quality Control Plate Results for Estradiol

| | Run 1 | Run 2 | Run 3 | Run 4 |
|---|---|---|---|---|
| Basal Production – Blank Wells | 158 pg/mL | 208 pg/mL | 182 pg/mL | 228 pg/mL |
| Basal Production – Solvent Control Wells | 153 pg/mL | 200 pg/mL | 179 pg/mL | 223 pg/mL |
| Induction (10 µM Forskolin) | 13.1 | 10.6 | 9.3 | 11.1 |
| Inhibition (1 µM Prochloraz) | 0.4 | 0.3 | 0.3 | 0.4 |

## TABLE 10 Octyl Salicylate – Results for Testosterone

| Concentration (µM) | Fold Change over SC Run 2 | | Fold Change over SC Run 3 | | Fold Change over SC Run 4 | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| 0.0001 | 0.96 | 0.04 | 0.96 | 0.03 | 0.99 | 0.03 |
| 0.001 | 0.98 | 0.02 | 0.95 | 0.01 | 0.99 | 0.02 |
| 0.01 | 0.96 | 0.01 | 0.99 | 0.04 | 1.01 | 0.03 |
| 0.1 | 0.99 | 0.02 | 1.00 | 0.02 | 1.03 | 0.02 |
| 1 | 1.01 | 0.05 | 0.98 | 0.01 | 1.04 | 0.04 |
| 10 | 0.97 | 0.03 | 0.98 | 0.03 | 0.99 | 0.01 |
| 100 | 1.11*† | 0.04 | 0.99† | 0.02 | 1.05† | 0.02 |

SC = Solvent Control
SD = Standard Deviation
*Denotes statistical significance ($p \leq 0.05$).
†Precipitation observed under the microscope after the 48 hour exposure at this concentration.

## TABLE 11 Octyl Salicylate – Results for Estradiol

| Concentration (µM) | Fold Change over SC Run 2 | | Fold Change over SC Run 3 | | Fold Change over SC Run 4 | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| 0.0001 | 0.89 | 0.02 | 0.91* | 0.01 | 0.97 | 0.03 |
| 0.001 | 0.92 | 0.07 | 0.94* | 0.01 | 0.95 | 0.00 |
| 0.01 | 0.94 | 0.04 | 0.95* | 0.01 | 0.97 | 0.01 |
| 0.1 | 0.95 | 0.05 | 0.97 | 0.00 | 1.03 | 0.06 |
| 1 | 1.02 | 0.04 | 0.93* | 0.02 | 0.98 | 0.03 |
| 10 | 1.14* | 0.04 | 1.04 | 0.02 | 1.05 | 0.02 |
| 100 | 1.97*† | 0.10 | 1.24*† | 0.02 | 1.34† | 0.05 |

SC = Solvent Control
SD = Standard Deviation
*Denotes statistical significance ($p \leq 0.05$).
†Precipitation observed under the microscope after the 48 hour exposure at this concentration.

## TABLE 12 Oxybenzone – Results for Testosterone

| Concentration (µM) | Fold Change over SC Run 2 | | Fold Change over SC Run 3 | | Fold Change over SC Run 4 | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| 0.0001 | 0.95 | 0.05 | 1.02 | 0.08 | 1.02 | 0.05 |
| 0.001 | 0.90 | 0.00 | 0.99 | 0.02 | 0.99 | 0.00 |
| 0.01 | 0.97 | 0.05 | 0.98 | 0.02 | 0.99 | 0.04 |
| 0.1 | 0.97 | 0.07 | 1.01 | 0.07 | 1.08 | 0.01 |
| 1 | 0.95 | 0.05 | 0.98 | 0.02 | 1.04 | 0.02 |
| 10 | 1.02 | 0.03 | 1.03 | 0.05 | 1.07 | 0.03 |
| 100 | 1.30* | 0.08 | 1.21*† | 0.05 | 1.32*† | 0.01 |

SC = Solvent Control
SD = Standard Deviation
*Denotes statistical significance ($p \leq 0.05$).
†Precipitation observed under the microscope after the 48 hour exposure at this concentration.

## TABLE 13 Oxybenzone – Results for Estradiol

| Concentration (µM) | Fold Change over SC Run 2 | | Fold Change over SC Run 3 | | Fold Change over SC Run 4 | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| 0.0001 | 0.93 | 0.01 | 0.97 | 0.03 | 0.99 | 0.02 |
| 0.001 | 0.90 | 0.02 | 0.98 | 0.01 | 1.00 | 0.02 |
| 0.01 | 0.97 | 0.06 | 0.97 | 0.02 | 1.01 | 0.05 |
| 0.1 | 0.92 | 0.02 | 0.98 | 0.04 | 1.11* | 0.04 |
| 1 | 1.07 | 0.07 | 1.06 | 0.03 | 1.13* | 0.00 |
| 10 | 1.52* | 0.05 | 1.43* | 0.02 | 1.52* | 0.06 |
| 100 | 2.27* | 0.17 | 1.79*† | 0.04 | 2.08*† | 0.08 |

SC = Solvent Control
SD = Standard Deviation
*Denotes statistical significance ($p \leq 0.05$).
†Precipitation observed under the microscope after the 48 hour exposure at this concentration.

## TABLE 14 Octocrylene – Results for Testosterone

| Concentration (µM) | Fold Change over SC Run 2 | | Fold Change over SC Run 3 | | Fold Change over SC Run 4 | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| 0.0001 | 1.05 | 0.10 | 0.97 | 0.02 | 0.95 | 0.03 |
| 0.001 | 1.00 | 0.06 | 0.93 | 0.05 | 0.94 | 0.03 |
| 0.01 | 0.94 | 0.01 | 1.02 | 0.05 | 0.95 | 0.02 |
| 0.1 | 1.00 | 0.06 | 1.04 | 0.02 | 0.96 | 0.02 |
| 1 | 1.03 | 0.03 | 0.97 | 0.04 | 0.98 | 0.03 |
| 10 | N/A** | N/A** | 0.74* | 0.03 | N/A** | N/A** |
| 100 | N/A**† | N/A** | 0.67*† | 0.03 | N/A** | N/A**† |

SC = Solvent Control
SD = Standard Deviation
*Denotes statistical significance ($p \leq 0.05$).
**N/A = concentration omitted from statistical analysis because of cytotoxicity greater than 20%
†Precipitation observed under the microscope after the 48 hour exposure at this concentration.

## TABLE 15 Octocrylene – Results for Estradiol

| Concentration (µM) | Fold Change over SC Run 2 | | Fold Change over SC Run 3 | | Fold Change over SC Run 4 | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| 0.0001 | 1.01 | 0.07 | 1.02 | 0.01 | 1.00 | 0.04 |
| 0.001 | 1.00 | 0.05 | 0.98 | 0.05 | 0.98 | 0.04 |
| 0.01 | 0.97 | 0.02 | 1.02 | 0.05 | 1.00 | 0.01 |
| 0.1 | 1.04 | 0.05 | 1.04 | 0.02 | 0.99 | 0.03 |
| 1 | 1.07 | 0.06 | 1.06 | 0.06 | 1.13* | 0.07 |
| 10 | N/A** | N/A** | 0.87* | 0.03 | N/A** | N/A** |
| 100 | N/A**† | N/A** | 0.75*† | 0.02 | N/A** | N/A**† |

SC = Solvent Control
SD = Standard Deviation
*Denotes statistical significance ($p \leq 0.05$).
**N/A = concentration omitted from statistical analysis because of cytotoxicity greater than 20%
†Precipitation observed under the microscope after the 48 hour exposure at this concentration.

## TABLE 16 Octylmethoxycinnamate – Results for Testosterone

| Concentration (µM) | Fold Change over SC Run 1 | | Fold Change over SC Run 2 | | Fold Change over SC Run 3 | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| 0.0001 | 0.96 | 0.15 | 1.13 | 0.06 | 1.00 | 0.12 |
| 0.001 | 0.98 | 0.01 | 1.04 | 0.11 | 1.01 | 0.11 |
| 0.01 | 0.98 | 0.04 | 0.99 | 0.05 | 0.91 | 0.06 |
| 0.1 | 0.94 | 0.01 | 1.05 | 0.12 | 0.91 | 0.04 |
| 1 | N/A** | N/A** | 1.13 | 0.09 | 0.96 | 0.01 |
| 10 | N/A** | N/A** | 0.97 | 0.07 | 0.90 | 0.12 |
| 100 | N/A** | N/A** | 1.02 | 0.14 | 0.92 | 0.09 |

SC = Solvent Control
SD = Standard Deviation
**N/A = concentration omitted from statistical analysis based on solubility results

## TABLE 17 Octylmethoxycinnamate – Results for Estradiol

| Concentration (µM) | Fold Change over SC Run 1 | | Fold Change over SC Run 2 | | Fold Change over SC Run 3 | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| 0.0001 | 0.90 | 0.13 | 1.11 | 0.04 | 1.02 | 0.10 |
| 0.001 | 0.95 | 0.03 | 1.03 | 0.09 | 1.01 | 0.10 |
| 0.01 | 0.97 | 0.04 | 1.06 | 0.05 | 0.95 | 0.06 |
| 0.1 | 0.97 | 0.06 | 1.07 | 0.16 | 0.96 | 0.05 |
| 1 | N/A** | N/A** | 1.13 | 0.03 | 1.04 | 0.02 |
| 10 | N/A** | N/A** | 1.04 | 0.12 | 1.03 | 0.09 |
| 100 | N/A** | N/A** | 1.14 | 0.16 | 1.15 | 0.11 |

SC = Solvent Control
SD = Standard Deviation
**N/A = concentration omitted from statistical analysis based on solubility results

## TABLE 18 Octyl Salicylate – Run 3 Results Normalized to Percent Viability

| Concentration (µM) | Testosterone Fold Change over SC Normalized to Percent Viability Run 3 | | Estradiol Fold Change over SC Normalized to Percent Viability Run 3 | |
|---|---|---|---|---|
| | Mean | SD | Mean | SD |
| 0.0001 | 1.03 | 0.04 | 0.98 | 0.02 |
| 0.001 | 1.03 | 0.02 | 1.02 | 0.02 |
| 0.01 | 1.04 | 0.03 | 1.00 | 0.02 |
| 0.1 | 1.03 | 0.02 | 1.00 | 0.03 |
| 1 | 1.04 | 0.02 | 0.98 | 0.03 |
| 10 | 1.07 | 0.05 | 1.13 | 0.04 |
| 100 | 1.07 | 0.01 | 1.34 | 0.01 |

SC = Solvent Control
SD = Standard Deviation

## TABLE 19 Octocrylene – Run 3 Results Normalized to Percent Viability

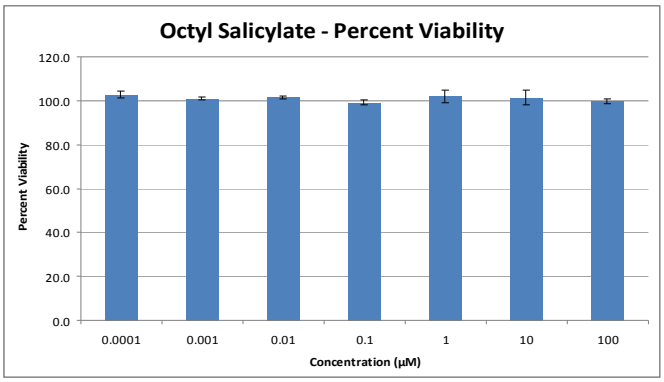
| Concentration (µM) | Testosterone Fold Change over SC Normalized to Percent Viability Run 3 | | Estradiol Fold Change over SC Normalized to Percent Viability Run 3 | |
|---|---|---|---|---|
| | Mean | SD | Mean | SD |
| 0.0001 | 1.06 | 0.01 | 1.11 | 0.04 |
| 0.001 | 0.99 | 0.07 | 1.04 | 0.07 |
| 0.01 | 1.05 | 0.06 | 1.05 | 0.07 |
| 0.1 | 1.04 | 0.02 | 1.05 | 0.02 |
| 1 | 1.03 | 0.07 | 1.13* | 0.09 |
| 10 | 0.92 | 0.02 | 1.08 | 0.03 |
| 100 | 0.76* | 0.06 | 0.84* | 0.04 |

SC = Solvent Control
SD = Standard Deviation
*Denotes statistical significance ($p \leq 0.05$)

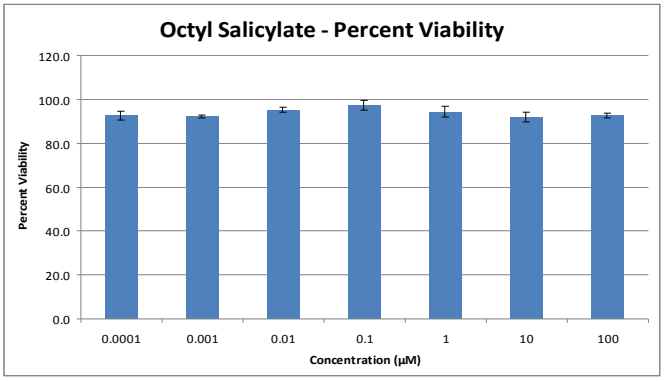
# FIGURES SECTION

## FIGURE 1 Octyl Salicylate – MTT Cell Viability Results

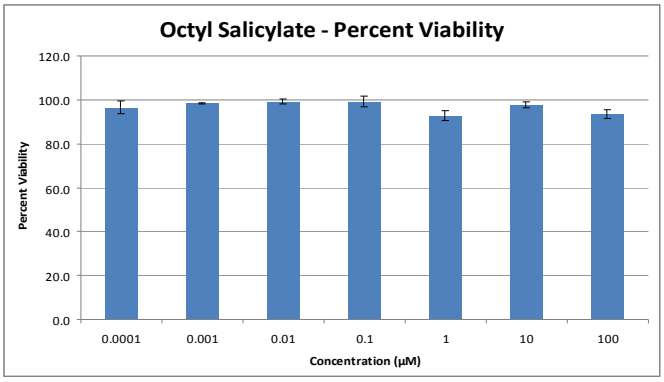
**Run 2**




**Run 3**

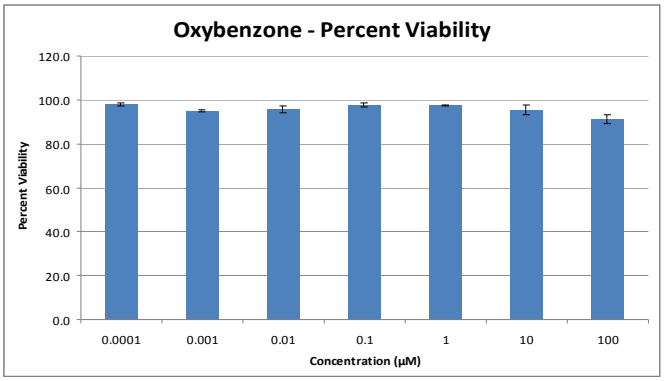



**Run 4**

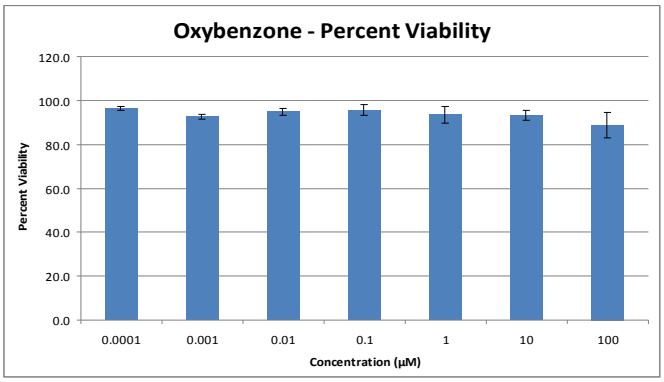


## FIGURE 2 Oxybenzone – MTT Cell Viability Results

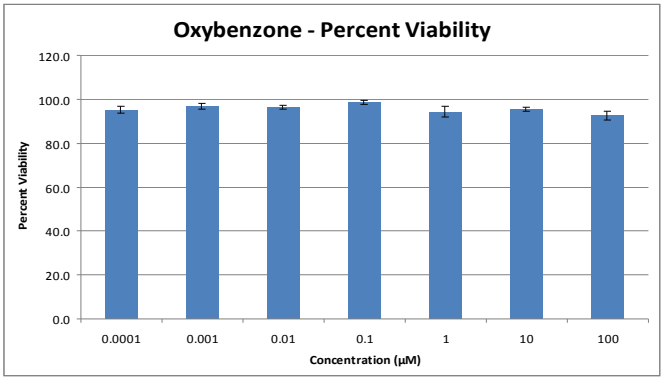
**Run 2**




**Run 3**

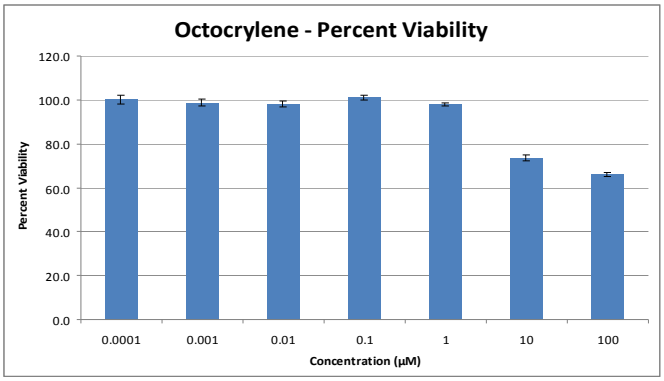



**Run 4**

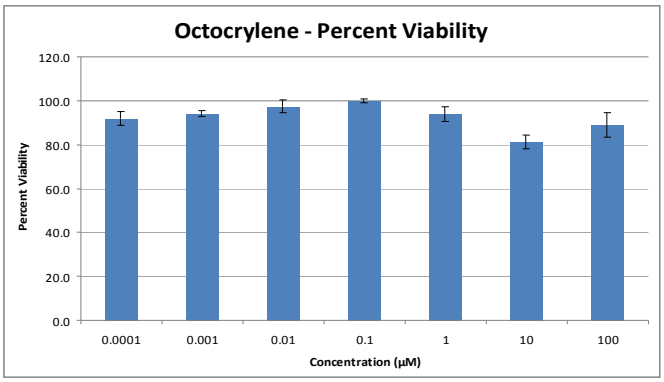


## FIGURE 3 Octocrylene – MTT Cell Viability Results

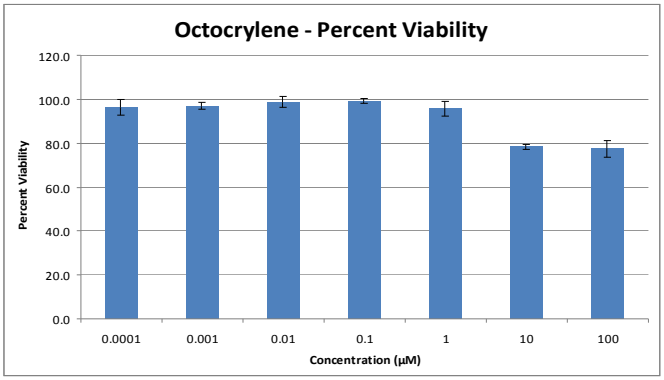
**Run 2**




**Run 3**

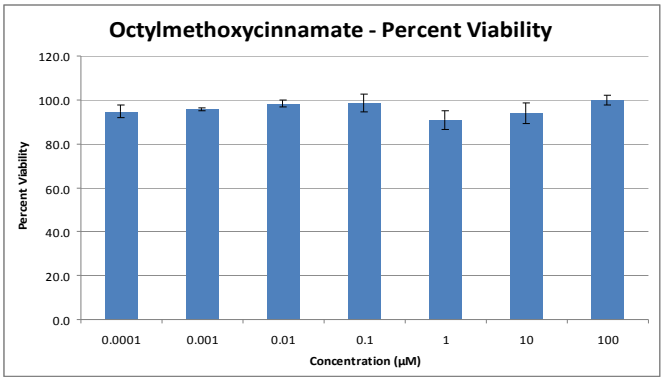



**Run 4**

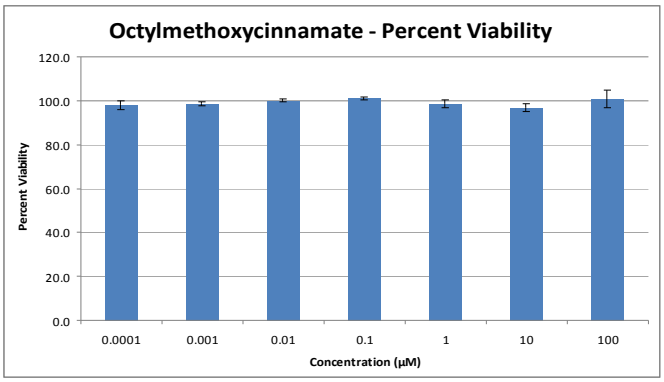


## FIGURE 4 Octylmethoxycinnamate – MTT Cell Viability Results

**Run 1**




**Run 2**




**Run 3**

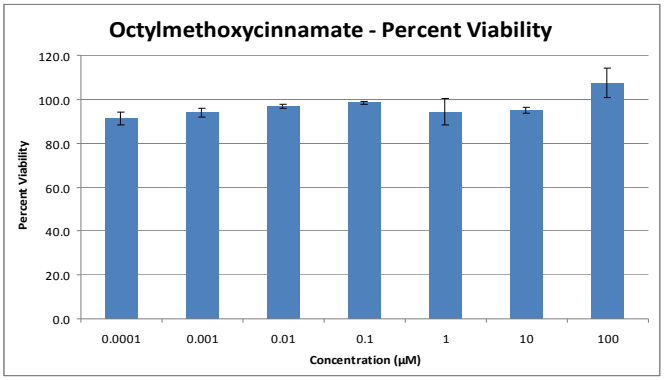
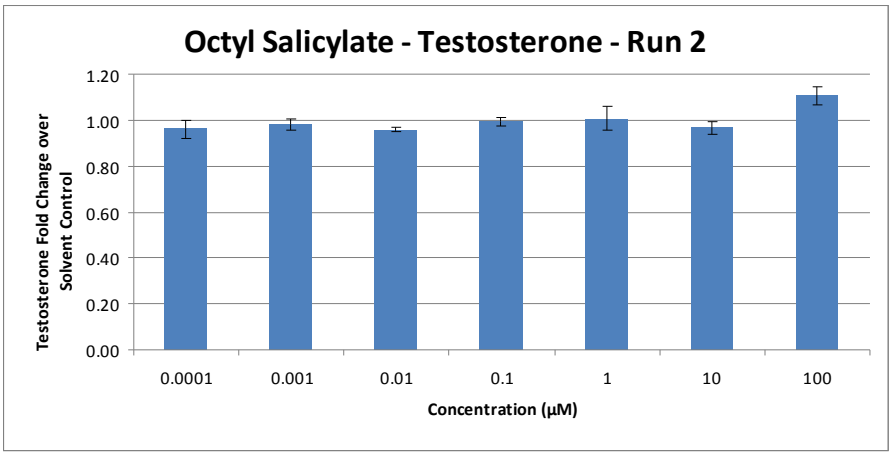
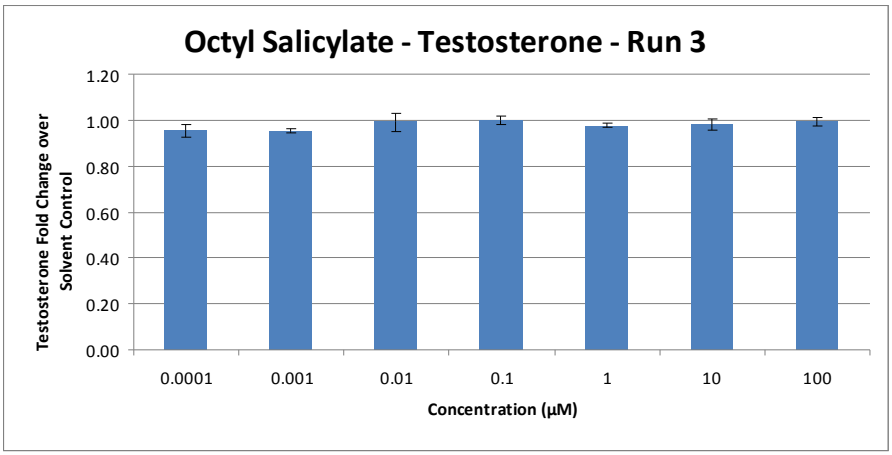


**FIGURE 5 Octyl Salicylate – Testosterone Fold Change**



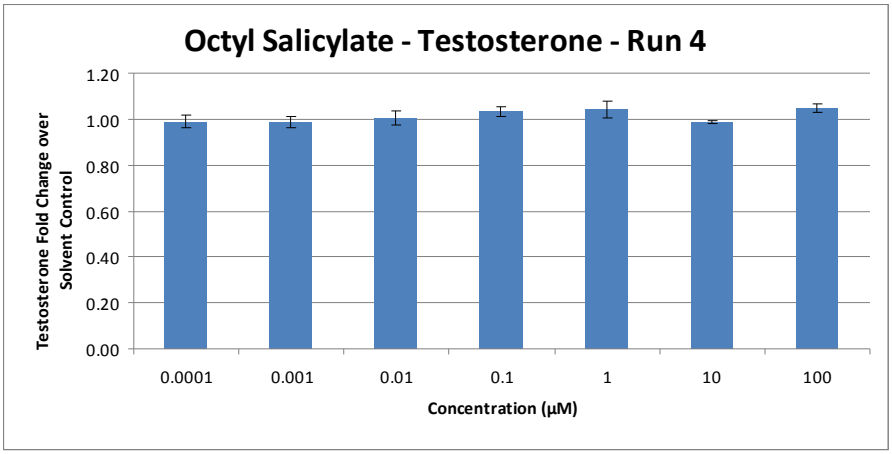
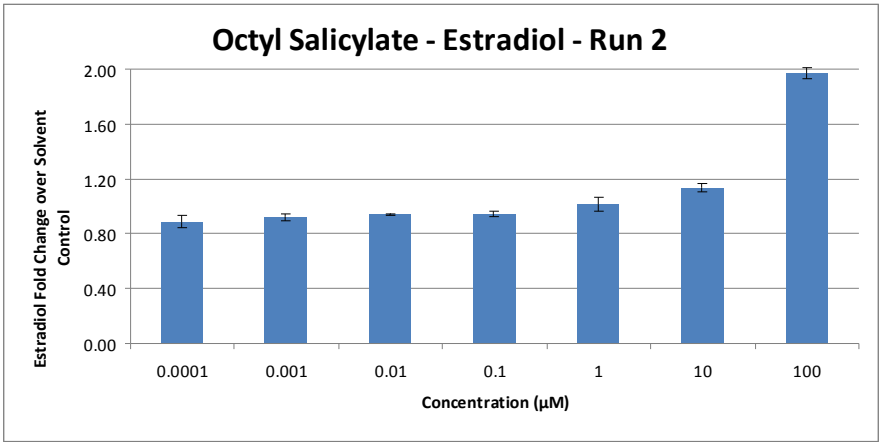
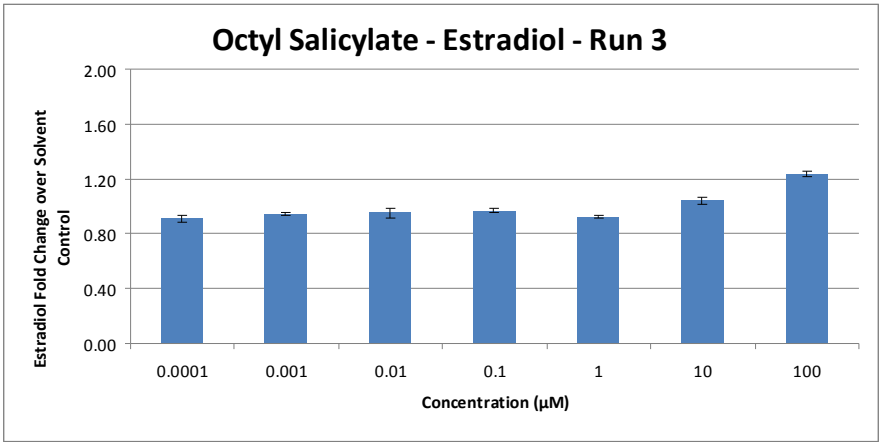



**FIGURE 6 Octyl Salicylate – Estradiol Fold Change**



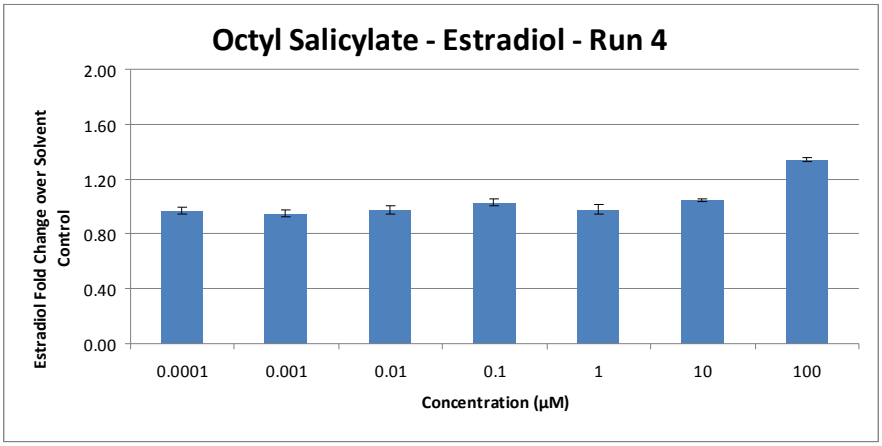
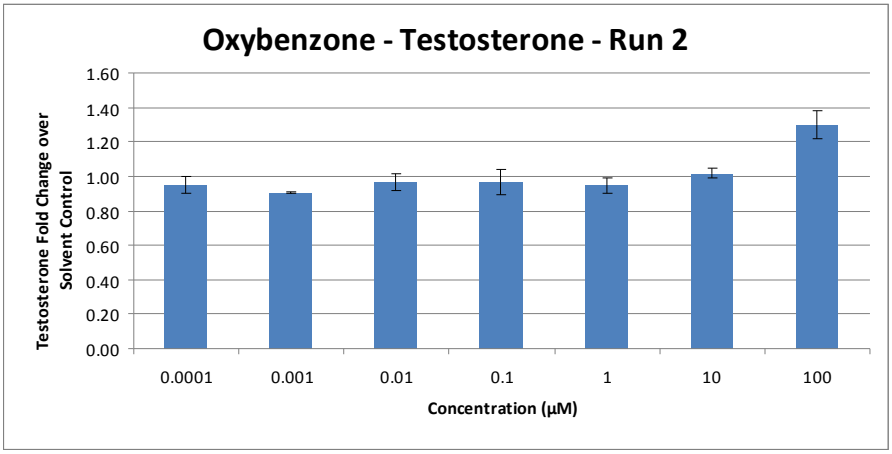
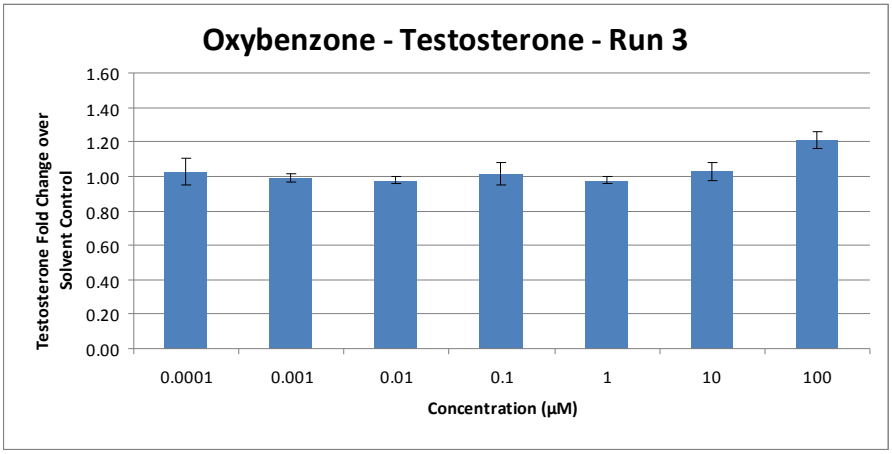



**FIGURE 7 Oxybenzone – Testosterone Fold Change**



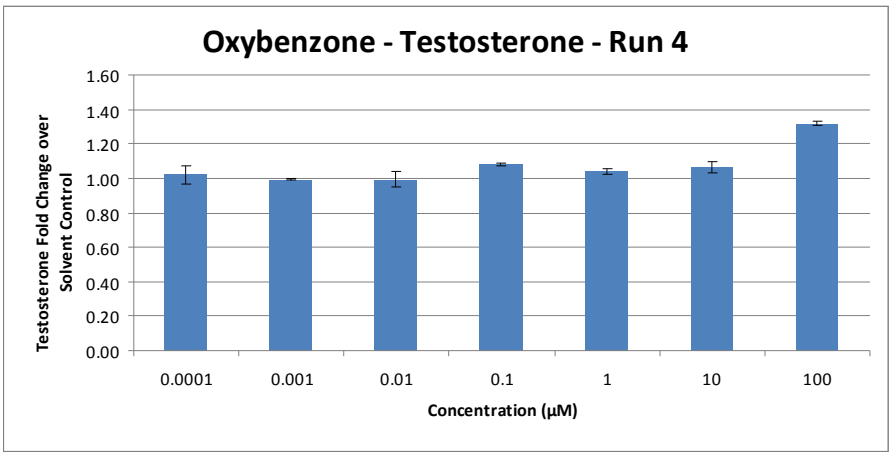
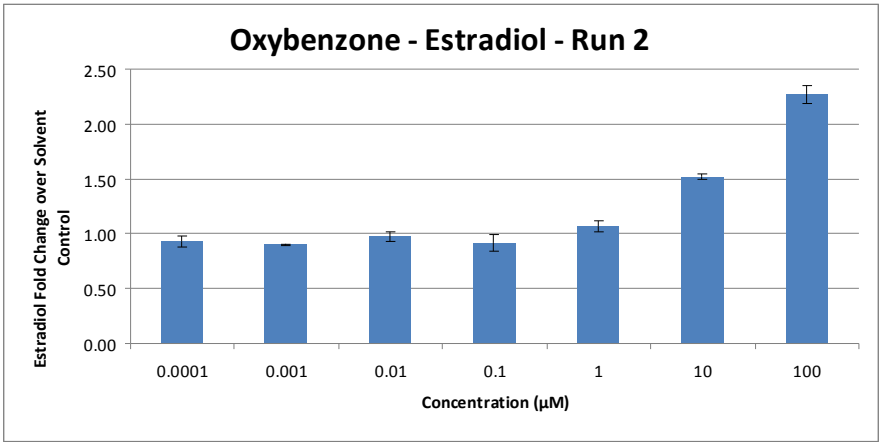
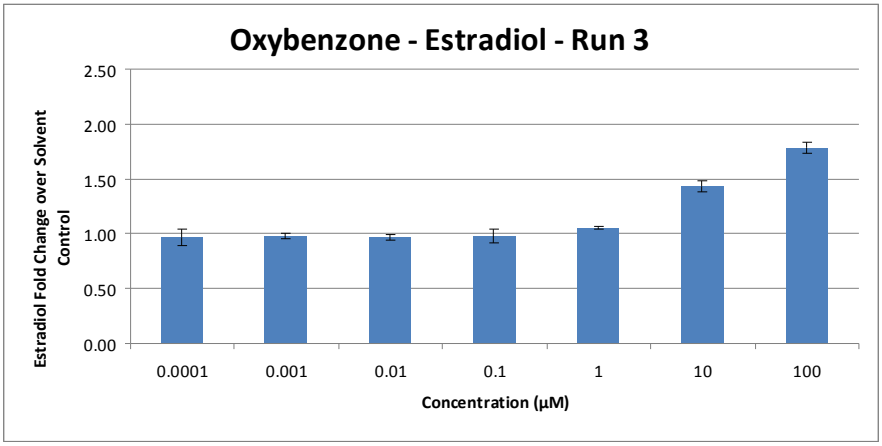
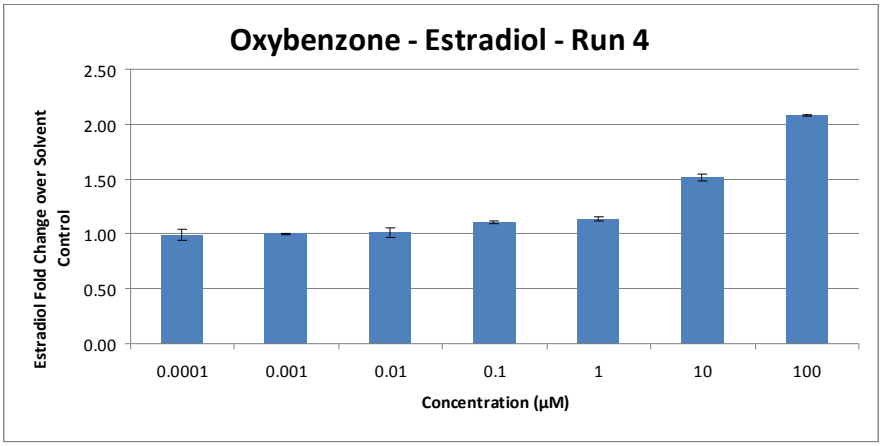



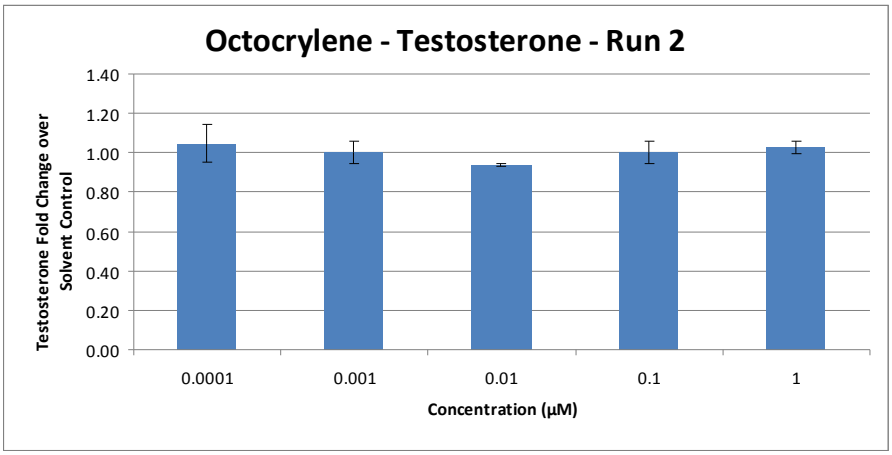
**FIGURE 8 Oxybenzone – Estradiol Fold Change**



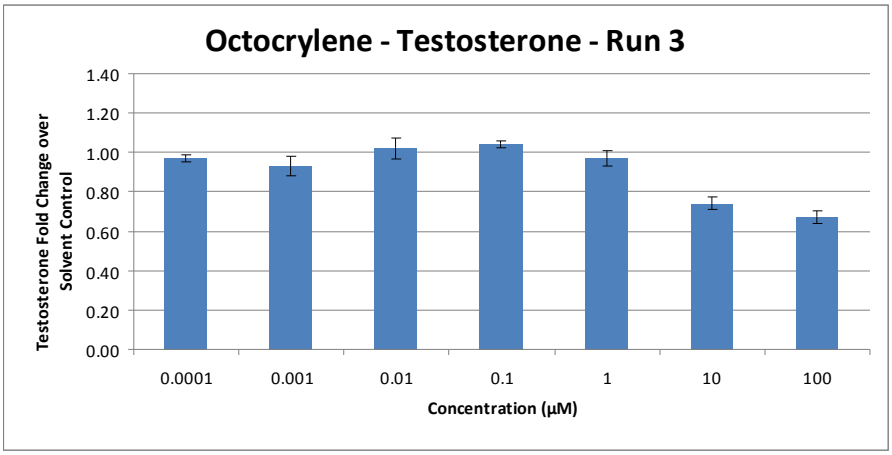



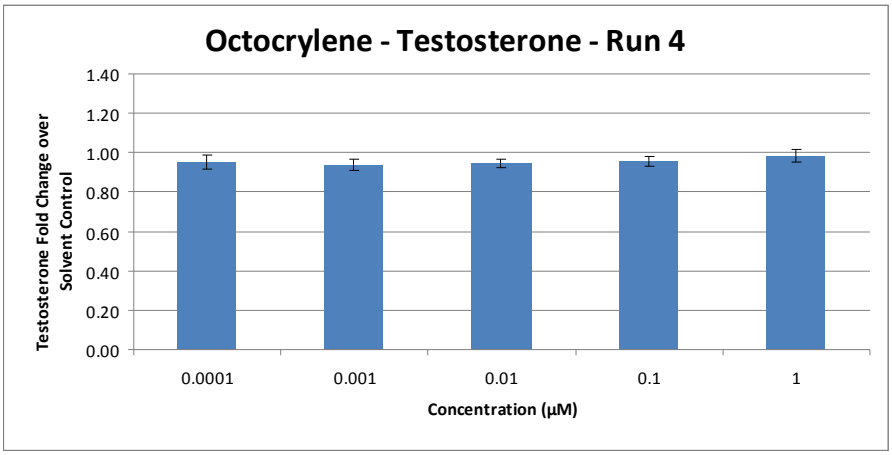



## FIGURE 9 Octocrylene – Testosterone Fold Change



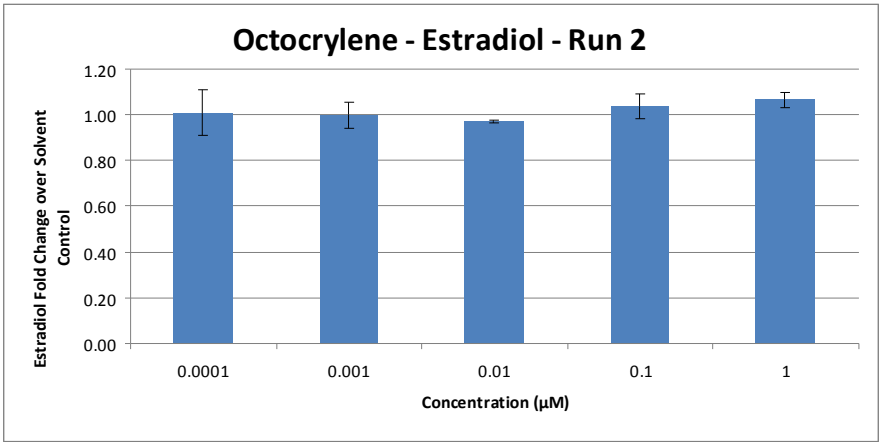

Concentrations 10 and 100 µM omitted due to cyotoxicity greater than 20%.



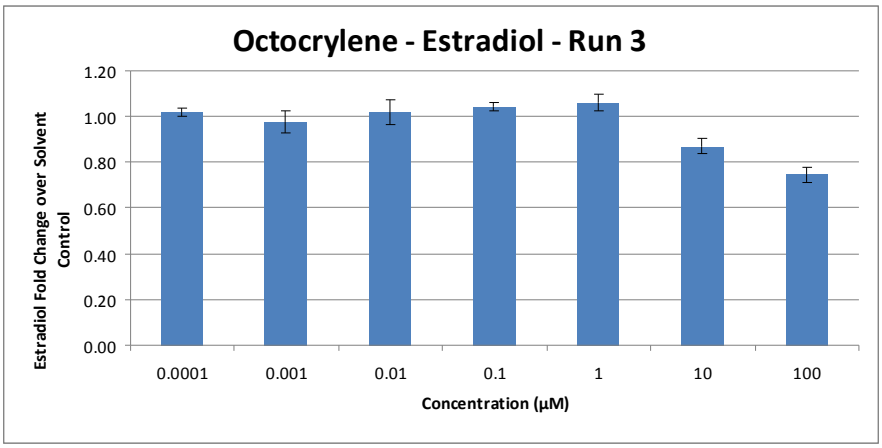



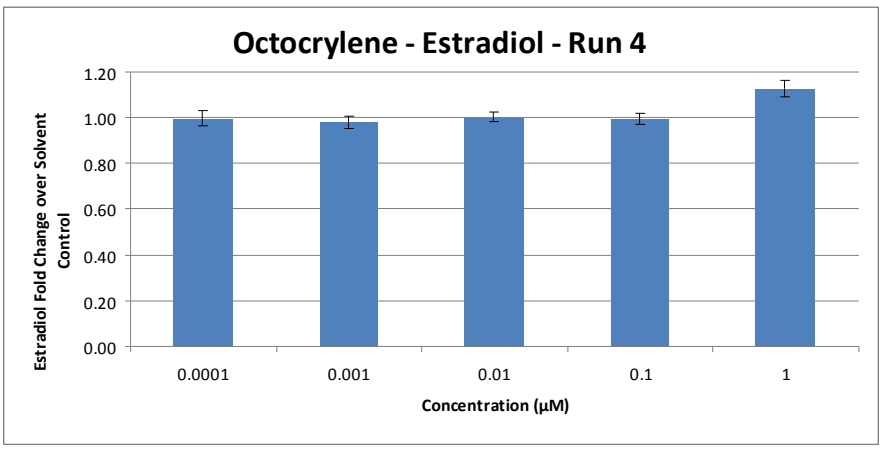

Concentrations 10 and 100 µM omitted due to cyotoxicity greater than 20%.

## FIGURE 10 Octocrylene – Estradiol Fold Change



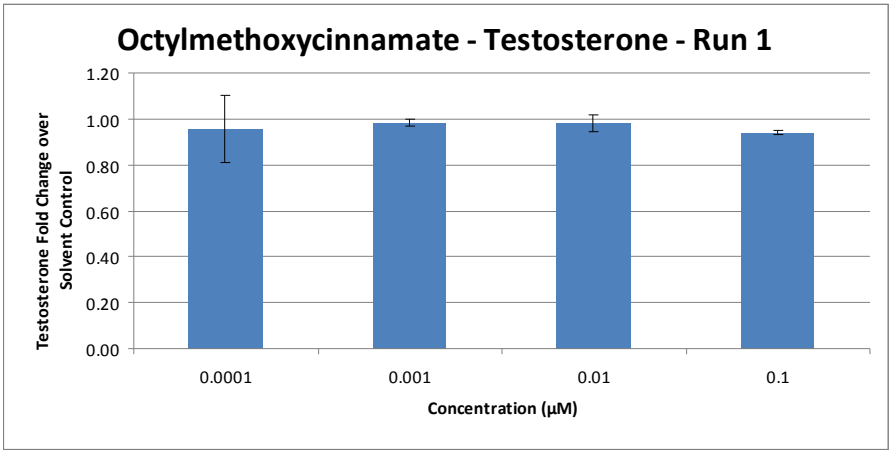

Concentrations 10 and 100 µM omitted due to cyotoxicity greater than 20%.



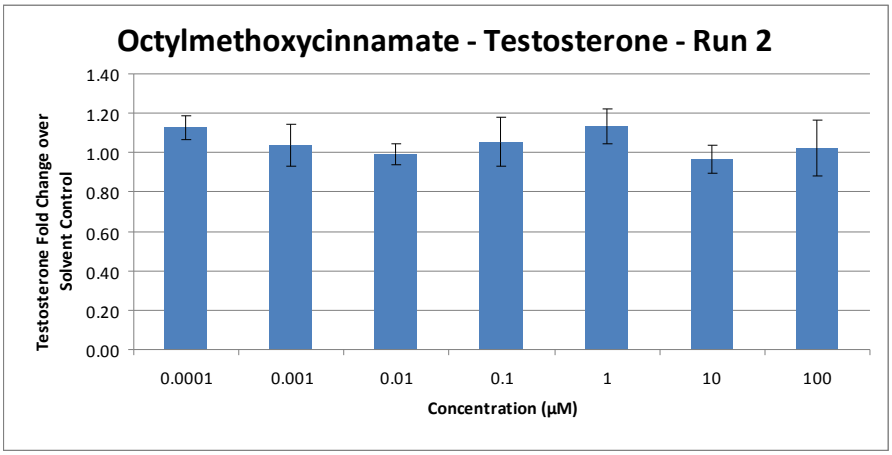



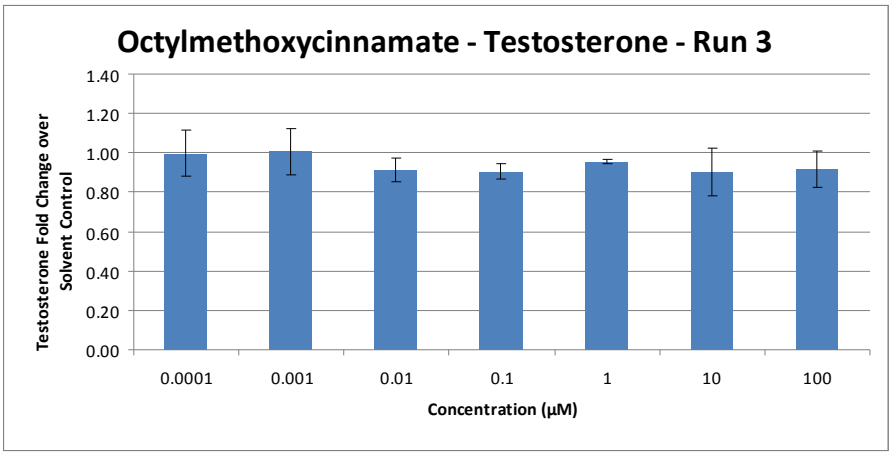

Concentrations 10 and 100 µM omitted due to cyotoxicity greater than 20%.

**FIGURE 11 Octylmethoxycinnamate – Testosterone Fold Change**

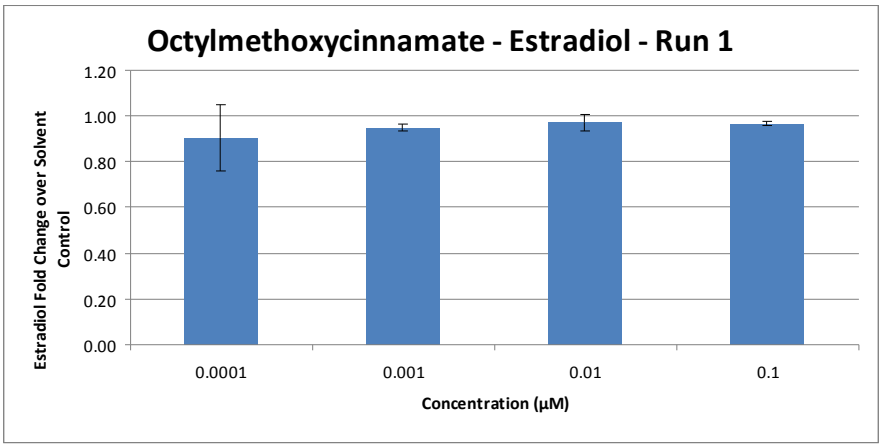



Concentrations 1, 10, and 100 µM omitted because of precipitation.

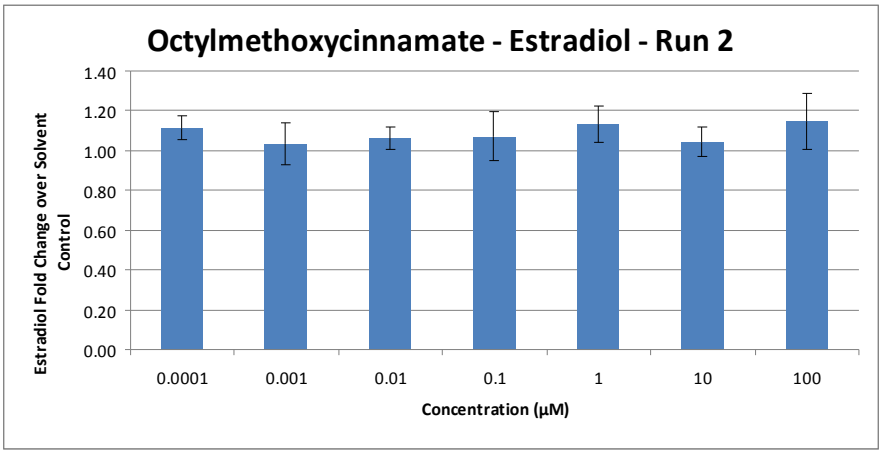


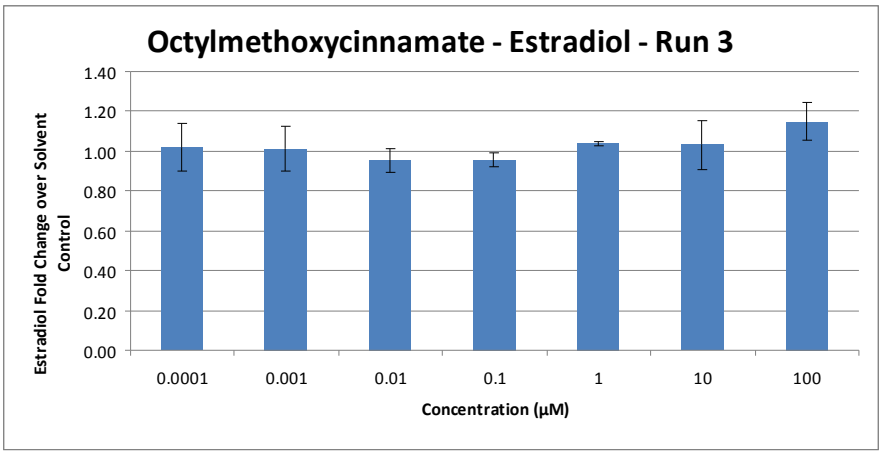



## FIGURE 12 Octylmethoxycinnamate – Estradiol Fold Change




Concentrations 1, 10, and 100 µM omitted because of precipitation.



# APPENDICES SECTION

## APPENDIX 1 Raw Data – Octyl Salicylate (Run 2)

**Testosterone**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | b | c | Avg. | SD | CV% |
| SC | 2,791 | 2,789 | 2,799 | 2,793 | 5.11 | 1.83E-01 |
| 0.0001 | 2,608 | 2,622 | 2,814 | 2,682 | 114.87 | 4.28E+00 |
| 0.001 | 2,811 | 2,677 | 2,730 | 2,739 | 67.55 | 2.47E+00 |
| 0.01 | 2,691 | 2,652 | 2,688 | 2,677 | 21.38 | 7.99E-01 |
| 0.1 | 2,835 | 2,722 | 2,765 | 2,774 | 57.31 | 2.07E+00 |
| 1 | 2,664 | 2,955 | 2,811 | 2,810 | 145.86 | 5.19E+00 |
| 10 | 2,778 | 2,626 | 2,691 | 2,699 | 76.61 | 2.84E+00 |
| 100 | 3,044 | 3,219 | 3,001 | 3,088 | 115.54 | 3.74E+00 |

**Estradiol**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | b | c | Avg. | SD | CV% |
| SC | 251 | 248 | 254 | 251 | 2.76 | 1.10E+00 |
| 0.0001 | 228 | 221 | 220 | 223 | 4.58 | 2.05E+00 |
| 0.001 | 250 | 228 | 216 | 231 | 17.22 | 7.45E+00 |
| 0.01 | 246 | 236 | 225 | 236 | 10.51 | 4.46E+00 |
| 0.1 | 253 | 235 | 226 | 238 | 13.72 | 5.77E+00 |
| 1 | 244 | 264 | 257 | 255 | 9.79 | 3.84E+00 |
| 10 | 278 | 281 | 296 | 285 | 9.72 | 3.41E+00 |
| 100 | 481 | 523 | 480 | 495 | 24.86 | 5.03E+00 |

**MTT**

| | Absorbance Values | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | B | c | Avg. | SD | CV% |
| SC | 0.463 | 0.443 | 0.447 | 0.451 | 0.01 | 2.35E+00 |
| 0.0001 | 0.466 | 0.456 | 0.469 | 0.464 | 0.01 | 1.47E+00 |
| 0.001 | 0.452 | 0.455 | 0.459 | 0.455 | 0.00 | 7.71E-01 |
| 0.01 | 0.462 | 0.456 | 0.458 | 0.459 | 0.00 | 6.66E-01 |
| 0.1 | 0.451 | 0.442 | 0.449 | 0.447 | 0.00 | 1.06E+00 |
| 1 | 0.450 | 0.456 | 0.475 | 0.460 | 0.01 | 2.84E+00 |
| 10 | 0.476 | 0.450 | 0.447 | 0.458 | 0.02 | 3.48E+00 |
| 100 | 0.455 | 0.450 | 0.445 | 0.450 | 0.01 | 1.11E+00 |

MTT values are subtracted values ($Absorbance_{570} - Absorbance_{650}$).

## APPENDIX 1 Raw Data – Octyl Salicylate (Run 3)

**Testosterone**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | b | c | Avg. | SD | CV% |
| SC | 1,926 | 1,835 | 2,043 | 1,935 | 104.50 | 5.40E+00 |
| 0.0001 | 1,903 | 1,797 | 1,849 | 1,850 | 52.84 | 2.86E+00 |
| 0.001 | 1,860 | 1,839 | 1,827 | 1,842 | 16.67 | 9.05E-01 |
| 0.01 | 2,002 | 1,854 | 1,900 | 1,919 | 75.88 | 3.95E+00 |
| 0.1 | 1,915 | 1,915 | 1,970 | 1,933 | 31.94 | 1.65E+00 |
| 1 | 1,883 | 1,873 | 1,909 | 1,888 | 18.89 | 1.00E+00 |
| 10 | 1,861 | 1,958 | 1,872 | 1,897 | 52.72 | 2.78E+00 |
| 100 | 1,915 | 1,892 | 1,965 | 1,924 | 37.37 | 1.94E+00 |

**Estradiol**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | b | c | Avg. | SD | CV% |
| SC | 171 | 162 | 178 | 170 | 7.81 | 4.59E+00 |
| 0.0001 | 156 | 152 | 157 | 155 | 2.25 | 1.45E+00 |
| 0.001 | 163 | 159 | 160 | 161 | 2.33 | 1.45E+00 |
| 0.01 | 162 | 161 | 163 | 162 | 1.33 | 8.20E-01 |
| 0.1 | 166 | 165 | 164 | 165 | 0.84 | 5.08E-01 |
| 1 | 154 | 160 | 159 | 158 | 2.99 | 1.90E+00 |
| 10 | 175 | 179 | 179 | 178 | 2.60 | 1.46E+00 |
| 100 | 211 | 208 | 214 | 211 | 3.13 | 1.49E+00 |

**MTT**

| | Absorbance Values | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | B | c | Avg. | SD | CV% |
| SC | 0.336 | 0.313 | 0.309 | 0.319 | 0.01 | 4.56E+00 |
| 0.0001 | 0.290 | 0.295 | 0.303 | 0.296 | 0.01 | 2.22E+00 |
| 0.001 | 0.292 | 0.296 | 0.296 | 0.295 | 0.00 | 7.84E-01 |
| 0.01 | 0.308 | 0.302 | 0.301 | 0.304 | 0.00 | 1.25E+00 |
| 0.1 | 0.302 | 0.313 | 0.316 | 0.310 | 0.01 | 2.38E+00 |
| 1 | 0.297 | 0.296 | 0.310 | 0.301 | 0.01 | 2.59E+00 |
| 10 | 0.302 | 0.289 | 0.290 | 0.294 | 0.01 | 2.46E+00 |
| 100 | 0.297 | 0.291 | 0.299 | 0.296 | 0.00 | 1.41E+00 |

MTT values are subtracted values ($Absorbance_{570} - Absorbance_{650}$).

## APPENDIX 1 Raw Data – Octyl Salicylate (Run 4)

**Testosterone**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | b | c | Avg. | SD | CV% |
| SC | 2,388 | 2,426 | 2,396 | 2,403 | 20.24 | 8.42E-01 |
| 0.0001 | 2,371 | 2,316 | 2,449 | 2,379 | 66.72 | 2.81E+00 |
| 0.001 | 2,397 | 2,408 | 2,308 | 2,371 | 54.65 | 2.31E+00 |
| 0.01 | 2,352 | 2,496 | 2,416 | 2,421 | 72.16 | 2.98E+00 |
| 0.1 | 2,425 | 2,526 | 2,504 | 2,485 | 53.24 | 2.14E+00 |
| 1 | 2,439 | 2,478 | 2,609 | 2,509 | 88.74 | 3.54E+00 |
| 10 | 2,391 | 2,355 | 2,374 | 2,373 | 18.00 | 7.58E-01 |
| 100 | 2,551 | 2,474 | 2,523 | 2,516 | 39.12 | 1.55E+00 |

**Estradiol**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | b | c | Avg. | SD | CV% |
| SC | 214 | 217 | 208 | 213 | 4.80 | 2.26E+00 |
| 0.0001 | 203 | 202 | 214 | 206 | 6.83 | 3.31E+00 |
| 0.001 | 203 | 201 | 202 | 202 | 1.05 | 5.19E-01 |
| 0.01 | 208 | 207 | 205 | 207 | 1.54 | 7.47E-01 |
| 0.1 | 206 | 231 | 221 | 219 | 12.53 | 5.71E+00 |
| 1 | 203 | 206 | 216 | 208 | 6.57 | 3.15E+00 |
| 10 | 218 | 226 | 225 | 223 | 4.26 | 1.91E+00 |
| 100 | 279 | 280 | 297 | 285 | 10.00 | 3.51E+00 |

**MTT**

| | Absorbance Values | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | b | c | Avg. | SD | CV% |
| SC | 0.432 | 0.431 | 0.438 | 0.434 | 0.00 | 8.73E-01 |
| 0.0001 | 0.405 | 0.420 | 0.430 | 0.418 | 0.01 | 3.01E+00 |
| 0.001 | 0.426 | 0.427 | 0.428 | 0.427 | 0.00 | 2.34E-01 |
| 0.01 | 0.435 | 0.427 | 0.428 | 0.430 | 0.00 | 1.01E+00 |
| 0.1 | 0.436 | 0.418 | 0.436 | 0.430 | 0.01 | 2.42E+00 |
| 1 | 0.412 | 0.393 | 0.404 | 0.403 | 0.01 | 2.37E+00 |
| 10 | 0.427 | 0.426 | 0.416 | 0.423 | 0.01 | 1.44E+00 |
| 100 | 0.410 | 0.396 | 0.412 | 0.406 | 0.01 | 2.15E+00 |

MTT values are subtracted values ($Absorbance_{570} - Absorbance_{650}$).

## APPENDIX 2 Raw Data – Oxybenzone (Run 2)

**Testosterone**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | b | c | Avg. | SD | CV% |
| SC | 3,333 | 2,887 | 2,822 | 3,014 | 277.96 | 9.22E+00 |
| 0.0001 | 2,790 | 2,786 | 3,046 | 2,874 | 148.85 | 5.18E+00 |
| 0.001 | 2,719 | 2,705 | 2,731 | 2,718 | 13.45 | 4.95E-01 |
| 0.01 | 3,076 | 2,797 | 2,868 | 2,914 | 145.24 | 4.99E+00 |
| 0.1 | 2,831 | 2,745 | 3,156 | 2,911 | 217.03 | 7.46E+00 |
| 1 | 2,722 | 2,844 | 3,004 | 2,857 | 141.20 | 4.94E+00 |
| 10 | 3,134 | 2,984 | 3,094 | 3,071 | 77.58 | 2.53E+00 |
| 100 | 4,147 | 3,662 | 3,943 | 3,917 | 243.70 | 6.22E+00 |

**Estradiol**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | b | c | Avg. | SD | CV% |
| SC | 294 | 263 | 263 | 273 | 18.14 | 6.64E+00 |
| 0.0001 | 258 | 251 | 254 | 254 | 3.38 | 1.33E+00 |
| 0.001 | 249 | 250 | 240 | 246 | 5.38 | 2.18E+00 |
| 0.01 | 285 | 262 | 252 | 266 | 16.79 | 6.30E+00 |
| 0.1 | 252 | 244 | 254 | 250 | 5.06 | 2.02E+00 |
| 1 | 273 | 292 | 312 | 292 | 19.31 | 6.61E+00 |
| 10 | 419 | 402 | 429 | 416 | 13.79 | 3.31E+00 |
| 100 | 663 | 569 | 629 | 620 | 47.73 | 7.70E+00 |

**MTT**

| | Absorbance Values | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | B | c | Avg. | SD | CV% |
| SC | 0.494 | 0.477 | 0.467 | 0.479 | 0.01 | 2.85E+00 |
| 0.0001 | 0.467 | 0.472 | 0.470 | 0.470 | 0.00 | 5.36E-01 |
| 0.001 | 0.458 | 0.453 | 0.456 | 0.456 | 0.00 | 5.52E-01 |
| 0.01 | 0.461 | 0.451 | 0.464 | 0.459 | 0.01 | 1.48E+00 |
| 0.1 | 0.472 | 0.471 | 0.463 | 0.469 | 0.00 | 1.05E+00 |
| 1 | 0.466 | 0.468 | 0.469 | 0.468 | 0.00 | 3.27E-01 |
| 10 | 0.468 | 0.448 | 0.457 | 0.458 | 0.01 | 2.19E+00 |
| 100 | 0.447 | 0.438 | 0.427 | 0.437 | 0.01 | 2.29E+00 |

MTT values are subtracted values ($Absorbance_{570} - Absorbance_{650}$).

## APPENDIX 2 Raw Data – Oxybenzone (Run 3)

**Testosterone**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | b | c | Avg. | SD | CV% |
| SC | 1,979 | 1,920 | 1,914 | 1,938 | 35.71 | 1.84E+00 |
| 0.0001 | 1,963 | 1,851 | 2,144 | 1,986 | 147.80 | 7.44E+00 |
| 0.001 | 1,951 | 1,940 | 1,870 | 1,920 | 43.76 | 2.28E+00 |
| 0.01 | 1,945 | 1,874 | 1,870 | 1,896 | 42.04 | 2.22E+00 |
| 0.1 | 2,108 | 1,876 | 1,902 | 1,962 | 126.97 | 6.47E+00 |
| 1 | 1,890 | 1,866 | 1,933 | 1,896 | 33.82 | 1.78E+00 |
| 10 | 2,104 | 1,967 | 1,912 | 1,994 | 98.76 | 4.95E+00 |
| 100 | 2,364 | 2,235 | 2,435 | 2,345 | 101.58 | 4.33E+00 |

**Estradiol**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | b | c | Avg. | SD | CV% |
| SC | 186 | 189 | 184 | 186 | 2.32 | 1.25E+00 |
| 0.0001 | 180 | 176 | 186 | 181 | 4.79 | 2.65E+00 |
| 0.001 | 184 | 182 | 180 | 182 | 1.70 | 9.34E-01 |
| 0.01 | 183 | 176 | 183 | 180 | 4.01 | 2.22E+00 |
| 0.1 | 190 | 178 | 181 | 183 | 6.55 | 3.58E+00 |
| 1 | 196 | 191 | 204 | 197 | 6.46 | 3.29E+00 |
| 10 | 270 | 265 | 262 | 266 | 4.14 | 1.56E+00 |
| 100 | 330 | 327 | 341 | 333 | 7.41 | 2.23E+00 |

**MTT**

| | Absorbance Values | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | B | c | Avg. | SD | CV% |
| SC | 0.308 | 0.307 | 0.317 | 0.311 | 0.01 | 1.77E+00 |
| 0.0001 | 0.297 | 0.302 | 0.298 | 0.299 | 0.00 | 8.85E-01 |
| 0.001 | 0.286 | 0.285 | 0.292 | 0.288 | 0.00 | 1.32E+00 |
| 0.01 | 0.298 | 0.289 | 0.297 | 0.295 | 0.00 | 1.67E+00 |
| 0.1 | 0.297 | 0.305 | 0.289 | 0.297 | 0.01 | 2.69E+00 |
| 1 | 0.292 | 0.302 | 0.278 | 0.291 | 0.01 | 4.15E+00 |
| 10 | 0.298 | 0.286 | 0.285 | 0.290 | 0.01 | 2.50E+00 |
| 100 | 0.278 | 0.256 | 0.292 | 0.275 | 0.02 | 6.59E+00 |

MTT values are subtracted values ($Absorbance_{570} - Absorbance_{650}$).

## APPENDIX 2 Raw Data – Oxybenzone (Run 4)

**Testosterone**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | b | c | Avg. | SD | CV% |
| SC | 2,481 | 2,389 | 2,381 | 2,417 | 55.16 | 2.28E+00 |
| 0.0001 | 2,596 | 2,354 | 2,442 | 2,464 | 122.64 | 4.98E+00 |
| 0.001 | 2,403 | 2,387 | 2,410 | 2,400 | 11.69 | 4.87E-01 |
| 0.01 | 2,513 | 2,379 | 2,302 | 2,398 | 106.68 | 4.45E+00 |
| 0.1 | 2,600 | 2,599 | 2,643 | 2,614 | 24.75 | 9.47E-01 |
| 1 | 2,528 | 2,541 | 2,460 | 2,509 | 43.30 | 1.73E+00 |
| 10 | 2,543 | 2,522 | 2,664 | 2,576 | 76.60 | 2.97E+00 |
| 100 | 3,183 | 3,168 | 3,214 | 3,188 | 23.45 | 7.36E-01 |

**Estradiol**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | b | c | Avg. | SD | CV% |
| SC | 222 | 222 | 228 | 224 | 3.18 | 1.42E+00 |
| 0.0001 | 223 | 218 | 227 | 222 | 4.55 | 2.04E+00 |
| 0.001 | 229 | 220 | 223 | 224 | 4.30 | 1.92E+00 |
| 0.01 | 231 | 234 | 214 | 227 | 10.92 | 4.82E+00 |
| 0.1 | 258 | 241 | 244 | 248 | 9.38 | 3.79E+00 |
| 1 | 253 | 254 | 254 | 254 | 1.02 | 4.01E-01 |
| 10 | 326 | 342 | 351 | 340 | 12.48 | 3.67E+00 |
| 100 | 462 | 451 | 485 | 466 | 17.33 | 3.72E+00 |

**MTT**

| | Absorbance Values | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | B | c | Avg. | SD | CV% |
| SC | 0.463 | 0.424 | 0.418 | 0.435 | 0.02 | 5.62E+00 |
| 0.0001 | 0.408 | 0.413 | 0.421 | 0.414 | 0.01 | 1.58E+00 |
| 0.001 | 0.427 | 0.416 | 0.420 | 0.421 | 0.01 | 1.32E+00 |
| 0.01 | 0.420 | 0.423 | 0.414 | 0.419 | 0.00 | 1.09E+00 |
| 0.1 | 0.434 | 0.426 | 0.428 | 0.429 | 0.00 | 9.70E-01 |
| 1 | 0.415 | 0.398 | 0.418 | 0.410 | 0.01 | 2.63E+00 |
| 10 | 0.417 | 0.418 | 0.410 | 0.415 | 0.00 | 1.05E+00 |
| 100 | 0.392 | 0.410 | 0.406 | 0.403 | 0.01 | 2.35E+00 |

MTT values are subtracted values ($Absorbance_{570} - Absorbance_{650}$).

## APPENDIX 3 Raw Data – Octocrylene (Run 2)

**Testosterone**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | b | c | Avg. | SD | CV% |
| SC | 2,634 | 2,958 | 2,733 | 2,775 | 165.78 | 5.97E+00 |
| 0.0001 | 3,215 | 2,717 | 2,788 | 2,906 | 269.53 | 9.27E+00 |
| 0.001 | 2,666 | 2,733 | 2,965 | 2,788 | 156.87 | 5.63E+00 |
| 0.01 | 2,609 | 2,576 | 2,616 | 2,600 | 21.43 | 8.24E-01 |
| 0.1 | 2,806 | 2,607 | 2,912 | 2,775 | 154.93 | 5.58E+00 |
| 1 | 2,771 | 2,847 | 2,950 | 2,856 | 89.78 | 3.14E+00 |
| 10 | 1,963 | 2,001 | 1,997 | 1,987 | 20.93 | 1.05E+00 |
| 100 | 1,528 | 1,618 | 1,667 | 1,605 | 70.60 | 4.40E+00 |

**Estradiol**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | b | c | Avg. | SD | CV% |
| SC | 234 | 265 | 243 | 247 | 16.10 | 6.52E+00 |
| 0.0001 | 267 | 233 | 248 | 249 | 16.63 | 6.67E+00 |
| 0.001 | 235 | 245 | 261 | 247 | 13.24 | 5.36E+00 |
| 0.01 | 244 | 239 | 235 | 240 | 4.77 | 1.99E+00 |
| 0.1 | 262 | 240 | 265 | 256 | 13.44 | 5.25E+00 |
| 1 | 252 | 259 | 278 | 263 | 13.75 | 5.23E+00 |
| 10 | 240 | 238 | 249 | 242 | 5.97 | 2.47E+00 |
| 100 | 151 | 166 | 175 | 164 | 12.05 | 7.36E+00 |

**MTT**

| | Absorbance Values | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | B | c | Avg. | SD | CV% |
| SC | 0.468 | 0.466 | 0.464 | 0.466 | 0.00 | 4.29E-01 |
| 0.0001 | 0.457 | 0.473 | 0.472 | 0.467 | 0.01 | 1.92E+00 |
| 0.001 | 0.462 | 0.453 | 0.466 | 0.460 | 0.01 | 1.45E+00 |
| 0.01 | 0.456 | 0.451 | 0.464 | 0.457 | 0.01 | 1.43E+00 |
| 0.1 | 0.472 | 0.476 | 0.465 | 0.471 | 0.01 | 1.18E+00 |
| 1 | 0.456 | 0.459 | 0.454 | 0.456 | 0.00 | 5.51E-01 |
| 10 | 0.338 | 0.342 | 0.350 | 0.343 | 0.01 | 1.78E+00 |
| 100 | 0.305 | 0.305 | 0.312 | 0.307 | 0.00 | 1.32E+00 |

MTT values are subtracted values ($Absorbance_{570} - Absorbance_{650}$).

## APPENDIX 3 Raw Data – Octocrylene (Run 3)

**Testosterone**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | b | c | Avg. | SD | CV% |
| SC | 1,948 | 1,863 | 2,115 | 1,975 | 128.03 | 6.48E+00 |
| 0.0001 | 1,923 | 1,878 | 1,951 | 1,917 | 36.91 | 1.92E+00 |
| 0.001 | 1,945 | 1,813 | 1,761 | 1,840 | 94.93 | 5.16E+00 |
| 0.01 | 1,979 | 1,938 | 2,132 | 2,017 | 102.51 | 5.08E+00 |
| 0.1 | 2,054 | 2,097 | 2,022 | 2,058 | 37.53 | 1.82E+00 |
| 1 | 1,959 | 1,830 | 1,961 | 1,917 | 75.06 | 3.92E+00 |
| 10 | 1,534 | 1,411 | 1,448 | 1,465 | 63.14 | 4.31E+00 |
| 100 | 1,397 | 1,328 | 1,268 | 1,331 | 64.53 | 4.85E+00 |

**Estradiol**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | b | c | Avg. | SD | CV% |
| SC | 169 | 164 | 180 | 171 | 8.11 | 4.74E+00 |
| 0.0001 | 172 | 175 | 176 | 175 | 1.92 | 1.10E+00 |
| 0.001 | 175 | 168 | 157 | 167 | 8.96 | 5.36E+00 |
| 0.01 | 168 | 172 | 184 | 174 | 8.37 | 4.80E+00 |
| 0.1 | 178 | 183 | 175 | 179 | 3.62 | 2.03E+00 |
| 1 | 181 | 173 | 192 | 182 | 9.69 | 5.33E+00 |
| 10 | 154 | 145 | 149 | 149 | 4.44 | 2.98E+00 |
| 100 | 129 | 130 | 124 | 128 | 3.41 | 2.66E+00 |

**MTT**

| | Absorbance Values | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | B | c | Avg. | SD | CV% |
| SC | 0.334 | 0.317 | 0.318 | 0.323 | 0.01 | 2.95E+00 |
| 0.0001 | 0.299 | 0.286 | 0.305 | 0.297 | 0.01 | 3.27E+00 |
| 0.001 | 0.299 | 0.308 | 0.306 | 0.304 | 0.00 | 1.55E+00 |
| 0.01 | 0.324 | 0.305 | 0.314 | 0.314 | 0.01 | 3.02E+00 |
| 0.1 | 0.325 | 0.323 | 0.319 | 0.322 | 0.00 | 9.48E-01 |
| 1 | 0.294 | 0.315 | 0.301 | 0.303 | 0.01 | 3.53E+00 |
| 10 | 0.273 | 0.258 | 0.254 | 0.262 | 0.01 | 3.83E+00 |
| 100 | 0.276 | 0.308 | 0.278 | 0.287 | 0.02 | 6.24E+00 |

MTT values are subtracted values ($Absorbance_{570} - Absorbance_{650}$).

## APPENDIX 3 Raw Data – Octocrylene (Run 4)

**Testosterone**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | b | c | Avg. | SD | CV% |
| SC | 2,566 | 2,394 | 2,353 | 2,437 | 113.07 | 4.64E+00 |
| 0.0001 | 2,372 | 2,224 | 2,361 | 2,319 | 82.41 | 3.55E+00 |
| 0.001 | 2,269 | 2,229 | 2,355 | 2,284 | 64.29 | 2.81E+00 |
| 0.01 | 2,326 | 2,250 | 2,347 | 2,308 | 50.88 | 2.20E+00 |
| 0.1 | 2,403 | 2,310 | 2,293 | 2,335 | 59.36 | 2.54E+00 |
| 1 | 2,387 | 2,317 | 2,483 | 2,395 | 83.05 | 3.47E+00 |
| 10 | 1,773 | 1,824 | 1,898 | 1,831 | 62.79 | 3.43E+00 |
| 100 | 1,794 | 1,727 | 1,640 | 1,720 | 77.13 | 4.48E+00 |

**Estradiol**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | b | c | Avg. | SD | CV% |
| SC | 206 | 204 | 212 | 207 | 3.90 | 1.88E+00 |
| 0.0001 | 216 | 200 | 205 | 207 | 8.06 | 3.90E+00 |
| 0.001 | 203 | 195 | 212 | 203 | 8.12 | 3.99E+00 |
| 0.01 | 209 | 206 | 209 | 208 | 1.50 | 7.21E-01 |
| 0.1 | 213 | 206 | 198 | 206 | 7.24 | 3.52E+00 |
| 1 | 231 | 219 | 250 | 234 | 15.48 | 6.63E+00 |
| 10 | 178 | 194 | 197 | 190 | 10.27 | 5.41E+00 |
| 100 | 161 | 163 | 152 | 159 | 5.59 | 3.52E+00 |

**MTT**

| | Absorbance Values | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | B | c | Avg. | SD | CV% |
| SC | 0.439 | 0.423 | 0.432 | 0.431 | 0.01 | 1.86E+00 |
| 0.0001 | 0.423 | 0.397 | 0.427 | 0.416 | 0.02 | 3.92E+00 |
| 0.001 | 0.416 | 0.425 | 0.412 | 0.418 | 0.01 | 1.59E+00 |
| 0.01 | 0.438 | 0.423 | 0.417 | 0.426 | 0.01 | 2.54E+00 |
| 0.1 | 0.429 | 0.431 | 0.422 | 0.427 | 0.00 | 1.11E+00 |
| 1 | 0.397 | 0.419 | 0.424 | 0.413 | 0.01 | 3.48E+00 |
| 10 | 0.342 | 0.332 | 0.340 | 0.338 | 0.01 | 1.57E+00 |
| 100 | 0.321 | 0.352 | 0.330 | 0.334 | 0.02 | 4.77E+00 |

MTT values are subtracted values ($Absorbance_{570} - Absorbance_{650}$).

## APPENDIX 4 Raw Data – Octylmethoxycinnamate (Run 1)

**Testosterone**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | b | c | Avg. | SD | CV% |
| SC | 2,603 | 2,555 | 2,372 | 2,510 | 121.79 | 4.85E+00 |
| 0.0001 | 2,531 | 1,990 | 2,690 | 2,404 | 366.71 | 1.53E+01 |
| 0.001 | 2,494 | NR | 2,442 | 2,468 | 36.85 | 1.49E+00 |
| 0.01 | 2,532 | NR | 2,403 | 2,468 | 91.31 | 3.70E+00 |
| 0.1 | 2,335 | 2,355 | 2,393 | 2,361 | 29.54 | 1.25E+00 |
| 1 | 2,648 | 2,642 | 2,753 | 2,681 | 62.52 | 2.33E+00 |
| 10 | 2,417 | 2,493 | 2,481 | 2,464 | 40.64 | 1.65E+00 |
| 100 | 2,456 | 2,635 | 2,971 | 2,687 | 261.29 | 9.72E+00 |

**Estradiol**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | b | c | Avg. | SD | CV% |
| SC | 187 | 190 | 171 | 183 | 9.92 | 5.43E+00 |
| 0.0001 | 183 | 137 | 175 | 165 | 24.57 | 1.49E+01 |
| 0.001 | 177 | NR | 170 | 173 | 4.91 | 2.83E+00 |
| 0.01 | 182 | NR | 172 | 177 | 7.03 | 3.96E+00 |
| 0.1 | 169 | 190 | 171 | 177 | 11.84 | 6.70E+00 |
| 1 | 188 | 181 | 187 | 185 | 3.62 | 1.96E+00 |
| 10 | 187 | 188 | 187 | 187 | 0.39 | 2.09E-01 |
| 100 | 222 | 241 | 250 | 237 | 14.30 | 6.02E+00 |

**MTT**

| | Absorbance Values | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | B | c | Avg. | SD | CV% |
| SC | 0.441 | 0.422 | 0.441 | 0.435 | 0.01 | 2.52E+00 |
| 0.0001 | 0.398 | 0.423 | 0.415 | 0.412 | 0.01 | 3.10E+00 |
| 0.001 | 0.414 | 0.419 | 0.415 | 0.416 | 0.00 | 6.36E-01 |
| 0.01 | 0.428 | 0.433 | 0.420 | 0.427 | 0.01 | 1.54E+00 |
| 0.1 | 0.414 | 0.424 | 0.447 | 0.428 | 0.02 | 3.95E+00 |
| 1 | 0.407 | 0.374 | 0.404 | 0.395 | 0.02 | 4.62E+00 |
| 10 | 0.431 | 0.405 | 0.391 | 0.409 | 0.02 | 4.96E+00 |
| 100 | 0.429 | 0.427 | 0.445 | 0.434 | 0.01 | 2.27E+00 |

MTT values are subtracted values ($Absorbance_{570} - Absorbance_{650}$).

## APPENDIX 4 Raw Data – Octylmethoxycinnamate (Run 2)

**Testosterone**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | b | c | Avg. | SD | CV% |
| SC | 2,283 | 2,564 | 2,255 | 2,367 | 170.67 | 7.21E+00 |
| 0.0001 | 2,598 | 2,573 | 2,836 | 2,669 | 145.13 | 5.44E+00 |
| 0.001 | 2,250 | 2,374 | 2,732 | 2,452 | 250.15 | 1.02E+01 |
| 0.01 | 2,398 | 2,207 | 2,452 | 2,352 | 128.70 | 5.47E+00 |
| 0.1 | 2,217 | 2,468 | 2,798 | 2,494 | 291.48 | 1.17E+01 |
| 1 | 2,924 | 2,618 | 2,510 | 2,684 | 214.62 | 8.00E+00 |
| 10 | 2,125 | 2,274 | 2,471 | 2,290 | 173.44 | 7.57E+00 |
| 100 | 2,651 | 2,579 | 2,042 | 2,424 | 332.81 | 1.37E+01 |

**Estradiol**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | b | c | Avg. | SD | CV% |
| SC | 194 | 221 | 190 | 202 | 17.12 | 8.49E+00 |
| 0.0001 | 222 | 219 | 234 | 225 | 8.27 | 3.68E+00 |
| 0.001 | 191 | 204 | 228 | 208 | 18.64 | 8.96E+00 |
| 0.01 | 216 | 204 | 222 | 214 | 9.23 | 4.31E+00 |
| 0.1 | 187 | 212 | 250 | 216 | 31.77 | 1.47E+01 |
| 1 | 235 | 230 | 222 | 229 | 6.60 | 2.89E+00 |
| 10 | 188 | 207 | 236 | 210 | 24.11 | 1.15E+01 |
| 100 | 237 | 259 | 196 | 231 | 31.92 | 1.38E+01 |

**MTT**

| | Absorbance Values | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | B | c | Avg. | SD | CV% |
| SC | 0.466 | 0.465 | 0.466 | 0.466 | 0.00 | 1.24E-01 |
| 0.0001 | 0.447 | 0.457 | 0.464 | 0.456 | 0.01 | 1.87E+00 |
| 0.001 | 0.459 | 0.456 | 0.463 | 0.459 | 0.00 | 7.65E-01 |
| 0.01 | 0.471 | 0.464 | 0.464 | 0.466 | 0.00 | 8.67E-01 |
| 0.1 | 0.469 | 0.470 | 0.474 | 0.471 | 0.00 | 5.62E-01 |
| 1 | 0.451 | 0.467 | 0.461 | 0.460 | 0.01 | 1.76E+00 |
| 10 | 0.460 | 0.450 | 0.444 | 0.451 | 0.01 | 1.79E+00 |
| 100 | 0.451 | 0.488 | 0.470 | 0.470 | 0.02 | 3.94E+00 |

MTT values are subtracted values ($Absorbance_{570} - Absorbance_{650}$).

## APPENDIX 4 Raw Data – Octylmethoxycinnamate (Run 3)

**Testosterone**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | b | c | Avg. | SD | CV% |
| SC | 1,921 | 1,772 | 2,325 | 2,006 | 286.22 | 1.43E+01 |
| 0.0001 | 2,192 | 2,082 | 1,732 | 2,002 | 239.91 | 1.20E+01 |
| 0.001 | 1,850 | 1,925 | 2,278 | 2,018 | 228.62 | 1.13E+01 |
| 0.01 | 1,844 | 1,701 | 1,937 | 1,827 | 118.87 | 6.51E+00 |
| 0.1 | 1,798 | 1,755 | 1,897 | 1,817 | 72.37 | 3.98E+00 |
| 1 | 1,945 | 1,898 | 1,909 | 1,917 | 24.28 | 1.27E+00 |
| 10 | 1,930 | 1,528 | 1,969 | 1,809 | 244.03 | 1.35E+01 |
| 100 | 2,017 | 1,862 | 1,645 | 1,842 | 186.81 | 1.01E+01 |

**Estradiol**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | b | c | Avg. | SD | CV% |
| SC | 173 | 155 | 186 | 171 | 15.78 | 9.21E+00 |
| 0.0001 | 190 | 178 | 155 | 174 | 17.86 | 1.02E+01 |
| 0.001 | 160 | 168 | 193 | 174 | 17.01 | 9.80E+00 |
| 0.01 | 158 | 156 | 176 | 163 | 11.08 | 6.78E+00 |
| 0.1 | 161 | 158 | 173 | 164 | 8.20 | 5.00E+00 |
| 1 | 176 | 176 | 182 | 178 | 3.66 | 2.06E+00 |
| 10 | 180 | 160 | 189 | 177 | 15.02 | 8.50E+00 |
| 100 | 216 | 197 | 178 | 197 | 18.81 | 9.55E+00 |

**MTT**

| | Absorbance Values | | | | | |
|---|---|---|---|---|---|---|
| Dose [uM] | a | B | c | Avg. | SD | CV% |
| SC | 0.332 | 0.313 | 0.308 | 0.318 | 0.01 | 3.99E+00 |
| 0.0001 | 0.284 | 0.285 | 0.301 | 0.290 | 0.01 | 3.29E+00 |
| 0.001 | 0.294 | 0.306 | 0.295 | 0.298 | 0.01 | 2.23E+00 |
| 0.01 | 0.305 | 0.310 | 0.307 | 0.307 | 0.00 | 8.19E-01 |
| 0.1 | 0.315 | 0.311 | 0.312 | 0.313 | 0.00 | 6.66E-01 |
| 1 | 0.288 | 0.321 | 0.290 | 0.300 | 0.02 | 6.17E+00 |
| 10 | 0.306 | 0.299 | 0.300 | 0.302 | 0.00 | 1.26E+00 |
| 100 | 0.338 | 0.365 | 0.322 | 0.342 | 0.02 | 6.36E+00 |

MTT values are subtracted values ($Absorbance_{570} - Absorbance_{650}$).

## APPENDIX 5 QC Plate Raw Data – MTT

**Run 1**

| | Absorbance Values | | | | | |
|---|---|---|---|---|---|---|
| **Dose [uM]** | **A** | **b** | **c** | **Avg.** | **SD** | **CV%** |
| Blank | 0.451 | 0.409 | 0.437 | 0.432 | 0.02 | 4.95E+00 |
| Background | 0.417 | 0.407 | 0.399 | 0.408 | 0.01 | 2.21E+00 |
| DMSO | 0.426 | 0.443 | 0.423 | 0.431 | 0.01 | 2.50E+00 |
| DMSO + MeOH | 0.048 | 0.031 | 0.038 | 0.039 | 0.01 | 2.19E+01 |
| Forskolin 1 µM | 0.433 | 0.443 | 0.440 | 0.439 | 0.01 | 1.17E+00 |
| Forskolin 10 µM | 0.399 | 0.435 | 0.426 | 0.420 | 0.02 | 4.46E+00 |
| Prochloraz 0.1 µM | 0.410 | 0.398 | 0.400 | 0.403 | 0.01 | 1.60E+00 |
| Prochloraz 1 µM | 0.415 | 0.390 | 0.410 | 0.405 | 0.01 | 3.27E+00 |

**Run 2**

| **Dose [uM]** | **A** | **b** | **c** | **Avg.** | **SD** | **CV%** |
|---|---|---|---|---|---|---|
| Blank | 0.496 | 0.475 | 0.474 | 0.482 | 0.01 | 2.58E+00 |
| Background | 0.462 | 0.435 | 0.425 | 0.441 | 0.02 | 4.34E+00 |
| DMSO | 0.461 | 0.451 | 0.474 | 0.462 | 0.01 | 2.50E+00 |
| DMSO + MeOH | 0.039 | 0.020 | 0.018 | 0.026 | 0.01 | 4.52E+01 |
| Forskolin 1 µM | 0.506 | 0.495 | 0.493 | 0.498 | 0.01 | 1.41E+00 |
| Forskolin 10 µM | 0.482 | 0.504 | 0.497 | 0.494 | 0.01 | 2.27E+00 |
| Prochloraz 0.1 µM | 0.463 | 0.456 | 0.443 | 0.454 | 0.01 | 2.24E+00 |
| Prochloraz 1 µM | 0.463 | 0.453 | 0.462 | 0.459 | 0.01 | 1.20E+00 |

## APPENDIX 5 QC Plate Raw Data – MTT (continued)

**Run 3**

| | Absorbance Values | | | | | |
|---|---|---|---|---|---|---|
| **Dose [uM]** | **A** | **b** | **c** | **Avg.** | **SD** | **CV%** |
| Blank | 0.345 | 0.327 | 0.338 | 0.337 | 0.01 | 2.70E+00 |
| Background | 0.324 | 0.334 | 0.334 | 0.331 | 0.01 | 1.75E+00 |
| DMSO | 0.327 | 0.309 | 0.332 | 0.323 | 0.01 | 3.75E+00 |
| DMSO + MeOH | 0.034 | 0.044 | 0.032 | 0.037 | 0.01 | 1.75E+01 |
| Forskolin 1 µM | 0.350 | 0.351 | 0.357 | 0.353 | 0.00 | 1.07E+00 |
| Forskolin 10 µM | 0.354 | 0.335 | 0.361 | 0.350 | 0.01 | 3.84E+00 |
| Prochloraz 0.1 µM | 0.327 | 0.325 | 0.328 | 0.327 | 0.00 | 4.68E-01 |
| Prochloraz 1 µM | 0.328 | 0.330 | 0.325 | 0.328 | 0.00 | 7.68E-01 |

**Run 4**

| | Absorbance Values | | | | | |
|---|---|---|---|---|---|---|
| **Dose [uM]** | **A** | **b** | **c** | **Avg.** | **SD** | **CV%** |
| Blank | 0.389 | 0.407 | 0.421 | 0.406 | 0.02 | 3.95E+00 |
| Background | 0.403 | 0.397 | 0.396 | 0.399 | 0.00 | 9.50E-01 |
| DMSO | 0.408 | 0.388 | 0.413 | 0.403 | 0.01 | 3.28E+00 |
| DMSO + MeOH | 0.021 | 0.021 | 0.020 | 0.021 | 0.00 | 2.79E+00 |
| Forskolin 1 µM | 0.425 | 0.416 | 0.428 | 0.423 | 0.01 | 1.48E+00 |
| Forskolin 10 µM | 0.416 | 0.401 | 0.421 | 0.413 | 0.01 | 2.52E+00 |
| Prochloraz 0.1 µM | 0.408 | 0.402 | 0.408 | 0.406 | 0.00 | 8.53E-01 |
| Prochloraz 1 µM | 0.395 | 0.412 | 0.396 | 0.401 | 0.01 | 2.38E+00 |

## APPENDIX 6 QC Plate Raw Data - Testosterone

**Testosterone – Run 1**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| **Dose [uM]** | **A** | **b** | **c** | **Avg.** | **SD** | **CV%** |
| Background | 1109 | 997 | 1063 | 1056 | 56.62 | 5.36E+00 |
| Blank | 2198 | 2212 | 2112 | 2174 | 54.21 | 2.49E+00 |
| DMSO | 2202 | 2128 | 2101 | 2143 | 58.73 | 2.74E+00 |
| DMSO | 2174 | 2053 | 2196 | | | |
| Forskolin 1 µM | 3008 | 2823 | 2960 | 2930 | 95.85 | 3.27E+00 |
| Forskolin 10 µM | 3281 | 3345 | 3420 | 3349 | 69.77 | 2.08E+00 |
| Prochloraz 0.1 µM | 1886 | 1906 | 1951 | 1914 | 33.50 | 1.75E+00 |
| Prochloraz 1 µM | 1030 | 982 | 1017 | 1009 | 24.57 | 2.43E+00 |

**Testosterone – Run 2**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| **Dose [uM]** | **A** | **b** | **c** | **Avg.** | **SD** | **CV%** |
| Background | 1199 | 1073 | 1134 | 1135 | 62.68 | 5.52E+00 |
| Blank | 2617 | 2555 | 2571 | 2581 | 32.52 | 1.26E+00 |
| DMSO | 2518 | 2520 | 2580 | 2511 | 40.42 | 1.61E+00 |
| DMSO | 2491 | 2458 | 2501 | | | |
| Forskolin 1 µM | 3798 | 3771 | 3723 | 3764 | 38.19 | 1.01E+00 |
| Forskolin 10 µM | 4611 | 4670 | 4972 | 4751 | 193.65 | 4.08E+00 |
| Prochloraz 0.1 µM | 1854 | 1772 | 1822 | 1816 | 41.51 | 2.29E+00 |
| Prochloraz 1 µM | 1205 | 1241 | 1118 | 1188 | 63.16 | 5.32E+00 |

## APPENDIX 6 QC Plate Raw Data - Testosterone

**Testosterone – Run 3**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| **Dose [uM]** | **A** | **b** | **c** | **Avg.** | **SD** | **CV%** |
| Background | 1121 | 1011 | 1088 | 1073 | 56.41 | 5.26E+00 |
| Blank | 1929 | 1829 | 1862 | 1873 | 51.36 | 2.74E+00 |
| DMSO | 1884 | 1875 | 1894 | 1874 | 15.52 | 8.28E-01 |
| DMSO | 1880 | 1857 | 1855 | | | |
| Forskolin 1 µM | 2568 | 2558 | 2658 | 2595 | 55.08 | 2.12E+00 |
| Forskolin 10 µM | 2977 | 3012 | 3067 | 3019 | 45.36 | 1.50E+00 |
| Prochloraz 0.1 µM | 1220 | 1092 | 1136 | 1149 | 64.69 | 5.63E+00 |
| Prochloraz 1 µM | 513 | 496 | 496 | 501 | 9.80 | 1.95E+00 |

**Testosterone – Run 4**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| **Dose [uM]** | **A** | **b** | **c** | **Avg.** | **SD** | **CV%** |
| Background | 1342 | 1208 | 1237 | 1262 | 70.61 | 5.59E+00 |
| Blank | 2403 | 2248 | 2261 | 2304 | 85.86 | 3.73E+00 |
| DMSO | 2335 | 2254 | 2535 | 2339 | 127.24 | 5.44E+00 |
| DMSO | 2443 | 2201 | 2265 | | | |
| Forskolin 1 µM | 3142 | 3082 | 3137 | 3120 | 33.06 | 1.06E+00 |
| Forskolin 10 µM | 3716 | 3532 | 4093 | 3780 | 285.62 | 7.56E+00 |
| Prochloraz 0.1 µM | 1897 | 1749 | 1808 | 1818 | 74.53 | 4.10E+00 |
| Prochloraz 1 µM | 1188 | 1133 | 1139 | 1153 | 30.34 | 2.63E+00 |

## APPENDIX 7 QC Plate Raw Data - Estradiol

**Estradiol – Run 1**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| **Dose [uM]** | **a** | **b** | **c** | **Avg.** | **SD** | **CV%** |
| Background | 76 | 66 | 73 | 72 | 5.15 | 7.18E+00 |
| Blank | 154 | 164 | 157 | 158 | 4.72 | 2.98E+00 |
| DMSO | 150 | 155 | 163 | 153 | 6.02 | 3.94E+00 |
| DMSO | 150 | 145 | 155 | | | |
| Forskolin 1 µM | 1795 | 1763 | 1802 | 1787 | 21.05 | 1.18E+00 |
| Forskolin 10 µM | 1977 | 2025 | 2004 | 2002 | 23.83 | 1.19E+00 |
| Prochloraz 0.1 µM | 143 | 152 | 149 | 148 | 4.43 | 2.99E+00 |
| Prochloraz 1 µM | 68 | 69 | 68 | 68 | 0.57 | 8.40E-01 |

**Estradiol – Run 2**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| **Dose [uM]** | **A** | **b** | **c** | **Avg.** | **SD** | **CV%** |
| Background | 45 | 41 | 38 | 41 | 3.41 | 8.27E+00 |
| Blank | 200 | 212 | 212 | 208 | 6.67 | 3.20E+00 |
| DMSO | 201 | 211 | 207 | 200 | 12.31 | 6.16E+00 |
| DMSO | 210 | 189 | 180 | | | |
| Forskolin 1 µM | 1287 | 1366 | 1375 | 1343 | 48.45 | 3.61E+00 |
| Forskolin 10 µM | 2027 | 2116 | 2206 | 2116 | 89.49 | 4.23E+00 |
| Prochloraz 0.1 µM | 144 | 128 | 127 | 133 | 9.55 | 7.17E+00 |
| Prochloraz 1 µM | 59 | 60 | 54 | 57 | 3.37 | 5.87E+00 |

## APPENDIX 7 QC Plate Raw Data - Estradiol

**Estradiol – Run 3**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| **Dose [uM]** | **A** | **b** | **c** | **Avg.** | **SD** | **CV%** |
| Background | 61 | 54 | 53 | 56 | 4.32 | 7.74E+00 |
| Blank | 179 | 181 | 184 | 182 | 2.52 | 1.39E+00 |
| DMSO | 173 | 179 | 185 | 179 | 5.19 | 2.90E+00 |
| DMSO | 183 | 181 | 173 | | | |
| Forskolin 1 µM | 1234 | 1235 | 1292 | 1254 | 32.95 | 2.63E+00 |
| Forskolin 10 µM | 1653 | 1676 | 1655 | 1661 | 12.95 | 7.80E-01 |
| Prochloraz 0.1 µM | 109 | 102 | 103 | 104 | 3.85 | 3.69E+00 |
| Prochloraz 1 µM | 51 | 51 | 54 | 52 | 1.56 | 3.00E+00 |

**Estradiol – Run 4**

| | pg/ml | | | | | |
|---|---|---|---|---|---|---|
| **Dose [uM]** | **A** | **b** | **c** | **Avg.** | **SD** | **CV%** |
| Background | 80 | 75 | 77 | 77 | 2.66 | 3.43E+00 |
| Blank | 231 | 222 | 230 | 228 | 4.99 | 2.19E+00 |
| DMSO | 225 | 213 | 231 | 223 | 7.75 | 3.48E+00 |
| DMSO | 230 | 214 | 222 | | | |
| Forskolin 1 µM | 1540 | 1509 | 1566 | 1538 | 28.44 | 1.85E+00 |
| Forskolin 10 µM | 2414 | 2389 | 2628 | 2477 | 131.32 | 5.30E+00 |
| Prochloraz 0.1 µM | 177 | 165 | 172 | 171 | 6.07 | 3.55E+00 |
| Prochloraz 1 µM | 83 | 85 | 82 | 83 | 1.61 | 1.93E+00 |

## APPENDIX 8 Statistics – Octyl Salicylate (Run 2)

| Testosterone | Test Statistic: | p-value: | Estradiol | Test Statistic: | p-value: |
|---|---|---|---|---|---|
| **Original Data Set:** | | | **Original Data Set:** | | |
| Shapiro-Wilk's (residuals) | 0.9503 | 0.2748 | Shapiro-Wilk's (residuals) | 0.9480 | 0.2453 |
| Levene's Test | 2.0366 | 0.1131 | Levene's Test | 2.7915 | 0.0422 |
| | | | | | |
| **Parametric Test (original data):** | | | **Parametric Test (original data):** | | |
| Dunnett's Test (original data) | Results Below | | Dunnett's Test (original data) | | |
| | | | | | |
| **Log Transformation:** | | | **Log Transformation:** | | |
| Shapiro-Wilk's (log residuals) | N/A | N/A | Shapiro-Wilk's (log residuals) | 0.9722 | 0.7220 |
| Levene's Test (transformed data) | N/A | N/A | Levene's Test (transformed data) | 1.4055 | 0.2696 |
| | | | | | |
| **Parametric Test (transformed data):** | | | **Parametric test (transformed data):** | | |
| Dunnett's Test (transformed data) | N/A | N/A | Dunnett's Test (transformed data) | Results Below | |
| | | | | | |
| | | | | | |
| **Non-parametric Test (original data):** | | | **Non-parametric Test (original data):** | | |
| Kruskal-Wallis (Dunn) | N/A | N/A | Kruskal-Wallis (Dunn) | N/A | N/A |

**Testosterone – Dunnett's Test Comparison of Treatment Groups to Solvent Controls:**

| Concentration (µM) | p-value: |
|---|---|
| 100 - 0 | 0.0047* |
| 1 - 0 | 1.0000 |
| 0.1 - 0 | 1.0000 |
| 0.001 - 0 | 0.9568 |
| 10 - 0 | 0.6555 |
| 0.0001 - 0 | 0.4996 |
| 0.01 - 0 | 0.4615 |

*Denotes statistical significance ($p \leq 0.05$).

**Estradiol – Dunnett's Test Comparison of Treatment Groups to Solvent Controls:**

| Concentration (µM) | p-value: |
|---|---|
| 100 - 0 | 0.0000** |
| 10 - 0 | 0.0340** |
| 1 - 0 | 0.9991 |
| 0.1 - 0 | 0.7358 |
| 0.01 - 0 | 0.6154 |
| 0.001 - 0 | 0.3600 |
| 0.0001 - 0 | 0.0997 |

*Denotes statistical significance ($p \leq 0.05$).

## APPENDIX 8 Statistics – Octyl Salicylate (Run 3)

| Testosterone | Test Statistic: | p-value: | Estradiol | Test Statistic: | p-value: |
|---|---|---|---|---|---|
| **Original Data Set:** | | | **Original Data Set:** | | |
| Shapiro-Wilk's (residuals) | 0.9649 | 0.5448 | Shapiro-Wilk's (residuals) | 0.9439 | 0.1991 |
| Levene's Test | 1.7375 | 0.1703 | Levene's Test | 1.8924 | 0.1377 |
| | | | | | |
| **Parametric Test (original data):** | | | **Parametric Test (original data):** | | |
| Dunnett's Test (original data) | Results Below | | Dunnett's Test (original data) | Results Below | |
| | | | | | |
| **Log Transformation:** | | | **Log Transformation:** | | |
| Shapiro-Wilk's (log residuals) | N/A | N/A | Shapiro-Wilk's (log residuals) | N/A | N/A |
| Levene's Test (transformed data) | N/A | N/A | Levene's Test (transformed data) | N/A | N/A |
| | | | | | |
| **Parametric Test (transformed data):** | | | **Parametric test (transformed data):** | | |
| Dunnett's Test (transformed data) | N/A | N/A | Dunnett's Test (transformed data) | N/A | N/A |
| | | | | | |
| | | | | | |
| **Non-parametric Test (original data):** | | | **Non-parametric Test (original data):** | | |
| Kruskal-Wallis (Dunn) | N/A | N/A | Kruskal-Wallis (Dunn) | N/A | N/A |

**Testosterone – Dunnett's Test Comparison of Treatment Groups to Solvent Controls:**

| Concentration (µM) | p-value: |
|---|---|
| 0.1 - 0 | 1.0000 |
| 100 - 0 | 1.0000 |
| 0.01 - 0 | 0.9994 |
| 10 - 0 | 0.9333 |
| 1 - 0 | 0.8466 |
| 0.0001 - 0 | 0.3275 |
| 0.001 - 0 | 0.2531 |

**Estradiol – Dunnett's Test Comparison of Treatment Groups to Solvent Controls:**

| Concentration (µM) | p-value: |
|---|---|
| 100 - 0 | 0.0000* |
| 10 - 0 | 0.1098 |
| 0.1 - 0 | 0.3279 |
| 0.01 - 0 | 0.0500* |
| 0.001 - 0 | 0.0228* |
| 1 - 0 | 0.0029* |
| 0.0001 - 0 | 0.0004* |

*Denotes statistical significance ($p \leq 0.05$).

## APPENDIX 8 Statistics – Octyl Salicylate (Run 3) - continued

Statistics for testosterone and estradiol normalized to percent viability:

| Testosterone | Test Statistic: | p-value: | Estradiol | Test Statistic: | p-value: |
|---|---|---|---|---|---|
| **Original Data Set:** | | | **Original Data Set:** | | |
| Shapiro-Wilk's (residuals) | 0.9551 | 0.3480 | Shapiro-Wilk's (residuals) | 0.9484 | 0.2502 |
| Levene's Test | 3.2359 | 0.0245 | Levene's Test | 4.0736 | 0.0095 |
| | | | | | |
| **Parametric Test (original data):** | | | **Parametric Test (original data):** | | |
| Dunnett's Test (original data) | N/A | N/A | Dunnett's Test (original data) | N/A | N/A |
| | | | | | |
| **Log Transformation:** | | | **Log Transformation:** | | |
| Shapiro-Wilk's (log residuals) | 0.9562 | 0.3666 | Shapiro-Wilk's (log residuals) | 0.9496 | 0.2659 |
| Levene's Test (transformed data) | 3.2706 | 0.0235 | Levene's Test (transformed data) | 4.0244 | 0.0100 |
| | | | | | |
| **Parametric Test (transformed data):** | | | **Parametric test (transformed data):** | | |
| Dunnett's Test (transformed data) | N/A | N/A | Dunnett's Test (transformed data) | N/A | N/A |
| | | | | | |
| | | | | | |
| **Non-parametric Test (original data):** | | | **Non-parametric Test (original data):** | | |
| Kruskal-Wallis (Dunn) | Results Below | | Kruskal-Wallis (Dunn) | Results Below | |

**Testosterone – Kruskal-Wallis (Dunn) Comparison of Treatment Groups to Solvent Controls:**

| Concentration (µM) | p-value: |
|---|---|
| 100 - 0 | 0.3972 |
| 10 - 0 | 1.0000 |
| 0.01 - 0 | 1.0000 |
| 0.001 - 0 | 1.0000 |
| 1 - 0 | 1.0000 |
| 0.1 - 0 | 1.0000 |
| 0.0001 - 0 | 1.0000 |

**Estradiol – Kruskal-Wallis (Dunn) Comparison of Treatment Groups to Solvent Controls:**

| Concentration (µM) | p-value: |
|---|---|
| 100 - 0 | 0.0656 |
| 10 - 0 | 0.2637 |
| 0.001 - 0 | 1.0000 |
| 0.01 - 0 | 1.0000 |
| 0.1 - 0 | 1.0000 |
| 1 - 0 | 1.0000 |
| 0.0001 - 0 | 1.0000 |

## APPENDIX 8 Statistics – Octyl Salicylate (Run 4)

| Testosterone | Test Statistic: | p-value: | Estradiol | Test Statistic: | p-value: |
|---|---|---|---|---|---|
| **Original Data Set:** | | | **Original Data Set:** | | |
| Shapiro-Wilk's (residuals) | 0.9610 | 0.4580 | Shapiro-Wilk's (residuals) | 0.9617 | 0.4742 |
| Levene's Test | 1.5491 | 0.2210 | Levene's Test | 2.9703 | 0.0338 |
| | | | | | |
| **Parametric Test (original data):** | | | **Parametric Test (original data):** | | |
| Dunnett's Test (original data) | Results Below | | Dunnett's Test (original data) | N/A | N/A |
| | | | | | |
| **Log Transformation:** | | | **Log Transformation:** | | |
| Shapiro-Wilk's (log residuals) | N/A | N/A | Shapiro-Wilk's (log residuals) | 0.9614 | 0.4664 |
| Levene's Test (transformed data) | N/A | N/A | Levene's Test (transformed data) | 2.7392 | 0.0451 |
| | | | | | |
| **Parametric Test (transformed data):** | | | **Parametric test (transformed data):** | | |
| Dunnett's Test (transformed data) | N/A | N/A | Dunnett's Test (transformed data) | N/A | N/A |
| | | | | | |
| | | | | | |
| **Non-parametric Test (original data):** | | | **Non-parametric Test (original data):** | | |
| Kruskal-Wallis (Dunn) | N/A | N/A | Kruskal-Wallis (Dunn) | Results Below | |

**Testosterone - Dunnett's Test Comparison of Treatment Groups to Solvent Controls:**

| Concentration (µM) | p-value: |
|---|---|
| 100 - 0 | 0.1232 |
| 1 - 0 | 0.1627 |
| 0.1 - 0 | 0.3715 |
| 0.01 - 0 | 0.9989 |
| 0.0001 - 0 | 0.9922 |
| 10 - 0 | 0.9770 |
| 0.001 - 0 | 0.9677 |

**Estradiol – Kruskal-Wallis (Dunn) Comparison of Treatment Groups to Solvent Controls:**

| Concentration (µM) | p-value: |
|---|---|
| 100 - 0 | 0.7418 |
| 10 - 0 | 1.0000 |
| 0.1 - 0 | 1.0000 |
| 1 - 0 | 1.0000 |
| 0.01 - 0 | 1.0000 |
| 0.0001 - 0 | 1.0000 |
| 0.001 - 0 | 0.4527 |

## APPENDIX 9 Statistics – Oxybenzone (Run 2)

| Testosterone | Test Statistic: | p-value: | Estradiol | Test Statistic: | p-value: |
|---|---|---|---|---|---|
| **Original Data Set:** | | | **Original Data Set:** | | |
| Shapiro-Wilk's (residuals) | 0.9604 | 0.4467 | Shapiro-Wilk's (residuals) | 0.9264 | 0.0809 |
| Levene's Test | 2.2734 | 0.0824 | Levene's Test | 3.2550 | 0.0240 |
| | | | | | |
| **Parametric Test (original data):** | | | **Parametric Test (original data):** | | |
| Dunnett's Test (original data) | Results Below | | Dunnett's Test (original data) | N/A | N/A |
| | | | | | |
| **Log Transformation:** | | | **Log Transformation:** | | |
| Shapiro-Wilk's (log residuals) | N/A | N/A | Shapiro-Wilk's (log residuals) | 0.9616 | 0.4712 |
| Levene's Test (transformed data) | N/A | N/A | Levene's Test (transformed data) | 1.9021 | 0.1359 |
| | | | | | |
| **Parametric Test (transformed data):** | | | **Parametric test (transformed data):** | | |
| Dunnett's Test (transformed data) | N/A | N/A | Dunnett's Test (transformed data) | Results Below | |
| | | | | | |
| | | | | | |
| **Non-parametric Test (original data):** | | | **Non-parametric Test (original data):** | | |
| Kruskal-Wallis (Dunn) | N/A | N/A | Kruskal-Wallis (Dunn) | N/A | N/A |

**Testosterone - Dunnett's Test Comparison of Treatment Groups to Solvent Controls:**

| Concentration (µM) | p-value: |
|---|---|
| 100 - 0 | 0.0001* |
| 10 - 0 | 0.9988 |
| 0.01 - 0 | 0.9695 |
| 0.1 - 0 | 0.9651 |
| 0.0001 - 0 | 0.8711 |
| 1 - 0 | 0.8032 |
| 0.001 - 0 | 0.2466 |

*Denotes statistical significance ($p \leq 0.05$).

**Estradiol – Dunnett's Test Comparison of Treatment Groups to Solvent Controls:**

| Concentration (µM) | p-value: |
|---|---|
| 100 - 0 | 0.0000* |
| 10 - 0 | 0.0000* |
| 1 - 0 | 0.4584 |
| 0.01 - 0 | 0.9839 |
| 0.0001 - 0 | 0.4060 |
| 0.1 - 0 | 0.2230 |
| 0.001 - 0 | 0.1188 |

*Denotes statistical significance ($p \leq 0.05$).

## APPENDIX 9 Statistics – Oxybenzone (Run 3)

| Testosterone | Test Statistic: | p-value: | Estradiol | Test Statistic: | p-value: |
|---|---|---|---|---|---|
| **Original Data Set:** | | | **Original Data Set:** | | |
| Shapiro-Wilk's (residuals) | 0.9650 | 0.5458 | Shapiro-Wilk's (residuals) | 0.9494 | 0.2625 |
| Levene's Test | 2.1618 | 0.0956 | Levene's Test | 1.4901 | 0.2398 |
| | | | | | |
| **Parametric Test (original data):** | | | **Parametric Test (original data):** | | |
| Dunnett's Test (original data) | Results Below | | Dunnett's Test (original data) | Results Below | |
| | | | | | |
| **Log Transformation:** | | | **Log Transformation:** | | |
| Shapiro-Wilk's (log residuals) | N/A | N/A | Shapiro-Wilk's (log residuals) | N/A | N/A |
| Levene's Test (transformed data) | N/A | N/A | Levene's Test (transformed data) | N/A | N/A |
| | | | | | |
| **Parametric Test (transformed data):** | | | **Parametric test (transformed data):** | | |
| Dunnett's Test (transformed data) | N/A | N/A | Dunnett's Test (transformed data) | N/A | N/A |
| | | | | | |
| | | | | | |
| **Non-parametric Test (original data):** | | | **Non-parametric Test (original data):** | | |
| Kruskal-Wallis (Dunn) | N/A | N/A | Kruskal-Wallis (Dunn) | N/A | N/A |

**Testosterone – Dunnett's Test Comparison of Treatment Groups to Solvent Controls:**

| Concentration (µM) | p-value: |
|---|---|
| 100 - 0 | 0.0003* |
| 10 - 0 | 0.9482 |
| 0.0001 - 0 | 0.9766 |
| 0.1 - 0 | 0.9995 |
| 0.001 - 0 | 1.0000 |
| 1 - 0 | 0.9890 |
| 0.01 - 0 | 0.9889 |

*Denotes statistical significance ($p \leq 0.05$).

**Estradiol – Dunnett's Test Comparison of Treatment Groups to Solvent Controls:**

| Concentration (µM) | p-value: |
|---|---|
| 100 - 0 | 0.0000* |
| 10 - 0 | 0.0000* |
| 1 - 0 | 0.1088 |
| 0.1 - 0 | 0.9223 |
| 0.001 - 0 | 0.8181 |
| 0.0001 - 0 | 0.6402 |
| 0.01 - 0 | 0.5705 |

*Denotes statistical significance ($p \leq 0.05$).

## APPENDIX 9 Statistics – Oxybenzone (Run 4)

| Testosterone | Test Statistic: | p-value: | Estradiol | Test Statistic: | p-value: |
|---|---|---|---|---|---|
| **Original Data Set:** | | | **Original Data Set:** | | |
| Shapiro-Wilk's (residuals) | 0.9517 | 0.2952 | Shapiro-Wilk's (residuals) | 0.9724 | 0.7255 |
| Levene's Test | 2.7045 | 0.0471 | Levene's Test | 2.9263 | 0.0357 |
| | | | | | |
| **Parametric Test (original data):** | | | **Parametric Test (original data):** | | |
| Dunnett's Test (original data) | N/A | N/A | Dunnett's Test (original data) | N/A | N/A |
| | | | | | |
| **Log Transformation:** | | | **Log Transformation:** | | |
| Shapiro-Wilk's (log residuals) | 0.9475 | 0.2386 | Shapiro-Wilk's (log residuals) | 0.9787 | 0.8703 |
| Levene's Test (transformed data) | 2.7303 | 0.0456 | Levene's Test (transformed data) | 2.3571 | 0.0737 |
| | | | | | |
| **Parametric Test (transformed data):** | | | **Parametric test (transformed data):** | | |
| Dunnett's Test (transformed data) | N/A | N/A | Dunnett's Test (transformed data) | Results Below | |
| | | | | | |
| | | | | | |
| **Non-parametric Test (original data):** | | | **Non-parametric Test (original data):** | | |
| Kruskal-Wallis (Dunn) | Results Below | | Kruskal-Wallis (Dunn) | N/A | N/A |

**Testosterone - Kruskal-Wallis (Dunn) Comparison of Treatment Groups to Solvent Controls:**

| Concentration (µM) | p-value: |
|---|---|
| 100 - 0 | 0.0391* |
| 0.1 - 0 | 0.2637 |
| 10 - 0 | 0.6585 |
| 1 - 0 | 1.0000 |
| 0.0001 - 0 | 1.0000 |
| 0.001 - 0 | 1.0000 |
| 0.01 - 0 | 1.0000 |

*Denotes statistical significance ($p \leq 0.05$).

**Estradiol – Dunnett's Test Comparison of Treatment Groups to Solvent Controls:**

| Concentration (µM) | p-value: |
|---|---|
| 100 - 0 | 0.0000* |
| 10 - 0 | 0.0000* |
| 1 - 0 | 0.0008* |
| 0.1 - 0 | 0.0060* |
| 0.01 - 0 | 0.9977 |
| 0.001 - 0 | 1.0000 |
| 0.0001 - 0 | 0.9999 |

*Denotes statistical significance ($p \leq 0.05$).

## APPENDIX 10 Statistics – Octocrylene (Run 2)

Concentration 10 and 100 µM omitted because of cytotoxicity.

| Testosterone | Test Statistic: | p-value: | Estradiol | Test Statistic: | p-value: |
|---|---|---|---|---|---|
| **Original Data Set:** | | | **Original Data Set:** | | |
| Shapiro-Wilk's (residuals) | 0.9527 | 0.4689 | Shapiro-Wilk's (residuals) | 0.9307 | 0.2002 |
| Levene's Test | 2.8599 | 0.0631 | Levene's Test | 0.8022 | 0.5692 |
| | | | | | |
| **Parametric Test (original data):** | | | **Parametric Test (original data):** | | |
| Dunnett's Test (original data) | Results Below | | Dunnett's Test (original data) | Results Below | |
| | | | | | |
| **Log Transformation:** | | | **Log Transformation:** | | |
| Shapiro-Wilk's (log residuals) | N/A | N/A | Shapiro-Wilk's (log residuals) | N/A | N/A |
| Levene's Test (transformed data) | N/A | N/A | Levene's Test (transformed data) | N/A | N/A |
| | | | | | |
| **Parametric Test (transformed data):** | | | **Parametric test (transformed data):** | | |
| Dunnett's Test (transformed data) | N/A | N/A | Dunnett's Test (transformed data) | N/A | N/A |
| | | | | | |
| | | | | | |
| **Non-parametric Test (original data):** | | | **Non-parametric Test (original data):** | | |
| Kruskal-Wallis (Dunn) | N/A | N/A | Kruskal-Wallis (Dunn) | N/A | N/A |

**Testosterone – Dunnett's Test Comparison of Treatment Groups to Solvent Controls:**

| Concentration (µM) | p-value: |
|---|---|
| 0.0001 - 0 | 0.7789 |
| 1 - 0 | 0.9555 |
| 0.001 - 0 | 1.0000 |
| 0.1 - 0 | 1.0000 |
| 0.01 - 0 | 0.5681 |

**Estradiol – Dunnett's Test Comparison of Treatment Groups to Solvent Controls:**

| Concentration (µM) | p-value: |
|---|---|
| 1 - 0 | 0.4877 |
| 0.1 - 0 | 0.8834 |
| 0.0001 - 0 | 0.9995 |
| 0.001 - 0 | 1.0000 |
| 0.01 - 0 | 0.9374 |

## APPENDIX 10 Statistics – Octocrylene (Run 3)

| Testosterone | Test Statistic: | p-value: | Estradiol | Test Statistic: | p-value: |
|---|---|---|---|---|---|
| **Original Data Set:** | | | **Original Data Set:** | | |
| Shapiro-Wilk's (residuals) | 0.9676 | 0.6079 | Shapiro-Wilk's (residuals) | 0.9651 | 0.5497 |
| Levene's Test | 1.3902 | 0.2754 | Levene's Test | 1.4571 | 0.2510 |
| | | | | | |
| **Parametric Test (original data):** | | | **Parametric Test (original data):** | | |
| Dunnett's Test (original data) | Results Below | | Dunnett's Test (original data) | Results Below | |
| | | | | | |
| **Log Transformation:** | | | **Log Transformation:** | | |
| Shapiro-Wilk's (log residuals) | N/A | N/A | Shapiro-Wilk's (log residuals) | N/A | N/A |
| Levene's Test (transformed data) | N/A | N/A | Levene's Test (transformed data) | N/A | N/A |
| | | | | | |
| **Parametric Test (transformed data):** | | | **Parametric test (transformed data):** | | |
| Dunnett's Test (transformed data) | N/A | N/A | Dunnett's Test (transformed data) | N/A | N/A |
| | | | | | |
| | | | | | |
| **Non-parametric Test (original data):** | | | **Non-parametric Test (original data):** | | |
| Kruskal-Wallis (Dunn) | N/A | N/A | Kruskal-Wallis (Dunn) | N/A | N/A |

**Testosterone – Dunnett's Test Comparison of Treatment Groups to Solvent Controls:**

| Concentration (µM) | p-value: |
|---|---|
| 0.1 - 0 | 0.6982 |
| 0.01 - 0 | 0.9820 |
| 0.0001 - 0 | 0.9110 |
| 1 - 0 | 0.9062 |
| 0.001 - 0 | 0.2397 |
| 10 - 0 | 0.0000* |
| 100 - 0 | 0.0000* |

*Denotes statistical significance ($p \leq 0.05$).

**Estradiol – Dunnett's Test Comparison of Treatment Groups to Solvent Controls:**

| Concentration (µM) | p-value: |
|---|---|
| 1 - 0 | 0.2863 |
| 0.1 - 0 | 0.5968 |
| 0.0001 - 0 | 0.9831 |
| 0.01 - 0 | 0.9847 |
| 0.001 - 0 | 0.9495 |
| 10 - 0 | 0.0052* |
| 100 - 0 | 0.0000* |

*Denotes statistical significance ($p \leq 0.05$).

## APPENDIX 10 Statistics – Octocrylene (Run 3) - continued

Statistics for testosterone and estradiol normalized to percent viability:

| Testosterone | Test Statistic: | p-value: | Estradiol | Test Statistic: | p-value: |
|---|---|---|---|---|---|
| **Original Data Set:** | | | **Original Data Set:** | | |
| Shapiro-Wilk's (residuals) | 0.9633 | 0.5074 | Shapiro-Wilk's (residuals) | 0.9688 | 0.6379 |
| Levene's Test | 2.7422 | 0.0449 | Levene's Test | 1.4218 | 0.2636 |
| | | | | | |
| **Parametric Test (original data):** | | | **Parametric Test (original data):** | | |
| Dunnett's Test (original data) | N/A | N/A | Dunnett's Test (original data) | Results Below | |
| | | | | | |
| **Log Transformation:** | | | **Log Transformation:** | | |
| Shapiro-Wilk's (log residuals) | 0.9647 | 0.5394 | Shapiro-Wilk's (log residuals) | N/A | N/A |
| Levene's Test (transformed data) | 2.6456 | 0.0507 | Levene's Test (transformed data) | N/A | N/A |
| | | | | | |
| **Parametric Test (transformed data):** | | | **Parametric test (transformed data):** | | |
| Dunnett's Test (transformed data) | Results Below | | Dunnett's Test (transformed data) | N/A | N/A |
| | | | | | |
| | | | | | |
| **Non-parametric Test (original data):** | | | **Non-parametric Test (original data):** | | |
| Kruskal-Wallis (Dunn) | N/A | N/A | Kruskal-Wallis (Dunn) | N/A | N/A |

**Testosterone – Dunnett's Test Comparison of Treatment Groups to Solvent Controls:**

| Concentration (µM) | p-value: |
|---|---|
| 0.0001 - 0 | 0.7102 |
| 0.01 - 0 | 0.8225 |
| 0.1 - 0 | 0.8740 |
| 1 - 0 | 0.9600 |
| 0.001 - 0 | 1.0000 |
| 10 - 0 | 0.3040 |
| 100 - 0 | 0.0001* |

*Denotes statistical significance ($p \leq 0.05$).

**Estradiol – Dunnett's Test Comparison of Treatment Groups to Solvent Controls:**

| Concentration (µM) | p-value: |
|---|---|
| 1 - 0 | 0.0497* |
| 0.0001 - 0 | 0.1218 |
| 10 - 0 | 0.4356 |
| 0.01 - 0 | 0.8195 |
| 0.1 - 0 | 0.8385 |
| 0.001 - 0 | 0.9462 |
| 100 - 0 | 0.0147* |

*Denotes statistical significance ($p \leq 0.05$).

## APPENDIX 10 Statistics – Octocrylene (Run 4)

Concentration 10 and 100 µM omitted because of cytotoxicity.

| Testosterone | Test Statistic: | p-value: | Estradiol | Test Statistic: | p-value: |
|---|---|---|---|---|---|
| **Original Data Set:** | | | **Original Data Set:** | | |
| Shapiro-Wilk's (residuals) | 0.9558 | 0.5237 | Shapiro-Wilk's (residuals) | 0.9699 | 0.7963 |
| Levene's Test | 0.8562 | 0.5369 | Levene's Test | 1.7656 | 0.1945 |
| | | | | | |
| **Parametric Test (original data):** | | | **Parametric Test (original data):** | | |
| Dunnett's Test (original data) | Results Below | | Dunnett's Test (original data) | Results Below | |
| | | | | | |
| **Log Transformation:** | | | **Log Transformation:** | | |
| Shapiro-Wilk's (log residuals) | N/A | N/A | Shapiro-Wilk's (log residuals) | N/A | N/A |
| Levene's Test (transformed data) | N/A | N/A | Levene's Test (transformed data) | N/A | N/A |
| | | | | | |
| **Parametric Test (transformed data):** | | | **Parametric test (transformed data):** | | |
| Dunnett's Test (transformed data) | N/A | N/A | Dunnett's Test (transformed data) | N/A | N/A |
| | | | | | |
| | | | | | |
| **Non-parametric Test (original data):** | | | **Non-parametric Test (original data):** | | |
| Kruskal-Wallis (Dunn) | N/A | N/A | Kruskal-Wallis (Dunn) | N/A | N/A |

**Testosterone – Dunnett's Test Comparison of Treatment Groups to Solvent Controls:**

| Concentration (µM) | p-value: |
|---|---|
| 1 - 0 | 0.9428 |
| 0.1 - 0 | 0.4022 |
| 0.0001 - 0 | 0.2826 |
| 0.01 - 0 | 0.2162 |
| 0.001 - 0 | 0.1195 |

**Estradiol – Dunnett's Test Comparison of Treatment Groups to Solvent Controls:**

| Concentration (µM) | p-value: |
|---|---|
| 1 - 0 | 0.0110* |
| 0.01 - 0 | 1.0000 |
| 0.0001 - 0 | 1.0000 |
| 0.1 - 0 | 0.9995 |
| 0.001 - 0 | 0.9680 |

*Denotes statistical significance ($p \leq 0.05$).

## APPENDIX 11 Statistics – Octylmethoxycinnamate (Run 1)

Concentrations 1, 10, and 100 µM excluded from statistical analysis based on solubility results.

| Testosterone | Test Statistic: | p-value: | Estradiol | Test Statistic: | p-value: |
|---|---|---|---|---|---|
| **Original Data Set:** | | | **Original Data Set:** | | |
| Shapiro-Wilk's (residuals) | 0.8933 | 0.1083 | Shapiro-Wilk's (residuals) | 0.9534 | 0.6507 |
| Levene's Test | 5.4353 | 0.0205 | Levene's Test | 3.4302 | 0.0649 |
| | | | | | |
| **Parametric Test (original data):** | | | **Parametric Test (original data):** | | |
| Dunnett's Test (original data) | N/A | N/A | Dunnett's Test (original data) | Results Below | |
| | | | | | |
| **Log Transformation:** | | | **Log Transformation:** | | |
| Shapiro-Wilk's (log residuals) | 0.8850 | 0.0833 | Shapiro-Wilk's (log residuals) | N/A | N/A |
| Levene's Test (transformed data) | 6.0076 | 0.0156 | Levene's Test (transformed data) | N/A | N/A |
| | | | | | |
| **Parametric Test (transformed data):** | | | **Parametric test (transformed data):** | | |
| Dunnett's Test (transformed data) | N/A | N/A | Dunnett's Test (transformed data) | N/A | N/A |
| | | | | | |
| | | | | | |
| **Non-parametric Test (original data):** | | | **Non-parametric Test (original data):** | | |
| Kruskal-Wallis (Dunn) | Results Below | | Kruskal-Wallis (Dunn) | N/A | N/A |

**Testosterone - Kruskal-Wallis (Dunn) Comparison of Treatment Groups to Solvent Controls:**

| Concentration (µM) | p-value: |
|---|---|
| 0.01 - 0 | 1.0000 |
| 0.0001 - 0 | 1.0000 |
| 0.001 - 0 | 1.0000 |
| 0.1 - 0 | 0.2989 |

**Estradiol – Dunnett's Test Comparison of Treatment Groups to Solvent Controls:**

| Concentration (µM) | p-value: |
|---|---|
| 0.01 - 0 | 0.9868 |
| 0.1 - 0 | 0.9668 |
| 0.001 - 0 | 0.9030 |
| 0.0001 - 0 | 0.4665 |

## APPENDIX 11 Statistics – Octylmethoxycinnamate (Run 2)

| Testosterone | Test Statistic: | p-value: | Estradiol | Test Statistic: | p-value: |
|---|---|---|---|---|---|
| **Original Data Set:** | | | **Original Data Set:** | | |
| Shapiro-Wilk's (residuals) | 0.9588 | 0.4156 | Shapiro-Wilk's (residuals) | 0.9766 | 0.8265 |
| Levene's Test | 0.9681 | 0.4859 | Levene's Test | 1.6436 | 0.1939 |
| | | | | | |
| **Parametric Test (original data):** | | | **Parametric Test (original data):** | | |
| Dunnett's Test (original data) | Results Below | | Dunnett's Test (original data) | Results Below | |
| | | | | | |
| **Log Transformation:** | | | **Log Transformation:** | | |
| Shapiro-Wilk's (log residuals) | N/A | N/A | Shapiro-Wilk's (log residuals) | N/A | N/A |
| Levene's Test (transformed data) | N/A | N/A | Levene's Test (transformed data) | N/A | N/A |
| | | | | | |
| **Parametric Test (transformed data):** | | | **Parametric test (transformed data):** | | |
| Dunnett's Test (transformed data) | N/A | N/A | Dunnett's Test (transformed data) | N/A | N/A |
| | | | | | |
| | | | | | |
| **Non-parametric Test (original data):** | | | **Non-parametric Test (original data):** | | |
| Kruskal-Wallis (Dunn) | N/A | N/A | Kruskal-Wallis (Dunn) | N/A | N/A |

**Testosterone – Dunnett's Test Comparison of Treatment Groups to Solvent Controls:**

| Concentration (µM) | p-value: |
|---|---|
| 1 - 0 | 0.3899 |
| 0.0001 - 0 | 0.4380 |
| 0.1 - 0 | 0.9690 |
| 0.001 - 0 | 0.9966 |
| 100 - 0 | 0.9999 |
| 0.01 - 0 | 1.0000 |
| 10 - 0 | 0.9980 |

**Estradiol – Dunnett's Test Comparison of Treatment Groups to Solvent Controls:**

| Concentration (µM) | p-value: |
|---|---|
| 100 - 0 | 0.3996 |
| 1 - 0 | 0.4728 |
| 0.0001 - 0 | 0.6203 |
| 0.1 - 0 | 0.9182 |
| 0.01 - 0 | 0.9607 |
| 10 - 0 | 0.9937 |
| 0.001 - 0 | 0.9991 |

## APPENDIX 11 Statistics – Octylmethoxycinnamate (Run 3)

| Testosterone | Test Statistic: | p-value: | Estradiol | Test Statistic: | p-value: |
|---|---|---|---|---|---|
| **Original Data Set:** | | | **Original Data Set:** | | |
| Shapiro-Wilk's (residuals) | 0.9815 | 0.9212 | Shapiro-Wilk's (residuals) | 0.9529 | 0.3133 |
| Levene's Test | 2.3581 | 0.0736 | Levene's Test | 0.8780 | 0.5446 |
| | | | | | |
| **Parametric Test (original data):** | | | **Parametric Test (original data):** | | |
| Dunnett's Test (original data) | Results Below | | Dunnett's Test (original data) | Results Below | |
| | | | | | |
| **Log Transformation:** | | | **Log Transformation:** | | |
| Shapiro-Wilk's (log residuals) | N/A | N/A | Shapiro-Wilk's (log residuals) | N/A | N/A |
| Levene's Test (transformed data) | N/A | N/A | Levene's Test (transformed data) | N/A | N/A |
| | | | | | |
| **Parametric Test (transformed data):** | | | **Parametric test (transformed data):** | | |
| Dunnett's Test (transformed data) | N/A | N/A | Dunnett's Test (transformed data) | N/A | N/A |
| | | | | | |
| | | | | | |
| **Non-parametric Test (original data):** | | | **Non-parametric Test (original data):** | | |
| Kruskal-Wallis (Dunn) | N/A | N/A | Kruskal-Wallis (Dunn) | N/A | N/A |

**Testosterone – Dunnett's Test Comparison of Treatment Groups to Solvent Controls:**

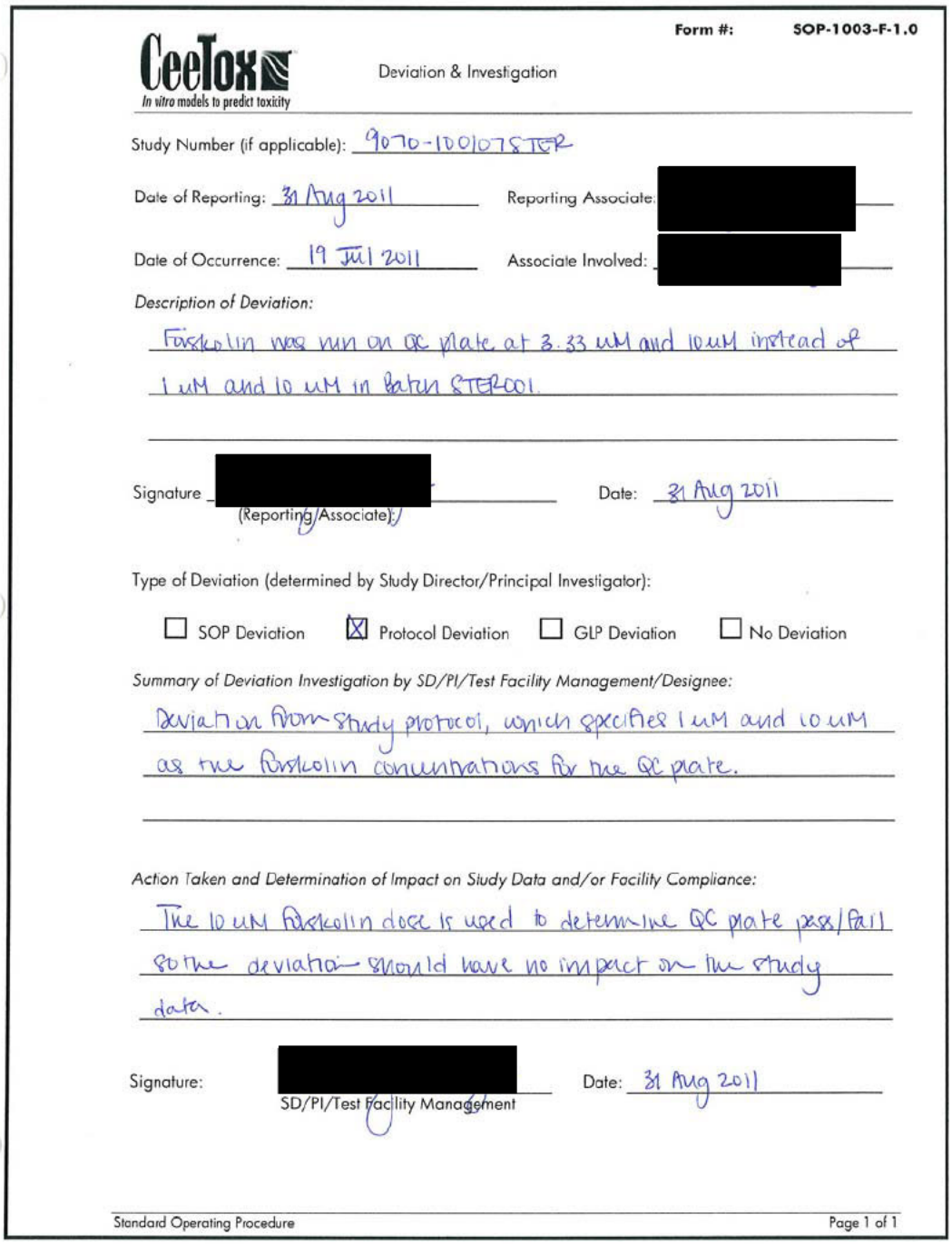
| Concentration (µM) | p-value: |
|---|---|
| 0.001 - 0 | 1.0000 |
| 0.0001 - 0 | 1.0000 |
| 1 - 0 | 0.9903 |
| 100 - 0 | 0.8351 |
| 0.01 - 0 | 0.7818 |
| 0.1 - 0 | 0.7398 |
| 10 - 0 | 0.7068 |

**Estradiol – Dunnett's Test Comparison of Treatment Groups to Solvent Controls:**

| Concentration (µM) | p-value: |
|---|---|
| 100 - 0 | 0.1890 |
| 1 - 0 | 0.9879 |
| 10 - 0 | 0.9967 |
| 0.0001 - 0 | 1.0000 |
| 0.001 - 0 | 1.0000 |
| 0.1 - 0 | 0.9837 |
| 0.01 - 0 | 0.9739 |

## APPENDIX 12 Deviation Forms

Protocol Deviations:



Form #: SOP-1003-F-1.0

CeeTox — In vitro models to predict toxicity

Deviation & Investigation

Study Number (if applicable): 9070-100107STER

Date of Reporting: 31 Aug 2011 Reporting Associate: [redacted]

Date of Occurrence: 19 Jul 2011 Associate Involved: [redacted]

*Description of Deviation:*

Forskolin was run on QC plate at 3.33 uM and 10uM instead of 1 uM and 10 uM in Batch STER001.

Signature [redacted] (Reporting Associate) Date: 31 Aug 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:*

Deviation from study protocol, which specifies 1 uM and 10 uM as the forskolin concentrations for the QC plate.

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

The 10 uM forskolin dose is used to determine QC plate pass/fail so the deviation should have no impact on the study data.

Signature: [redacted] SD/PI/Test Facility Management Date: 31 Aug 2011

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Form #: SOP-1003-F-1.0

CeeTox
*In vitro models to predict toxicity*

Deviation & Investigation

Study Number (if applicable): 9070-100107STER

Date of Reporting: 26 Aug 2011 Reporting Associate: [redacted]

Date of Occurrence: 19 Jul 2011 20 Jul 2011 20 Jul 2011 21 Jul 2011 Associate Involved: [redacted]

*Description of Deviation:*

Oxybenzone (2-hydroxy-4-methoxybenzone) lot number in the protocol was 20080801. Lot supplied by sponsor was 20100801. No C of A was provided for lot 20100801.

Signature [redacted] (Reporting Associate): Date: 26 Aug 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:*

Compound lot supplied by sponsor differs from lot number indicated in protocol.

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

Request proper C of A for lot 20100801 from sponsor.

Signature: [redacted] SD/PI/Test Facility Management Date: 26 Aug 2011

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## APPENDIX 12 Deviation Forms (continued)

Form #: SOP-1003-F-1.0

CeeTox
*In vitro* models to predict toxicity

Deviation & Investigation

Study Number (if applicable): 9070-100107STER

Date of Reporting: 29Sep2011 Reporting Associate: [redacted]

Date of Occurrence: 19Jul2011, 20Jul2011, 26Jul2011, 27Jul2011 Associate Involved: [redacted]

*Description of Deviation:*

Purity used for octylmethoxycinnamate was 98%. Actual purity according to certificate of analysis is 99.8%.

Signature [redacted] Date: 07 Feb 2012

(Reporting Associate)

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

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

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

Protocol deviation. All stock solution concentrations for octylmethoxycinnamate differ by approximately 2% from the indicated concentration.

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

The deviation has no impact on the study data. Each stock solution concentration is approximately 2% off From the indicated concentration.

Signature: [redacted] Date: 07 Feb 2012

SD/PI/Test Facility Management

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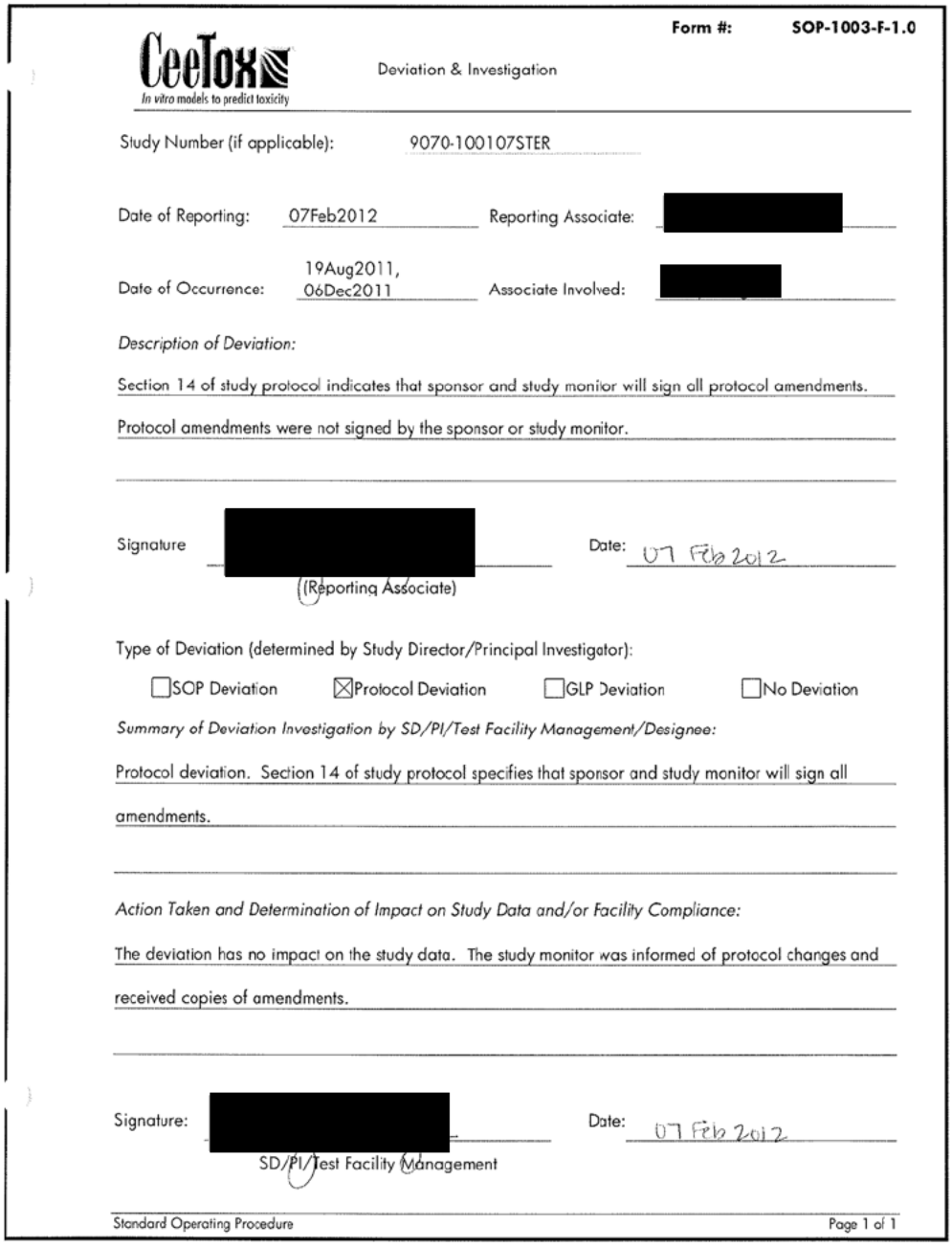
## APPENDIX 12 Deviation Forms (continued)



Form #: SOP-1003-F-1.0

CeeTox
In vitro models to predict toxicity

Deviation & Investigation

Study Number (if applicable): 9070-100107STER

Date of Reporting: 07Feb2012 Reporting Associate: [redacted]

Date of Occurrence: 19Aug2011, 06Dec2011 Associate Involved: [redacted]

*Description of Deviation:*

Section 14 of study protocol indicates that sponsor and study monitor will sign all protocol amendments.

Protocol amendments were not signed by the sponsor or study monitor.

Signature [redacted] Date: 07 Feb 2012

(Reporting Associate)

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

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

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

Protocol deviation. Section 14 of study protocol specifies that sponsor and study monitor will sign all amendments.

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

The deviation has no impact on the study data. The study monitor was informed of protocol changes and received copies of amendments.

Signature: [redacted] Date: 07 Feb 2012

SD/PI/Test Facility Management

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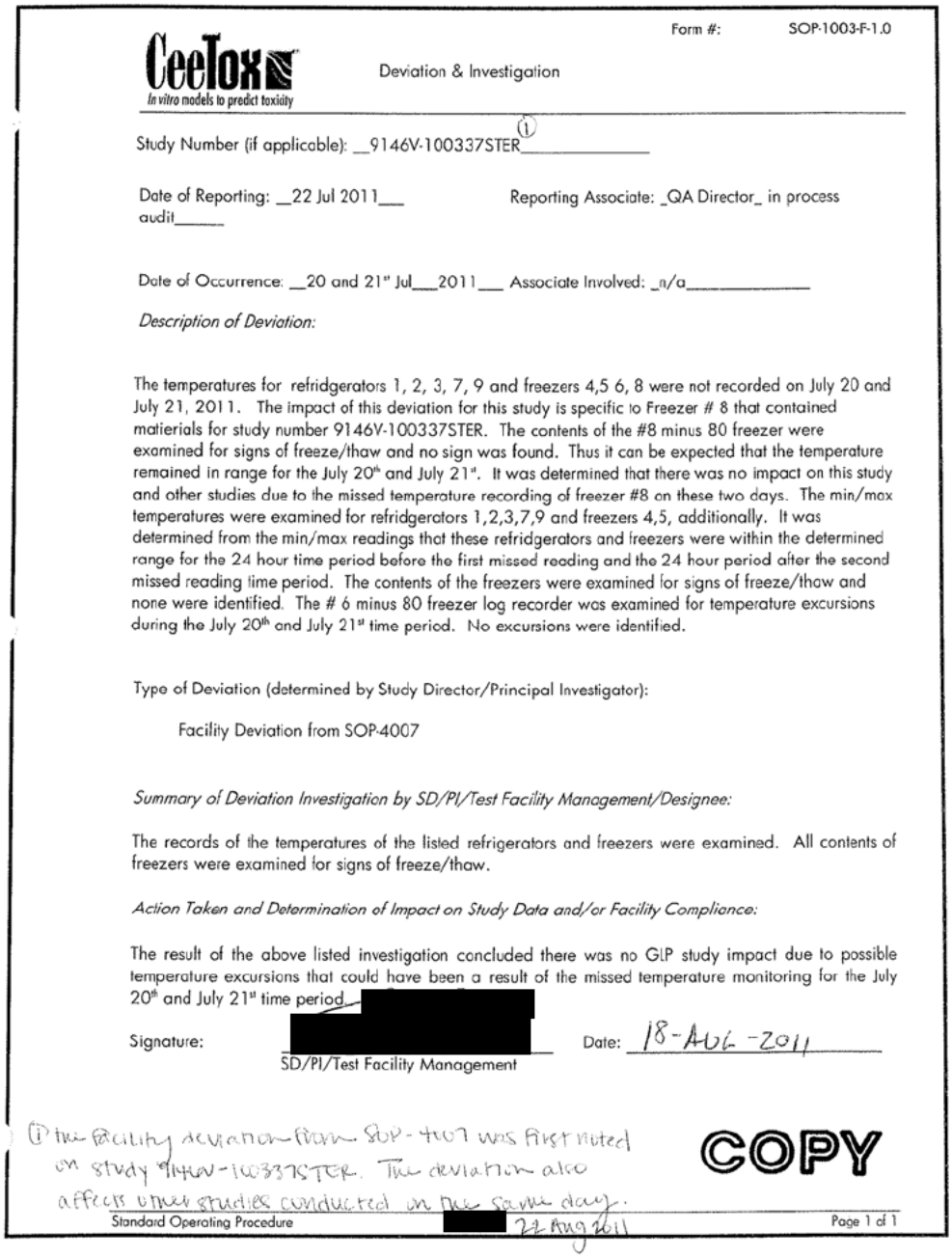
## APPENDIX 12 Deviation Forms (continued)

Facility Deviation:



Form #: SOP-1003-F-1.0

CeeTox
In vitro models to predict toxicity

Deviation & Investigation

Study Number (if applicable): __9146V-100337STER (1)_____

Date of Reporting: __22 Jul 2011___ Reporting Associate: _QA Director_ in process audit_____

Date of Occurrence: __20 and 21st Jul___2011___ Associate Involved: _n/a_____

*Description of Deviation:*

The temperatures for refridgerators 1, 2, 3, 7, 9 and freezers 4,5 6, 8 were not recorded on July 20 and July 21, 2011. The impact of this deviation for this study is specific to Freezer # 8 that contained matierials for study number 9146V-100337STER. The contents of the #8 minus 80 freezer were examined for signs of freeze/thaw and no sign was found. Thus it can be expected that the temperature remained in range for the July 20th and July 21st. It was determined that there was no impact on this study and other studies due to the missed temperature recording of freezer #8 on these two days. The min/max temperatures were examined for refridgerators 1,2,3,7,9 and freezers 4,5, additionally. It was determined from the min/max readings that these refridgerators and freezers were within the determined range for the 24 hour time period before the first missed reading and the 24 hour period after the second missed reading time period. The contents of the freezers were examined for signs of freeze/thaw and none were identified. The # 6 minus 80 freezer log recorder was examined for temperature excursions during the July 20th and July 21st time period. No excursions were identified.

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

Facility Deviation from SOP-4007

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

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

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

The result of the above listed investigation concluded there was no GLP study impact due to possible temperature excursions that could have been a result of the missed temperature monitoring for the July 20th and July 21st time period.

Signature: [redacted] Date: 18-Aug-2011

SD/PI/Test Facility Management

(1) The facility deviation from SOP-4007 was first noted on study 9146V-100337STER. The deviation also affects other studies conducted on the same day.

COPY

Standard Operating Procedure [redacted] 22 Aug 2011 Page 1 of 1

## APPENDIX 13 Certificate of Analysis – Octyl Salicylate



# Certificate of Analysis

SIGMA-ALDRICH

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

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

[redacted]

[redacted] Supervisor
Quality Control
Milwaukee, Wisconsin USA

## APPENDIX 13 Certificate of Analysis – Oxybenzone

**IVYCHEM**

IVY FINE CHEMICALS

http://www.ivychem.com

**CERTICATE OF ANALYSIS**

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

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

## APPENDIX 13 Certificate of Analysis – Octocrylene



# Certificate of Analysis

SIGMA-ALDRICH

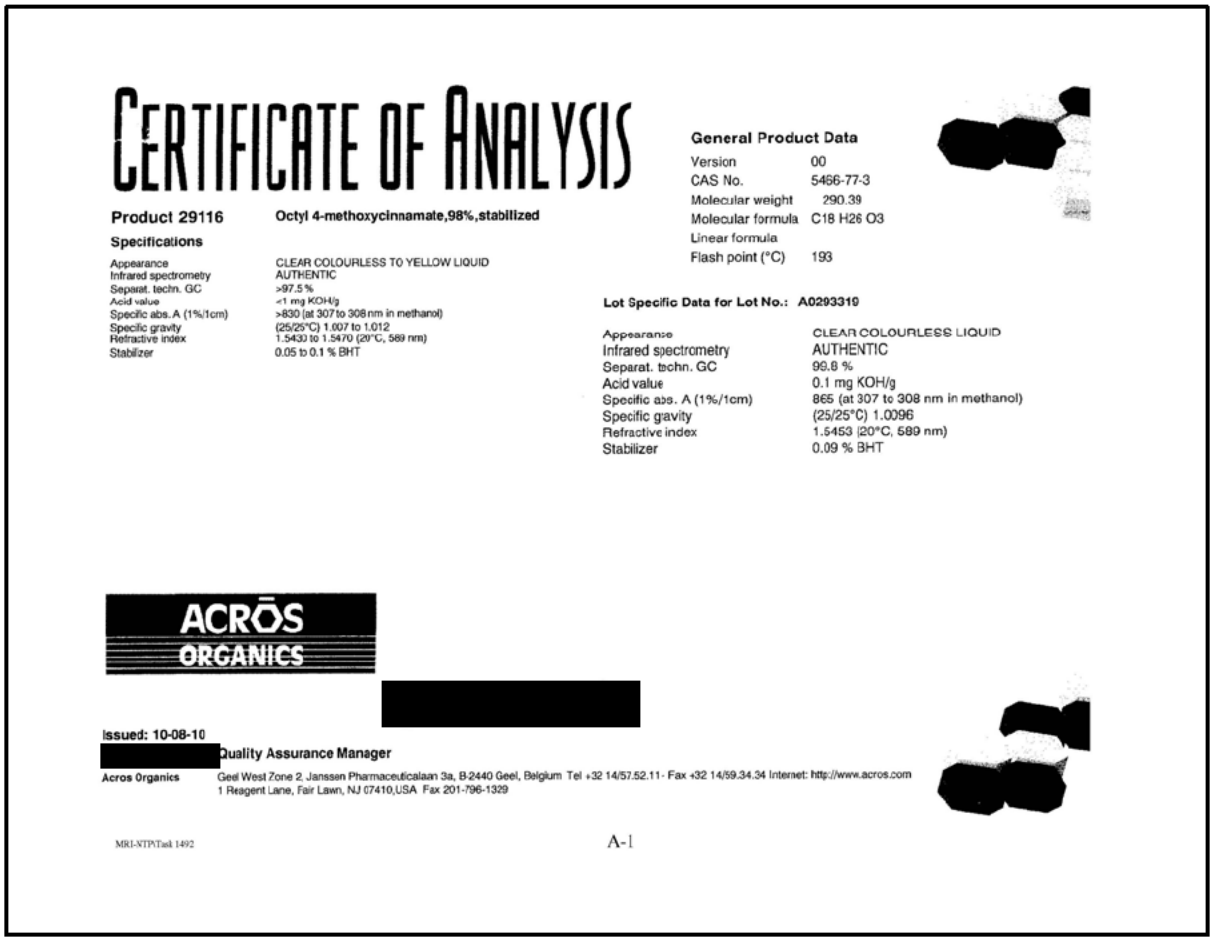
| | |
|---|---|
| **Product Name** | 2-Ethylhexyl 2-cyano-3,3-diphenylacrylate, 97% |
| **Product Number** | 415820 |
| **Product Brand** | ALDRICH |
| **CAS Number** | 6197-30-4 |
| **Molecular Formula** | $(C_6H_5)_2C=C(CN)CO_2CH_2CH(C_2H_5)(CH_2)_3CH_3$ |
| **Molecular Weight** | 361.48 |

| TEST | SPECIFICATION | LOT 01697MJ RESULTS |
|---|---|---|
| **Appearance (Color)** | Yellow | Yellow |
| **Appearance (Form)** | Viscous Liquid | Viscous Liquid |
| **Infrared spectrum** | Conforms to Structure | Conforms |
| **Purity (GC)** | ≥96.5 % | 99.2 % |
| **Specification Date:** | | OCT 2009 |
| **Date of QC Release:** | | OCT 2009 |
| **Print Date:** | | OCT 22 2008 |

[redacted]

[redacted] Supervisor
Quality Control
Milwaukee, Wisconsin USA

## APPENDIX 13 Certificate of Analysis – Octylmethoxycinnamate



# CERTIFICATE OF ANALYSIS

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

**Specifications**

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

**General Product Data**

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

**Lot Specific Data for Lot No.: A0293319**

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

ACROS ORGANICS

[redacted]

**Issued: 10-08-10**

[redacted] **Quality Assurance Manager**

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

## APPENDIX 14 Principal Investigator Report – OpAns, LLC

**Title:**

Determination of Testosterone and Estradiol in H295R Supplemented Medium Specimens from the Study Entitled, "H295R Steroidogenesis Assay"

Study Number: 9070-100107STER

Document Number: OPR-CTX-0004.02

| **Author:** | **Signature:** | **Date:** |
|---|---|---|
| [redacted]<br>Analyst | [redacted] | 24OCT11 |
| **Approved by:**<br>Kenneth C Lewis, PhD<br>Principal Investigator,<br>Opans, LLC | **Signature:**<br>[redacted] | **Effective Date:**<br>24Oct11 |

## Revisions

| Version | Effective Date | Description |
|---|---|---|
| 01 | 3 Oct 2011 | Initial document |
| 02 | 24 Oct 2011 | Revised Calibration Standard Tables to correct intercept values, corrected typographical errors, enlarged example chromatogram in the appendix |

## Contents

# PRINCIPAL INVESTIGATOR'S STATEMENT OF COMPLIANCE

This phase (i.e., HPLC/MS-MS measurement of testosterone and estradiol) of the study was conducted in accordance with US Environmental Protection Agency's Good Laboratory Practice Regulations as set forth in Title 40 of the CFR Part 160 (October 16, 1989) and the OECD Principles of Good Laboratory Practice (as revised in 1997).

Nothing occurred to affect adversely the quality or integrity of these experimental data.

I consider the data generated to be valid.

Kenneth C Lewis, PhD
Principal Investigator
OpAns, LLC.

**Signature:** [redacted]

**Date:** 24Oct11

# QUALITY ASSURANCE STATEMENT

## Study Number: 9070-100107STER

## Report Title: Determination of Testosterone and Estradiol in H295R Supplemented Medium Specimens from the Study Entitled, "H295R Steroidogenesis Assay"

The phases, processes and documents relating to this portion of the study conducted by OpAns were audited and the results of the audits were reported to the Principal Investigator, Study Director and Management. The methods and results presented in the parts of the report prepared by OpAns accurately reflect the raw data.

Associated laboratories and support functions are subject to regular audits.

| Audit Phase | Audit Date(s) | Date(s) Reported to PI/SD/Management |
|---|---|---|
| Protocol | 04 Aug 2011 | No Findings |
| Standard / QC / Sample Preparation | 02 Aug 2011 | No Findings |
| Report | 23, 26 Sep 2011 | 30 Sep 2011* |
| Report Revision | 21 Oct 2011 | 21 Oct 2011 |

* Report included summary of previously conducted audits resulting in no findings.

**Auditor:** [redacted]
Director Quality Assurance
OpAns, LLC

**Signature:** [redacted]

**Date:** 24 Oct 2011

## Key Study Details

| | |
|---|---|
| Sponsor: | NIEHS<br>530 Davis Drive, MD K2-12<br>PO Box 12233<br>Durham, NC 27713 (USA) |
| Test Facility: | CeeTox<br>4717 Campus Drive<br>Kalamazoo, MI 49008 (USA) |
| Study Director: | [redacted]<br>CeeTox, Inc |
| Test Site: | OpAns, LLC<br>4134 South Alston Ave, Suite 101<br>Durham, NC 27713-1879 (USA) |
| Principal Investigator: | Kenneth C Lewis, PhD |
| Study Phase: | HPLC/MS-MS measurement of testosterone and estradiol |
| Analyst Involved: | [redacted] |
| Date of First Sample Analysis: | 28 July 2011 |
| Date of Last Sample Analysis: | 26 August 2011 |
| Primary Applications Used to Acquire Data: | Agilent MassHunter Workstation Data Acquisition for Triple Quad B.03.01 (B2065)<br>Agilent MassHunter Quantitative Analysis for QQQ (B.04.00/Build 4.0.225.0.)<br>Excel 2007 |

### Location of Records:

The signed original of this report and the raw data (or exact copies there of) generated as a result of this phase of the study will be retained at OpAns or an approved archive facility contracted by OpAns for a period up to 1 year following completion of the study (i.e., final report issue date) or until returned to the Sponsor. OpAns reserves the right to retain exact copies of these records for purposes of maintaining the capability of addressing test facility regulatory requirements.

# 1. OBJECTIVES AND PROCEDURES

The objective of this phase of the study was to determine the levels of testosterone and estradiol in H295R supplemented medium using HPLC/MS-MS and report the results to the Study Director.

# 2. ANALYTICAL METHOD

## 2.1. Analytical Method

Testosterone and estradiol were extracted from H295R supplemented medium by liquid liquid extraction using methyl tert-butyl ether (MTBE) after the addition of [$^2H_5$]-testosterone and [$^2H_5$]-estradiol as internal standards. Extracts were analysed by HPLC-MS/MS using positive ion multiple reaction monitoring. This method (OPM-OPP-0008, see summary in Appendix 1) was validated over the range 100 to 100000 pg/mL for testosterone and 10 to 10000 pg/mL for estradiol [OpAns Document Number OPR-OPP-0006]. The lower limit of quantification (LLQ) was 100 pg/mL for testosterone and 10 pg/mL for estradiol using a 300 μL aliquot of H295R supplemented medium.

Freshly prepared calibration standards (n = 7) were prepared for each run. QC samples at three concentrations (2 replicates per concentration), were included with each run. All chromatograms, from each analytical run, were reviewed to verify that the appropriate peaks had been identified and correctly integrated. Representative calibration standard chromatograms are presented in Figure 1.

## 2.2. Calibration Standards Acceptance Criteria

Matrix-based calibration standards were deemed acceptable if the back-calculated concentration fell within ±15%, except for LLOQ, when it fell within ±20% for at least 75% (or a minimum of six standards) of the calibration standards. Values falling outside of these limits may have been discarded, provided they did not change the established model. Results for the back-calculated calibration standards for each accepted analytical run are presented in Tables 1 and 2.

## 2.3. Quality Control Acceptance Criteria

Quality Control (QC) samples replicated (at least once) at a minimum of three concentrations (one with 3x of the LLOQ (QC 30 300), one in the midrange (QC 800 8000), and one approaching the high end of the range (QC 8000 80000) were incorporated into each run. The results of the QC samples provided the basis for accepting or rejecting the analytical run. At least 67% (four out of six) of the QC samples were within 15% of their respective nominal (theoretical) values. The minimum number of QC samples (in multiples of three) was at least 5% of the total number of unknowns, or at least six, whichever was greater.

Results of QC samples analysed within the study, along with precision and accuracy data are presented in Tables 3 and 4.

## 2.4. Preparation and Storage of Quality Control Samples

QC samples were prepared for testosterone and estradiol at three concentrations (300, 8000, and 80000 pg/mL for testosterone and 30, 800, and 8000 pg/mL for estradiol). The QC samples were prepared on 11 July 2011 and stored frozen at -80°C with the study samples.

# 3. STUDY SPECIMENS

## 3.1. Specimen Management

All study specimens were received in acceptable condition (dry ice (solid $CO_2$).) Specimens were stored at -80°C then thawed at room temperature prior to analysis.

## 3.2. Data Analysis

HPLC-MS/MS data were acquired using proprietary software application MassHunter Workstation Acquisition (Version B.03.01 (B2065), Agilent Technologies, Inc.). Data were processed (integrated) using the software application MassHunter Quantitative Analysis for QQQ (version B.04.00/Build 4.0.225.0. Agilent Technologies, Inc.) Calibration plots of area ratio versus testosterone and estradiol concentrations were constructed and a weighted $1/x^2$ linear regression applied to the data using MassHunter Quantitative Analysis for QQQ. Statistical calculations such as means, standard deviations, etc. were performed using Excel 2007. Sample results are presented in Table 5.

## 3.3. Repeat Analyses

No study samples were re-analysed during this study.

# 4. REFERENCES

OpAns Document Number OPR-OPP-0006.01 Study No. OPP-OPP-0003. The validation of a method for the determination of testosterone and estradiol and in H295R cell medium by HPLC/MS-MS.

# TABLES

## Table 1 Summary of Back-Calculated Calibration Testosterone Standards for Study 9070-100107STER

| Analytical Run ID (Plate ID) | Calibration Standard Concentration *pg/mL* | | | | | | | Slope | Intercept | Corr. Coeff. |
|---|---|---|---|---|---|---|---|---|---|---|
| | 100 | 200 | 1000 | 5000 | 10000 | 50000 | 100000 | | | |
| STER007 Run 1 (STER007_QC) | 101 | 206 | 941 | 4986 | 10385 | 50082 | 102372 | 0.000153 | 0.000106 | 0.998812 |
| | 100 | 196 | 982 | 4750 | 9949 | 51954 | 101979 | | | |
| STER007 Run 2 (STER013_QC, STER019_QC) | 97 | 192 | 945 | 4950 | 10086 | 51286 | 104654 | 0.000155 | -0.000259 | 0.998198 |
| | 104 | 209 | 936 | 4960 | 9926 | 50592 | 103743 | | | |
| STER007 Run 3 (STER001_QC) | 96 | 186 | 963 | 4946 | 9992 | 51441 | 104692 | 0.000153 | 0.000623 | 0.995975 |
| | 113 | 184 | 953 | 4952 | 10234 | 52079 | 103165 | | | |
| 9070-100107STER Run 1 (STER007-Plate 5 rows A-D, STER013-Plate 5 rows A-D, STER019-Plate 5 rows A-D) | 101 | 190 | 962 | 5102 | 9926 | 51057 | 101186 | 0.000152 | -0.000251 | 0.999191 |
| | 103 | 196 | 989 | 5017 | 9882 | 50359 | 103401 | | | |
| 9070-100107STER Run 2 (STER007-Plate 5 rows E-H, STER013-Plate 5 rows E-H, STER019-Plate 5 rows E-H) | 99 | 195 | 978 | 5102 | 10250 | 50953 | 95390 | 0.000148 | -0.000099 | 0.999217 |
| | 104 | 195 | 1003 | 4996 | 10059 | 49776 | 102353 | | | |
| 9070-100107STER Run 3 (STER007-Plate 6 rows A-D, STER013-Plate 6 rows A-D, STER019-Plate 6 rows A-D) | 98 | 191 | 959 | 5021 | 9572 | 52161 | 97135 | 0.000154 | -0.000580 | 0.998318 |
| | 104 | 206 | 965 | 5091 | 10400 | 52395 | 99569 | | | |
| 9070-100107STER Run 4 (STER007-Plate 6 rows E-H, STER013-Plate 6 rows E-H, STER019-Plate 6 rows G-H) | 97 | 185 | 965 | 5020 | 10052 | 50834 | 101666 | 0.000152 | 0.000116 | 0.997555 |
| | 109 | 192 | 952 | 4958 | 10333 | 51942 | 102500 | | | |
| 9070-100107STER Run 5 (STER001-Plate 6) | 107 | 189 | 983 | 4932 | 10029 | 51024 | 102298 | 0.000153 | 0.000523 | 0.997792 |
| | 100 | 187 | 938 | 4939 | 10240 | 51952 | 104763 | | | |

| Summary Statistics | Calibration Standard Concentration *pg/mL* | | | | | | | Slope | Intercept | Corr. Coeff. |
|---|---|---|---|---|---|---|---|---|---|---|
| | 100 | 200 | 1000 | 5000 | 10000 | 50000 | 100000 | | | |
| Mean | 102 | 194 | 963 | 4982 | 10082 | 51243 | 101929 | 0.00015 | 0.00002 | 0.99813 |
| Standard Deviation | 4.68 | 7.62 | 19.34 | 84.68 | 217.82 | 793.44 | 2618.51 | 0.000002 | 0.000407 | 0.001062 |
| Precision (%) | 4.59 | 3.94 | 2.01 | 1.70 | 2.16 | 1.55 | 2.57 | -- | -- | -- |
| Accuracy (%) | 101.9 | 96.8 | 96.3 | 99.6 | 100.8 | 102.5 | 101.9 | -- | -- | -- |
| n | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 8 | 8 | 8 |

Statistics calculated from non-rounded data.
*Excluded from calculations, did not meet acceptance criteria.

## Table 2 Summary of Back-Calculated Estradiol Calibration Standards for Study 9070-100107STER

| Analytical Run ID | Calibration Standard Concentration *pg/mL* | | | | | | | Slope | Intercept | Corr. Coeff. |
|---|---|---|---|---|---|---|---|---|---|---|
| | 10 | 20 | 100 | 500 | 1000 | 5000 | 10000 | | | |
| STER007 Run 1 (STER007_QC) | 9.7 | 19.2 | 90.2 | 493.1 | 1006.7 | 5026.2 | 10220.0 | 0.00203 | 0.00027 | 0.99685 |
| | 10.2 | 21.8 | 98.6 | 472.4 | 998.4 | 5356.7 | 10408.1 | | | |
| STER007 Run 2 (STER013_QC, STER019_QC) | 10.3 | 20.3 | 94.4 | 477.3 | 992.8 | 5195.0 | 10514.1 | 0.00186 | 0.00067 | 0.99813 |
| | *14.3 | 18.8 | 98.0 | 491.9 | 995.1 | 5159.1 | 10423.0 | | | |
| STER007 Run 3 (STER001_QC) | 10.2 | 20.7 | 96.2 | 491.1 | 976.4 | 5092.3 | 10625.7 | 0.00172 | -0.00012 | 0.99715 |
| | 10.1 | 18.4 | 95.2 | 493.0 | 948.1 | 5309.7 | 10639.0 | | | |
| 9070-100107STER Run 1 (STER007-Plate 5 rows A-D, STER013-Plate 5 rows A-D, STER019-Plate 5 rows A-D) | 8.9 | 19.6 | 98.2 | 504.5 | 982.5 | 5126.5 | 10058.3 | 0.00186 | 0.02501 | 0.99589 |
| | 10.6 | 22.7 | 97.1 | 485.4 | 988.7 | 4844.8 | 10328.4 | | | |
| 9070-100107STER Run 2 (STER007-Plate 5 rows E-H, STER013-Plate 5 rows E-H, STER019-Plate 5 rows E-H) | 10.4 | 20.9 | 99.6 | 512.2 | 1018.6 | 5164.9 | 9878.6 | 0.00176 | 0.00443 | 0.99763 |
| | *16.9 | 17.8 | 96.8 | 502.6 | 965.7 | 4919.3 | 10471.3 | | | |
| 9070-100107STER Run 3 (STER007-Plate 6 rows A-D, STER013-Plate 6 rows A-D, STER019-Plate 6 rows A-D) | 10.5 | 19.6 | 92.5 | 485.4 | 945.9 | 5083.3 | 9677.8 | 0.00195 | 0.00216 | 0.99754 |
| | 9.7 | 20.0 | 98.6 | 528.3 | 1042.5 | 5317.6 | 10274.2 | | | |
| 9070-100107STER Run 4 (STER007-Plate 6 rows E-H, STER013-Plate 6 rows E-H, STER019-Plate 6 rows G-H) | 10.1 | *24.4 | 96.6 | 487.5 | 1002.4 | 5218.6 | 10298.8 | 0.00189 | 0.00139 | 0.99860 |
| | 10.1 | 19.6 | 92.7 | 491.0 | 1013.8 | 5051.4 | 10484.5 | | | |
| 9070-100107STER Run 5 (STER001-Plate 6) | 10.6 | 17.6 | 96.4 | 492.4 | 985.1 | 5115.2 | 10504.6 | 0.00162 | 0.00229 | 0.99641 |
| | 10.3 | 19.2 | 96.5 | 486.2 | 1009.8 | 5192.5 | 10796.2 | | | |

| Summary Statistics | Calibration Standard Concentration *pg/mL* | | | | | | | Slope | Intercept | Corr. Coeff. |
|---|---|---|---|---|---|---|---|---|---|---|
| | 10 | 20 | 100 | 500 | 1000 | 5000 | 10000 | | | |
| Mean | 10.1 | 19.7 | 96.1 | 493.4 | 992.0 | 5135.8 | 10350.2 | 0.00184 | 0.00451 | 0.99727 |
| Standard Deviation | 0.44 | 1.41 | 2.55 | 13.44 | 25.25 | 137.33 | 287.04 | 0.000132 | 0.008406 | 0.000889 |
| Precision (%) | 4.35 | 7.15 | 2.66 | 2.72 | 2.55 | 2.67 | 2.77 | -- | -- | -- |
| Accuracy (%) | 101.2 | 98.7 | 96.1 | 98.7 | 99.2 | 102.7 | 103.5 | -- | -- | -- |
| n | 14 | 15 | 16 | 16 | 16 | 16 | 16 | 8 | 8 | 8 |

Statistics calculated from non-rounded data.
*Excluded from calculations, did not meet acceptance criteria.

**Table 3 Summary of Testosterone Quality Control Data for Study 9070-100107STER**

| Analytical Run ID (Plate IDs) | Quality Control Sample Concentration *pg/mL* | | | | | |
|---|---|---|---|---|---|---|
| | 300 | Accuracy (%) | 800 | Accuracy (%) | 8000 | Accuracy (%) |
| STER007 Run 1 (STER007_QC) | 277 | 92 | 8431 | 105 | 80885 | 101 |
| | 285 | 95 | 8183 | 102 | 80959 | 101 |
| STER007 Run 2 (STER013_QC, STER019_QC) | 290 | 97 | 7816 | 98 | 82040 | 103 |
| | 298 | 99 | 7908 | 99 | 82640 | 103 |
| STER007 Run 3 (STER001_QC) | 276 | 92 | 7809 | 98 | 81056 | 101 |
| | 286 | 95 | 7956 | 99 | 81044 | 101 |
| 9070-100107STER Run 1 (STER007-Plate 5 rows A-D, STER013-Plate 5 rows A-D, STER019-Plate 5 rows A-D) | 289 | 96 | 7949 | 99 | 81720 | 102 |
| | 283 | 94 | 8021 | 100 | 80798 | 101 |
| 9070-100107STER Run 2 (STER007-Plate 5 rows E-H, STER013-Plate 5 rows E-H, STER019-Plate 5 rows E-H) | 262 | 87 | 7810 | 98 | 77945 | 97 |
| | 287 | 96 | 7687 | 96 | 77167 | 96 |
| 9070-100107STER Run 3 (STER007-Plate 6 rows A-D, STER013-Plate 6 rows A-D, STER019-Plate 6 rows A-D) | 287 | 96 | 7651 | 96 | 78865 | 99 |
| | 281 | 94 | 7840 | 98 | 78838 | 99 |
| 9070-100107STER Run 4 (STER007-Plate 6 rows E-H, STER013-Plate 6 rows E-H, STER019-Plate 6 rows G-H) | 304 | 101 | 7695 | 96 | 80155 | 100 |
| | 292 | 97 | 8055 | 101 | 81477 | 102 |
| 9070-100107STER Run 5 (STER001-Plate 6) | 277 | 92 | 8015 | 100 | 79425 | 99 |
| | 286 | 95 | 7763 | 97 | 80142 | 100 |

| Summary Statistics | Quality Control Sample Concentration *pg/mL* | | |
|---|---|---|---|
| | 300 | 8000 | 80000 |
| Mean | 285.0 | 7911.8 | 80322.2 |
| Standard Deviation | 9.58 | 202.09 | 1514.77 |
| Precision (%) | 3.36 | 2.55 | 1.89 |
| Accuracy (%) | 95.0 | 98.9 | 100.4 |
| n | 16 | 16 | 16 |
| Statistics calculated from non-rounded data. | | | |

**Table 4 Summary of Estradiol Quality Control Data for Study 9070-100107STER**

| Analytical Run ID (Plate IDs) | Quality Control Sample Concentration *pg/mL* | | | | | |
|---|---|---|---|---|---|---|
| | 30 | Accuracy (%) | 800 | Accuracy (%) | 8000 | Accuracy (%) |
| STER007 Run 1 (STER007_QC) | 26.2 | 87.4 | 744.1 | 93.0 | 7410.0 | 92.6 |
| | 26.9 | 89.8 | 732.4 | 91.6 | 7370.3 | 92.1 |
| STER007 Run 2 (STER013_QC, STER019_QC) | 28.7 | 95.7 | 780.0 | 97.5 | 8394.8 | 104.9 |
| | 30.5 | 101.7 | 784.5 | 98.1 | 8485.4 | 106.1 |
| STER007 Run 3 (STER001_QC) | 31.1 | 103.6 | 786.8 | 98.4 | 8344.5 | 104.3 |
| | 31.3 | 104.2 | 795.3 | 99.4 | 8501.3 | 106.3 |
| 9070-100107STER Run 1 (STER007-Plate 5 rows A-D, STER013-Plate 5 rows A-D, STER019-Plate 5 rows A-D) | 29.3 | 97.5 | 791.0 | 98.9 | 8051.8 | 100.6 |
| | 30.6 | 102.0 | 756.8 | 94.6 | 7873.5 | 98.4 |
| 9070-100107STER Run 2 (STER007-Plate 5 rows E-H, STER013-Plate 5 rows E-H, STER019-Plate 5 rows E-H) | 25.7 | 85.7 | 749.4 | 93.7 | 7671.8 | 95.9 |
| | 26.6 | 88.6 | 741.4 | 92.7 | 7350.7 | 91.9 |
| 9070-100107STER Run 3 (STER007-Plate 6 rows A-D, STER013-Plate 6 rows A-D, STER019-Plate 6 rows A-D) | 26.5 | 88.4 | 705.5 | 88.2 | 7341.8 | 91.8 |
| | 27.8 | 92.6 | 723.3 | 90.4 | 7459.0 | 93.2 |
| 9070-100107STER Run 4 (STER007-Plate 6 rows E-H, STER013-Plate 6 rows E-H, STER019-Plate 6 rows G-H) | 33.0 | 110.2 | 734.3 | 91.8 | 7820.6 | 97.8 |
| | 28.6 | 95.2 | 735.9 | 92.0 | 7775.0 | 97.2 |
| 9070-100107STER Run 5 (STER001-Plate 6) | 30.5 | 101.8 | 816.9 | 102.1 | 8405.2 | 105.1 |
| | 29.3 | 97.8 | 801.6 | 100.2 | 8535.0 | 106.7 |

| Summary Statistics | Quality Control Sample Concentration *pg/mL* | | |
|---|---|---|---|
| | 30 | 800 | 8000 |
| Mean | 28.9 | 761.2 | 7924.4 |
| Standard Deviation | 2.15 | 32.58 | 463.45 |
| Precision (%) | 7.45 | 4.28 | 5.85 |
| Accuracy (%) | 96.4 | 95.2 | 99.1 |
| n | 16 | 16 | 16 |
| Statistics calculated from non-rounded data. | | | |

## Table 5 Testosterone and Estradiol Concentrations in H295R Media Samples

| Sample Plate | Sample Name | Testosterone Conc. (pg/mL) | Estradiol Conc. (pg/mL) |
|---|---|---|---|
| STER007_QC | Background A7 | 1199 | 44.6 |
| STER007_QC | Background A9 | 1073 | 41.2 |
| STER007_QC | Background A11 | 1134 | 37.8 |
| STER007_QC | Blank A1 | 2617 | 200.5 |
| STER007_QC | Blank A3 | 2555 | 211.8 |
| STER007_QC | Blank A5 | 2571 | 212.2 |
| STER007_QC | DMSO B1 | 2518 | 201.2 |
| STER007_QC | DMSO B3 | 2520 | 211.0 |
| STER007_QC | DMSO B5 | 2580 | 206.7 |
| STER007_QC | DMSO B7 | 2491 | 209.6 |
| STER007_QC | DMSO B9 | 2458 | 189.2 |
| STER007_QC | DMSO B11 | 2501 | 180.5 |
| STER007_QC | Forskolin 1uM C1 | 3798 | 1286.9 |
| STER007_QC | Forskolin 1uM C3 | 3771 | 1366.0 |
| STER007_QC | Forskolin 1uM C5 | 3723 | 1374.9 |
| STER007_QC | Forskolin 10uM D1 | 4611 | 2026.9 |
| STER007_QC | Forskolin 10uM D3 | 4670 | 2116.4 |
| STER007_QC | Forskolin 10uM D5 | 4972 | 2205.9 |
| STER007_QC | Prochloraz 0.1uM C7 | 1854 | 144.3 |
| STER007_QC | Prochloraz 0.1uM C9 | 1772 | 128.5 |
| STER007_QC | Prochloraz 0.1uM C11 | 1822 | 127.1 |
| STER007_QC | Prochloraz 1uM D7 | 1205 | 59.1 |
| STER007_QC | Prochloraz 1uM D9 | 1241 | 59.6 |
| STER007_QC | Prochloraz 1uM D11 | 1118 | 53.5 |
| STER013_QC | Background A7 | 1121 | 60.8 |
| STER013_QC | Background A9 | 1011 | 53.6 |
| STER013_QC | Background A11 | 1088 | 53.0 |
| STER013_QC | Blank A1 | 1929 | 179.2 |
| STER013_QC | Blank A3 | 1829 | 181.2 |
| STER013_QC | Blank A5 | 1862 | 184.2 |
| STER013_QC | DMSO B1 | 1884 | 172.6 |
| STER013_QC | DMSO B3 | 1875 | 178.7 |
| STER013_QC | DMSO B5 | 1894 | 185.1 |
| STER013_QC | DMSO B7 | 1880 | 183.4 |
| STER013_QC | DMSO B9 | 1857 | 180.6 |
| STER013_QC | DMSO B11 | 1855 | 173.2 |

| Sample Plate | Sample Name | Testosterone Conc. (pg/mL) | Estradiol Conc. (pg/mL) |
|---|---|---|---|
| STER013_QC | Forskolin 1uM C1 | 2568 | 1234.5 |
| STER013_QC | Forskolin 1uM C3 | 2558 | 1235.3 |
| STER013_QC | Forskolin 1uM C5 | 2658 | 1292.0 |
| STER013_QC | Forskolin 10uM D1 | 2977 | 1652.5 |
| STER013_QC | Forskolin 10uM D3 | 3012 | 1676.1 |
| STER013_QC | Forskolin 10uM D5 | 3067 | 1654.9 |
| STER013_QC | Prochloraz 0.1uM C7 | 1220 | 108.8 |
| STER013_QC | Prochloraz 0.1uM C9 | 1092 | 101.5 |
| STER013_QC | Prochloraz 0.1uM C11 | 1136 | 103.0 |
| STER013_QC | Prochloraz 1uM D7 | 513 | 51.0 |
| STER013_QC | Prochloraz 1uM D9 | 496 | 51.3 |
| STER013_QC | Prochloraz 1uM D11 | 496 | 53.8 |
| STER019_QC | Background A7 | 1342 | 80.4 |
| STER019_QC | Background A9 | 1208 | 75.2 |
| STER019_QC | Background A11 | 1237 | 76.8 |
| STER019_QC | Blank A1 | 2403 | 231.4 |
| STER019_QC | Blank A3 | 2248 | 222.3 |
| STER019_QC | Blank A5 | 2261 | 230.3 |
| STER019_QC | DMSO B1 | 2335 | 225.3 |
| STER019_QC | DMSO B3 | 2254 | 212.8 |
| STER019_QC | DMSO B5 | 2535 | 230.6 |
| STER019_QC | DMSO B7 | 2443 | 230.5 |
| STER019_QC | DMSO B9 | 2201 | 214.2 |
| STER019_QC | DMSO B11 | 2265 | 222.0 |
| STER019_QC | Forskolin 1uM C1 | 3142 | 1539.7 |
| STER019_QC | Forskolin 1uM C3 | 3082 | 1509.4 |
| STER019_QC | Forskolin 1uM C5 | 3137 | 1566.3 |
| STER019_QC | Forskolin 10uM D1 | 3716 | 2413.6 |
| STER019_QC | Forskolin 10uM D3 | 3532 | 2388.6 |
| STER019_QC | Forskolin 10uM D5 | 4093 | 2627.5 |
| STER019_QC | Prochloraz 0.1uM C7 | 1897 | 176.9 |
| STER019_QC | Prochloraz 0.1uM C9 | 1749 | 164.8 |
| STER019_QC | Prochloraz 0.1uM C11 | 1808 | 171.6 |
| STER019_QC | Prochloraz 1uM D7 | 1188 | 82.8 |
| STER019_QC | Prochloraz 1uM D9 | 1133 | 84.9 |
| STER019_QC | Prochloraz 1uM D11 | 1139 | 81.8 |
| STER001_QC | Background A7 | 1109 | 76.0 |
| STER001_QC | Background A9 | 997 | 66.0 |

| Sample Plate | Sample Name | Testosterone Conc. (pg/mL) | Estradiol Conc. (pg/mL) |
|---|---|---|---|
| STER001_QC | Background A11 | 1063 | 73.2 |
| STER001_QC | Blank A1 | 2198 | 154.2 |
| STER001_QC | Blank A3 | 2212 | 163.5 |
| STER001_QC | Blank A5 | 2112 | 157.4 |
| STER001_QC | DMSO B1 | 2202 | 149.6 |
| STER001_QC | DMSO B3 | 2128 | 154.9 |
| STER001_QC | DMSO B5 | 2101 | 162.5 |
| STER001_QC | DMSO B7 | 2174 | 150.0 |
| STER001_QC | DMSO B9 | 2053 | 145.0 |
| STER001_QC | DMSO B11 | 2196 | 154.6 |
| STER001_QC | Forskolin 3.33uM C1 | 3008 | 1795.4 |
| STER001_QC | Forskolin 3.33uM C3 | 2823 | 1762.7 |
| STER001_QC | Forskolin 3.33uM C5 | 2960 | 1802.0 |
| STER001_QC | Forskolin 10uM D1 | 3281 | 1977.2 |
| STER001_QC | Forskolin 10uM D3 | 3345 | 2024.6 |
| STER001_QC | Forskolin 10uM D5 | 3420 | 2004.4 |
| STER001_QC | Prochloraz 0.1uM C7 | 1886 | 143.4 |
| STER001_QC | Prochloraz 0.1uM C9 | 1906 | 152.1 |
| STER001_QC | Prochloraz 0.1uM C11 | 1951 | 149.2 |
| STER001_QC | Prochloraz 1uM D7 | 1030 | 67.6 |
| STER001_QC | Prochloraz 1uM D9 | 982 | 68.6 |
| STER001_QC | Prochloraz 1uM D11 | 1017 | 68.5 |
| STER007-Plate 5 (Rows A-D) | 20110720 DMSO A1 | 2791 | 250.9 |
| STER007-Plate 5 (Rows A-D) | 20110720 DMSO A3 | 2789 | 248.1 |
| STER007-Plate 5 (Rows A-D) | 20110720 DMSO A5 | 2799 | 253.6 |
| STER007-Plate 5 (Rows A-D) | 20110720 100uM B1 | 3044 | 480.8 |
| STER007-Plate 5 (Rows A-D) | 20110720 100uM B3 | 3219 | 523.2 |
| STER007-Plate 5 (Rows A-D) | 20110720 100uM B5 | 3001 | 479.6 |
| STER007-Plate 5 (Rows A-D) | 20110720 10uM C1 | 2778 | 277.6 |
| STER007-Plate 5 (Rows A-D) | 20110720 10uM C3 | 2626 | 281.3 |
| STER007-Plate 5 (Rows A-D) | 20110720 10uM C5 | 2691 | 296.0 |
| STER007-Plate 5 (Rows A-D) | 20110720 1uM D1 | 2664 | 244.3 |
| STER007-Plate 5 (Rows A-D) | 20110720 1uM D3 | 2955 | 263.5 |
| STER007-Plate 5 (Rows A-D) | 20110720 1uM D5 | 2811 | 257.0 |
| STER007-Plate 5 (Rows A-D) | 20110720 0.1uM A7 | 2835 | 252.7 |
| STER007-Plate 5 (Rows A-D) | 20110720 0.1uM A9 | 2722 | 235.2 |
| STER007-Plate 5 (Rows A-D) | 20110720 0.1uM A11 | 2765 | 225.6 |
| STER007-Plate 5 (Rows A-D) | 20110720 0.01uM B7 | 2691 | 246.5 |

| Sample Plate | Sample Name | Testosterone Conc. (pg/mL) | Estradiol Conc. (pg/mL) |
|---|---|---|---|
| STER007-Plate 5 (Rows A-D) | 20110720 0.01uM B9 | 2652 | 235.7 |
| STER007-Plate 5 (Rows A-D) | 20110720 0.01uM B11 | 2688 | 225.4 |
| STER007-Plate 5 (Rows A-D) | 20110720 0.001uM C7 | 2811 | 249.9 |
| STER007-Plate 5 (Rows A-D) | 20110720 0.001uM C9 | 2677 | 228.2 |
| STER007-Plate 5 (Rows A-D) | 20110720 0.001uM C11 | 2730 | 215.9 |
| STER007-Plate 5 (Rows A-D) | 20110720 0.0001uM D7 | 2608 | 228.1 |
| STER007-Plate 5 (Rows A-D) | 20110720 0.0001uM D9 | 2622 | 221.1 |
| STER007-Plate 5 (Rows A-D) | 20110720 0.0001uM D11 | 2814 | 219.5 |
| STER013-Plate 5 (Rows A-D) | 20110726 DMSO A1 | 1926 | 170.7 |
| STER013-Plate 5 (Rows A-D) | 20110726 DMSO A3 | 1835 | 162.3 |
| STER013-Plate 5 (Rows A-D) | 20110726 DMSO A5 | 2043 | 177.9 |
| STER013-Plate 5 (Rows A-D) | 20110726 100uM B1 | 1915 | 210.5 |
| STER013-Plate 5 (Rows A-D) | 20110726 100uM B3 | 1892 | 207.6 |
| STER013-Plate 5 (Rows A-D) | 20110726 100uM B5 | 1965 | 213.9 |
| STER013-Plate 5 (Rows A-D) | 20110726 10uM C1 | 1861 | 174.5 |
| STER013-Plate 5 (Rows A-D) | 20110726 10uM C3 | 1958 | 179.3 |
| STER013-Plate 5 (Rows A-D) | 20110726 10uM C5 | 1872 | 178.7 |
| STER013-Plate 5 (Rows A-D) | 20110726 1uM D1 | 1883 | 154.4 |
| STER013-Plate 5 (Rows A-D) | 20110726 1uM D3 | 1873 | 160.0 |
| STER013-Plate 5 (Rows A-D) | 20110726 1uM D5 | 1909 | 159.0 |
| STER013-Plate 5 (Rows A-D) | 20110726 0.1uM A7 | 1915 | 165.9 |
| STER013-Plate 5 (Rows A-D) | 20110726 0.1uM A9 | 1915 | 164.5 |
| STER013-Plate 5 (Rows A-D) | 20110726 0.1uM A11 | 1970 | 164.4 |
| STER013-Plate 5 (Rows A-D) | 20110726 0.01uM B7 | 2002 | 161.7 |
| STER013-Plate 5 (Rows A-D) | 20110726 0.01uM B9 | 1854 | 160.6 |
| STER013-Plate 5 (Rows A-D) | 20110726 0.01uM B11 | 1900 | 163.3 |
| STER013-Plate 5 (Rows A-D) | 20110726 0.001uM C7 | 1860 | 163.3 |
| STER013-Plate 5 (Rows A-D) | 20110726 0.001uM C9 | 1839 | 158.8 |
| STER013-Plate 5 (Rows A-D) | 20110726 0.001uM C11 | 1827 | 160.1 |
| STER013-Plate 5 (Rows A-D) | 20110726 0.0001uM D7 | 1903 | 155.6 |
| STER013-Plate 5 (Rows A-D) | 20110726 0.0001uM D9 | 1797 | 152.4 |
| STER013-Plate 5 (Rows A-D) | 20110726 0.0001uM D11 | 1849 | 156.7 |
| STER019-Plate 5 (Rows A-D) | 20110727 DMSO A1 | 2388 | 214.1 |
| STER019-Plate 5 (Rows A-D) | 20110727 DMSO A3 | 2426 | 217.1 |
| STER019-Plate 5 (Rows A-D) | 20110727 DMSO A5 | 2396 | 207.7 |
| STER019-Plate 5 (Rows A-D) | 20110727 100uM B1 | 2551 | 279.1 |
| STER019-Plate 5 (Rows A-D) | 20110727 100uM B3 | 2474 | 280.1 |
| STER019-Plate 5 (Rows A-D) | 20110727 100uM B5 | 2523 | 296.9 |

| Sample Plate | Sample Name | Testosterone Conc. (pg/mL) | Estradiol Conc. (pg/mL) |
|---|---|---|---|
| STER019-Plate 5 (Rows A-D) | 20110727 10uM C1 | 2391 | 217.9 |
| STER019-Plate 5 (Rows A-D) | 20110727 10uM C3 | 2355 | 225.8 |
| STER019-Plate 5 (Rows A-D) | 20110727 10uM C5 | 2374 | 224.5 |
| STER019-Plate 5 (Rows A-D) | 20110727 1uM D1 | 2439 | 203.4 |
| STER019-Plate 5 (Rows A-D) | 20110727 1uM D3 | 2478 | 205.9 |
| STER019-Plate 5 (Rows A-D) | 20110727 1uM D5 | 2609 | 215.8 |
| STER019-Plate 5 (Rows A-D) | 20110727 0.1uM A7 | 2425 | 205.9 |
| STER019-Plate 5 (Rows A-D) | 20110727 0.1uM A9 | 2526 | 230.7 |
| STER019-Plate 5 (Rows A-D) | 20110727 0.1uM A11 | 2504 | 221.4 |
| STER019-Plate 5 (Rows A-D) | 20110727 0.01uM B7 | 2352 | 207.6 |
| STER019-Plate 5 (Rows A-D) | 20110727 0.01uM B9 | 2496 | 207.5 |
| STER019-Plate 5 (Rows A-D) | 20110727 0.01uM B11 | 2416 | 204.9 |
| STER019-Plate 5 (Rows A-D) | 20110727 0.001uM C7 | 2397 | 203.3 |
| STER019-Plate 5 (Rows A-D) | 20110727 0.001uM C9 | 2408 | 201.2 |
| STER019-Plate 5 (Rows A-D) | 20110727 0.001uM C11 | 2308 | 202.1 |
| STER019-Plate 5 (Rows A-D) | 20110727 0.0001uM D7 | 2371 | 202.9 |
| STER019-Plate 5 (Rows A-D) | 20110727 0.0001uM D9 | 2316 | 202.1 |
| STER019-Plate 5 (Rows A-D) | 20110727 0.0001uM D11 | 2449 | 214.3 |
| STER007-Plate 5 (Rows E-H) | 20110720 DMSO E1 | 3333 | 294.3 |
| STER007-Plate 5 (Rows E-H) | 20110720 DMSO E3 | 2887 | 262.7 |
| STER007-Plate 5 (Rows E-H) | 20110720 DMSO E5 | 2822 | 263.0 |
| STER007-Plate 5 (Rows E-H) | 20110720 100uM F1 | 4147 | 662.8 |
| STER007-Plate 5 (Rows E-H) | 20110720 100uM F3 | 3662 | 568.7 |
| STER007-Plate 5 (Rows E-H) | 20110720 100uM F5 | 3943 | 629.1 |
| STER007-Plate 5 (Rows E-H) | 20110720 10uM G1 | 3134 | 418.6 |
| STER007-Plate 5 (Rows E-H) | 20110720 10uM G3 | 2984 | 401.6 |
| STER007-Plate 5 (Rows E-H) | 20110720 10uM G5 | 3094 | 428.9 |
| STER007-Plate 5 (Rows E-H) | 20110720 1uM H1 | 2722 | 272.9 |
| STER007-Plate 5 (Rows E-H) | 20110720 1uM H3 | 2844 | 292.4 |
| STER007-Plate 5 (Rows E-H) | 20110720 1uM H5 | 3004 | 311.6 |
| STER007-Plate 5 (Rows E-H) | 20110720 0.1uM E7 | 2831 | 252.0 |
| STER007-Plate 5 (Rows E-H) | 20110720 0.1uM E9 | 2745 | 244.4 |
| STER007-Plate 5 (Rows E-H) | 20110720 0.1uM E11 | 3156 | 254.0 |
| STER007-Plate 5 (Rows E-H) | 20110720 0.01uM F7 | 3076 | 285.0 |
| STER007-Plate 5 (Rows E-H) | 20110720 0.01uM F9 | 2797 | 261.8 |
| STER007-Plate 5 (Rows E-H) | 20110720 0.01uM F11 | 2868 | 252.4 |
| STER007-Plate 5 (Rows E-H) | 20110720 0.001uM G7 | 2719 | 248.7 |
| STER007-Plate 5 (Rows E-H) | 20110720 0.001uM G9 | 2705 | 250.2 |

| Sample Plate | Sample Name | Testosterone Conc. (pg/mL) | Estradiol Conc. (pg/mL) |
|---|---|---|---|
| STER007-Plate 5 (Rows E-H) | 20110720 0.001uM G11 | 2731 | 240.2 |
| STER007-Plate 5 (Rows E-H) | 20110720 0.0001uM H7 | 2790 | 257.5 |
| STER007-Plate 5 (Rows E-H) | 20110720 0.0001uM H9 | 2786 | 250.8 |
| STER007-Plate 5 (Rows E-H) | 20110720 0.0001uM H11 | 3046 | 254.5 |
| STER013-Plate 5 (Rows E-H) | 20110726 DMSO E1 | 1979 | 186.1 |
| STER013-Plate 5 (Rows E-H) | 20110726 DMSO E3 | 1920 | 188.8 |
| STER013-Plate 5 (Rows E-H) | 20110726 DMSO E5 | 1914 | 184.1 |
| STER013-Plate 5 (Rows E-H) | 20110726 100uM F1 | 2364 | 329.9 |
| STER013-Plate 5 (Rows E-H) | 20110726 100uM F3 | 2235 | 327.2 |
| STER013-Plate 5 (Rows E-H) | 20110726 100uM F5 | 2435 | 341.2 |
| STER013-Plate 5 (Rows E-H) | 20110726 10uM G1 | 2104 | 270.2 |
| STER013-Plate 5 (Rows E-H) | 20110726 10uM G3 | 1967 | 265.4 |
| STER013-Plate 5 (Rows E-H) | 20110726 10uM G5 | 1912 | 262.0 |
| STER013-Plate 5 (Rows E-H) | 20110726 1uM H1 | 1890 | 195.8 |
| STER013-Plate 5 (Rows E-H) | 20110726 1uM H3 | 1866 | 190.7 |
| STER013-Plate 5 (Rows E-H) | 20110726 1uM H5 | 1933 | 203.5 |
| STER013-Plate 5 (Rows E-H) | 20110726 0.1uM E7 | 2108 | 190.2 |
| STER013-Plate 5 (Rows E-H) | 20110726 0.1uM E9 | 1876 | 177.6 |
| STER013-Plate 5 (Rows E-H) | 20110726 0.1uM E11 | 1902 | 180.7 |
| STER013-Plate 5 (Rows E-H) | 20110726 0.01uM F7 | 1945 | 182.6 |
| STER013-Plate 5 (Rows E-H) | 20110726 0.01uM F9 | 1874 | 175.8 |
| STER013-Plate 5 (Rows E-H) | 20110726 0.01uM F11 | 1870 | 182.8 |
| STER013-Plate 5 (Rows E-H) | 20110726 0.001uM G7 | 1951 | 183.7 |
| STER013-Plate 5 (Rows E-H) | 20110726 0.001uM G9 | 1940 | 181.9 |
| STER013-Plate 5 (Rows E-H) | 20110726 0.001uM G11 | 1870 | 180.3 |
| STER013-Plate 5 (Rows E-H) | 20110726 0.0001uM H7 | 1963 | 180.1 |
| STER013-Plate 5 (Rows E-H) | 20110726 0.0001uM H9 | 1851 | 176.4 |
| STER013-Plate 5 (Rows E-H) | 20110726 0.0001uM H11 | 2144 | 185.9 |
| STER019-Plate 5 (Rows E-H) | 20110727 DMSO E1 | 2481 | 222.2 |
| STER019-Plate 5 (Rows E-H) | 20110727 DMSO E3 | 2389 | 222.3 |
| STER019-Plate 5 (Rows E-H) | 20110727 DMSO E5 | 2381 | 227.8 |
| STER019-Plate 5 (Rows E-H) | 20110727 100uM F1 | 3183 | 462.1 |
| STER019-Plate 5 (Rows E-H) | 20110727 100uM F3 | 3168 | 451.0 |
| STER019-Plate 5 (Rows E-H) | 20110727 100uM F5 | 3214 | 485.0 |
| STER019-Plate 5 (Rows E-H) | 20110727 10uM G1 | 2543 | 326.5 |
| STER019-Plate 5 (Rows E-H) | 20110727 10uM G3 | 2522 | 342.0 |
| STER019-Plate 5 (Rows E-H) | 20110727 10uM G5 | 2664 | 351.2 |
| STER019-Plate 5 (Rows E-H) | 20110727 1uM H1 | 2528 | 252.5 |

| Sample Plate | Sample Name | Testosterone Conc. (pg/mL) | Estradiol Conc. (pg/mL) |
|---|---|---|---|
| STER019-Plate 5 (Rows E-H) | 20110727 1uM H3 | 2541 | 254.4 |
| STER019-Plate 5 (Rows E-H) | 20110727 1uM H5 | 2460 | 254.2 |
| STER019-Plate 5 (Rows E-H) | 20110727 0.1uM E7 | 2600 | 258.4 |
| STER019-Plate 5 (Rows E-H) | 20110727 0.1uM E9 | 2599 | 240.7 |
| STER019-Plate 5 (Rows E-H) | 20110727 0.1uM E11 | 2643 | 244.0 |
| STER019-Plate 5 (Rows E-H) | 20110727 0.01uM F7 | 2513 | 231.5 |
| STER019-Plate 5 (Rows E-H) | 20110727 0.01uM F9 | 2379 | 234.4 |
| STER019-Plate 5 (Rows E-H) | 20110727 0.01uM F11 | 2302 | 214.2 |
| STER019-Plate 5 (Rows E-H) | 20110727 0.001uM G7 | 2403 | 228.6 |
| STER019-Plate 5 (Rows E-H) | 20110727 0.001uM G9 | 2387 | 220.2 |
| STER019-Plate 5 (Rows E-H) | 20110727 0.001uM G11 | 2410 | 222.9 |
| STER019-Plate 5 (Rows E-H) | 20110727 0.0001uM H7 | 2596 | 222.7 |
| STER019-Plate 5 (Rows E-H) | 20110727 0.0001uM H9 | 2354 | 217.8 |
| STER019-Plate 5 (Rows E-H) | 20110727 0.0001uM H11 | 2442 | 226.9 |
| STER007-Plate 6 (Rows A-D) | 20110720 DMSO A1 | 2634 | 233.6 |
| STER007-Plate 6 (Rows A-D) | 20110720 DMSO A3 | 2958 | 264.9 |
| STER007-Plate 6 (Rows A-D) | 20110720 DMSO A5 | 2733 | 242.5 |
| STER007-Plate 6 (Rows A-D) | 20110720 100uM B1 | 1528 | 150.9 |
| STER007-Plate 6 (Rows A-D) | 20110720 100uM B3 | 1618 | 165.5 |
| STER007-Plate 6 (Rows A-D) | 20110720 100uM B5 | 1667 | 174.8 |
| STER007-Plate 6 (Rows A-D) | 20110720 10uM C1 | 1963 | 240.0 |
| STER007-Plate 6 (Rows A-D) | 20110720 10uM C3 | 2001 | 237.8 |
| STER007-Plate 6 (Rows A-D) | 20110720 10uM C5 | 1997 | 249.1 |
| STER007-Plate 6 (Rows A-D) | 20110720 1uM D1 | 2771 | 252.0 |
| STER007-Plate 6 (Rows A-D) | 20110720 1uM D3 | 2847 | 258.7 |
| STER007-Plate 6 (Rows A-D) | 20110720 1uM D5 | 2950 | 278.4 |
| STER007-Plate 6 (Rows A-D) | 20110720 0.1uM A7 | 2806 | 262.1 |
| STER007-Plate 6 (Rows A-D) | 20110720 0.1uM A9 | 2607 | 240.5 |
| STER007-Plate 6 (Rows A-D) | 20110720 0.1uM A11 | 2912 | 265.1 |
| STER007-Plate 6 (Rows A-D) | 20110720 0.01uM B7 | 2609 | 244.4 |
| STER007-Plate 6 (Rows A-D) | 20110720 0.01uM B9 | 2576 | 239.4 |
| STER007-Plate 6 (Rows A-D) | 20110720 0.01uM B11 | 2616 | 234.9 |
| STER007-Plate 6 (Rows A-D) | 20110720 0.001uM C7 | 2666 | 235.2 |
| STER007-Plate 6 (Rows A-D) | 20110720 0.001uM C9 | 2733 | 244.6 |
| STER007-Plate 6 (Rows A-D) | 20110720 0.001uM C11 | 2965 | 261.3 |
| STER007-Plate 6 (Rows A-D) | 20110720 0.0001uM D7 | 3215 | 266.6 |
| STER007-Plate 6 (Rows A-D) | 20110720 0.0001uM D9 | 2717 | 233.5 |
| STER007-Plate 6 (Rows A-D) | 20110720 0.0001uM D11 | 2788 | 248.2 |

| Sample Plate | Sample Name | Testosterone Conc. (pg/mL) | Estradiol Conc. (pg/mL) |
|---|---|---|---|
| STER013-Plate 6 (Rows A-D) | 20110726 DMSO A1 | 1948 | 169.1 |
| STER013-Plate 6 (Rows A-D) | 20110726 DMSO A3 | 1863 | 164.2 |
| STER013-Plate 6 (Rows A-D) | 20110726 DMSO A5 | 2115 | 180.1 |
| STER013-Plate 6 (Rows A-D) | 20110726 100uM B1 | 1397 | 129.5 |
| STER013-Plate 6 (Rows A-D) | 20110726 100uM B3 | 1328 | 130.1 |
| STER013-Plate 6 (Rows A-D) | 20110726 100uM B5 | 1268 | 123.9 |
| STER013-Plate 6 (Rows A-D) | 20110726 10uM C1 | 1534 | 153.6 |
| STER013-Plate 6 (Rows A-D) | 20110726 10uM C3 | 1411 | 144.8 |
| STER013-Plate 6 (Rows A-D) | 20110726 10uM C5 | 1448 | 148.7 |
| STER013-Plate 6 (Rows A-D) | 20110726 1uM D1 | 1959 | 180.5 |
| STER013-Plate 6 (Rows A-D) | 20110726 1uM D3 | 1830 | 172.7 |
| STER013-Plate 6 (Rows A-D) | 20110726 1uM D5 | 1961 | 192.0 |
| STER013-Plate 6 (Rows A-D) | 20110726 0.1uM A7 | 2054 | 178.4 |
| STER013-Plate 6 (Rows A-D) | 20110726 0.1uM A9 | 2097 | 182.6 |
| STER013-Plate 6 (Rows A-D) | 20110726 0.1uM A11 | 2022 | 175.4 |
| STER013-Plate 6 (Rows A-D) | 20110726 0.01uM B7 | 1979 | 167.7 |
| STER013-Plate 6 (Rows A-D) | 20110726 0.01uM B9 | 1938 | 171.8 |
| STER013-Plate 6 (Rows A-D) | 20110726 0.01uM B11 | 2132 | 183.8 |
| STER013-Plate 6 (Rows A-D) | 20110726 0.001uM C7 | 1945 | 175.1 |
| STER013-Plate 6 (Rows A-D) | 20110726 0.001uM C9 | 1813 | 168.4 |
| STER013-Plate 6 (Rows A-D) | 20110726 0.001uM C11 | 1761 | 157.4 |
| STER013-Plate 6 (Rows A-D) | 20110726 0.0001uM D7 | 1923 | 172.5 |
| STER013-Plate 6 (Rows A-D) | 20110726 0.0001uM D9 | 1878 | 174.9 |
| STER013-Plate 6 (Rows A-D) | 20110726 0.0001uM D11 | 1951 | 176.3 |
| STER019-Plate 6 (Rows A-D) | 20110727 DMSO A1 | 2566 | 206.1 |
| STER019-Plate 6 (Rows A-D) | 20110727 DMSO A3 | 2394 | 204.1 |
| STER019-Plate 6 (Rows A-D) | 20110727 DMSO A5 | 2353 | 211.6 |
| STER019-Plate 6 (Rows A-D) | 20110727 100uM B1 | 1794 | 161.3 |
| STER019-Plate 6 (Rows A-D) | 20110727 100uM B3 | 1727 | 162.6 |
| STER019-Plate 6 (Rows A-D) | 20110727 100uM B5 | 1640 | 152.3 |
| STER019-Plate 6 (Rows A-D) | 20110727 10uM C1 | 1773 | 178.1 |
| STER019-Plate 6 (Rows A-D) | 20110727 10uM C3 | 1824 | 194.3 |
| STER019-Plate 6 (Rows A-D) | 20110727 10uM C5 | 1898 | 197.1 |
| STER019-Plate 6 (Rows A-D) | 20110727 1uM D1 | 2387 | 231.4 |
| STER019-Plate 6 (Rows A-D) | 20110727 1uM D3 | 2317 | 219.2 |
| STER019-Plate 6 (Rows A-D) | 20110727 1uM D5 | 2483 | 249.9 |
| STER019-Plate 6 (Rows A-D) | 20110727 0.1uM A7 | 2403 | 212.8 |
| STER019-Plate 6 (Rows A-D) | 20110727 0.1uM A9 | 2310 | 205.8 |

| Sample Plate | Sample Name | Testosterone Conc. (pg/mL) | Estradiol Conc. (pg/mL) |
|---|---|---|---|
| STER019-Plate 6 (Rows A-D) | 20110727 0.1uM A11 | 2293 | 198.4 |
| STER019-Plate 6 (Rows A-D) | 20110727 0.01uM B7 | 2326 | 208.6 |
| STER019-Plate 6 (Rows A-D) | 20110727 0.01uM B9 | 2250 | 206.2 |
| STER019-Plate 6 (Rows A-D) | 20110727 0.01uM B11 | 2347 | 209.0 |
| STER019-Plate 6 (Rows A-D) | 20110727 0.001uM C7 | 2269 | 203.1 |
| STER019-Plate 6 (Rows A-D) | 20110727 0.001uM C9 | 2229 | 195.3 |
| STER019-Plate 6 (Rows A-D) | 20110727 0.001uM C11 | 2355 | 211.5 |
| STER019-Plate 6 (Rows A-D) | 20110727 0.0001uM D7 | 2372 | 215.7 |
| STER019-Plate 6 (Rows A-D) | 20110727 0.0001uM D9 | 2224 | 200.0 |
| STER019-Plate 6 (Rows A-D) | 20110727 0.0001uM D11 | 2361 | 204.7 |
| STER001-Plate 6 (Rows A-D) | 9070-100107-3 DMSO A1 | 2433 | 183.8 |
| STER001-Plate 6 (Rows A-D) | 9070-100107-3 DMSO A3 | 2577 | 193.5 |
| STER001-Plate 6 (Rows A-D) | 9070-100107-3 DMSO A5 | 2580 | 202.3 |
| STER001-Plate 6 (Rows A-D) | 9070-100107-3 100uM E1 | 1860 | 170.6 |
| STER001-Plate 6 (Rows A-D) | 9070-100107-3 100uM B3 | 2034 | 196.2 |
| STER001-Plate 6 (Rows A-D) | 9070-100107-3 100uM B5 | 1889 | 182.7 |
| STER001-Plate 6 (Rows A-D) | 9070-100107-3 10uM C1 | 1722 | 145.2 |
| STER001-Plate 6 (Rows A-D) | 9070-100107-3 10uM C3 | 1689 | 141.6 |
| STER001-Plate 6 (Rows A-D) | 9070-100107-3 10uM C5 | 1739 | 147.5 |
| STER001-Plate 6 (Rows A-D) | 9070-100107-3 1uM D1 | 2684 | 206.9 |
| STER001-Plate 6 (Rows A-D) | 9070-100107-3 1uM D3 | 2406 | 185.3 |
| STER001-Plate 6 (Rows A-D) | 9070-100107-3 1uM D5 | 2569 | 196.3 |
| STER001-Plate 6 (Rows A-D) | 9070-100107-3 0.1uM A7 | 2494 | 195.2 |
| STER001-Plate 6 (Rows A-D) | 9070-100107-3 0.1uM A9 | 2417 | 185.1 |
| STER001-Plate 6 (Rows A-D) | 9070-100107-3 0.1uM A11 | 2506 | 188.6 |
| STER001-Plate 6 (Rows A-D) | 9070-100107-3 0.01uM B7 | 2409 | 175.0 |
| STER001-Plate 6 (Rows A-D) | 9070-100107-3 0.01uM B9 | 2402 | 183.7 |
| STER001-Plate 6 (Rows A-D) | 9070-100107-3 0.01uM B11 | 2398 | 174.3 |
| STER001-Plate 6 (Rows A-D) | 9070-100107-3 0.001uM C7 | 2581 | 186.9 |
| STER001-Plate 6 (Rows A-D) | 9070-100107-3 0.001uM C9 | 2357 | 174.1 |
| STER001-Plate 6 (Rows A-D) | 9070-100107-3 0.001uM C11 | 2448 | 175.2 |
| STER001-Plate 6 (Rows A-D) | 9070-100107-3 0.0001uM D7 | 2472 | 189.7 |
| STER001-Plate 6 (Rows A-D) | 9070-100107-3 0.0001uM D9 | 2402 | 171.7 |
| STER001-Plate 6 (Rows A-D) | 9070-100107-3 0.0001uM D11 | 2587 | 182.1 |
| STER007-Plate 6 (Rows E-H) | 20110720 DMSO E1 | 2283 | 194.2 |
| STER007-Plate 6 (Rows E-H) | 20110720 DMSO E3 | 2564 | 221.3 |
| STER007-Plate 6 (Rows E-H) | 20110720 DMSO E5 | 2255 | 189.7 |
| STER007-Plate 6 (Rows E-H) | 20110720 100uM F1 | 2651 | 237.1 |

| Sample Plate | Sample Name | Testosterone Conc. (pg/mL) | Estradiol Conc. (pg/mL) |
|---|---|---|---|
| STER007-Plate 6 (Rows E-H) | 20110720 100uM F3 | 2579 | 259.1 |
| STER007-Plate 6 (Rows E-H) | 20110720 100uM F5 | 2042 | 196.2 |
| STER007-Plate 6 (Rows E-H) | 20110720 10uM G1 | 2125 | 188.4 |
| STER007-Plate 6 (Rows E-H) | 20110720 10uM G3 | 2274 | 206.7 |
| STER007-Plate 6 (Rows E-H) | 20110720 10uM G5 | 2471 | 236.2 |
| STER007-Plate 6 (Rows E-H) | 20110720 1uM H1 | 2924 | 234.8 |
| STER007-Plate 6 (Rows E-H) | 20110720 1uM H3 | 2618 | 229.6 |
| STER007-Plate 6 (Rows E-H) | 20110720 1uM H5 | 2510 | 221.7 |
| STER007-Plate 6 (Rows E-H) | 20110720 0.1uM E7 | 2217 | 187.0 |
| STER007-Plate 6 (Rows E-H) | 20110720 0.1uM E9 | 2468 | 211.7 |
| STER007-Plate 6 (Rows E-H) | 20110720 0.1uM E11 | 2798 | 250.1 |
| STER007-Plate 6 (Rows E-H) | 20110720 0.01uM F7 | 2398 | 215.6 |
| STER007-Plate 6 (Rows E-H) | 20110720 0.01uM F9 | 2207 | 204.2 |
| STER007-Plate 6 (Rows E-H) | 20110720 0.01uM F11 | 2452 | 222.4 |
| STER007-Plate 6 (Rows E-H) | 20110720 0.001uM G7 | 2250 | 191.5 |
| STER007-Plate 6 (Rows E-H) | 20110720 0.001uM G9 | 2374 | 204.5 |
| STER007-Plate 6 (Rows E-H) | 20110720 0.001uM G11 | 2732 | 228.2 |
| STER007-Plate 6 (Rows E-H) | 20110720 0.0001uM H7 | 2598 | 221.7 |
| STER007-Plate 6 (Rows E-H) | 20110720 0.0001uM H9 | 2573 | 218.5 |
| STER007-Plate 6 (Rows E-H) | 20110720 0.0001uM H11 | 2836 | 234.2 |
| STER013-Plate 6 (Rows E-H) | 20110726 DMSO E1 | 1921 | 173.2 |
| STER013-Plate 6 (Rows E-H) | 20110726 DMSO E3 | 1772 | 154.7 |
| STER013-Plate 6 (Rows E-H) | 20110726 DMSO E5 | 2325 | 186.1 |
| STER013-Plate 6 (Rows E-H) | 20110726 100uM F1 | 2017 | 215.8 |
| STER013-Plate 6 (Rows E-H) | 20110726 100uM F3 | 1862 | 197.0 |
| STER013-Plate 6 (Rows E-H) | 20110726 100uM F5 | 1645 | 178.2 |
| STER013-Plate 6 (Rows E-H) | 20110726 10uM G1 | 1930 | 180.4 |
| STER013-Plate 6 (Rows E-H) | 20110726 10uM G3 | 1528 | 160.1 |
| STER013-Plate 6 (Rows E-H) | 20110726 10uM G5 | 1969 | 189.5 |
| STER013-Plate 6 (Rows E-H) | 20110726 1uM H1 | 1945 | 175.7 |
| STER013-Plate 6 (Rows E-H) | 20110726 1uM H3 | 1898 | 176.2 |
| STER013-Plate 6 (Rows E-H) | 20110726 1uM H5 | 1909 | 182.3 |
| STER013-Plate 6 (Rows E-H) | 20110726 0.1uM E7 | 1798 | 161.4 |
| STER013-Plate 6 (Rows E-H) | 20110726 0.1uM E9 | 1755 | 157.7 |
| STER013-Plate 6 (Rows E-H) | 20110726 0.1uM E11 | 1897 | 173.4 |
| STER013-Plate 6 (Rows E-H) | 20110726 0.01uM F7 | 1844 | 158.4 |
| STER013-Plate 6 (Rows E-H) | 20110726 0.01uM F9 | 1701 | 155.8 |
| STER013-Plate 6 (Rows E-H) | 20110726 0.01uM F11 | 1937 | 176.2 |

| Sample Plate | Sample Name | Testosterone Conc. (pg/mL) | Estradiol Conc. (pg/mL) |
|---|---|---|---|
| STER013-Plate 6 (Rows E-H) | 20110726 0.001uM G7 | 1850 | 159.9 |
| STER013-Plate 6 (Rows E-H) | 20110726 0.001uM G9 | 1925 | 168.2 |
| STER013-Plate 6 (Rows E-H) | 20110726 0.001uM G11 | 2278 | 192.6 |
| STER013-Plate 6 (Rows E-H) | 20110726 0.0001uM H7 | 2192 | 189.8 |
| STER013-Plate 6 (Rows E-H) | 20110726 0.0001uM H9 | 2082 | 178.3 |
| STER013-Plate 6 (Rows E-H) | 20110726 0.0001uM H11 | 1732 | 154.8 |
| STER019-Plate 6 (Rows E-H) | 20110727 DMSO E1 | NS | NS |
| STER019-Plate 6 (Rows E-H) | 20110727 DMSO E3 | NS | NS |
| STER019-Plate 6 (Rows E-H) | 20110727 DMSO E5 | NS | NS |
| STER019-Plate 6 (Rows E-H) | 20110727 100uM F1 | NS | NS |
| STER019-Plate 6 (Rows E-H) | 20110727 100uM F3 | NS | NS |
| STER019-Plate 6 (Rows E-H) | 20110727 100uM F5 | NS | NS |
| STER019-Plate 6 (Rows E-H) | 20110727 10uM G1 | 1648 | 153.0 |
| STER019-Plate 6 (Rows E-H) | 20110727 10uM G3 | 2413 | 218.9 |
| STER019-Plate 6 (Rows E-H) | 20110727 10uM G5 | 2090 | 192.1 |
| STER019-Plate 6 (Rows E-H) | 20110727 1uM H1 | 2404 | 219.1 |
| STER019-Plate 6 (Rows E-H) | 20110727 1uM H3 | 2326 | 196.9 |
| STER019-Plate 6 (Rows E-H) | 20110727 1uM H5 | 2390 | 219.9 |
| STER019-Plate 6 (Rows E-H) | 20110727 0.1uM E7 | NS | NS |
| STER019-Plate 6 (Rows E-H) | 20110727 0.1uM E9 | NS | NS |
| STER019-Plate 6 (Rows E-H) | 20110727 0.1uM E11 | NS | NS |
| STER019-Plate 6 (Rows E-H) | 20110727 0.01uM F7 | NS | NS |
| STER019-Plate 6 (Rows E-H) | 20110727 0.01uM F9 | NS | NS |
| STER019-Plate 6 (Rows E-H) | 20110727 0.01uM F11 | NS | NS |
| STER019-Plate 6 (Rows E-H) | 20110727 0.001uM G7 | 2049 | 193.2 |
| STER019-Plate 6 (Rows E-H) | 20110727 0.001uM G9 | 2182 | 204.0 |
| STER019-Plate 6 (Rows E-H) | 20110727 0.001uM G11 | 2384 | 204.7 |
| STER019-Plate 6 (Rows E-H) | 20110727 0.0001uM H7 | 2307 | 205.8 |
| STER019-Plate 6 (Rows E-H) | 20110727 0.0001uM H9 | 2295 | 211.5 |
| STER019-Plate 6 (Rows E-H) | 20110727 0.0001uM H11 | 2343 | 211.1 |
| STER001-Plate 6 (Rows E-H) | 9070-100107-4 DMSO E1 | 2603 | 186.9 |
| STER001-Plate 6 (Rows E-H) | 9070-100107-4 DMSO E3 | 2555 | 189.5 |
| STER001-Plate 6 (Rows E-H) | 9070-100107-4 DMSO E5 | 2372 | 171.2 |
| STER001-Plate 6 (Rows E-H) | 9070-100107-4 100uM F1 | 2456 | 221.7 |
| STER001-Plate 6 (Rows E-H) | 9070-100107-4 100uM F3 | 2635 | 241.2 |
| STER001-Plate 6 (Rows E-H) | 9070-100107-4 100uM F5 | 2971 | 249.5 |
| STER001-Plate 6 (Rows E-H) | 9070-100107-4 10uM G1 | 2417 | 187.4 |
| STER001-Plate 6 (Rows E-H) | 9070-100107-4 10uM G3 | 2493 | 187.7 |

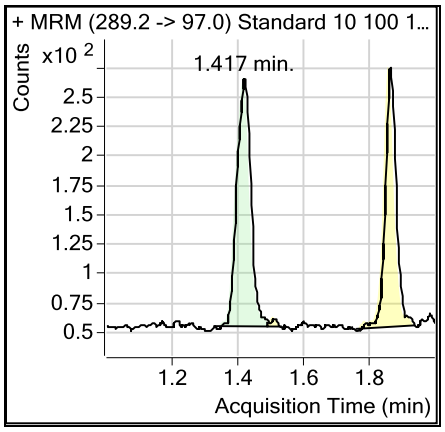
| Sample Plate | Sample Name | Testosterone Conc. (pg/mL) | Estradiol Conc. (pg/mL) |
|---|---|---|---|
| STER001-Plate 6 (Rows E-H) | 9070-100107-4 10uM G5 | 2481 | 186.9 |
| STER001-Plate 6 (Rows E-H) | 9070-100107-4 1uM H1 | 2648 | 187.5 |
| STER001-Plate 6 (Rows E-H) | 9070-100107-4 1uM H3 | 2642 | 181.1 |
| STER001-Plate 6 (Rows E-H) | 9070-100107-4 1uM H5 | 2753 | 187.2 |
| STER001-Plate 6 (Rows E-H) | 9070-100107-4 0.1uM E7 | 2335 | 168.9 |
| STER001-Plate 6 (Rows E-H) | 9070-100107-4 0.1uM E9 | 2355 | 190.3 |
| STER001-Plate 6 (Rows E-H) | 9070-100107-4 0.1uM E11 | 2393 | 170.7 |
| STER001-Plate 6 (Rows E-H) | 9070-100107-4 0.01uM F7 | 2532 | 182.4 |
| STER001-Plate 6 (Rows E-H) | 9070-100107-4 0.01uM F9 | NR | NR |
| STER001-Plate 6 (Rows E-H) | 9070-100107-4 0.01uM F11 | 2403 | 172.5 |
| STER001-Plate 6 (Rows E-H) | 9070-100107-4 0.001uM G7 | 2494 | 176.8 |
| STER001-Plate 6 (Rows E-H) | 9070-100107-4 0.001uM G9 | NR | NR |
| STER001-Plate 6 (Rows E-H) | 9070-100107-4 0.001uM G11 | 2442 | 169.8 |
| STER001-Plate 6 (Rows E-H) | 9070-100107-4 0.0001uM H7 | 2531 | 183.0 |
| STER001-Plate 6 (Rows E-H) | 9070-100107-4 0.0001uM H9 | 1990 | 137.1 |
| STER001-Plate 6 (Rows E-H) | 9070-100107-4 0.0001uM H11 | 2690 | 175.1 |

NR: Not Reportable due to a pipetting error
NS: No Sample

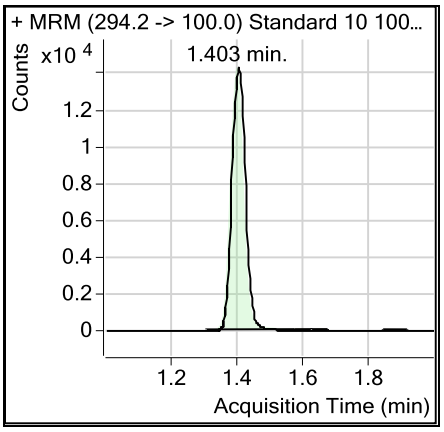
# FIGURES

**Figure 1 Representative Chromatograms: Standards at LLOQ - 10 pg/mL (Estradiol) 100 pg/mL (Testosterone)**

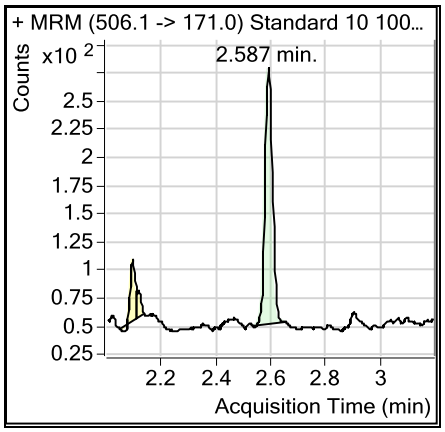
Testosterone



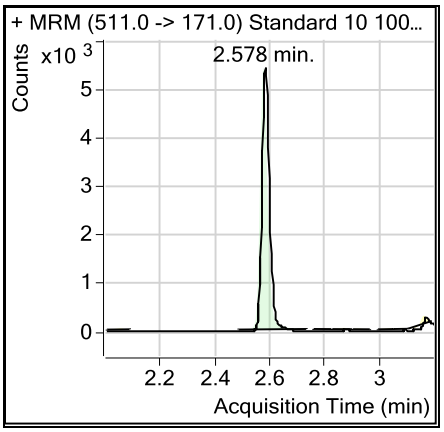

[$^2H_5$]-Testosterone




Estradiol




[$^2H_5$]-Estradiol



# APPENDICES

### Appendix 1 Summary of Analytical Method OPM-OPP-0008

## Analytes:

| | |
|---|---|
| Analytes | Testosterone (free alcohol) |
| | Estradiol (free alcohol) |
| Internal Standards (I.S.) | $d^5$-Testosterone<br>$d^5$-Estradiol |

Compounds are to be accurately weighed and corrected for purity and salt as necessary.

## Matrices:

H295R supplemented medium

Control H295R supplemented medium is to be centrifuged for approximately 5 minutes at 4000 g prior to use if necessary.

## Instrumentation Requirements:

### HPLC-MS/MS

Reverse phase C18 gradient elution with electrospray positive ionization and MS/MS detection

### Preparation of Calibration Standards

Calibration standards are to be prepared as follows and thoroughly mixed.

| Standard Concentration (Estradiol/Testosterone pg/mL) | Volume of Working Solution (µL) | | | | | | | Total Volume in Control Matrix (mL) |
|---|---|---|---|---|---|---|---|---|
| | STDWS-200 | STDWS-100 | STDWS-20 | STDWS-10 | STDWS-2 | STDWS-0.4 | STDWS-0.2 | |
| 10/100 | - | - | - | - | - | - | 50 | 1 |
| 20/200 | - | - | - | - | - | 50 | - | 1 |
| 100/1000 | - | - | - | - | 50 | - | - | 1 |
| 500/5000 | - | - | - | 50 | - | - | - | 1 |

| Standard Concentration (Estradiol/Testosterone pg/mL) | Volume of Working Solution (µL) | | | | | | | Total Volume in Control Matrix (mL) |
|---|---|---|---|---|---|---|---|---|
| | STDWS-200 | STDWS-100 | STDWS-20 | STDWS-10 | STDWS-2 | STDWS-0.4 | STDWS-0.2 | |
| 1000/10000 | - | - | 50 | - | - | - | - | 1 |
| 5000/50000 | - | 50 | - | - | - | - | - | 1 |
| 10000/100000 | 50 | - | - | - | - | - | - | 1 |

### Preparation of Quality Control Samples

Quality controls (QC) are to be prepared as follows and thoroughly mixed. .

| QC Concentration (Estradiol/Testosterone pg/mL) | Volume of Spiking Solution (µL) | | | | | Total Volume in Control Matrix (mL) |
|---|---|---|---|---|---|---|
| | QCWS-400 | QCWS -200 | QCWS -20 | QCWS -2 | QCWS -0.2 | |
| 10/100 | - | - | - | - | 250 | 5 |
| 30/300 | - | - | - | 375 | - | 25 |
| 800/8000 | - | - | 1000 | - | - | 25 |
| 8000/80000 | - | 1000 | - | - | - | 25 |
| 20000/200000 | 250 | - | - | - | - | 5 |

The total volumes prepared may be scaled up or down as required.

## Sample Preparation:

### Extraction Procedure

Aliquot sample into tube or well, and add internal standard working solution. Seal and mix for approximately 1 minute. Perform liquid/liquid extraction on samples and centrifuge for approximately 5 minutes at 4000 rpm. Transfer aliquot for analysis and evaporate to dryness. Add derivatization solution and mix. Centrifuge for approximately 1 minute at approximately 4000 rpm and inject for HPLC-MS/MS analysis.

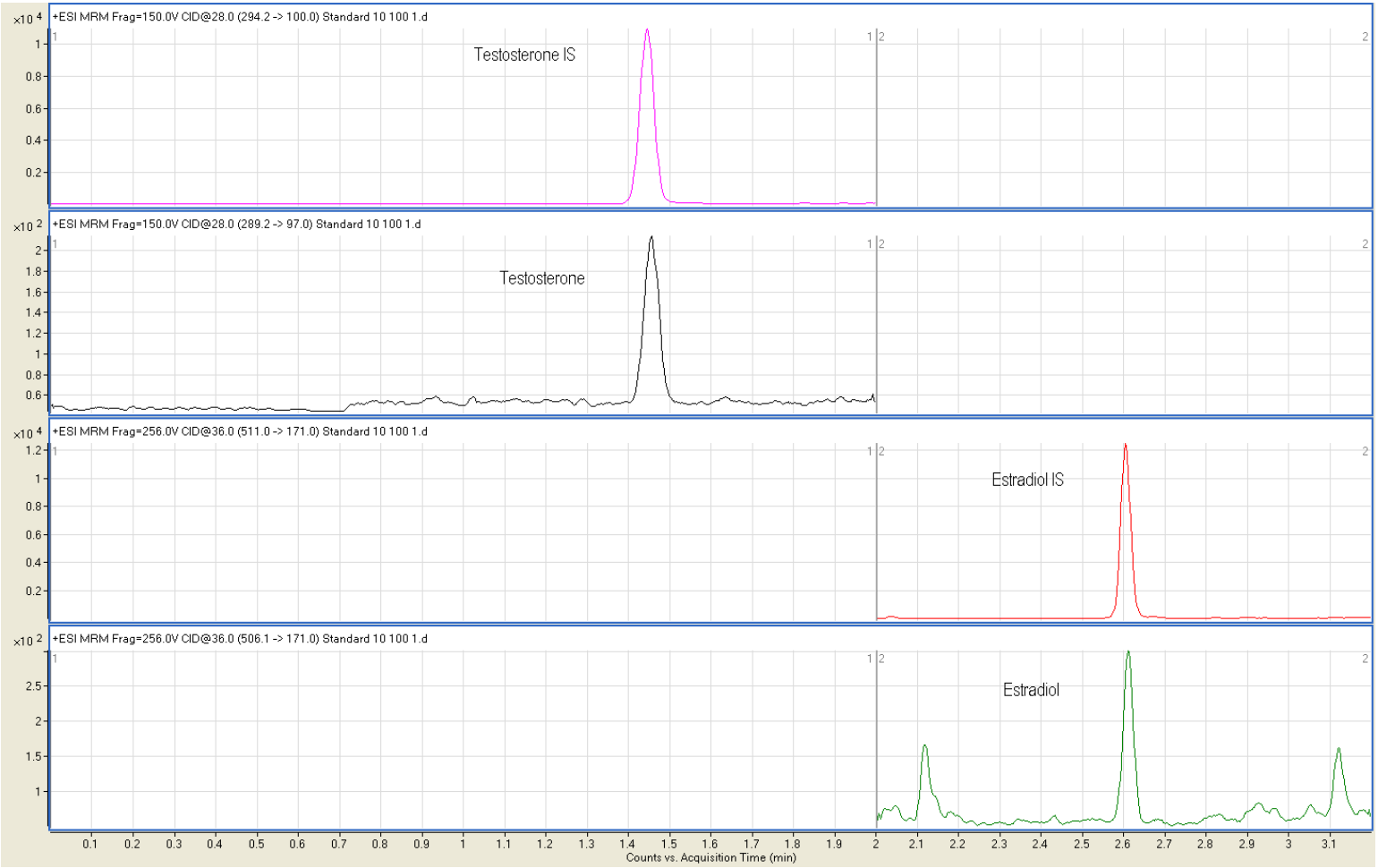
## Data Analysis:

### Regression Model

Use peak area ratios with $1/x^2$ weighted linear regression for all components.

## Representative Chromatograms:

Example chromatogram of testosterone, estradiol, and their internal standards in H295R Supplemented Medium at an approximate concentration of 100 and 10 pg/mL, respectively:



## APPENDIX 15 Study Protocol and Protocol Amendments

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

CeeTox®
*In vitro* models to predict toxicity

**FINAL PROTOCOL**

# H295R Steroidogenesis Assay

Data Requirements: *OPPTS 890.1550*

Author
[redacted]

*Study Number:*
*9070-100107STER*

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

Test Facility:
CeeTox
4717 Campus Drive
Kalamazoo, MI 49008

# TEST PROTOCOL

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

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

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

## Table of Contents

## Signatures

[redacted]
Study Sponsor

6/29/11
Date

[redacted]
Study Monitor

6/29/11
Date

[redacted]
Study Director

7/12/2011
Date

## 1. Title of Study

H295R Steroidogenesis Assay

## 2. Purpose of Study

The purpose of the H295R Steroidogenesis Assay is intended to identify test substances that affect the steroidogenic pathway occurring after the gonadotropin hormone receptors (FSHR and LHR) through the production of testosterone and estradiol/estrone. The steroidogenic assay is not intended to identify substances that affect steroidogenesis due to effects on the hypothalamus or pituitary gland.

## 3. Compliance Statement

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

## 4. Quality Assurance

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

## 5. Regulatory Citations and Guidelines

OPPTS 890.1550: Steroidogenesis (Human Cell Line-H295R). 2009.

## 6. Test Facility and Test Site

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

Test Site
OpAns LLC
4134 South Alston, Suite 101
Durham, NC 27713
Phone 919-323-4299

Principal Investigator
Kenneth Lewis

## 7. Test & Positive Substance(s)

**Note:** A certificate of analysis will be provided by the sponsor and will be stored in the study data and appended to the study report. Confirmation of the identity of the test substance, characterization and stability will be verified by the sponsor. Test substance will be either returned to the Sponsor or destroyed following finalization of the study report.

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

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

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

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

*7.3 Test Substance: Octyl Salicylate (Octylsalate)*

| | |
|---|---|
| CAS No. | 118-60-5 |
| Source: | Sigma-Aldrich |

| | |
|---|---|
| Lot/Batch No.: | 44698PJ |
| ILS Repository No.: | 11-30 |
| Formula: | $C_{15}H_{22}O_3$ |
| Description: | Colorless liquid |
| Storage | Room Temperature |

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

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

*7.5 Preparation of Test Substance*

Test substance(s) will be formulated in appropriate buffer or dimethylsulfoxide (DMSO). The total volume of test substance formulation used in each assay will result in no more than 0.1% DMSO in order to minimize the potential of the solvent to inhibit the cell based assay. Fresh dilutions of the stock solution will be prepared in the same solvent as the stock solution on the day of use. Dose concentrations of test and control substances will not be verified using analytical methods.

7.6 *Positive Substance*

All information regarding supplier, lot numbers and purity will be included in the study reports

**Forskolin**

CAS No: 66575-29-9

Molecular Formula/Weight: MW=410.50

Supplier/source: Sigma Chemical

**Prochloraz**

CAS No: 67747-09-5

Molecular Formula/Weight: MW=376.67

Supplier/source: Sigma Chemical

Note: Copies of the Certificates of analysis for Forskolin and Prochloraz will be provided in the study notebook and also as appendix in the report

**Vehicle Control: 0.1% DMSO in cell medium.**

Test and Control Substance Preparation:

- Prepare a 100 mM stock solution of forskolin, prochloraz, and all test substances in DMSO or appropriate vehicle. Weigh out an appropriate amount of forskolin, prochloraz, and test substances in tared vials. Add of the appropriate amount of DMSO to the vials to prepare a 100 mM stock solution. Cap and vortex the vials. For all test substances, this results in the Stock 1 Test Solution.
- Dilute the control and test substance stock solutions as follows:
- Forskolin: Dilute 100 mM stock solution 1:10 (10 µl of 100 mM Stock 1 + 90 µl DMSO) to make 100 µl of a 10 mM solution. Dilute 10 µl of this 10 mM solution 1:10 to make 100 µl of a 1 mM solution.
- Prochloraz: Dilute 100 mM stock solution 1:10 (10 µl of 100 mM Stock 1 + 90 µl DMSO) to make 100 µl of a 10 mM solution. Dilute 10 µl of this 10 mM solution 1:10 to make a 1 mM solution. Dilute 10 µl of this 1 mM solution 1:10 to make a 0.1 mM solution.
- Test Substances: Dilute Stock 1 1:10 (10 µl of 100 mM Stock 1 + 90 µl DMSO) to make 100 µl of Stock 2 solution. Continue the 1:10 serial dilutions until a total of seven dilutions have been made (Stock 1 – Stock 7).

## 8. Test System

As per the guideline (OPPTS 890.1550) the NCI-H295R, a human adreno-carcinoma cell line will be used in this study.

## 9. Cells

The cells used for the steroidogenesis assay are the H295R human adrenocortical carcinoma cells (ATCC CRL-2128).

After initiation from an ATCC batch, cells are grown for five passages. Passage five cells are then frozen in liquid nitrogen. Cells started from frozen batches are cultured for at least four additional passages before they are used to conduct the assay. The maximum passage number used in the assay is passage 10.

The H295R cells are maintained according to the Endocrine Disruptor Screening Program Test Guidelines OPPTS 890.1550: Steroidogenesis and CeeTox SOP 3039. The cells are grown in supplemented media containing a DMEM:F12 media base with ITS + Premix (insulin, transferrin, selenium, BSA, and linoleic acid) and Nu-Serum.

Note: The passage number used in the assay will be provided in the report.

## 10. Pre-test Requirements

Quality Control Plate

A quality control (QC) plate will be assayed in order to assess the performance of the H295R cell line for potential changes in hormone production as a function of cell age prior to using a new ATCC batch or after using a previously frozen stock of cells for the first time (unless previous proficiency data with that batch is available). To verify that the performance of the H295R cells under Standard Culture Conditions is meeting the QC requirements, a subset of passage five cells is run in a QC plate. If passage 5 cryopreserved cells are used for the study, the cells will be thawed and analyzed at passage 3 and used in the QC run.

The quality control (QC) plate will be incubated, exposed to control substances and assessed in the same manner as test plates. The cells will be exposed with a known inducer (forskolin) and inhibitor (prochloraz) of E2 (estradiol) and T (testosterone) synthesis. Exposure concentrations for forskolin will be 1 and 10 µM and 1 and 0.1 µM for prochloraz (see Table 1):

**Table 1: Quality Control Plate Layout**

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| A | Blank | Blank | Blank | Blank + MeOH | Blank + MeOH | Blank + MeOH |
| B | *DMSO (0.1%) | *DMSO (0.1%) | *DMSO (0.1%) | *DMSO (0.1%) + MeOH | *DMSO (0.1%) + MeOH | *DMSO (0.1%) + MeOH |
| C | Forskolin 1 µM | Forskolin 1 µM | Forskolin 1 µM | Prochloraz 0.1 µM | Prochloraz 0.1 µM | Prochloraz 0.1 µM |
| D | Forskolin 10 µM | Forskolin 10 µM | Forskolin 10 µM | Prochloraz 1 µM | Prochloraz 1 µM | Prochloraz 1 µM |

Blank wells receive medium only
Methanol (MeOH) is used as a positive control for toxicity
*Or alternative vehicle control

The QC plate criteria are presented in Table 2.

**Table 2: Quality Control Plate Criteria**

| | **Testosterone** | **Estradiol** |
|---|---|---|
| Minimum Basal Production | 500 pg/ml | 40 pg/ml |
| Basal Production | ≥ 5 times method detection limit | ≥ 2.5 times method detection limit |
| Induction (10 µM forskolin) | ≥ 2 times solvent control | ≥ 7.5 times solvent control |
| Inhibition (1 µM prochloraz) | ≤ 0.5 times solvent control | ≤ 0.5 times solvent control |

If basal E2 production does not meet the minimum basal production level specified in Table 2, 22-R hydroxycholesterol may be added to the supplemented medium to increase basal production.

Hormone Measurement System Evaluation

Analysis of the production of testosterone and estradiol by H295R cells will be conducted by HPLC/MS/MS.

## 11. Test Conditions and Methods

*Plating and Pre-Incubation of Cells*

The H295R cells are plated in supplemented media at a density of ~250,000 cells/ml in a 24 well plate (1 ml of cell suspended in supplemented media per well is added to the plate) to achieve 50-60% confluency in the wells at ~24 hours.

*Exposure of Cells*

Cells are cultured and plated in 24 well plates according to the cell culture procedures described earlier in this protocol.

Prior to exposure, a mastermix will be prepared of each test substance stock solution prepared in section 7.1 by adding 4 µl of the test substance stock solution to 3.996 ml of supplemented medium. Also, a mastermix will be prepared containing 4 µl of DMSO (or alternative vehicle) and 3.996 ml of supplemented medium. This solution will be used to expose the vehicle control wells. The final DMSO (or appropriate vehicle) concentration in all solutions will be ≤0.1%.

At the time of exposure, the dilutions will be visually observed for precipitation. The stock solutions will be visually observed in vehicle. The dilutions in media on the cell plate will also be visually observed.

An identical 24 well plate will be used to assess potential MTT reduction in the absence of cells by the test substance (Table 4). Solutions of test substance will be added to a 24 well plate (no cells) and an MTT assay will be performed.

After ~24 hour pre-incubation of plated cells, the plates will be removed from the incubator and checked for attachment and morphology prior to test substance exposure. Observations will be recorded.

Remove old medium, add dosing medium (1 ml/well of the appropriate mastermix to the appropriate wells using the dosing scheme presented in Table 4), and place into incubator.

**Table 4: Dosing Scheme for Exposure of H295R Cells to Test Substances in a 24-Well Plate**

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| A | DMSO | DMSO | DMSO | Stock 4 | Stock 4 | Stock 4 |
| B | Stock 1 | Stock 1 | Stock 1 | Stock 5 | Stock 5 | Stock 5 |
| C | Stock 2 | Stock 2 | Stock 2 | Stock 6 | Stock 6 | Stock 6 |
| D | Stock 3 | Stock 3 | Stock 3 | Stock 7 | Stock 7 | Stock 7 |

Test substance exposed cells will be incubated at approximately 37°C with 5% $CO_2$ for approximately 48 hours.

After the approximately 48 hour exposure, plates will be removed from the incubator and every well checked under the microscope for cell condition. Observations will be recorded.

Media will be split from each well into two equal aliquots and transferred onto two separate plates.

Media will be frozen at approximately -80°C until further processing (HPLC/MS/MS analytical detection methods for T and E2).

Immediately after removing media, an MTT cell viability test will be conducted on each exposure plate.

MTT Viability Assay

Following the full ~48 hour exposure period, an MTT assay will performed by adding 0.5 ml MTT medium (0.5 mg/ml) to each well. After ~3 hours MTT incubation at approximately 37°C, 5% $CO_2$ in a humidified incubator, the blue formazan salt will be extracted with 0.5 ml isopropanol per well for ~20 minutes at room temperature with shaking. After the extraction, the optical density of the extracted formazan will be determined using a spectrophotometer (570 nm). Viable cells will have the greatest amount of MTT reduction and hence the highest absorbance values. Relative cell viability will be calculated for each tissue as a % of the mean of the vehicle control wells.

*Hormone Measurement*

HPLC/MS/MS

Samples will be split into two portions. One or both portions will be shipped to OpAns for determination of estradiol and testosterone levels. Hormone concentrations will be measured using bioanalytical methods validated by OpAns. References to the validated methods will be included in the final report.

## 12. Test Results and Data Analysis

Results will be expressed as change in hormone production relative to the mean solvent control for the assay. Data will be expressed as mean ± standard deviation. All doses that exhibit cytotoxicity greater than 20% are omitted from further evaluation.

Relative changes are calculated using the equation below:

Equation 1: Relative Change = [Hormone] in each well ÷ [Hormone] of mean solvent (Vehicle control)

**Table 5. Data Categorization Parameters for the Analysis of Results obtained with the H295R Steroidogenesis Assay**

| Parameter | Criterion |
|---|---|
| Statistical Significance | Difference from the Solvent Control has $p \leq 0.05$. |
| Dose Response | Data is expected to follow a dose response type profile at non-cytotoxic doses, or doses that do not interfere with the hormone measurement assay (note: response can be bi-phasic such as an increase at lower and a decrease at higher doses, but changes randomly observed only at a few concentrations within the dose range are to be excluded) |
| Solubility | The results at concentrations for which cloudiness or a precipitate is observed are not included. |
| Cell Viability | Only non-cytotoxic concentrations (>80% viability) will be included. |

Data Reporting and Analysis

Data processing and Statistics: To evaluate the relative increase/decrease in chemically altered hormone production the results will be normalized to the mean solvent control value for each assay (i. e., each 24-well plate of cells used to test a given substance), and results will be expressed as changes relative to the SC in each exposure plate. Data will be expressed as mean values ± standard deviation (SD). All doses that exhibit cytotoxicity greater than 20% by MTT assay are omitted for further evaluation. Relative changes are calculated using equation 1 (see above)

Prior to conducting statistical analyses, the assumptions of normality and variance homogeneity are evaluated. Normality will be evaluated using standard probability plots or any other appropriate statistical method (e.g., Shapiro-Wilk's test). If the data are not normally distributed, the data will be transformed to

approximate a normal distribution. If the data are normally distributed or approximate normal distribution, differences between substance treatments and solvent controls (SCs) are analyzed using parametric test (e.g., Dunnett's Test). If data are not normally distributed, an appropriate non-parametric test is used (e.g., Kruskal Wallis, Steel's Many-one rank test). Differences are considered significant at $p \leq 0.05$.

A summary of criteria for the evaluation of data has been provided in Table 5.

Data Interpretation

A test substance will be judged to be positive if the fold induction is statistically different from the solvent control (vehicle control). Results exceeding the limits of solubility or at cytotoxic concentrations will be excluded from the interpreted results. MTT interference of the test substance will be monitored.

## 13. Study Reports

The data to be reported in the interim data summary and final report will be determined per Standard Operating Procedure (SOP) and will include (but will not be limited to) the following information: assay date and run number, laboratory personnel involved in the study, chemical/test substance information (including but not limited to substance name, code, molecular weight, concentrations tested, notes regarding solubility), data, mean values, SEM, and other data. The Principal Investigator from OpAns will provide a signed report describing the sample analysis as well as all concentration results. A summary of the results will be included. The study report issued by the Study Director and the entire phase report will be included as an appendix.

## 14. Alterations of the Study Design

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

## 15. 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
- Final Report

CeeTox

**Protocol Amendment**

**Study Number:** 9070V-100107STER

**Title of Study to be Amended:** H295R Steroidogenesis Assay

**Reason for Amendment to Protocol:** Protocol updated to reflect procedural changes based on the results of recent laboratory proficiency runs in the steroidogenesis assay.

**Change:**
In section 10, the quality control plate will not be run prior to using this batch of cells as laboratory proficiency data has already been collected with this batch.

In section 10, the quality control plate layout has been changed to allow for the addition of background wells that will not be dosed with 22R-hydroxycholesterol.

- The background wells will allow for the evaluation of the baseline production of testosterone and estradiol in the H295R cells without the addition of 22R-hydroxycholesterol at the time of dosing. The amended plate layout is as follows:

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| A | Blank | Blank | Blank | Background | Background | Background |
| B | DMSO | DMSO | DMSO | DMSO + MeOH | DMSO + MeOH | DMSO + MeOH |
| C | Forskolin 1 μM | Forskolin 1 μM | Forskolin 1 μM | Prochloraz 0.1 μM | Prochloraz 0.1 μM | Prochloraz 0.1 μM |
| D | Forskolin 10 μM | Forskolin 10 μM | Forskolin 10 μM | Prochloraz 1 μM | Prochloraz 1 μM | Prochloraz 1 μM |

In section 10, the estradiol passing criteria has been changed from 40 pg/ml to 25 pg/ml.

- This modification was made based on the "Correction and clarifications on technical aspects of the test guidelines for the EDSP Tier 1 Assays," supplied by the EPA on March 3, 2011. This document allows for the reduction in

the estradiol passing criteria as long as basal production of the hormone is at least 2.5 times the method detection limit.

- The detection limit for estradiol in the hormone measurement system is 10 pg/ml. As a result, to achieve basal production of 2.5 times the method detection limit, the minimum production required is 25 pg/ml.

**Signature**

**CeeTox, Inc.**

[redacted]

Study Director

19 Aug 2011

Date

CeeTox

**Protocol Amendment**

**Study Number:** 9070V-100107STER

**Title of Study to be Amended:** H295R Steroidogenesis Assay

**Reason for Amendment to Protocol:** Protocol updated to reflect procedural changes based on the results of recent laboratory proficiency runs in the steroidogenesis assay.

**Change:**
In section 7, the vehicle control will be 0.05% DMSO in cell medium containing 10 $\mu$M 22R-hydroxycholesterol. The compound preparation procedure has been modified to allow for use of 22R-hydroxycholesterol prepared in ethanol at time of dosing while maintaining the total vehicle concentration at ≤0.1%.

Section 10 states that 22R-hydroxycholesterol may be added to the supplemented medium to increase basal hormone production. Based on the results of laboratory proficiency studies, 22R-hydroxycholesterol will be used at time of plating, dosing, and harvest at a concentration of 10 $\mu$M.

In section 11, cells will be plated at 300,000 cells per ml instead of 250,000 cells per ml.

- This density falls within the range allowed in the test guideline (OPPTS 890.1550: Steroidogenesis) and was chosen based on the results of preliminary experiments conducted at CeeTox.

In section 11, the mastermix preparation procedure has been modified to allow for the use of 22R-hydroxycholesterol at time of dosing while maintaining the total vehicle concentration at ≤0.1%.

In section 11, the test for MTT reduction by the test article in the absence of cells will not be conducted. In section 12, MTT interference of the test article will not be monitored.

- This is not required per the EDSP Test Guideline (OPPTS 890.1550: Steroidogenesis).

**Signature**

**CeeTox, Inc.**

[redacted]

Study Director

19 Aug 2011

Date

CeeTox

**Protocol Amendment**

**Study Number:** 9070-100107STER

**Title of Study to be Amended:** H295R Steroidogenesis Assay

**Reason for Amendment to Protocol:** Client requested amendment

**Change:**
Section Data Retention and Archiving will now state:

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

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

**Signature**

**CeeTox, Inc.**

[redacted]
Study Monitor [redacted]

12-6-11
Date

[redacted]
Study Director (Project Manager)

6 Dec 2011
Date