FINAL REPORT

Study Title

The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Test Guideline

OPPTS 890.1400
OECD 441

ILS Project-Study Numbers

10005.0103

Author

Jeffrey P. Davis, B.S., LATG

Performing Laboratory

Integrated Laboratory Systems, Inc.
635 Davis Drive, Suite 600
Morrisville, NC 27560 USA

Sponsor

RTI International
3040 Cornwallis Road
Research Triangle Park, NC 27709 USA

Date of Submission

31 March 2017

STATEMENT OF NO DATA CLAIM OF CONFIDENTIALITY

No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA sec. 10(d)(1)(A), (B), or (C) and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provisions regarding disclosure to multinational entities under FIFRA 10(g).

Submitter Signature:	Sherry L Black
Date:	3/31/17
Name of Submitter:	Sherry L. Black
Name of Company:	RTI International

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The following is a detailed description of all differences between the practices used in the study and those required by 40 CFR 160:

Flutamide and testosterone propionate dose formulations were not analyzed as stated in 40 CFR 160.113(a)(1) of the U.S. EPA GLP requirements; a positive response in the test system following flutamide and/or testosterone propionate administration was evident following statistical analysis of the tissue weights.

Study Director Signature:	
Date:	31 March 2017
Name of Study Director:	Jeffrey P. Davis, B.S., LATG
Name of Company:	Integrated Laboratory Systems, Inc.

Sponsor Signature:	Sherry L Black
Date:	3/31/17
Name of Sponsor:	Sherry L. Black
Name of Company:	RTI International

Submitter Signature:	Sherry L Black
Date:	3/31/17
Name of Submitter:	Sherry L. Black
Name of Company:	RTI International

QUALITY ASSURANCE INSPECTION STATEMENT

ILS Project - Study No.: 10005.0103
Test Article: 2-Ethylhexyl Paraben
ILS Repository No.: 15-172
Study Title: The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

This study was inspected by one or more persons of the QA Unit of ILS, Durham, NC, USA, and written status reports were submitted on the following dates:

Inspection/Audit:	Date(s) Performed:	Dates Reported to Study Director / Management:
Study Protocol	07 Jan 2016	07 Jan 2016 / 07 Jan 2016
Clinical Observations	01 Feb 2016	01 Feb 2016 / 01 Feb 2016
Dose Administration	01 Feb 2016	01 Feb 2016 / 01 Feb 2016
Necropsy	09 Feb 2016	09 Feb 2016 / 09 Feb 2016
Study Data/Draft Report	06-08 June 2016	08 June 2016 / 08 June 2016
Final Report	31 Mar 2017	31 Mar 2017 / 31 Mar 2017

Jeanne deWard, B.S., LATG, RQAP-GLP
Quality Assurance Auditor
Integrated Laboratory Systems, Inc.

31 March 2017
Date

TABLE OF CONTENTS

Tables

Appendices:

EXECUTIVE SUMMARY

The Hershberger Assay consists of androgenic and anti-androgenic components. To screen for potential androgenic activity, 2-ethylhexyl paraben (99.8% a.i., lot/batch number 7CZZO) in corn oil was administered daily via oral gavage to postnatal day (PND) 59/60, castrated male Sprague Dawley rats, eight animals per group, at dose levels of 0 (vehicle), 250, 500, 750, or 1000 mg/kg/day. An androgenic positive control group consisted of eight castrated rats exposed to 0.4 mg/kg/day testosterone propionate (TP) by subcutaneous injection (s.c.).

To screen for potential anti-androgenic activity, 2-ethylhexyl paraben (99.8% a.i., lot/batch number 7CZZO) in corn oil was administered daily via oral gavage to PND 59/60, castrated male Sprague Dawley rats, eight animals per group, at dose levels of 0 (vehicle), 250, 500, 750, or 1000 mg/kg/day and co-administered with a daily dose of reference androgen TP at 0.4 mg/kg/day by s.c. injection. An anti-androgenic positive control group consisted of eight castrated rats exposed to 0.4 mg/kg/day (s.c.) TP and 3.0 mg/kg/day (oral gavage) flutamide (FT). TP alone (s.c.) was used as the anti-androgenic negative control.

For both components of the assay, the animals were dosed for ten consecutive days and necropsied approximately 24 hours after the final dose administration to determine weights of the five androgen-dependent tissues (glans penis, ventral prostate, levator ani plus bulbocavernous muscle [LABC], Cowper's glands, and seminal vesicles with coagulating fluid). Body weight measurements were collected daily prior to dose administration and prior to humane euthanasia with body weight gains calculated for the study period. Clinical observations were recorded prior to daily dose administration with cage-side observations recorded one-hour following dose administration.

In the agonist assay, all animals survived until the scheduled termination. Animals administered 0, 250, or 500 mg/kg/day 2-ethylhexyl paraben did not exhibit abnormal clinical signs during the course of the study. One animal administered 750 mg/kg/day 2-ethylhexyl paraben was found to have decreased movement post-dose on Study Day 10. Administration of 1000 mg/kg/day 2-ethylhexyl paraben resulted in one animal exhibiting rales on Study Day 5 and 6 and two animals with decreased movement post-dose on Study Day 9. Body weight gain of animals administered 1000 mg/kg/day 2-ethylhexyl paraben was significantly decreased (34% decrease from control) compared to the concurrent negative control group. Final body weights averaged 97.2%, 99.5%, 94.0%, and 92.7% of controls in animals administered 250, 500, 750, or 1000 mg/kg/day 2-ethylhexyl paraben, respectively. Administration of 2-ethylhexyl paraben up to and including 1000 mg/kg/day did not statistically affect glans penis, Cowper's gland, LABC, ventral prostate, or seminal vesicle weights.

In the antagonist assay, all animals survived to the scheduled termination. No abnormal clinical observations were found in animals co-administered 0, 250, or 750 mg/kg/day 2-ethylhexyl paraben with TP during the course of the study. One animal administered 500 mg/kg/day 2-ethylhexyl paraben with TP was found to have a scab at the dorsal neck region (area of s.c. injection) from Study Day 5 through termination. Administration of 1000 mg/kg/day 2-ethylhexyl paraben with TP resulted in one animal with decreased movement post-dose on Study Day 10 and a rough coat the following day (Study Day 11), while another animal

exhibited rales on Study Day 9. Body weight gain of animals administered 1000 mg/kg/day 2-ethylhexyl paraben plus TP was significantly decreased (26% decrease from control) compared to the concurrent negative control group. Final body weights averaged 96.1%, 98.2%, 96.4%, and 92.9% of controls in animals administered 250, 500, 750, or 1000 mg/kg/day 2-ethylhexyl paraben, respectively. Administration of 2-ethylhexyl paraben up to and including 1000 mg/kg/day with TP did not statistically affect glans penis, Cowper's glands, or seminal vesicle weights. Compared to the negative control group (Group 6), LABC weights were decreased by 10% (statistically significant) at 500 mg/kg/day, 9% (not statistically significant) at 750 mg/kg/day, and 12% (statistically significant) at 1000 mg/kg/day 2-ethylhexyl paraben with TP, however, there was no clear dose-response relationship. Ventral prostate weights were statistically decreased following co-administration of 1000 mg/kg/day 2-ethylhexyl paraben plus TP (decrease of 20% from the concurrent control).

The positive control in the agonist assay, TP, induced a statistically significant increase in glans penis, Cowper's glands, seminal vesicle, ventral prostate, and LABC weights compared to the negative control group. In the antagonist assay, the positive control FT (co-administered with TP) induced a statistically significant reduction in glans penis, Cowper's glands, seminal vesicle, ventral prostate, and LABC weights compared to the control group (TP only).

Based on these findings, using the castrated rat model Hershberger Bioassay, the oral gavage administration of 2-ethylhexyl paraben up to the limit dose of 1000 mg/kg/day did not produce any evidence of androgen agonist activity.

In the androgen antagonist assay, statistically significant decreases were noted in two of the five tissue weights (LABC and ventral prostate) which could be indicative of androgen antagonist activity. Relatively small reductions in LABC weights were observed in the top three dose groups (statistically significant at 500 and 1000 mg/kg/day) and ventral prostate weights were statistically significantly decreased at 1000 mg/kg/day. However, it is not clear if the reductions in LABC weights are related to 2-ethyhexyl paraben administration in the three highest dose groups (500, 750, and 1000 mg/kg/day) because they are of similar magnitude, showed no clear dose-response relationship, and are not all statistically significant. Further, glans penis, Cowper's gland, and seminal vesicle weights showed no degree of reduced growth.

INTRODUCTION

1.1 Background

Endocrine Disruptor Screening Program (EDSP) Tier 1 screening assays will be used to identify substances that have the potential to interact with the estrogen, androgen, or thyroid hormone systems (test guidelines in the OPPTS 890 series). The determination of a chemical's ability to interact with hormone systems will be made on a weight-of-evidence basis, taking into account data from the Tier 1 assays and other scientifically relevant information available. If a substance interacts with a hormone system, it does not imply that when used it will cause adverse effects in humans or ecological systems. The Hershberger Bioassay (U.S. EPA, 2009 and OECD, 2009) is used as an *in vivo* screening assay for androgen agonists, androgen antagonists, and 5α-reductase inhibitors and is one of four *in vivo* mammalian assays in the EDSP Tier 1 battery of assays.

EPA has requested *in vivo* mammalian studies to bridge data gaps regarding a chemical's potential endocrine effects; the data from these studies may also be used to evaluate and refine computational models that predict *in vivo* responses from *in vitro* assays.

1.2 Purpose

The purpose of the Hershberger Bioassay was to screen 2-ethylhexyl paraben for its androgen agonist/antagonist activity and 5α-reductase inhibition properties using a castrated rat model (U.S. EPA, 2009 and OECD, 2009).

1.3 Sponsor

RTI International
3040 Cornwallis Road
Research Triangle Park, NC 27709 USA

Sponsor Representative

Sherry Black, B.S.
Telephone No.: (919) 541-7353
E-mail: sherryb@rti.org

1.4 Testing Facility

Integrated Laboratory Systems, Inc. (ILS)

Shipping Address: 635 Davis Drive, Suite 600
Morrisville, NC 27560 USA

Mailing Address: P.O. Box 13501

Research Triangle Park, NC 27709 USA

Study Director

Jeffrey P. Davis, B.S., LATG
Telephone No.: (919) 281-1110 ext. 720
Facsimile No.: (919) 281-1118
E-mail: jdavis@ils-inc.com

1.5 Study Dates

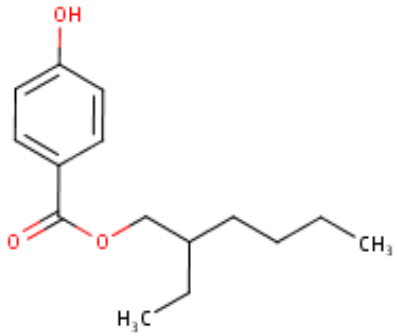
Study Initiation Date:	21 January 2016
Animal Arrival Date:	21 January 2016
Experimental Start Date:	29 January 2016
Experimental In-Life Termination Date:	09 February 2016
Experimental Termination Date:	09 February 2016

TEST SUBSTANCE, REFERENCE SUBSTANCES, VEHICLES

2.1 Test Substance: 2-Ethylhexyl Paraben

CAS No.: 5153-25-3

Molecular Structure:



Source: Tokyo Chemistry Industry Co., Ltd. (Tokyo, Japan)

Lot/Batch No.: 7CZZO

ILS Repository No.: 15-172

Formula: $C_{15}H_{22}O_3$

Description: Colorless, clear liquid

Purity:	99.8%
Expiration Date:	None given on Certificate of Analysis
Dose Formulation:	2-Ethylhexyl paraben was prepared at ILS in corn oil once at dose levels of 50, 100, 150, and 200 mg/mL and dispensed into vials used daily during the study.
Storage:	
Test Substance:	Room temperature and protected from light
Dose Formulation:	Between 1 and 10°C
Stability:	
Dose Formulation:	Concentrations of 1 and 200 mg/mL stored between 1 and 10°C were shown to be stable for 20 days (Appendix VI).

2.2 Reference Substance: Testosterone Propionate (androgen agonist)

CAS No.:	57-85-2
Source:	Spectrum Chemicals (New Brunswick, NJ)
Lot/Batch No.:	1EL0103
ILS Repository No.:	16-18
Formula:	$C_{22}H_{32}O_3$
Description:	White to off-white powder
Purity:	99.7%
Expiration Date:	31 July 2020
Dose Formulation:	TP was prepared in corn oil once at a dose level of 0.8 mg/mL and dispensed into vials used daily during the study.
Storage:	
Reference Substance:	Room temperature and protected from light

Dose Formulation: Between 1 and 10°C

Stability:

Dose Formulation: TP in corn oil held between 1-10°C was shown to be stable for 14 days (Smith, 2011).

2.3 Reference Substance: Flutamide (androgen antagonist)

CAS No.: 13311-84-7

Source: Spectrum Chemicals (New Brunswick, NJ)

Lot/Batch No.: 2EL0006

ILS Repository No.: 15-212

Formula: $C_{11}H_{11}F_3N_2O_3$

Description: Yellow powder

Purity: 99.9%

Expiration Date: None given on Certificate of Analysis

Dose Formulation: FT was prepared at ILS in corn oil once at a dose level of 0.6 mg/mL and dispensed into vials used daily during the study.

Storage:

Reference Substance: Room temperature and protected from light

Dose Formulation: Between 1 and 10°C

Stability:

Dose Formulation: FT in corn oil held between 1-10°C was shown to be stable for 42 days (Graves, 2001).

2.4 Vehicle Corn oil

Used in dose formulations of 2-ethylhexyl paraben and flutamide.

CAS No.: 8001-30-7

Source: Animal Health International (Greeley, CO)

Lot/Batch No.:	16303-100175
ILS Repository No.:	15-206
Formula:	$C_{27}H_{50}O_6$
Description:	Yellow oil
Storage:	Room temperature and protected from light
Justification:	Corn oil was selected as the vehicle due to its previous use in a dose range finding study performed at ILS.

2.5 Vehicle **Corn oil**

Used in dose formulation of TP. National Formulary grade for use in injections.

CAS No.:	8001-30-7
Source:	Spectrum Chemicals (New Brunswick, NJ)
Lot/Batch No.:	2EJ0098
ILS Repository No.:	16-09
Formula:	$C_{27}H_{50}O_6$
Description:	Yellow oil
Storage:	Room temperature and protected from light

2.6 Archival Samples

An approximate 1 mg sample of neat test and reference substances and 1 mL of the vehicle and dose formulations for each preparation are stored between 0 and -30°C. After acceptance of the study report by the Sponsor, archival dose formulation samples will be discarded. Test and reference substances will be maintained by ILS for five years following finalization of the study report.

2.7 Dose Formulation Analysis

Dose formulations were prepared at ILS, with only 2-ethylhexyl paraben dose formulations sent to and analyzed at Smithers Viscient, LLC (Wareham, MA) in accordance with GLP regulations as promulgated by the U.S. EPA GLP Regulations (40 CFR Part 160).

Principal Investigator- Xianai Wu, Ph.D., DABT
Smithers Viscient, LLC
790 Main Street
Wareham, MA 02571-1075

Samples of dose formulations prepared on 25 January 2016 were collected from the top, middle, and bottom of the formulation and sent to Smithers Viscient, LLC for analysis. Smithers Viscient, LLC analyzed duplicate samples received for concentration and homogeneity.

Concentration results were acceptable if the mean concentration was within 15% of the target concentration. Homogeneity results were acceptable if the coefficient of variation was less than 15% of the target concentration.

EXPERIMENTAL DESIGN

3.1 Test System

Species:	Rat, *Rattus norvegicus*
Strain:	Sprague Dawley Crl:CD®(SD) IGS
Source:	Charles River Laboratories International, Inc. (Raleigh, NC)
Number/Sex:	88/Castrated males. Surgical manipulation on PND 49 was performed by Charles River Laboratories International, Inc.
Date of birth:	01 December 2015
Age at arrival:	Postnatal day (PND) 51 Note: PND 0 is the date of birth
Acclimation:	Animals were acclimated in the study room for at least 7 days
Age at initial dose administration:	PND 59/60
Weight at initial dose administration:	229.3 – 322.5 grams
Identification:	Each animal was uniquely identified by ear punch prior to dose administration. Until the animals were ear punched, they were identified by the temporary numbers located on the animals' cages.
Justification:	Animal model used is in accordance with the test guidelines (U.S. EPA, 2009 and OECD, 2009).

3.2 Animal Husbandry

All procedures are in compliance with the Animal Welfare Act Regulations, 9 CFR 1-4 and animals were handled and treated according to the *Guide for the Care and Use of Laboratory Animals* (ILAR, 2011).

Housing (pre-allocation):	Two per cage
Housing (post-allocation):	Two per cage
Cage Type:	Polycarbonate with micro-isolator tops
Cage Size:	23 cm wide by 44 cm long (1012 cm^2 area) and 21 cm high
Bedding:	Absorbent heat-treated hardwood bedding (Northeastern Products Corp., Warrensburg, NY)
Cage Changes:	Twice per week
Diet:	Teklad Global 16% Protein Rodent Diet (Teklad Diets, Madison WI) *ad libitum.* Prior to shipment, rats were fed Autoclaved Purina 5L79 Rat and Mouse diet *ad libitum* at Charles River Laboratories International, Inc. A copy of the diet composition is included in the study records.
Analysis:	The manufacturer's analytical results are included in the study records and were reviewed prior to animal arrival. The total genistein equivalent of genistein plus daidzein (as described by Owens et al., 2003) in Teklad Global 16% Protein Rodent Dietwas determined to be 5.4 µg/g of feed.
Archival:	A sample of the diet (~200 g) was retained and stored between 0 and -30°C.
Water:	Reverse osmosis treated tap water (City of Durham, NC) *ad libitum*
Supplied:	Glass water bottles with stainless steel sipper tube
Analysis:	The results of the current annual comprehensive chemical analyses of water from National Testing Laboratories, Inc. (Cleveland, OH) were reviewed prior to initiation of the study and are included in the study records.
Water Bottle Changes:	Once per week

Animal Room Conditions:

Temperature: 20.0 – 25.1°C

Humidity 29.9 – 71.2%

Lighting: 12/12 hour light/dark cycle

Animal Enrichment: None

3.3 Allocation

The animals were assigned to a dose group within Provantis® (electronic data capture software) one day prior to initial dose administration using a procedure that stratifies animals across groups by body weight into randomized blocks (e.g. dose groups) such that mean body weight of each group was not statistically different from any other group using analysis of variance [ANOVA Statistical Analysis System (SAS) version 9.2, SAS Institute, Cary, NC]. All animals used in the allocation process were clinically healthy and had undergone preputial separation.

3.4 Group Designation

Table 1. Androgen Agonist- Group Designation, Animal Identification, and Dose Levels

Group Number	Animal Identification	Dose Group[a]	Test Substance Dose Level (mg/kg/day)
1	01-08	2-Ethylhexyl Paraben (Vehicle Control)[b]	0
2	09-16	2-Ethylhexyl Paraben	250
3	17-24	2-Ethylhexyl Paraben	500
4	25-32	2-Ethylhexyl Paraben	750
5	33-40	2-Ethylhexyl Paraben	1000

[a]2-Ethylyhexyl paraben was administered by oral gavage
[b]Negative (vehicle) control group

Table 2. Androgen Antagonist- Group Designation, Animal Identification, Dose Group Levels

Group Number	Animal Identification	Dose Group[a]	Test or Reference Substance Dose Level (mg/kg/day)
6[b]	41-48	2-Ethylhexyl Paraben + Testosterone Propionate	0 + 0.4
7	49-56	2-Ethylhexyl Paraben + Testosterone Propionate	250 + 0.4
8	57-64	2-Ethylhexyl Paraben + Testosterone Propionate	500 + 0.4
9	65-72	2-Ethylhexyl Paraben + Testosterone Propionate	750 + 0.4
10	73-80	2-Ethylhexyl Paraben + Testosterone Propionate	1000 + 0.4
11[c]	81-88	Flutamide + Testosterone Propionate	3.0 + 0.4

[a]2-Ethylhexyl paraben and flutamide were administered via oral gavage; testosterone propionate was administered via subcutaneous injection
[b]Negative (vehicle) control (vehicle + testosterone propionate only) for antagonist assay (Table 2) and positive control for the agonist assay (Table 1)
[c]Positive control

3.5 Dose Administration

The test substance, FT (reference substance), and vehicle control (corn oil) dose formulations were administered by oral gavage at a dose volume of 5 mL/kg body weight. TP dose formulations were administered by subcutaneous injection into the dorsoscapular region at a dose volume of 0.5 mL/kg body weight. In co-administered animals, oral gavage preceded subcutaneous injections.

The dose formulations were administered on a staggered start for 10 consecutive days (PND 59/60 through PND 68/69). The first four animals from each group were dosed beginning on PND 59 and the second four from each group on PND 60. Dosing occurred 24 hours (± 2 hours) from the previous dose. Dose volume was determined on individual animal daily body weight. The dosing sequence was stratified across dose groups; one animal from each group and then repeated until all animals were dosed.

3.5.1 Justification of Route of Administration

Selection of the route of administration was in accordance with the test guidelines (U.S. EPA, 2009 and OECD, 2009).

3.5.2 Justification of Dose Levels

A dose range finding study was conducted to select a maximum tolerated dose (MTD) for this assay. Intact Sprague Dawley male rats were administered 0, 200, 400, 600, 800, or 1000 mg/kg/day 2-ethylhexyl paraben in corn oil via oral gavage from PND 36 through 49 (n=4 per dose group). All rats survived to the scheduled termination. Two animals administered 1000 mg/kg/day 2-ethylhexyl paraben were lethargic three hours following dosing on Study Day 1, but were normal thereafter. One animal administered 800 mg/kg/day 2-ethylhexyl paraben was observed with an ungroomed appearance on Study Day 7 both before and after dose administration which coincided with a 21.6 g body weight loss from the previous day.

Animals were humanely euthanized approximately 24 hours following the final dose administration. Final body weight and body weight gain were assessed following ten days of dose administration and were not significantly different in rats administered 2-ethylhexyl paraben as compared to the vehicle control group. Body weights following ten days of dosing averaged 91.8%, 95.1%, 101.5%, 90.7% (94.2% if an animal with a marked decrease is excluded), and 93.4% of controls in animals administered 200, 400, 600, 800, or 1000 mg/kg/day 2-ethylhexyl paraben, respectively.

Based on the results described above and the current test guideline recommendations, the limit dose of 1000 mg/kg/day was selected as the top dose to be evaluated.

TP and FT dose levels were in accordance with the test guidelines (U.S. EPA, 2009; OECD, 2009).

3.5.3 Disposal of Dose Formulations

Dose formulations were disposed of as hazardous material following dosing each day.

3.6 In-Life Animal Observations

Mortality/Moribundity: Twice daily on weekdays, once daily on weekends/holidays

Clinical Observations:	Observed within two days of arrival, again for allocation of animals to study groups, daily prior to dose administration, and prior to euthanasia.
Cage-Side Observations:	Observed 1 hour (± 30 minutes) following doing each day.
Body Weights:	Collected within two days of arrival, again for allocation of animals to study groups, daily prior to dose administration, and prior to euthanasia.

3.7 Termination

Scheduled:	Twenty-four hours (± two hours) after the final dose administration, animals were humanely euthanized by carbon dioxide asphyxiation with death confirmed by cervical dislocation in the same order as they were dosed.
Tissue Collection:	Gross observations of the tissues that were excised for tissue weights were recorded.
Tissue Weights:	The following androgen-dependent tissues were excised, trimmed of excess adhering tissue and fat, and weighed to the nearest 0.1 mg: 1. Ventral prostate 2. Seminal vesicle with coagulating gland with fluid 3. Levator ani plus bulbocavernous muscle complex 4. Cowper's glands (weighed as a pair) 5. Glans penis

3.8 Statistical Analysis

Descriptive statistics (mean, standard deviation, coefficient of variance, and sample size) were calculated using Provantis version 9.3.1 (Instem, Philadelphia, PA). Each data set was analyzed using Statistical Analysis System version 9.2 (SAS Institute, Cary, NC). In the androgen agonist phase, data collected from animals administered 2-ethylhexyl paraben were compared to the negative control group (0 mg/kg/day 2-ethylhexyl paraben). In the androgen antagonist phase, data collected from animals co-administered 2-ethylhexyl paraben and TP were compared to the animals co-administered 0 mg/kg/day 2-ethylhexyl paraben and TP.

Studentized residual plots were used to detect possible outliers and Levene's test was used to assess homogeneity of variance. Final body weight, body weight gain, and tissue weights were analyzed by one-way ANOVA followed by pairwise comparisons using a Dunnett's one-tailed t-test (tissue weights) and Dunnett's two-tailed t-test (final body

weight and body weight gain). Statistically significant effects were reported when $p<0.05$.

Final body weight, body weight gain, and tissue weights for animals administered the vehicle control and TP or TP co-administered with FT were compared to their concurrent control group by appropriate t-tests. Statistically significant effects were reported when $p<0.05$.

3.9 Record Retention

Upon acceptance of the final report, dose formulation samples and the feed diet sample will be discarded. All original data [including the original signed study protocol, test substance information, animal receipt records, animal caretaker records, body weight records, clinical observations, etc.] and the original final report will be maintained by ILS for five years following finalization of the study report. Transfer of study records may be requested by the Sponsor prior to the end of the five-year archival period. At the end of the five-year archival period, the Sponsor will be notified for direction of appropriate disposition of study records remaining at ILS.

RESULTS

4.1 Dose Formulation Analysis

Dose formulations concentration and homogeneity results were within the acceptable criteria (Appendix VI).

Table 3. Dose Formulation Concentration and Homogeneity Results
Preparation Date: 25 January 2016

Dose Group	Nominal Dose Concentration (mg/mL)	Actual Dose Concentration* (mg/mL) [Percent of Nominal]	Percent CV* (Homogeneity)	Nominal Dose Level (mg/kg/day)
2-Ethylhexyl Paraben	50	48.3 [96.5]	1.13	250
2-Ethylhexyl Paraben	100	95.2 [95.2]	1.26	500
2-Ethylhexyl Paraben	150	139 [92.4]	2.33	750
2-Ethylhexyl Paraben	200	181 [90.4]	6.20	1000

Abbreviation: CV - coefficient of variation
*See Appendix VI

4.2 In-Life Animal Observations

Mortality/Moribundity

Androgen Agonist

All animals survived to the scheduled termination with no animals showing signs of moribundity.

Androgen Antagonist

All animals survived to the scheduled termination with no animals showing signs of moribundity.

Clinical and Cage-Side Observations

Cage-side (1 hour) and clinical observations (~24 hours) were performed post-dose administration. Individual animal data are listed in Appendix I.

Androgen Agonist

No 2-ethylhexyl paraben dose-related abnormal clinical observations were noted in animals administered 0, 250, or 500 mg/kg/day. One animal (#32) administered 750 mg/kg/day exhibited decreased movement post dose on Study Day 10, with no other abnormal findings in the dose group. Three animals administered 1000 mg/kg/day 2-ethylhexyl paraben were observed with abnormal findings: animal #33 exhibited rales on Study Days 5 and 6, animals #38 and #40 exhibited decreased movement post-dose on Study Day 9. The remaining five animals in the group were normal throughout the dosing period.

Androgen Antagonist

Animals administered 0, 250, or 750 mg/kg/day 2-ethylhexyl paraben plus TP were normal during the dosing period. One animal (#59) administered 500 mg/kg/day 2-ethylhexyl paraben plus TP had a scab on the dorsal neck from Study Day 5 through termination. Two animals administered 1000 mg/kg/day 2-ethylhexyl paraben plus TP exhibited abnormal clinical observations: animal #78 showed decreased movement post-dose on Study Day 10 followed with a rough coat on Study Day 11, while animal #80 exhibited rales on Study Day 9.

Body Weights

Group mean initial and final body weights and body weight changes for animals euthanized following ten consecutive days of 2-ethylhexyl paraben administration are presented in Table 4 (agonist assay) and Table 5 (antagonist assay). Individual animal data are listed in Appendix II.

Androgen Agonist (Table 4)

There were no statistically significant changes in final body weight or body weight gain in animals administered 250, 500, or 750 mg/kg/day 2-ethylhexyl paraben compared to the concurrent negative control group. There was a statistically significant decrease in body weight gain (decrease of 34% from control), but not final body weight, in animals administered 1000 mg/kg/day 2-ethylhexyl paraben compared to the negative control group (Group 1). A statistically significant increase in body weight gain was observed in animals administered the positive control compared to the negative control group (Group 1).

Androgen Antagonist (Table 5)

There were no statistically significant changes in final body weight or body weight gain in animals administered 250, 500, or 750 mg/kg/day 2-ethylhexyl paraben plus TP compared to the concurrent negative control group (Group 6). There was a statistically significant decrease in body weight gain (decrease of 26% from control) in animals administered 1000 mg/kg/day 2-ethylhexyl paraben plus TP, but not final body weight, compared to the negative control group (Group 6). Administration of the positive control (FT plus TP) did not lead to a significantly altered final body weight or body weight gain compared to the concurrent control group.

Table 4. Androgen Agonist; Body Weight Changes in Male Sprague Dawley Rats

Group Number	Dose Group[a]	Test or Reference Substance Dose Level (mg/kg/day)	n	Initial Mean Body Weight (g) ± SD	Final Mean Body Weight (g) ± SD	Mean Body Weight Gain (g) ± SD	Final Body Weight Percent of Control
1	2-Ethylhexyl Paraben[b] (Vehicle Control)	0	8	285.6 ± 23.4	345.3 ± 33.1	59.8 ± 13.3	-
2	2-Ethylhexyl Paraben	250	8	275.3 ± 29.7	335.7 ± 39.7	60.4 ± 12.2	97.2
3	2-Ethylhexyl Paraben	500	8	282.5 ± 16.7	343.5 ± 13.2	61.0 ± 8.2	99.5
4	2-Ethylhexyl Paraben	750	8	273.9 ± 22.8	324.6 ± 23.7	50.6 ± 7.4	94.0
5	2-Ethylhexyl Paraben	1000	8	281.1 ± 23.2	320.3 ± 25.8	**39.2 ± 10.8***	92.7
6	2-Ethylhexyl Paraben + TP[c] (Positive Control	0 + 0.4	8	279.0 ± 20.4	370.3 ± 29.4	**91.3 ± 12.8**[†]	-

Abbreviation: SD - standard deviation, TP - testosterone propionate
[a]2-Ethylhexyl paraben was administered via oral gavage; TP was administered via subcutaneous injection
[b]Negative (vehicle) control
[c]Positive control (antagonist negative control)
*Statistically significant ($p<0.05$) compared to the vehicle control mean (Dunnett's test)
[†]Statistically significant ($p<0.05$) compared to the vehicle control mean (t-test)

Table 5. Androgen Antagonist; Body Weight Changes in Male Sprague Dawley Rats

Group Number	Dose Group[a]	Test or Reference Substance Dose Level (mg/kg/day)	n	Initial Mean Body Weight (g) ± SD	Final Mean Body Weight (g) ± SD	Mean Body Weight Gain (g) ± SD	Final Body Weight Percent of Control
6	2-Ethylhexyl Paraben + TP[b]	0 + 0.4	8	279.0 ± 20.4	370.3 ± 29.4	91.3 ± 12.8	-
7	2-Ethylhexyl Paraben + TP	250 + 0.4	8	273.5 ± 25.4	355.8 ± 28.7	82.3 ± 12.6	96.1
8	2-Ethylhexyl Paraben + TP	500 + 0.4	8	275.3 ± 22.0	363.8 ± 24.8	88.5 ± 7.8	98.2
9	2-Ethylhexyl Paraben + TP	750 + 0.4	8	274.3 ± 24.9	357.0 ± 26.6	82.6 ± 16.7	96.4
10	2-Ethylhexyl Paraben + TP	1000 + 0.4	8	276.6 ± 22.3	344.0 ± 31.4	**67.3 ± 24.3***	92.9
11	FT + TP[c] (Positive Control)	3.0 + 0.4	8	278.0 ± 23.7	358.7 ± 19.8	80.6 ± 11.9	96.8

Abbreviation: SD - standard deviation, FT - flutamide, TP - testosterone propionate
[a]2-Ethylhexyl paraben and flutamide were administered via oral gavage; TP was administered via subcutaneous injection
[b]Negative control (agonist positive control)
[c]Positive control
*Statistically significant ($p<0.05$) compared to the vehicle control mean (Dunnett's test)

4.3 Necropsy Procedures

Gross Observations

There were no abnormal gross observations noted in the five androgen-dependent tissues in either the agonist or antagonist phases of the study at necropsy.

Group data are listed in Appendix III.

Tissue Weights

Group mean weights of glans penis, Cowper's glands, LABC, ventral prostate, and seminal vesicle for animals euthanized following ten consecutive days administration are presented in Table 6 (agonist assay) and Table 7 (antagonist assay). Individual animal tissue weight data are listed in Appendix IV.

Androgen Agonist (Table 6)

No statistically significant effect was observed on glans penis, Cowper's glands, LABC, ventral prostate, and seminal vesicle tissue weights in animals administered up to 1000 mg/kg/day 2-ethylhexyl paraben compared to the negative control group (Group 1).

The five androgen-dependent tissue weights were significantly increased in the positive control group (Group 6) as compared to the negative control group.

Androgen Antagonist (Table 7)

No statistically significant effect was observed on glans penis, Cowper's glands, and seminal vesicle tissue weights in animals co-administered 2-ethylhexyl paraben up to 1000 mg/kg/day plus TP compared to the negative control group (Group 6). Compared to the negative control group (Group 6), LABC weights were decreased from control by 10% (statistically significant) at 500 mg/kg/day, by 9% (not statistically significant) at 750 mg/kg/day, and by 12% (statistically significant) at 1000 mg/kg/day 2-ethylhexyl paraben with TP, however, there was no clear dose-response relationship.

Ventral prostate weights were statistically decreased after dose administration of 1000 mg/kg/day 2-ethylhexyl paraben with TP (20% decrease from control) when compared to the negative control group (Group 6).

The five androgen-dependent tissues weights were significantly decreased in the positive control group (Group 11) compared to the negative control group.

Table 6. Androgen Agonist; Androgen Dependent Tissue Weights in Male Sprague Dawley rats

Group Number	Dose Group[a]	Test or Reference Substance Dose Level (mg/kg/day)	n	Mean Glans Penis Weight (mg) ± SD (CV)	Mean Cowper's Gland Weight (mg) ± SD (CV)	Mean LABC Weight (mg) ± SD (CV)	Mean Ventral Prostate Weight (mg) ± SD (CV)	Mean Seminal Vesicle Weight (mg) ± SD (CV)
1	2-Ethylhexyl Paraben[b] (Vehicle Control)	0	8	59.3 ± 6.7 (11)	9.3 ± 2.6 (28)	190.7 ± 42.1 (22)	21.0 ± 2.2 (10)	68.6 ± 14.1 (21)
2	2-Ethylhexyl Paraben	250	8	55.8 ± 6.1 (10)	8.8 ± 3.3 (38)	192.6 ± 35.7 (19)	19.2 ± 4.8 (25)	67.2 ± 18.9 (28)
3	2-Ethylhexyl Paraben	500	8	56.6 ± 5.5 (10)	9.2 ± 2.3 (24)	211.3 ± 34.2 (16)	20.0 ± 4.2 (21)	72.4 ± 14.7 (20)
4	2-Ethylhexyl Paraben	7500	8	58.6 ± 7.7 (13)	9.4 ± 3.0 (32)	201.6 ± 9.2 (05)	19.6 ± 2.7 (14)	67.1 ± 9.1 (14)
5	2-Ethylhexyl Paraben	1000	8	61.9 ± 6.2 (10)	8.8 ± 2.6 (29)	204.8 ± 22.9 (11)	20.0 ± 3.3 (17)	59.3 ± 5.7 (10)
6	2-Ethylhexyl Paraben + TP[c] (Positive Control)	0 + 0.4	8	**94.0 ± 5.9†** (06)	**59.1 ± 8.2†** (14)	**656.6 ± 49.8†** (08)	**217.3 ± 29.5†** (14)	**835.3 ± 76.8†** (09)

Abbreviations: SD - standard deviation; LABC - levator ani plus bulbocavernous muscle complex; CV- coefficient of variation, TP - testosterone propionate

[a]2-Ethylhexyl paraben was administered via oral gavage; TP was administered via subcutaneous injection

[b]Negative (vehicle) control

[c]Positive control (antagonist negative control)

†Statistically significant ($p<0.05$) compared to the vehicle control mean (t-test)

Table 7. Androgen Antagonist; Androgen Dependent Tissue Weights in Male Sprague Dawley Rats

Group Number	Dose Group[a]	Test or Reference Substance Dose Level (mg/kg/day)	n	Mean Glans Penis Weight (mg) ± SD (CV)	Mean Cowper's Gland Weight (mg) ± SD (CV)	Mean LABC Weight (mg) ± SD (CV)	Mean Ventral Prostate Weight (mg) ± SD (CV)	Mean Seminal Vesicle Weight (mg) ± SD (CV)
6	2-Ethylhexyl Paraben + TP[b]	0 + 0.4	8	94.0 ± 5.9 (06)	59.1 ± 8.2 (14)	656.6 ± 49.8 (08)	217.3 ± 29.5 (14)	835.3 ± 76.8 (09)
7	2-Ethylhexyl Paraben + TP	250 + 0.4	8	88.2 ± 9.9 (11)	52.6 ± 10.4 (20)	618.4 ± 45.4 (07)	211.4 ± 41.8 (20)	856.9 ± 122.2 (14)
8	2-Ethylhexyl Paraben +TP	500 + 0.4	8	93.4 ± 12.3 (13)	59.2 ± 8.5 (14)	**590.1 ± 60.3*** (10)	202.4 ± 42.3 (21)	810.9 ± 129.0 (16)
9	2-Ethylhexyl Paraben +TP	750 + 0.4	8	87.5 ± 10.3 (12)	52.9 ± 5.9 (11)	599.6 ± 73.1 (12)	201.4 ± 34.4 (17)	850.1 ± 133.4 (16)
10	2-Ethylhexyl Paraben +TP	1000 + 0.4	8	96.5 ± 6.2 (06)	50.0 ± 8.9 (18)	**575.9 ± 62.7*** (11)	**174.7 ± 25.6*** (15)	867.9 ± 98.8 (11)
11	FT + TP[c] (Positive Control)	3.0 + 0.4	8	**71.0 ± 7.2†** (10)	**23.4 ± 4.5†** (19)	**296.0 ± 69.1†** (23)	**67.5 ± 14.4†** (21)	**208.9 ± 45.0†** (22)

Abbreviations: SD - standard deviation; LABC - levator ani plus bulbocavernous muscle complex; CV- coefficient of variation; TP - testosterone propionate; FT - flutamide
[a]2-Ethylhexyl Paraben and flutamide were administered via oral gavage; TP was administered via subcutaneous injection
[b]Negative control (agonist positive control)
[c]Positive control
*Statistically significant ($p<0.05$) compared to the vehicle control mean (Dunnett's test)
†Statistically significant ($p<0.05$) compared to the vehicle control mean (t-test)

4.4 Performance Criteria

Agonist

The coefficient of variation (CV) for all androgen-dependent tissues for the negative control group (Group 1) and 1000 mg/kg/day 2-ethylhexyl paraben administered animals (Group 5) were below the maximum allowable limits (Table 8).

Antagonist

All androgen-dependent tissue CVs for the negative control group (Group 6) and 1000 mg/kg/day 2-ethylhexyl paraben plus TP administered animals (Group 10) were below the maximum allowable limit (Table 8).

Table 8. Maximum Allowable and ILS Coefficient of Variations

Tissue	EPA Maximum Allowable CV* Androgen Agonist	Androgen Agonist CV** (Control / High Dose)	EPA Maximum Allowable CV* Androgen Antagonist	Androgen Antagonist CV*** (Control / High Dose)
Glans Penis	22%	11% / 10%	17%	06% / 06%
Cowper's Glands	55%	28% / 29%	35%	14% / 18%
LABC	30%	22% / 11%	20%	08% / 11%
Ventral Prostate	45%	10% / 17%	40%	14% / 15%
Seminal Vesicle	40%	21% / 10%	40%	09% / 11%

*Source: U.S. EPA (2009), OECD (2009)
**See Table 6.
***See Table 7.

CONCLUSION

2-Ethylhexyl paraben was evaluated in the Hershberger Bioassay to investigate its potential androgen agonist/antagonist activity and 5α-reductase inhibition properties using a castrated Sprague Dawley rat model. Tissue weight performance criteria were met. 2-Ethylhexyl paraben was evaluated up to, and including the limit dose (1000 mg/kg/day).

Based on these findings, using the castrated rat model Hershberger Bioassay, the oral gavage administration of 2-ethylhexyl paraben up to the limit dose of 1000 mg/kg/day did not produce any evidence of androgen agonist activity.

In the androgen antagonist assay, statistically significant decreases were noted in two of the five tissue weights (LABC and ventral prostate) which could be indicative of androgen antagonist activity. Relatively small reductions in LABC weights were observed in the top three dose groups (statistically significant at 500 and 1000 mg/kg/day) and ventral prostate weights were statistically significantly decreased at 1000 mg/kg/day. However, it is not clear if the reductions in LABC weights are related to 2-ethyhexyl paraben administration in the three highest dose groups (500, 750, and 1000 mg/kg/day) because they are of similar magnitude, showed no clear dose-response relationship, and are not all statistically significant. Further, glans penis, Cowper's gland, and seminal vesicle weights showed no degree of reduced growth.

REFERENCES

Institute of Laboratory Animal Resources. (2011). *Guide for the Care and Use of Laboratory Animals.* National Academy Press, Washington, DC.

Graves, S. (2001). Dose Formulation Development Study Report Flutamide. Study Project Number Project Number: G004110-AXG. Unpublished study report prepared by Battelle.

OECD (Organisation for Economic Co-operation and Development). (2009). Hershberger Bioassay in Rats: A Short-term Screening Assay for (Anti)Androgenic Properties. OECD Guideline for the Testing of Chemicals 441.

Owens, W., Ashby, J., Odum, J., and Onyon, L. (2003). The OECD Program to Validate the Rat Uterotrophic Bioassay. Phase 2: Dietary Phytoestrogen Analyses. 111: 1559-1567.

Smith, R. (2011). Storage Stability of Testosterone Propionate in Corn Oil. Unpublished study report prepared by Smithers Viscient, LLC. Study No. 13974.6106.

U.S. EPA (Environmental Protection Agency). 2009. Endocrine Disruptor Screening Program Test Guidelines. OPPTS 890.1400: Hershberger Bioassay. EPA 740-C-09-008. Office of Prevention, Pesticides and Toxic Substances, U.S. EPA., Washington, DC.

KEY PERSONNEL

Study Director:	Jeffrey P. Davis, B.S., LATG
Vice President, Research and Development:	Leslie Recio, Ph.D., DABT
Director of Toxicology:	Cheryl Hobbs, Ph.D.
Investigative Toxicology Study Manager:	Eileen Phillips, B.S.
Formulations Manager:	Carol Swartz, Ph.D., DVM
Necropsy Manager:	John Pope, B.S.
Attending Veterinarian:	Alyssa McIntyre, D.V.M., DACLAM
Health and Safety Officer:	Michael Streicker, B.S., LATG

APPENDIX I: Individual Animal Clinical and Cage-Side Observation Data

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	1 1-Hour Post-Dose	2	2 1-Hour Post-Dose	3	3 1-Hour Post-Dose	4
1	m	01	No Abnormalities Detected		X	X	X	X	X	X	X
		02	No Abnormalities Detected		X	X	X	X	X	X	X
		03	No Abnormalities Detected		X	X	X	X	X	X	X
		04	No Abnormalities Detected		X	X	X	X	X	X	X
		05	No Abnormalities Detected		X	X	X	X	X	X	X
		06	No Abnormalities Detected		X	X	X	X	X	X	X
		07	No Abnormalities Detected		X	X	X	X	X	X	X
		08	No Abnormalities Detected		X	X	X	X	X	X	X

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP Group 2 - 250 mg/kg/day 2EHP Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP Group 5 - 1000 mg/kg/day 2EHP Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP Group 8 - 500 mg/kg/day 2EHP Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	4 1-Hour Post-Dose	5	5 1-Hour Post-Dose	6	6 1-Hour Post-Dose	7	7 1-Hour Post-Dose
1	m	01	No Abnormalities Detected		X	X	X	X	X	X	X
		02	No Abnormalities Detected		X	X	X	X	X	X	X
		03	No Abnormalities Detected		X	X	X	X	X	X	X
		04	No Abnormalities Detected		X	X	X	X	X	X	X
		05	No Abnormalities Detected		X	X	X	X	X	X	X
		06	No Abnormalities Detected		X	X	X	X	X	X	X
		07	No Abnormalities Detected		X	X	X	X	X	X	X
		08	No Abnormalities Detected		X	X	X	X	X	X	X

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP Group 2 - 250 mg/kg/day 2EHP Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP Group 5 - 1000 mg/kg/day 2EHP Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP Group 8 - 500 mg/kg/day 2EHP Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	8	8 1-Hour Post-Dose	9	9 1-Hour Post-Dose	10	10 1-Hour Post-Dose	11
1	m	01	No Abnormalities Detected		X	X	X	X	X	X	X
		02	No Abnormalities Detected		X	X	X	X	X	X	X
		03	No Abnormalities Detected		X	X	X	X	X	X	X
		04	No Abnormalities Detected		X	X	X	X	X	X	X
		05	No Abnormalities Detected		X	X	X	X	X	X	X
		06	No Abnormalities Detected		X	X	X	X	X	X	X
		07	No Abnormalities Detected		X	X	X	X	X	X	X
		08	No Abnormalities Detected		X	X	X	X	X	X	X

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP Group 2 - 250 mg/kg/day 2EHP Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP Group 5 - 1000 mg/kg/day 2EHP Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP Group 8 - 500 mg/kg/day 2EHP Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	1 1-Hour Post-Dose	2	2 1-Hour Post-Dose	3	3 1-Hour Post-Dose	4
2	m	09	No Abnormalities Detected		X	X	X	X	X	X	X
		10	No Abnormalities Detected		X	X	X	X	X	X	X
		11	No Abnormalities Detected		X	X	X	X	X	X	X
		12	No Abnormalities Detected		X	X	X	X	X	X	X
		13	No Abnormalities Detected		X	X	X	X	X	X	X
		14	No Abnormalities Detected		X	X	X	X	X	X	X
		15	No Abnormalities Detected		X	X	X	X	X	X	X
		16	No Abnormalities Detected		X	X	X	X	X	X	X

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP
Group 2 - 250 mg/kg/day 2EHP
Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP
Group 5 - 1000 mg/kg/day 2EHP
Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP
Group 8 - 500 mg/kg/day 2EHP
Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP
Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	4 1-Hour Post-Dose	5	5 1-Hour Post-Dose	6	6 1-Hour Post-Dose	7	7 1-Hour Post-Dose
2	m	09	No Abnormalities Detected		X	X	X	X	X	X	X
		10	No Abnormalities Detected		X	X	X	X	X	X	X
		11	No Abnormalities Detected		X	X	X	X	X	X	X
		12	No Abnormalities Detected		X	X	X	X	X	X	X
		13	No Abnormalities Detected		X	X	X	X	X	X	X
		14	No Abnormalities Detected		X	X	X	X	X	X	X
		15	No Abnormalities Detected		X	X	X	X	X	X	X
		16	No Abnormalities Detected		X	X	X	X	X	X	X

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP Group 2 - 250 mg/kg/day 2EHP Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP Group 5 - 1000 mg/kg/day 2EHP Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP Group 8 - 500 mg/kg/day 2EHP Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	8	8 1-Hour Post-Dose	9	9 1-Hour Post-Dose	10	10 1-Hour Post-Dose	11
2	m	09	No Abnormalities Detected		X	X	X	X	X	X	X
		10	No Abnormalities Detected		X	X	X	X	X	X	X
		11	No Abnormalities Detected		X	X	X	X	X	X	X
		12	No Abnormalities Detected		X	X	X	X	X	X	X
		13	No Abnormalities Detected		X	X	X	X	X	X	X
		14	No Abnormalities Detected		X	X	X	X	X	X	X
		15	No Abnormalities Detected		X	X	X	X	X	X	X
		16	No Abnormalities Detected		X	X	X	X	X	X	X

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP
Group 2 - 250 mg/kg/day 2EHP
Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP
Group 5 - 1000 mg/kg/day 2EHP
Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP
Group 8 - 500 mg/kg/day 2EHP
Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP
Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	1 1-Hour Post-Dose	2	2 1-Hour Post-Dose	3	3 1-Hour Post-Dose	4
3	m	17	No Abnormalities Detected		X	X	X	X	X	X	X
		18	No Abnormalities Detected		X	X	X	X	X	X	X
			Scab	Left Ear	.	.	.	.	.	.	.
			Scab	Right Ear	.	.	.	.	.	.	.
		19	No Abnormalities Detected		X	X	X	X	X	X	X
		20	No Abnormalities Detected		X	X	X	X	X	X	X
		21	No Abnormalities Detected		X	X	X	X	X	X	X
		22	No Abnormalities Detected		X	X	X	X	X	X	X
		23	No Abnormalities Detected		X	X	X	X	X	X	X
		24	No Abnormalities Detected		X	X	X	X	X	X	X

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP Group 2 - 250 mg/kg/day 2EHP Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP Group 5 - 1000 mg/kg/day 2EHP Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP Group 8 - 500 mg/kg/day 2EHP Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	4 1-Hour Post-Dose	5	5 1-Hour Post-Dose	6	6 1-Hour Post-Dose	7	7 1-Hour Post-Dose
3	m	17	No Abnormalities Detected		X	X	X	X	X	X	X
		18	No Abnormalities Detected		X	.	.	.	.	X	X
			Scab	Left Ear	.	X	X	X	X	.	.
			Scab	Right Ear	.	X	X	X	X	.	.
		19	No Abnormalities Detected		X	X	X	X	X	X	X
		20	No Abnormalities Detected		X	X	X	X	X	X	X
		21	No Abnormalities Detected		X	X	X	X	X	X	X
		22	No Abnormalities Detected		X	X	X	X	X	X	X
		23	No Abnormalities Detected		X	X	X	X	X	X	X
		24	No Abnormalities Detected		X	X	X	X	X	X	X

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP Group 2 - 250 mg/kg/day 2EHP Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP Group 5 - 1000 mg/kg/day 2EHP Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP Group 8 - 500 mg/kg/day 2EHP Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	8	8 1-Hour Post-Dose	9	9 1-Hour Post-Dose	10	10 1-Hour Post-Dose	11
3	m	17	No Abnormalities Detected		X	X	X	X	X	X	X
		18	No Abnormalities Detected		X	X	X	X	X	X	X
			Scab	Left Ear	.	.	.	.	.	.	.
			Scab	Right Ear	.	.	.	.	.	.	.
		19	No Abnormalities Detected		X	X	X	X	X	X	X
		20	No Abnormalities Detected		X	X	X	X	X	X	X
		21	No Abnormalities Detected		X	X	X	X	X	X	X
		22	No Abnormalities Detected		X	X	X	X	X	X	X
		23	No Abnormalities Detected		X	X	X	X	X	X	X
		24	No Abnormalities Detected		X	X	X	X	X	X	X

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP Group 2 - 250 mg/kg/day 2EHP Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP Group 5 - 1000 mg/kg/day 2EHP Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP Group 8 - 500 mg/kg/day 2EHP Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	1 1-Hour Post-Dose	2	2 1-Hour Post-Dose	3	3 1-Hour Post-Dose	4
4	m	25	No Abnormalities Detected		X	X	X	X	X	X	X
		26	No Abnormalities Detected		X	X	X	X	X	X	X
		27	No Abnormalities Detected		X	X	X	X	X	X	X
		28	No Abnormalities Detected		X	X	X	X	X	X	X
		29	No Abnormalities Detected		X	X	X	X	X	X	X
		30	No Abnormalities Detected		X	X	X	X	X	X	X
		31	No Abnormalities Detected		X	X	X	X	X	X	X
		32	No Abnormalities Detected		X	X	X	X	X	X	X
			Decreased Movement		.	.	.	.	.	.	.

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP
Group 2 - 250 mg/kg/day 2EHP
Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP
Group 5 - 1000 mg/kg/day 2EHP
Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP
Group 8 - 500 mg/kg/day 2EHP
Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP
Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	4 1-Hour Post-Dose	5	5 1-Hour Post-Dose	6	6 1-Hour Post-Dose	7	7 1-Hour Post-Dose
4	m	25	No Abnormalities Detected		X	X	X	X	X	X	X
		26	No Abnormalities Detected		X	X	X	X	X	X	X
		27	No Abnormalities Detected		X	X	X	X	X	X	X
		28	No Abnormalities Detected		X	X	X	X	X	X	X
		29	No Abnormalities Detected		X	X	X	X	X	X	X
		30	No Abnormalities Detected		X	X	X	X	X	X	X
		31	No Abnormalities Detected		X	X	X	X	X	X	X
		32	No Abnormalities Detected		X	X	X	X	X	X	X
			Decreased Movement		.	.	.	.	.	.	.

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP
Group 2 - 250 mg/kg/day 2EHP
Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP
Group 5 - 1000 mg/kg/day 2EHP
Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP
Group 8 - 500 mg/kg/day 2EHP
Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP
Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	8	8 1-Hour Post-Dose	9	9 1-Hour Post-Dose	10	10 1-Hour Post-Dose	11
4	m	25	No Abnormalities Detected		X	X	X	X	X	X	X
		26	No Abnormalities Detected		X	X	X	X	X	X	X
		27	No Abnormalities Detected		X	X	X	X	X	X	X
		28	No Abnormalities Detected		X	X	X	X	X	X	X
		29	No Abnormalities Detected		X	X	X	X	X	X	X
		30	No Abnormalities Detected		X	X	X	X	X	X	X
		31	No Abnormalities Detected		X	X	X	X	X	X	X
		32	No Abnormalities Detected		X	X	X	X	X	.	X
			Decreased Movement		.	.	.	.	.	X	.

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP
Group 2 - 250 mg/kg/day 2EHP
Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP
Group 5 - 1000 mg/kg/day 2EHP
Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP
Group 8 - 500 mg/kg/day 2EHP
Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP
Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	1 1-Hour Post-Dose	2	2 1-Hour Post-Dose	3	3 1-Hour Post-Dose	4
5	m	33	No Abnormalities Detected		X	X	X	X	X	X	X
			Rales		.	.	.	.	.	.	.
		34	No Abnormalities Detected		X	X	X	X	X	X	X
		35	No Abnormalities Detected		X	X	X	X	X	X	X
		36	No Abnormalities Detected		X	X	X	X	X	X	X
		37	No Abnormalities Detected		X	X	X	X	X	X	X
		38	No Abnormalities Detected		X	X	X	X	X	X	X
			Decreased Movement		.	.	.	.	.	.	.
		39	No Abnormalities Detected		X	X	X	X	X	X	X
		40	No Abnormalities Detected		X	X	X	X	X	X	X
			Decreased Movement		.	.	.	.	.	.	.

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP
Group 2 - 250 mg/kg/day 2EHP
Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP
Group 5 - 1000 mg/kg/day 2EHP
Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP
Group 8 - 500 mg/kg/day 2EHP
Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP
Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	4 1-Hour Post-Dose	5	5 1-Hour Post-Dose	6	6 1-Hour Post-Dose	7	7 1-Hour Post-Dose
5	m	33	No Abnormalities Detected		X	.	.	.	.	X	X
			Rales		.	X	X	X	X	.	.
		34	No Abnormalities Detected		X	X	X	X	X	X	X
		35	No Abnormalities Detected		X	X	X	X	X	X	X
		36	No Abnormalities Detected		X	X	X	X	X	X	X
		37	No Abnormalities Detected		X	X	X	X	X	X	X
		38	No Abnormalities Detected		X	X	X	X	X	X	X
			Decreased Movement		.	.	.	.	.	.	.
		39	No Abnormalities Detected		X	X	X	X	X	X	X
		40	No Abnormalities Detected		X	X	X	X	X	X	X
			Decreased Movement		.	.	.	.	.	.	.

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP Group 2 - 250 mg/kg/day 2EHP Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP Group 5 - 1000 mg/kg/day 2EHP Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP Group 8 - 500 mg/kg/day 2EHP Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	8	8 1-Hour Post-Dose	9	9 1-Hour Post-Dose	10	10 1-Hour Post-Dose	11
5	m	33	No Abnormalities Detected		X	X	X	X	X	X	X
			Rales		.	.	.	.	.	.	.
		34	No Abnormalities Detected		X	X	X	X	X	X	X
		35	No Abnormalities Detected		X	X	X	X	X	X	X
		36	No Abnormalities Detected		X	X	X	X	X	X	X
		37	No Abnormalities Detected		X	X	X	X	X	X	X
		38	No Abnormalities Detected		X	X	X	.	X	X	X
			Decreased Movement		.	.	.	X	.	.	.
		39	No Abnormalities Detected		X	X	X	X	X	X	X
		40	No Abnormalities Detected		X	X	X	.	X	X	X
			Decreased Movement		.	.	.	X	.	.	.

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP
Group 2 - 250 mg/kg/day 2EHP
Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP
Group 5 - 1000 mg/kg/day 2EHP
Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP
Group 8 - 500 mg/kg/day 2EHP
Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP
Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	1 1-Hour Post-Dose	2	2 1-Hour Post-Dose	3	3 1-Hour Post-Dose	4
6	m	41	No Abnormalities Detected		X	X	X	X	X	X	X
		42	No Abnormalities Detected		X	X	X	X	X	X	X
		43	No Abnormalities Detected		X	X	X	X	X	X	X
		44	No Abnormalities Detected		X	X	X	X	X	X	X
		45	No Abnormalities Detected		X	X	X	X	X	X	X
		46	No Abnormalities Detected		X	X	X	X	X	X	X
		47	No Abnormalities Detected		X	X	X	X	X	X	X
		48	No Abnormalities Detected		X	X	X	X	X	X	X

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP Group 2 - 250 mg/kg/day 2EHP Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP Group 5 - 1000 mg/kg/day 2EHP Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP Group 8 - 500 mg/kg/day 2EHP Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	4 1-Hour Post-Dose	5	5 1-Hour Post-Dose	6	6 1-Hour Post-Dose	7	7 1-Hour Post-Dose
6	m	41	No Abnormalities Detected		X	X	X	X	X	X	X
		42	No Abnormalities Detected		X	X	X	X	X	X	X
		43	No Abnormalities Detected		X	X	X	X	X	X	X
		44	No Abnormalities Detected		X	X	X	X	X	X	X
		45	No Abnormalities Detected		X	X	X	X	X	X	X
		46	No Abnormalities Detected		X	X	X	X	X	X	X
		47	No Abnormalities Detected		X	X	X	X	X	X	X
		48	No Abnormalities Detected		X	X	X	X	X	X	X

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP Group 2 - 250 mg/kg/day 2EHP Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP Group 5 - 1000 mg/kg/day 2EHP Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP Group 8 - 500 mg/kg/day 2EHP Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	8	8 1-Hour Post-Dose	9	9 1-Hour Post-Dose	10	10 1-Hour Post-Dose	11
6	m	41	No Abnormalities Detected		X	X	X	X	X	X	X
		42	No Abnormalities Detected		X	X	X	X	X	X	X
		43	No Abnormalities Detected		X	X	X	X	X	X	X
		44	No Abnormalities Detected		X	X	X	X	X	X	X
		45	No Abnormalities Detected		X	X	X	X	X	X	X
		46	No Abnormalities Detected		X	X	X	X	X	X	X
		47	No Abnormalities Detected		X	X	X	X	X	X	X
		48	No Abnormalities Detected		X	X	X	X	X	X	X

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP Group 2 - 250 mg/kg/day 2EHP Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP Group 5 - 1000 mg/kg/day 2EHP Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP Group 8 - 500 mg/kg/day 2EHP Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	1 1-Hour Post-Dose	2	2 1-Hour Post-Dose	3	3 1-Hour Post-Dose	4
7	m	49	No Abnormalities Detected		X	X	X	X	X	X	X
		50	No Abnormalities Detected		X	X	X	X	X	X	X
		51	No Abnormalities Detected		X	X	X	X	X	X	X
		52	No Abnormalities Detected		X	X	X	X	X	X	X
		53	No Abnormalities Detected		X	X	X	X	X	X	X
		54	No Abnormalities Detected		X	X	X	X	X	X	X
		55	No Abnormalities Detected		X	X	X	X	X	X	X
		56	No Abnormalities Detected		X	X	X	X	X	X	X

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP Group 2 - 250 mg/kg/day 2EHP Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP Group 5 - 1000 mg/kg/day 2EHP Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP Group 8 - 500 mg/kg/day 2EHP Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	4 1-Hour Post-Dose	5	5 1-Hour Post-Dose	6	6 1-Hour Post-Dose	7	7 1-Hour Post-Dose
7	m	49	No Abnormalities Detected		X	X	X	X	X	X	X
		50	No Abnormalities Detected		X	X	X	X	X	X	X
		51	No Abnormalities Detected		X	X	X	X	X	X	X
		52	No Abnormalities Detected		X	X	X	X	X	X	X
		53	No Abnormalities Detected		X	X	X	X	X	X	X
		54	No Abnormalities Detected		X	X	X	X	X	X	X
		55	No Abnormalities Detected		X	X	X	X	X	X	X
		56	No Abnormalities Detected		X	X	X	X	X	X	X

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP Group 2 - 250 mg/kg/day 2EHP Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP Group 5 - 1000 mg/kg/day 2EHP Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP Group 8 - 500 mg/kg/day 2EHP Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	8	8 1-Hour Post-Dose	9	9 1-Hour Post-Dose	10	10 1-Hour Post-Dose	11
7	m	49	No Abnormalities Detected		X	X	X	X	X	X	X
		50	No Abnormalities Detected		X	X	X	X	X	X	X
		51	No Abnormalities Detected		X	X	X	X	X	X	X
		52	No Abnormalities Detected		X	X	X	X	X	X	X
		53	No Abnormalities Detected		X	X	X	X	X	X	X
		54	No Abnormalities Detected		X	X	X	X	X	X	X
		55	No Abnormalities Detected		X	X	X	X	X	X	X
		56	No Abnormalities Detected		X	X	X	X	X	X	X

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP Group 2 - 250 mg/kg/day 2EHP Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP Group 5 - 1000 mg/kg/day 2EHP Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP Group 8 - 500 mg/kg/day 2EHP Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	1 1-Hour Post-Dose	2	2 1-Hour Post-Dose	3	3 1-Hour Post-Dose	4
8	m	57	No Abnormalities Detected		X	X	X	X	X	X	X
		58	No Abnormalities Detected		X	X	X	X	X	X	X
		59	No Abnormalities Detected		X	X	X	X	X	X	X
			Scab	Dorsal Neck	.	.	.	.	.	.	.
		60	No Abnormalities Detected		X	X	X	X	X	X	X
		61	No Abnormalities Detected		X	X	X	X	X	X	X
		62	No Abnormalities Detected		X	X	X	X	X	X	X
		63	No Abnormalities Detected		X	X	X	X	X	X	X
		64	No Abnormalities Detected		X	X	X	X	X	X	X

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP Group 2 - 250 mg/kg/day 2EHP Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP Group 5 - 1000 mg/kg/day 2EHP Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP Group 8 - 500 mg/kg/day 2EHP Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	4 1-Hour Post-Dose	5	5 1-Hour Post-Dose	6	6 1-Hour Post-Dose	7	7 1-Hour Post-Dose
8	m	57	No Abnormalities Detected		X	X	X	X	X	X	X
		58	No Abnormalities Detected		X	X	X	X	X	X	X
		59	No Abnormalities Detected		X	.	.	.	.	.	.
			Scab	Dorsal Neck	.	X	X	X	X	X	X
		60	No Abnormalities Detected		X	X	X	X	X	X	X
		61	No Abnormalities Detected		X	X	X	X	X	X	X
		62	No Abnormalities Detected		X	X	X	X	X	X	X
		63	No Abnormalities Detected		X	X	X	X	X	X	X
		64	No Abnormalities Detected		X	X	X	X	X	X	X

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP
Group 2 - 250 mg/kg/day 2EHP
Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP
Group 5 - 1000 mg/kg/day 2EHP
Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP
Group 8 - 500 mg/kg/day 2EHP
Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP
Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	8	8 1-Hour Post-Dose	9	9 1-Hour Post-Dose	10	10 1-Hour Post-Dose	11
8	m	57	No Abnormalities Detected		X	X	X	X	X	X	X
		58	No Abnormalities Detected		X	X	X	X	X	X	X
		59	No Abnormalities Detected		.	.	.	.	.	.	.
			Scab	Dorsal Neck	X	X	X	X	X	X	X
		60	No Abnormalities Detected		X	X	X	X	X	X	X
		61	No Abnormalities Detected		X	X	X	X	X	X	X
		62	No Abnormalities Detected		X	X	X	X	X	X	X
		63	No Abnormalities Detected		X	X	X	X	X	X	X
		64	No Abnormalities Detected		X	X	X	X	X	X	X

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP Group 2 - 250 mg/kg/day 2EHP Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP Group 5 - 1000 mg/kg/day 2EHP Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP Group 8 - 500 mg/kg/day 2EHP Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	1 1-Hour Post-Dose	2	2 1-Hour Post-Dose	3	3 1-Hour Post-Dose	4
9	m	65	No Abnormalities Detected		X	X	X	X	X	X	X
		66	No Abnormalities Detected		X	X	X	X	X	X	X
		67	No Abnormalities Detected		X	X	X	X	X	X	X
		68	No Abnormalities Detected		X	X	X	X	X	X	X
		69	No Abnormalities Detected		X	X	X	X	X	X	X
		70	No Abnormalities Detected		X	X	X	X	X	X	X
		71	No Abnormalities Detected		X	X	X	X	X	X	X
		72	No Abnormalities Detected		X	X	X	X	X	X	X

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP
Group 2 - 250 mg/kg/day 2EHP
Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP
Group 5 - 1000 mg/kg/day 2EHP
Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP
Group 8 - 500 mg/kg/day 2EHP
Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP
Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	4 1-Hour Post-Dose	5	5 1-Hour Post-Dose	6	6 1-Hour Post-Dose	7	7 1-Hour Post-Dose
9	m	65	No Abnormalities Detected		X	X	X	X	X	X	X
		66	No Abnormalities Detected		X	X	X	X	X	X	X
		67	No Abnormalities Detected		X	X	X	X	X	X	X
		68	No Abnormalities Detected		X	X	X	X	X	X	X
		69	No Abnormalities Detected		X	X	X	X	X	X	X
		70	No Abnormalities Detected		X	X	X	X	X	X	X
		71	No Abnormalities Detected		X	X	X	X	X	X	X
		72	No Abnormalities Detected		X	X	X	X	X	X	X

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP
Group 2 - 250 mg/kg/day 2EHP
Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP
Group 5 - 1000 mg/kg/day 2EHP
Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP
Group 8 - 500 mg/kg/day 2EHP
Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP
Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	8	8 1-Hour Post-Dose	9	9 1-Hour Post-Dose	10	10 1-Hour Post-Dose	11
9	m	65	No Abnormalities Detected		X	X	X	X	X	X	X
		66	No Abnormalities Detected		X	X	X	X	X	X	X
		67	No Abnormalities Detected		X	X	X	X	X	X	X
		68	No Abnormalities Detected		X	X	X	X	X	X	X
		69	No Abnormalities Detected		X	X	X	X	X	X	X
		70	No Abnormalities Detected		X	X	X	X	X	X	X
		71	No Abnormalities Detected		X	X	X	X	X	X	X
		72	No Abnormalities Detected		X	X	X	X	X	X	X

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP
Group 2 - 250 mg/kg/day 2EHP
Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP
Group 5 - 1000 mg/kg/day 2EHP
Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP
Group 8 - 500 mg/kg/day 2EHP
Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP
Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	1 1-Hour Post-Dose	2	2 1-Hour Post-Dose	3	3 1-Hour Post-Dose	4
10	m	73	No Abnormalities Detected		X	X	X	X	X	X	X
		74	No Abnormalities Detected		X	X	X	X	X	X	X
		75	No Abnormalities Detected		X	X	X	X	X	X	X
		76	No Abnormalities Detected		X	X	X	X	X	X	X
		77	No Abnormalities Detected		X	X	X	X	X	X	X
		78	No Abnormalities Detected		X	X	X	X	X	X	X
			Decreased Movement		.	.	.	.	.	.	.
			Rough Coat		.	.	.	.	.	.	.
		79	No Abnormalities Detected		X	X	X	X	X	X	X
		80	No Abnormalities Detected		X	X	X	X	X	X	X
			Rales		.	.	.	.	.	.	.

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP
Group 2 - 250 mg/kg/day 2EHP
Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP
Group 5 - 1000 mg/kg/day 2EHP
Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP
Group 8 - 500 mg/kg/day 2EHP
Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP
Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	4 1-Hour Post-Dose	5	5 1-Hour Post-Dose	6	6 1-Hour Post-Dose	7	7 1-Hour Post-Dose
10	m	73	No Abnormalities Detected		X	X	X	X	X	X	X
		74	No Abnormalities Detected		X	X	X	X	X	X	X
		75	No Abnormalities Detected		X	X	X	X	X	X	X
		76	No Abnormalities Detected		X	X	X	X	X	X	X
		77	No Abnormalities Detected		X	X	X	X	X	X	X
		78	No Abnormalities Detected		X	X	X	X	X	X	X
			Decreased Movement		.	.	.	.	.	.	.
			Rough Coat		.	.	.	.	.	.	.
		79	No Abnormalities Detected		X	X	X	X	X	X	X
		80	No Abnormalities Detected		X	X	X	X	X	X	X
			Rales		.	.	.	.	.	.	.

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP Group 2 - 250 mg/kg/day 2EHP Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP Group 5 - 1000 mg/kg/day 2EHP Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP Group 8 - 500 mg/kg/day 2EHP Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	8	8 1-Hour Post-Dose	9	9 1-Hour Post-Dose	10	10 1-Hour Post-Dose	11
10	m	73	No Abnormalities Detected		X	X	X	X	X	X	X
		74	No Abnormalities Detected		X	X	X	X	X	X	X
		75	No Abnormalities Detected		X	X	X	X	X	X	X
		76	No Abnormalities Detected		X	X	X	X	X	X	X
		77	No Abnormalities Detected		X	X	X	X	X	X	X
		78	No Abnormalities Detected		X	X	X	X	X	.	.
			Decreased Movement		.	.	.	.	.	X	.
			Rough Coat		.	.	.	.	.	.	X
		79	No Abnormalities Detected		X	X	X	X	X	X	X
		80	No Abnormalities Detected		X	X	.	.	X	X	X
			Rales		.	.	X	X	.	.	.

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP
Group 2 - 250 mg/kg/day 2EHP
Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP
Group 5 - 1000 mg/kg/day 2EHP
Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP
Group 8 - 500 mg/kg/day 2EHP
Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP
Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	1 1-Hour Post-Dose	2	2 1-Hour Post-Dose	3	3 1-Hour Post-Dose	4
11	m	81	No Abnormalities Detected		X	X	X	X	X	X	X
		82	No Abnormalities Detected		X	X	X	X	X	X	X
		83	No Abnormalities Detected		X	X	X	X	X	X	X
		84	No Abnormalities Detected		X	X	X	X	X	X	X
		85	No Abnormalities Detected		X	X	X	X	X	X	X
		86	No Abnormalities Detected		X	X	X	X	X	X	X
		87	No Abnormalities Detected		X	X	X	X	X	X	X
		88	No Abnormalities Detected		X	X	X	X	X	X	X

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP Group 2 - 250 mg/kg/day 2EHP Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP Group 5 - 1000 mg/kg/day 2EHP Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP Group 8 - 500 mg/kg/day 2EHP Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	4 1-Hour Post-Dose	5	5 1-Hour Post-Dose	6	6 1-Hour Post-Dose	7	7 1-Hour Post-Dose
11	m	81	No Abnormalities Detected		X	X	X	X	X	X	X
		82	No Abnormalities Detected		X	X	X	X	X	X	X
		83	No Abnormalities Detected		X	X	X	X	X	X	X
		84	No Abnormalities Detected		X	X	X	X	X	X	X
		85	No Abnormalities Detected		X	X	X	X	X	X	X
		86	No Abnormalities Detected		X	X	X	X	X	X	X
		87	No Abnormalities Detected		X	X	X	X	X	X	X
		88	No Abnormalities Detected		X	X	X	X	X	X	X

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP Group 2 - 250 mg/kg/day 2EHP Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP Group 5 - 1000 mg/kg/day 2EHP Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP Group 8 - 500 mg/kg/day 2EHP Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	8	8 1-Hour Post-Dose	9	9 1-Hour Post-Dose	10	10 1-Hour Post-Dose	11
11	m	81	No Abnormalities Detected		X	X	X	X	X	X	X
		82	No Abnormalities Detected		X	X	X	X	X	X	X
		83	No Abnormalities Detected		X	X	X	X	X	X	X
		84	No Abnormalities Detected		X	X	X	X	X	X	X
		85	No Abnormalities Detected		X	X	X	X	X	X	X
		86	No Abnormalities Detected		X	X	X	X	X	X	X
		87	No Abnormalities Detected		X	X	X	X	X	X	X
		88	No Abnormalities Detected		X	X	X	X	X	X	X

Severity Codes: X = Present

Group 1 - 0 mg/kg/day 2 EHP Group 2 - 250 mg/kg/day 2EHP Group 3 - 500 mg/kg/day 2EHP
Group 4 - 750 mg/kg/day 2EHP Group 5 - 1000 mg/kg/day 2EHP Group 6 - 0 mg/kg/day 2EHP
Group 7 - 250 mg/kg/day 2EHP Group 8 - 500 mg/kg/day 2EHP Group 9 - 750 mg/kg/day 2EHP
Group 10 - 1000 mg/kg/day 2EHP Group 11 - 3.0 mg/kg/day Flutamide

Clinical Observations - Clinical Signs with Site by Animal

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

ƒƒƒ
Key Page
ƒƒƒƒƒƒƒƒ

APPENDIX II: Individual Animal and Group Mean Body Weight Data

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

0 mg/kg/day 2 EHP	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)
	1	2	3	4	5	6	7
01	247.1	252.4	258.7	265.0	269.5	271.0	271.5
02	283.9	286.5	295.1	301.9	306.1	310.7	314.5
03	262.1	264.8	270.5	280.0	285.8	289.7	296.1
04	314.1	319.1	326.9	334.4	341.3	343.6	353.4
05	272.6	280.4	288.0	294.3	298.0	303.3	304.5
06	303.5	312.7	318.4	324.6	330.4	338.9	345.8
07	305.9	303.3	317.3	324.0	333.3	334.5	340.2
08	295.3	288.1	304.2	303.1	306.8	309.2	312.2
Mean	285.56	288.41	297.39	303.41	308.90	312.61	317.28
SD	23.44	22.91	24.11	23.73	24.86	25.32	27.74
N	8	8	8	8	8	8	8

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

0 mg/kg/day 2 EHP	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Bodyweight Gain (g)
	8	9	10	11	1 → 11
01	277.9	269.1	291.2	293.7	46.6
02	324.0	308.0 [1]	336.3	341.7	57.8
03	303.4	308.9	314.8	324.5	62.4
04	363.5	370.0	382.8	397.1	83.0
05	313.3	317.6	319.5	326.4	53.8
06	351.0	354.1	357.6	364.4	60.9
07	346.8	356.7	368.5	378.0	72.1
08	321.1	321.3	331.1	336.8	41.5
Mean	325.13	325.71	337.73	345.33	59.76
SD	27.96	32.98	30.34	33.07	13.34
N	8	8	8	8	8

1 [RC:Water bottle issue.]

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

250 mg/kg/day 2EHP	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)
	1	2	3	4	5	6	7
09	290.9	294.8	305.6	312.1	314.4	327.4	329.4
10	251.3	250.2	261.8	266.6	266.5	273.0	277.2
11	251.6	256.0	261.8	269.8	275.0	280.1	283.0
12	291.3	298.1	303.9	313.3	318.8	324.3	329.2
13	322.4	330.0	335.5	346.3	350.1	355.2	361.8
14	229.3	232.7	236.4	244.6	251.0	249.7	257.9
15	277.1	281.0	292.4	297.7	307.0	309.9	321.5
16	288.8	294.4	304.8	308.1	313.8	316.8	324.1
Mean	275.34	279.65	287.78	294.81	299.58	304.55	310.51
SD	29.66	31.52	31.97	32.56	32.64	34.35	34.39
N	8	8	8	8	8	8	8
%Diff	-3.6	-3.0	-3.2	-2.8	-3.0	-2.6	-2.1

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

250 mg/kg/day 2EHP	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Bodyweight Gain (g)
	8	9	10	11	1 → 11
09	339.6	349.5	355.4	366.7	75.8
10	284.8	284.8	291.8	296.2	44.9
11	291.9	290.7	296.0	303.5	51.9
12	335.4	338.4	344.7	354.4	63.1
13	369.4	376.1	384.0	391.7	69.3
14	262.2	267.8	269.9	275.6	46.3
15	329.1	336.9	344.5	351.4	74.3
16	326.5	335.6	342.1	346.1	57.3
Mean	317.36	322.48	328.55	335.70	60.36
SD	34.85	37.15	38.44	39.66	12.18
N	8	8	8	8	8
%Diff	-2.4	-1.0	-2.7	-2.8	.

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

500 mg/kg/day 2EHP	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)
	1	2	3	4	5	6	7
17	307.3	312.4	318.3	325.6	334.5	334.2	337.0
18	276.5	279.7	284.7	291.9	297.1	301.5	303.3
19	290.4	291.6	301.3	307.7	313.3	315.8	321.9
20	296.2	298.5	305.1	308.4	313.6	318.2	322.5
21	287.8	294.4	305.6	311.0	316.6	323.1	328.7
22	257.2	265.9	273.6	278.6	284.9	292.4	298.9
23	263.3	268.4	279.5	283.2	288.0	293.7	300.7
24	281.3	287.1	299.9	301.5	307.8	312.3	318.4
Mean	282.50	287.25	296.00	300.99	306.98	311.40	316.43
SD	16.65	15.57	15.20	15.61	16.39	14.62	14.00
N	8	8	8	8	8	8	8
%Diff	-1.1	-0.4	-0.5	-0.8	-0.6	-0.4	-0.3

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

500 mg/kg/day 2EHP	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Bodyweight Gain (g)
	8	9	10	11	1 → 11
17	344.8	350.1	357.4	368.2	60.9
18	311.4	313.3	318.8	331.3	54.8
19	327.1	332.2	339.9	348.4	58.0
20	329.2	330.4	340.3	341.9	45.7
21	330.9	342.3	344.6	351.2	63.4
22	301.1	312.1	321.1	329.2	72.0
23	291.3	313.1	322.3	330.9	67.6
24	320.1	329.2	341.2	347.1	65.8
Mean	319.49	327.84	335.70	343.53	61.03
SD	17.41	14.18	13.61	13.21	8.24
N	8	8	8	8	8
%Diff	-1.7	0.7	-0.6	-0.5	.

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

750 mg/kg/day 2EHP	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)
	1	2	3	4	5	6	7
25	305.7	311.2	319.0	326.4	329.6	336.8	342.3
26	281.1	283.1	288.7	296.2	302.4	306.5	309.9
27	271.8	275.2	278.9	284.6	288.5	292.4	296.7
28	239.3	242.2	249.3	256.2	262.8	264.9	271.8
29	280.0	285.2	292.2	297.7	305.0	305.3	314.8
30	256.0	261.0	271.3	275.8	283.5	289.3	299.3
31	300.6	305.3	309.7	311.1	320.0	323.9	327.2
32	256.9	261.2	267.5	268.5	275.1	280.7	284.9
Mean	273.93	278.05	284.58	289.56	295.86	299.98	305.86
SD	22.75	23.26	22.79	23.00	22.61	23.21	22.70
N	8	8	8	8	8	8	8
%Diff	-4.1	-3.6	-4.3	-4.6	-4.2	-4.0	-3.6

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

750 mg/kg/day 2EHP	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Bodyweight Gain (g)
	8	9	10	11	1 → 11
25	347.3	353.8	360.4	364.3	58.6
26	310.7	317.9	317.6	325.7	44.6
27	305.5	309.8	315.6	322.2	50.4
28	278.9	285.9	290.2	298.7	59.4
29	321.7	327.0	331.5	335.6	55.6
30	304.1	305.7	309.4	308.5	52.5
31	333.3	337.8	345.5	345.9	45.3
32	289.9	296.7	305.6	295.5	38.6
Mean	311.43	316.83	321.98	324.55	50.63
SD	22.30	22.19	22.73	23.71	7.35
N	8	8	8	8	8
%Diff	-4.2	-2.7	-4.7	-6.0	.

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

1000 mg/kg/day 2EHP	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)
	1	2	3	4	5	6	7
33	269.5	270.0	272.4	277.4	254.1	261.2	267.4
34	296.5	291.5	299.9	306.4	309.9	313.2	321.2
35	269.0	275.1	282.2	285.9	290.8	293.4	300.2
36	264.9	262.9	268.0	275.4	280.3	281.2	287.2
37	292.2	294.1	302.2	304.9	309.2	313.6	320.3
38	313.5	312.2	320.5	326.1	330.4	321.7	332.0
39	300.2	300.6	303.1	316.2	320.5	318.8	318.2
40	243.1	242.7	254.7	258.7	265.5	267.6	271.6
Mean	281.11	281.14	287.88	293.88	295.09	296.34	302.26
SD	23.18	22.65	22.08	23.08	27.00	23.99	24.52
N	8	8	8	8	8	8	8
%Diff	-1.6	-2.5	-3.2	-3.1	-4.5	-5.2	-4.7

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

1000 mg/kg/day 2EHP	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Bodyweight Gain (g)
	8	9	10	11	1 → 11
33	271.1	273.7	281.5	290.0	20.5
34	328.2	331.5	334.6	345.1	48.6
35	304.8	305.6	311.0	320.3	51.3
36	290.9	299.6	305.1	312.9	48.0
37	326.0	333.8	334.1	332.1	39.9
38	330.2	329.2	336.0	342.5	29.0
39	323.5	325.3	335.9	342.9	42.7
40	275.4	283.6	265.8	276.3	33.2
Mean	306.26	310.29	313.00	320.26	39.15
SD	24.40	23.22	27.39	25.82	10.79
N	8	8	8	8	8
%Diff	-5.8	-4.7	-7.3	-7.3	.

Comments and Markers

Page	Day	Group	Sex	Subject	Measurement	Type	Marker
2	9	1	Male	02	Body Weight	Result	

Comment: Water bottle issue.

Key Page

Measurement Descriptions

Headings Used	Description
Body Weight	Bodyweight
Bodyweight Gain	Bodyweight Gain

Unit Descriptions

Headings Used	Description
g	g

Measurement/Statistics

Measurement	Descriptive
Body Weight	Mean
	Standard Deviation
	Count (N)
	% Difference from Control
Bodyweight Gain	Mean
	Standard Deviation
	Count (N)

Group Information

Short Name	Long Name	Report Headings 1-4		
1	Vehicle Control	0	mg/kg/day	2 EHP
2	Agonist- low dose	250	mg/kg/day	2EHP
3	Agonist- mid-low	500	mg/kg/day	2EHP
4	Agonist- mid-high	750	mg/kg/day	2EHP
5	Agonist-high dos	1000	mg/kg/day	2EHP

Key Page

Comment Abbreviations

RC = Result Comment

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

0 mg/kg/day 2EHP plus TP	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)
	1	2	3	4	5	6	7
41	302.4	307.7	324.2	335.9	348.8	356.8	365.6
42	292.5	298.9	309.2	320.5	328.5	336.2	343.6
43	249.3	259.3	266.5	274.6	285.8	292.2	298.3
44	248.7	256.3	267.1	273.3	285.2	290.8	298.6
45	275.1	287.1	295.3	302.3	314.8	324.4	332.9
46	290.6	299.5	303.8	316.6	320.8	331.7	332.8
47	279.4	291.7	305.6	319.5	300.5	332.6	341.9
48	294.1	301.3	316.7	329.3	311.6	337.4	347.9
Mean	279.01	287.73	298.55	309.00	312.00	325.26	332.70
SD	20.38	19.49	21.40	23.72	21.54	22.80	23.49
N	8	8	8	8	8	8	8

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

0 mg/kg/day 2EHP plus TP	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Bodyweight Gain (g)
	8	9	10	11	1 → 11
41	376.1	386.2	397.5	409.1	106.7
42	357.5	365.0	377.8	385.8	93.3
43	307.7	310.7	326.3	330.5	81.2
44	311.3	312.9	323.7	325.6	76.9
45	344.5	356.1	360.2	369.8	94.7
46	343.0	352.4	359.7	364.5	73.9
47	355.6	366.9	376.2	386.2	106.8
48	363.5	371.4	380.8	391.1	97.0
Mean	344.90	352.70	362.78	370.33	91.31
SD	24.23	27.21	26.21	29.39	12.75
N	8	8	8	8	8

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

250 mg/kg/day 2EHP plus TP	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)
	1	2	3	4	5	6	7
49	283.8	291.1	301.4	313.6	322.6	329.1	336.6
50	277.4	286.2	294.5	305.5	316.3	321.8	329.8
51	257.0	261.4	274.2	283.1	293.8	295.3	306.3
52	263.0	266.4	274.5	281.5	289.0	288.1	296.4
53	249.5	250.1	250.5	255.7	289.9	301.5	313.8
54	241.3	242.5	254.6	262.9	270.3	275.7	285.6
55	312.5	319.7	335.1	343.3	346.6	351.6	375.0
56	303.3	309.6	314.2	331.2	331.6	335.7	345.4
Mean	273.48	278.38	287.38	297.10	307.51	312.35	323.61
SD	25.43	27.84	29.32	31.54	25.74	26.17	29.04
N	8	8	8	8	8	8	8
%Diff	-2.0	-3.2	-3.7	-3.9	-1.4	-4.0	-2.7

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

250 mg/kg/day 2EHP plus TP	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Bodyweight Gain (g)
	8	9	10	11	1 → 11
49	347.7	352.8	363.7	372.3	88.5
50	340.5	351.4	361.6	372.3	94.9
51	314.6	322.1	330.2	339.0	82.0
52	301.1	306.9	316.5	323.9	60.9
53	322.3	333.9	340.7	348.2	98.7
54	290.9	303.6	311.4	315.7	74.4
55	374.7	386.7	393.3	399.2	86.7
56	353.5	360.0	368.8	375.4	72.1
Mean	330.66	339.68	348.28	355.75	82.28
SD	28.36	28.42	28.33	28.73	12.59
N	8	8	8	8	8
%Diff	-4.1	-3.7	-4.0	-3.9	.

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

500 mg/kg/day 2EHP plus TP	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)
	1	2	3	4	5	6	7
57	260.0	263.1	273.9	288.8	297.7	310.2	313.2
58	300.5	306.7	313.0	325.3	333.3	339.6	344.8
59	279.6	292.5	301.2	318.6	324.0	337.7	345.9
60	242.6	255.0	262.6	271.3	281.1	294.4	300.6
61	250.9	258.3	269.8	280.5	286.3	291.4	301.7
62	283.3	291.6	297.9	311.0	319.6	328.6	340.3
63	283.9	289.0	304.1	317.7	328.6	331.2	344.7
64	301.5	303.7	319.1	328.4	338.1	345.2	355.6
Mean	275.29	282.49	292.70	305.20	313.59	322.29	330.85
SD	21.96	20.63	21.12	21.84	22.08	20.91	22.01
N	8	8	8	8	8	8	8
%Diff	-1.3	-1.8	-2.0	-1.2	0.5	-0.9	-0.6

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

500 mg/kg/day 2EHP plus TP	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Bodyweight Gain (g)
	8	9	10	11	1 → 11
57	324.2	332.3	339.6	351.3	91.3
58	353.8	360.1	366.8	376.8	76.3
59	357.2	365.6	374.0	378.7	99.1
60	312.1	328.4	325.5	324.2	81.6
61	307.4	318.4	327.9	332.6	81.7
62	351.5	360.1	368.5	374.1	90.8
63	354.2	356.4	371.0	378.6	94.7
64	368.2	375.3	385.9	393.7	92.2
Mean	341.08	349.58	357.40	363.75	88.46
SD	22.98	20.37	22.96	24.81	7.75
N	8	8	8	8	8
%Diff	-1.1	-0.9	-1.5	-1.8	.

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

750 mg/kg/day 2EHP plus TP	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)
	1	2	3	4	5	6	7
65	245.0	248.8	260.2	269.2	273.6	279.2	285.7
66	285.1	289.3	303.5	310.7	323.6	335.1	337.9
67	251.7	253.0	256.0	260.9	267.2	276.7	281.7
68	275.6	280.9	293.4	304.9	315.6	326.4	337.2
69	289.4	294.9	304.4	316.4	320.5	328.4	340.9
70	266.3	269.0	280.9	289.0	295.4	307.8	319.2
71	259.1	265.1	278.1	287.5	294.8	303.5	307.3
72	322.5	331.0	338.6	348.9	359.0	365.0	346.6
Mean	274.34	279.00	289.39	298.44	306.21	315.26	319.56
SD	24.88	26.59	26.80	28.12	29.79	29.63	25.54
N	8	8	8	8	8	8	8
%Diff	-1.7	-3.0	-3.1	-3.4	-1.9	-3.1	-3.9

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

750 mg/kg/day 2EHP plus TP	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Bodyweight Gain (g)
	8	9	10	11	1 → 11
65	291.2	303.7	312.0	318.8	73.8
66	352.8	365.4	367.6	378.4	93.3
67	294.5	302.6	310.8	321.2	69.5
68	348.7	361.1	370.8	387.4	111.8
69	345.9	358.8	362.6	371.7	82.3
70	321.6	335.4	343.7	354.3	88.0
71	320.1	330.3	335.7	345.0	85.9
72	352.3	361.6	368.3	378.8	56.3
Mean	328.39	339.86	346.44	356.95	82.61
SD	25.45	26.03	24.96	26.63	16.68
N	8	8	8	8	8
%Diff	-4.8	-3.6	-4.5	-3.6	.

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

1000 mg/kg/day 2EHP plus TP	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)
	1	2	3	4	5	6	7
73	302.8	311.8	316.2	328.0	341.8	351.8	359.7
74	242.2	247.8	252.6	260.9	272.3	276.7	280.1
75	250.2	257.3	259.9	266.2	275.5	283.2	292.7
76	278.3	282.7	286.6	299.3	303.6	309.9	315.9
77	292.2	299.9	311.7	316.7	325.9	334.8	346.2
78	299.4	302.3	313.2	320.7	328.1	332.0	338.4
79	265.7	271.5	282.6	290.4	298.4	302.5	309.2
80	282.1	288.9	297.9	306.5	316.7	325.1	325.6
Mean	276.61	282.78	290.09	298.59	307.79	314.50	320.98
SD	22.30	22.53	24.29	24.73	25.03	26.17	26.98
N	8	8	8	8	8	8	8
%Diff	-0.9	-1.7	-2.8	-3.4	-1.4	-3.3	-3.5

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

1000 mg/kg/day 2EHP plus TP	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Bodyweight Gain (g)
	8	9	10	11	1 → 11
73	370.8	383.7	388.5	399.8	97.0
74	289.8	295.7	301.1	310.2	68.0
75	300.1	311.7	314.4	324.1	73.9
76	320.5	327.8	336.5	343.6	65.3
77	354.2	364.6	373.2	381.9	89.7
78	339.9	349.7	348.6	316.9	17.5
79	320.7	327.3	331.6	338.9	73.2
80	317.4	318.4	326.2	336.2	54.1
Mean	326.68	334.86	340.01	343.95	67.34
SD	27.01	29.12	29.20	31.42	24.26
N	8	8	8	8	8
%Diff	-5.3	-5.1	-6.3	-7.1	.

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

3.0 mg/kg/day Flutamide plus TP	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)
	1	2	3	4	5	6	7
81	261.4	267.2	278.9	288.8	294.5	303.5	309.1
82	301.2	298.8	314.8	321.4	330.2	335.3	341.2
83	310.3	311.0	323.9	327.6	324.6	330.0	343.3
84	284.1	289.6	294.6	303.9	304.4	315.4	320.5
85	292.2	301.3	316.6	323.3	325.5	333.3	349.3
86	240.2	247.4	257.1	265.6	270.4	280.2	294.5
87	277.2	277.7	292.6	297.3	307.6	314.9	317.0
88	257.5	265.3	277.0	288.5	295.2	302.8	309.4
Mean	278.01	282.29	294.44	302.05	306.55	314.43	323.04
SD	23.74	21.60	23.04	21.36	20.11	18.75	19.52
N	8	8	8	8	8	8	8
%Diff	-0.4	-1.9	-1.4	-2.2	-1.7	-3.3	-2.9

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

3.0 mg/kg/day Flutamide plus TP	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Bodyweight Gain (g)
	8	9	10	11	1 → 11
81	321.4	327.9	338.7	346.9	85.5
82	354.3	357.9	370.6	376.4	75.2
83	348.2	359.9	369.7	379.1	68.8
84	328.2	333.7	344.5	351.0	66.9
85	355.6	368.1	381.0	389.1	96.9
86	304.9	313.9	325.3	337.3	97.1
87	328.0	338.0	342.7	349.9	72.7
88	313.6	323.5	330.4	339.5	82.0
Mean	331.78	340.36	350.36	358.65	80.64
SD	19.02	19.45	20.63	19.84	11.85
N	8	8	8	8	8
%Diff	-3.8	-3.5	-3.4	-3.2	.

Key Page

Measurement Descriptions

Headings Used	Description
Body Weight	Bodyweight
Bodyweight Gain	Bodyweight Gain

Unit Descriptions

Headings Used	Description
g	g

Measurement/Statistics

Measurement	Descriptive
Body Weight	Mean
	Standard Deviation
	Count (N)
	% Difference from Control
Bodyweight Gain	Mean
	Standard Deviation
	Count (N)

Group Information

Short Name	Long Name	Report Headings 1-4			
6	Antagonist- Neg Cont	0	mg/kg/day	2EHP	plus TP
7	Antagonist-low dose	250	mg/kg/day	2EHP	plus TP
8	Antagonist- mid-low	500	mg/kg/day	2EHP	plus TP
9	Antagonist- mid-high	750	mg/kg/day	2EHP	plus TP
10	Antagonist high dose	1000	mg/kg/day	2EHP	plus TP

Key Page

Group Information (Continued)

Short Name	Long Name	Report Headings 1-4			
11	Antagonist positive	3.0	mg/kg/day	Flutamide	plus TP

APPENDIX III: Individual Animal Necropsy Gross Observations

Pathology - Intergroup Comparison of Pathology Observations

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Removal Reason: ALL	Male						
	0 mg/kg/day 2 EHP	250 mg/kg/day 2EHP	500 mg/kg/day 2EHP	750 mg/kg/day 2EHP	1000 mg/kg/day 2EHP	0 mg/kg/day 2EHP plus TP	
Number of Animals:	8	8	8	8	8	8	
Number of Completed Animals:	8	8	8	8	8	8	
SEMINAL VESICLES							
Submitted	8	8	8	8	8	8	
Normal	8	8	8	8	8	8	
GLANS PENIS							
Submitted	8	8	8	8	8	8	
Normal	8	8	8	8	8	8	
LABC MUSCLE							
Submitted	8	8	8	8	8	8	
Normal	8	8	8	8	8	8	
COWPERS GLANDS							
Submitted	8	8	8	8	8	8	
Normal	8	8	8	8	8	8	
PROSTATE GLAND, VENTRAL							
Submitted	8	8	8	8	8	8	
Normal	8	8	8	8	8	8	

Pathology - Intergroup Comparison of Pathology Observations

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Removal Reason: ALL	Male					
	250 mg/kg/day 2EHP plus TP	500 mg/kg/day 2EHP plus TP	750 mg/kg/day 2EHP plus TP	1000 mg/kg/day 2EHP plus TP	3.0 mg/kg/day Flutamide plus TP	
Number of Animals:	8	8	8	8	8	
Number of Completed Animals:	8	8	8	8	8	
SEMINAL VESICLES						
Submitted	8	8	8	8	8	
Normal	8	8	8	8	8	
GLANS PENIS						
Submitted	8	8	8	8	8	
Normal	8	8	8	8	8	
LABC MUSCLE						
Submitted	8	8	8	8	8	
Normal	8	8	8	8	8	
COWPERS GLANDS						
Submitted	8	8	8	8	8	
Normal	8	8	8	8	8	
PROSTATE GLAND, VENTRAL						
Submitted	8	8	8	8	8	
Normal	8	8	8	8	8	

Key Page

Measurement/Statistics

Measurement	Descriptive	Comparative	Arithmetic/Adjusted	Transformation
Pathology Observation	Count Positives			

Group Information

Short Name	Long Name	Report Headings			
1	Vehicle Control	0	mg/kg/day	2 EHP	
2	Agonist- low dose	250	mg/kg/day	2EHP	
3	Agonist- mid-low	500	mg/kg/day	2EHP	
4	Agonist- mid-high	750	mg/kg/day	2EHP	
5	Agonist-high dos	1000	mg/kg/day	2EHP	
6	Antagonist- Neg Cont	0	mg/kg/day	2EHP	plus TP
7	Antagonist-low dose	250	mg/kg/day	2EHP	plus TP
8	Antagonist- mid-low	500	mg/kg/day	2EHP	plus TP
9	Antagonist- mid-high	750	mg/kg/day	2EHP	plus TP
10	Antagonist high dose	1000	mg/kg/day	2EHP	plus TP
11	Antagonist positive	3.0	mg/kg/day	Flutamide	plus TP

Key Page

Removal Reason Grouping

Grouping Name	Abbreviation	Removal Reasons
Killed Terminal	Term	Killed Terminal
Killed Clinical	Clin	Killed Clinical
Killed Interim	Int	Killed Interim
Killed Moribund	Mori	Killed Moribund
Found Dead	FD	Found Dead
Mechanically Killed	Mech	Mechanically Killed
Other, See Text Comments	Oth	Other, See Text Comments

APPENDIX IV: Individual Animal and Group Mean Tissue Weight Data

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

0 mg/kg/day 2 EHP	Glans Penis Weight (g)	VP Gland Weight (g)	SV Weight (g)	Cowper's Gland Weight (g)	LABC Weight (g)
	11	11	11	11	11
01	0.0697	0.0204	0.0545 <	0.0114	0.1876
02	0.0635	0.0202	0.0942	0.0130	0.0957 <
03	0.0577	0.0223	0.0493 <	0.0069	0.1826
04	0.0524	0.0241	0.0796	0.0067	0.2243
05	0.0634	0.0189	0.0707	0.0068	0.1928
06	0.0493	0.0239	0.0696	0.0107	0.2004
07	0.0555	0.0183	0.0687	0.0115	0.2315
08	0.0625	0.0200	0.0625	0.0071	0.2109
Mean	0.05925	0.02101	0.06864	0.00926	0.19073
SD	0.00673	0.00218	0.01409	0.00263	0.04209
N	8	8	8	8	8

General Footnote: [< > markers indicates weight below/above flagging range. Data is valid.]

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

250 mg/kg/day 2EHP	Glans Penis Weight (g)	VP Gland Weight (g)	SV Weight (g)	Cowper's Gland Weight (g)	LABC Weight (g)
	11	11	11	11	11
09	0.0667	0.0279	0.1038	0.0129	0.2368
10	0.0568	0.0143	0.0618	0.0142	0.1681
11	0.0549	0.0190	0.0513 <	0.0061	0.1358
12	0.0617	0.0215	0.0698	0.0100	0.2161
13	0.0506	0.0175	0.0733	0.0079	0.2132
14	0.0474	0.0127	0.0437 <	0.0064	0.1679
15	0.0544	0.0225	0.0788	0.0050	0.2281
16	0.0538	0.0183	0.0552 <	0.0078	0.1748
Mean	0.05579	0.01921	0.06721	0.00879	0.19260
SD	0.00608	0.00481	0.01888	0.00331	0.03574
N	8	8	8	8	8

General Footnote: [< > markers indicates weight below/above flagging range. Data is valid.]

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

500 mg/kg/day 2EHP	Glans Penis Weight (g)	VP Gland Weight (g)	SV Weight (g)	Cowper's Gland Weight (g)	LABC Weight (g)
	11	11	11	11	11
17	0.0656	0.0288	0.0883	0.0078	0.2173
18	0.0574	0.0189	0.0641	0.0102	0.2480
19	0.0539	0.0205	0.0826	0.0123	0.2649
20	0.0644	0.0145	0.0665	0.0049	0.1860
21	0.0515	0.0186	0.0849	0.0102	0.1625
22	0.0518	0.0174	0.0731	0.0107	0.2202
23	0.0548	0.0188	0.0429 <	0.0082	0.1823
24	0.0535	0.0222	0.0764	0.0092	0.2092
Mean	0.05661	0.01996	0.07235	0.00919	0.21130
SD	0.00550	0.00421	0.01466	0.00225	0.03423
N	8	8	8	8	8

General Footnote: [< > markers indicates weight below/above flagging range. Data is valid.]

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

750 mg/kg/day 2EHP	Glans Penis Weight (g)	VP Gland Weight (g)	SV Weight (g)	Cowper's Gland Weight (g)	LABC Weight (g)
	11	11	11	11	11
25	0.0611	0.0226	0.0723	0.0084	0.2051
26	0.0684	0.0224	0.0816	0.0132	0.2034
27	0.0646	0.0178	0.0592 <	0.0063	0.1971
28	0.0646	0.0175	0.0643	0.0052	0.2113
29	0.0515	0.0185	0.0657	0.0128	0.2074
30	0.0458	0.0163	0.0523 <	0.0078	0.2092
31	0.0534	0.0183	0.0673	0.0116	0.1960
32	0.0591	0.0230	0.0740	0.0099	0.1831
Mean	0.05856	0.01955	0.06709	0.00940	0.20158
SD	0.00771	0.00267	0.00907	0.00297	0.00922
N	8	8	8	8	8

General Footnote: [< > markers indicates weight below/above flagging range. Data is valid.]

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

1000 mg/kg/day 2EHP	Glans Penis Weight (g)	VP Gland Weight (g)	SV Weight (g)	Cowper's Gland Weight (g)	LABC Weight (g)
	11	11	11	11	11
33	0.0563	0.0172	0.0536 <	0.0083	0.2172
34	0.0553	0.0236	0.0647	0.0129	0.2443
35	0.0714	0.0184	0.0563 <	0.0067	0.2043
36	0.0573	0.0154	0.0491 <	0.0102	0.1965
37	0.0626	0.0226	0.0611	0.0090	0.2205
38	0.0585	0.0237	0.0634	0.0114	0.2031
39	0.0638	0.0219	0.0641	0.0066	0.1803
40	0.0699	0.0171	0.0622	0.0055	0.1724
Mean	0.06189	0.01999	0.05931	0.00883	0.20483
SD	0.00616	0.00332	0.00568	0.00256	0.02294
N	8	8	8	8	8

General Footnote: [< > markers indicates weight below/above flagging range. Data is valid.]

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

0 mg/kg/day 2EHP plus TP	Glans Penis Weight (g)	VP Gland Weight (g)	SV Weight (g)	Cowper's Gland Weight (g)	LABC Weight (g)
	11	11	11	11	11
41	0.0880	0.2062	0.9290	0.0732 >	0.6478 >
42	0.0914	0.2006	0.9391	0.0538 >	0.6861 >
43	0.1022	0.2383	0.8365	0.0668 >	0.6660 >
44	0.0865	0.1920	0.7965	0.0579 >	0.5575
45	0.0946	0.2065	0.8027	0.0532 >	0.6709 >
46	0.0934	0.2825	0.8530	0.0640 >	0.7237 >
47	0.0931	0.2084	0.6982	0.0487 >	0.6794 >
48	0.1027	0.2042	0.8276	0.0551 >	0.6214 >
Mean	0.09399	0.21734	0.83533	0.05909	0.65660
SD	0.00590	0.02952	0.07680	0.00820	0.04975
N	8	8	8	8	8

General Footnote: [< > markers indicates weight below/above flagging range. Data is valid.]

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

250 mg/kg/day 2EHP plus TP	Glans Penis Weight (g)	VP Gland Weight (g)	SV Weight (g)	Cowper's Gland Weight (g)	LABC Weight (g)
	11	11	11	11	11
49	0.0866	0.1966	0.9463	0.0552 >	0.6310 >
50	0.0919	0.1757	0.9216	0.0664 >	0.6237 >
51	0.1007	0.2174	0.7602	0.0398 >	0.6236 >
52	0.0801	0.1962	0.8981	0.0482 >	0.5683
53	0.0798	0.1599	0.6064	0.0423 >	0.5574
54	0.0905	0.2112	0.8964	0.0540 >	0.6479 >
55	0.0743	0.2391	0.8397	0.0467 >	0.5955
56	0.1018	0.2953 >	0.9861	0.0680 >	0.6994 >
Mean	0.08821	0.21143	0.85685	0.05258	0.61835
SD	0.00994	0.04178	0.12220	0.01042	0.04536
N	8	8	8	8	8

General Footnote: [< > markers indicates weight below/above flagging range. Data is valid.]

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

500 mg/kg/day 2EHP plus TP	Glans Penis Weight (g)	VP Gland Weight (g)	SV Weight (g)	Cowper's Gland Weight (g)	LABC Weight (g)
	11	11	11	11	11
57	0.0953	0.2318	0.7485	0.0561 >	0.5779
58	0.1119 >	0.2257	0.7849	0.0725 >	0.6616 >
59	0.1090	0.2391	1.0239	0.0625 >	0.5958
60	0.0969	0.1452	0.8749	0.0585 >	0.6194 >
61	0.0887	0.1539	0.6267	0.0453 >	0.4771
62	0.0847	0.1976	0.9416	0.0607 >	0.5365
63	0.0796	0.1688	0.7089	0.0512 >	0.6489 >
64	0.0807	0.2572	0.7777	0.0665 >	0.6034 >
Mean	0.09335	0.20241	0.81089	0.05916	0.59008
SD	0.01225	0.04231	0.12904	0.00854	0.06027
N	8	8	8	8	8

General Footnote: [< > markers indicates weight below/above flagging range. Data is valid.]

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

750 mg/kg/day 2EHP plus TP	Glans Penis Weight (g)	VP Gland Weight (g)	SV Weight (g)	Cowper's Gland Weight (g)	LABC Weight (g)
	11	11	11	11	11
65	0.0950	0.1496	0.5743	0.0487 >	0.5459
66	0.1007	0.2644	1.0019	0.0557 >	0.6702 >
67	0.0944	0.2116	0.8392	0.0565 >	0.5114
68	0.0726	0.1687	0.9445	0.0501 >	0.6663 >
69	0.0791	0.1947	0.7649	0.0587 >	0.6257 >
70	0.0777	0.1966	0.8593	0.0603 >	0.5862
71	0.0955	0.2049	0.8733	0.0428 >	0.5050
72	0.0852	0.2210	0.9430	0.0502 >	0.6864 >
Mean	0.08753	0.20144	0.85005	0.05288	0.59964
SD	0.01025	0.03441	0.13344	0.00590	0.07313
N	8	8	8	8	8

General Footnote: [< > markers indicates weight below/above flagging range. Data is valid.]

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

1000 mg/kg/day 2EHP plus TP	Glans Penis Weight (g)	VP Gland Weight (g)	SV Weight (g)	Cowper's Gland Weight (g)	LABC Weight (g)
	11	11	11	11	11
73	0.0959	0.1645	0.8130	0.0528 >	0.6096 >
74	0.0939	0.1805	0.7587	0.0437 >	0.4855
75	0.1010	0.1567	0.7930	0.0572 >	0.4904
76	0.1020	0.2156	1.0166	0.0653 >	0.6130 >
77	0.0991	0.2034	0.9373	0.0522 >	0.5888
78	0.0964	0.1733	0.8038	0.0362 >	0.6262 >
79	0.0830	0.1689	0.8294	0.0461 >	0.5426
80	0.1009	0.1348	0.9912	0.0465 >	0.6514 >
Mean	0.09653	0.17471	0.86788	0.05000	0.57594
SD	0.00616	0.02560	0.09876	0.00891	0.06268
N	8	8	8	8	8

General Footnote: [< > markers indicates weight below/above flagging range. Data is valid.]

Generalised Results - Animals by Mixed Parameter / Time

10005.0103 - The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

3.0 mg/kg/day Flutamide plus TP	Glans Penis Weight (g)	VP Gland Weight (g)	SV Weight (g)	Cowper's Gland Weight (g)	LABC Weight (g)
	11	11	11	11	11
81	0.0701	0.0477	0.1559	0.0223	0.2564
82	0.0846	0.0767	0.2971	0.0280	0.2137
83	0.0724	0.0545	0.2068	0.0282	0.1947
84	0.0777	0.0645	0.2452	0.0272	0.3132
85	0.0627	0.0573	0.1844	0.0164	0.2959
86	0.0651	0.0905	0.2078	0.0217	0.3577
87	0.0678	0.0684	0.2054	0.0254	0.3792
88	0.0675	0.0804	0.1682	0.0182	0.3573
Mean	0.07099	0.06750	0.20885	0.02343	0.29601
SD	0.00716	0.01442	0.04501	0.00452	0.06906
N	8	8	8	8	8

General Footnote: [< > markers indicates weight below/above flagging range. Data is valid.]

Comments and Markers

Page	Day	Group	Sex	Subject	Measurement	Type	Marker
1	11	1	Male	01	SV Weight	Out of Range	<
1	11	1	Male	03	SV Weight	Out of Range	<
1	11	1	Male	02	LABC Weight	Out of Range	<
2	11	2	Male	11	SV Weight	Out of Range	<
2	11	2	Male	14	SV Weight	Out of Range	<
2	11	2	Male	16	SV Weight	Out of Range	<
3	11	3	Male	23	SV Weight	Out of Range	<
4	11	4	Male	27	SV Weight	Out of Range	<
4	11	4	Male	30	SV Weight	Out of Range	<
5	11	5	Male	33	SV Weight	Out of Range	<
5	11	5	Male	35	SV Weight	Out of Range	<
5	11	5	Male	36	SV Weight	Out of Range	<
6	11	6	Male	41	Cowper's Gland Weight	Out of Range	>
6	11	6	Male	41	LABC Weight	Out of Range	>
6	11	6	Male	42	Cowper's Gland Weight	Out of Range	>
6	11	6	Male	42	LABC Weight	Out of Range	>
6	11	6	Male	43	Cowper's Gland Weight	Out of Range	>
6	11	6	Male	43	LABC Weight	Out of Range	>
6	11	6	Male	44	Cowper's Gland Weight	Out of Range	>
6	11	6	Male	45	Cowper's Gland Weight	Out of Range	>
6	11	6	Male	45	LABC Weight	Out of Range	>
6	11	6	Male	46	Cowper's Gland Weight	Out of Range	>
6	11	6	Male	46	LABC Weight	Out of Range	>
6	11	6	Male	47	Cowper's Gland Weight	Out of Range	>
6	11	6	Male	47	LABC Weight	Out of Range	>
6	11	6	Male	48	Cowper's Gland Weight	Out of Range	>
6	11	6	Male	48	LABC Weight	Out of Range	>
7	11	7	Male	56	VP Gland Weight	Out of Range	>
7	11	7	Male	49	Cowper's Gland Weight	Out of Range	>
7	11	7	Male	49	LABC Weight	Out of Range	>

Comments and Markers

Page	Day	Group	Sex	Subject	Measurement	Type	Marker
7	11	7	Male	50	Cowper's Gland Weight	Out of Range	>
7	11	7	Male	50	LABC Weight	Out of Range	>
7	11	7	Male	51	Cowper's Gland Weight	Out of Range	>
7	11	7	Male	51	LABC Weight	Out of Range	>
7	11	7	Male	52	Cowper's Gland Weight	Out of Range	>
7	11	7	Male	53	Cowper's Gland Weight	Out of Range	>
7	11	7	Male	54	Cowper's Gland Weight	Out of Range	>
7	11	7	Male	54	LABC Weight	Out of Range	>
7	11	7	Male	55	Cowper's Gland Weight	Out of Range	>
7	11	7	Male	56	Cowper's Gland Weight	Out of Range	>
7	11	7	Male	56	LABC Weight	Out of Range	>
8	11	8	Male	57	Cowper's Gland Weight	Out of Range	>
8	11	8	Male	58	Glans Penis Weight	Out of Range	>
8	11	8	Male	58	Cowper's Gland Weight	Out of Range	>
8	11	8	Male	58	LABC Weight	Out of Range	>
8	11	8	Male	59	Cowper's Gland Weight	Out of Range	>
8	11	8	Male	60	Cowper's Gland Weight	Out of Range	>
8	11	8	Male	60	LABC Weight	Out of Range	>
8	11	8	Male	61	Cowper's Gland Weight	Out of Range	>
8	11	8	Male	62	Cowper's Gland Weight	Out of Range	>
8	11	8	Male	63	Cowper's Gland Weight	Out of Range	>
8	11	8	Male	63	LABC Weight	Out of Range	>
8	11	8	Male	64	Cowper's Gland Weight	Out of Range	>
8	11	8	Male	64	LABC Weight	Out of Range	>
9	11	9	Male	65	Cowper's Gland Weight	Out of Range	>
9	11	9	Male	66	Cowper's Gland Weight	Out of Range	>
9	11	9	Male	66	LABC Weight	Out of Range	>
9	11	9	Male	67	Cowper's Gland Weight	Out of Range	>
9	11	9	Male	68	Cowper's Gland Weight	Out of Range	>
9	11	9	Male	68	LABC Weight	Out of Range	>
9	11	9	Male	69	Cowper's Gland Weight	Out of Range	>

Comments and Markers

Page	Day	Group	Sex	Subject	Measurement	Type	Marker
9	11	9	Male	69	LABC Weight	Out of Range	>
9	11	9	Male	70	Cowper's Gland Weight	Out of Range	>
9	11	9	Male	71	Cowper's Gland Weight	Out of Range	>
9	11	9	Male	72	Cowper's Gland Weight	Out of Range	>
9	11	9	Male	72	LABC Weight	Out of Range	>
10	11	10	Male	73	Cowper's Gland Weight	Out of Range	>
10	11	10	Male	73	LABC Weight	Out of Range	>
10	11	10	Male	74	Cowper's Gland Weight	Out of Range	>
10	11	10	Male	75	Cowper's Gland Weight	Out of Range	>
10	11	10	Male	76	Cowper's Gland Weight	Out of Range	>
10	11	10	Male	76	LABC Weight	Out of Range	>
10	11	10	Male	77	Cowper's Gland Weight	Out of Range	>
10	11	10	Male	78	Cowper's Gland Weight	Out of Range	>
10	11	10	Male	78	LABC Weight	Out of Range	>
10	11	10	Male	79	Cowper's Gland Weight	Out of Range	>
10	11	10	Male	80	Cowper's Gland Weight	Out of Range	>
10	11	10	Male	80	LABC Weight	Out of Range	>

Key Page

General Footnotes

< > markers indicates weight below/above flagging range. Data is valid.

Measurement Descriptions

Headings Used	Description
Glans Penis Weight	Glans Penis Weight
VP Gland Weight	Prostate Gland, Ventral Weight
SV Weight	Seminal Vesicles Weight
Cowper's Gland Weight	Cowper's Gland Weight
LABC Weight	LABC Muscle Weight

Unit Descriptions

Headings Used	Description
g	g

Time-Points/Ranges

Measurement	From	To	Report As
VP Gland Weight	-9,999	9,999	11
SV Weight	-9,999	9,999	11

Measurement/Statistics

Measurement	Descriptive
Glans Penis Weight	Mean
	Standard Deviation
	Count (N)

Key Page

Measurement/Statistics (Continued)

Measurement	Descriptive
VP Gland Weight	Mean
	Standard Deviation
	Count (N)
SV Weight	Mean
	Standard Deviation
	Count (N)
Cowper's Gland Weight	Mean
	Standard Deviation
	Count (N)
LABC Weight	Mean
	Standard Deviation
	Count (N)

Group Information

Short Name	Long Name	Report Headings 1-4			
1	Vehicle Control	0	mg/kg/day	2 EHP	
2	Agonist- low dose	250	mg/kg/day	2EHP	
3	Agonist- mid-low	500	mg/kg/day	2EHP	
4	Agonist- mid-high	750	mg/kg/day	2EHP	
5	Agonist-high dos	1000	mg/kg/day	2EHP	
6	Antagonist- Neg Cont	0	mg/kg/day	2EHP	plus TP
7	Antagonist-low dose	250	mg/kg/day	2EHP	plus TP
8	Antagonist- mid-low	500	mg/kg/day	2EHP	plus TP
9	Antagonist- mid-high	750	mg/kg/day	2EHP	plus TP
10	Antagonist high dose	1000	mg/kg/day	2EHP	plus TP
11	Antagonist positive	3.0	mg/kg/day	Flutamide	plus TP

APPENDIX V: Certificates of Analysis and Identity and Purity Screen of 2-Ethylhexyl Paraben

Certificate of Analysis

Sep 25, 2015 (JST)

TOKYO CHEMICAL INDUSTRY CO.,LTD.
4-10-1 Nihonbashi-Honcho, Chuo-ku, Tokyo 103-0023 Japan

Chemical Name: 2-Ethylhexyl 4-Hydroxybenzoate		
Product Number: H0506 CAS: 5153-25-3	Lot: 7CZZO	

Tests	Results	Specifications
Purity(HPLC)	99.3 area%	min. 98.0 area%
Purity(Neutralization titration)	99.8 %	min. 98.0 %
Specific gravity (20/20)	1.0382	1.0360 to 1.0390
Refractive Index n20/D	1.5210	1.5190 to 1.5220

TCI Lot numbers are 4-5 characters in length.
Characters listed after the first 4-5 characters are control numbers for internal purpose only.

Customer service:

TCI AMERICA
Tel: +1-800-423-8616 / +1-503-283-1681
Fax: +1-888-520-1075 / +1-503-283-1987
E-mail: Sales-US@TCIchemicals.com

spectrum® CHEMICAL MFG CORP

Certificate Of Analysis

Item Number	T1315	Lot Number	1EL0103
Item	Testosterone Propionate (CIII), Micronized, USP		
CAS Number	57-85-2		
Molecular Formula	$C_{22}H_{32}O_3$	Molecular Weight	344.49

Test	Specification		Result
	min	max	
ASSAY ($C_{22}H_{32}O_3$; DRIED BASIS)	97.0 - 103.0 %		99.7 %
MELTING RANGE	118° - 123°C		120 - 121°C
SPECIFIC ROTATION, $[a]_D25$	+83° to +90°		+83°
LOSS ON DRYING		0.5 %	0.0 %
IDENTIFICATION		TO PASS TEST	PASSES TEST
EXPIRATION DATE			31-JUL-2020
RESIDUAL SOLVENTS		TO PASS TEST	.
CLASS 2 (SOLVENT) / METHANOL			<3000 ppm
CLASS 2 (solvent) / METHYLENE CHLORIDE			<600 ppm
CLASS 2 (solvent) / PYRIDINE			<200 ppm
APPEARANCE			WHITE POWDER
MANUFACTURE DATE			01-AUG-2015

Spectrum Chemical Mfg Corp
14422 South San Pedro Street
Gardena 90248 CA

Certificate of Analysis Results Certified By:

Adan Hernandez
Quality Control Manager
Spectrum Chemicals & Laboratory Products

CERTIFICATE OF ANALYSIS

Printed: 12/7/2015
Customer No : 55620
Customer : INTEGRATED LABORATORY SYSTEMS
Customer PO : ILS151688RE
Order Number : 2851563
Delivery # : 41517075
Catalog : F3518
Flutamide
Lot : 2EL0006

Chemical Formula : $C_{11}H_{11}F_3N_2O_3$
CAS# : 13311-84-7
Formula Weight : 276.21

Test	Limit Min.	Limit Max.	Results
ASSAY (GC)	98 %		99.9 %
DATE OF MANUFACTURE			30-NOV-2015

Read and understand label and MSDS/SDS before handling any chemical. All Spectrum's chemicals are for manufacturing, processing, repacking or research purposes by experienced personnel only. The customer must ensure to provide its users adequate hazardous material training and appropriate protective gears before handling our chemicals.

Certificate of Analysis Results Certified By:

Mulbah F. Dwanah
Laboratory Supervisor

spectrum™ CHEMICAL MFG CORP
SpectrumChemical.com
AN ISO 9001:2008 REGISTERED COMPANY

Corporate Office:
769 Jersey Ave.
New Brunswick, NJ 08901
(732) 214-1300
Fax: (732) 246-7132

West Coast Plant:
14422 S. San Pedro St.
Gardena, CA 90248
(310) 516-8000
Fax: (310) 516-9843

2-ETHYLHEXYL PARABEN

Identity and Purity Screen

Amended Report

SUBMITTED TO:

Integrated Laboratory Systems, Inc.
635 Davis Drive, Suite 600
Morrisville, NC 27560 USA

PERFORMED BY:

RTI International*
3040 Cornwallis Road
P.O. Box 12194
Research Triangle Park, NC 27709-2194

RTI Study Numbers: 0213211.013.002.001 and 0213211.013.002.006

ILS Study Numbers: 10005.0103 and 10005.0102

September 22, 2016

Prepared by:

Sherry L Black 9/20/16

Sherry Black Date
Task Leader

Approved by:

for Navarro 9/22/16

Hernan Navarro Date
Principal Investigator

RTI International is a registered trademark and a trade name of Research Triangle Institute.

RTI INTERNATIONAL | **Memorandum**

Date: 09/22/16

To: Sherry Black, Senior Research Chemist

From: Phillip S. Anderson, Quality Assurance Specialist

Subject: Review of amended 2-Ethylhexyl Paraben report

The QAU performed a data and report audit of the 2-Ethylhexyl Paraben Identity and Purity Screen report dated September 9, 2016. The data audited against the project quality system documents and applicable SOPs. This work was not conducted in compliance with EPA FIFRA GLPs (40 CFR160).

Phillip S. Anderson

Quality Assurance Specialist

09.22.16

Date

turning knowledge into practice

This amended report is issued to correct the following typographic error:

Section 1.0

Original Text:

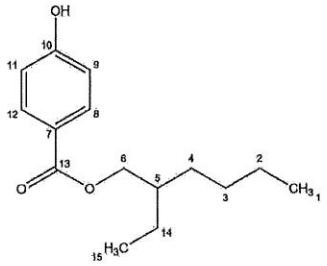
The test article 2-ethylhexyl paraben was analyzed by NMR to confirm identity and by HPLC with UV detection to confirm purity prior to use in the Hershberger and Male Pubertal Assays. Purity of the bulk material was assessed again near the end of the in vitro period of the assays.

Revised Text:

The test article 2-ethylhexyl paraben was analyzed by NMR to confirm identity and by HPLC with UV detection to confirm purity prior to use in the Hershberger and Male Pubertal Assays. Purity of the bulk material was assessed again near the end of the in **vivo** period of the assays.

2.1.2 NMR Results

Structure and Assignments:

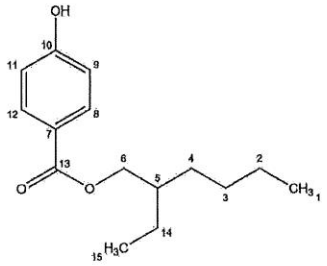


Assignment	Chemical Shift (ppm)	Integral Ratio
1, 15	0.9 - 1	6H
2, 3, 4, 14	1.3-1.5	8H
5	1.7-1.8	1H
6	4.2-4.3	2H
9, 11	7.0	2H
8, 12	8.0	2H
solvent	7.3	

The observed proton spectrum (Figure 1) shows chemical shift and splitting patterns consistent with the structure of 2-ethylhexyl paraben and with the predicted spectrum generated using ACD/C + H NMR Predictors and DB (V.10.02).

2.1.2 NMR Results

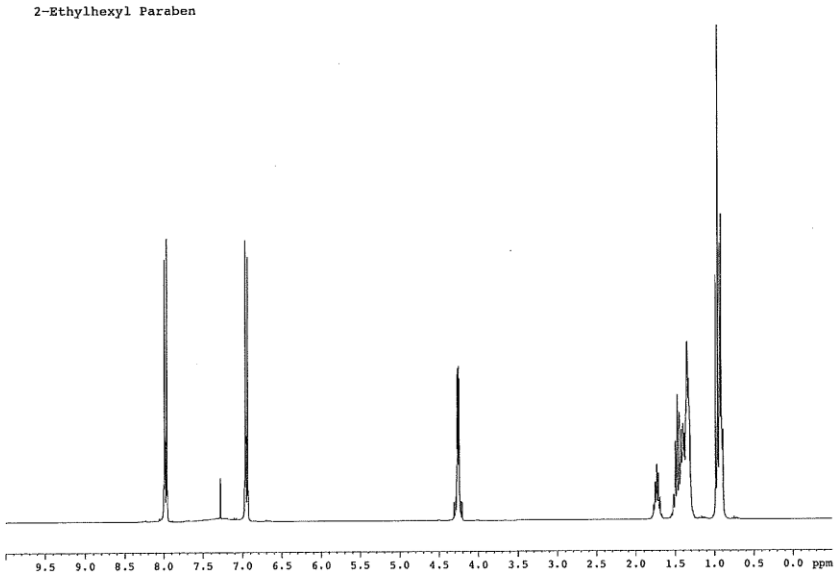
Structure and Assignments:



Assignment	Chemical Shift (ppm)	Integral Ratio
1, 15	0.9 - 1	6H
2, 3, 4, 14	1.3-1.5	8H
5	1.7-1.8	1H
6	4.2-4.3	2H
9, 11	7.0	2H
8, 12	8.0	2H
solvent	7.3	

The observed proton spectrum (Figure 1) shows chemical shift and splitting patterns consistent with the structure of 2-ethylhexyl paraben and with the predicted spectrum generated using ACD/C + H NMR Predictors and DB (V.10.02).

Figure 1. ^{1}H NMR of 2-Ethylhexyl Paraben (Lot # 7CZZO) in $CDCl_3$



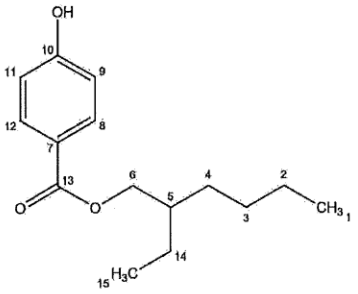
2.2 Nuclear Magnetic Resonance (Carbon)

2.2.1 NMR Instrument Parameters

System	Bruker Avance DPX-300 NMR
Software	Topspin 1.3
Sweep Frequency	75 mHz
Sweep Width	23810 Hz
Pulse Width	5.8 µsec
Solvent	$CDCl_3$
Reference	Solvent

2.2.2 NMR Results

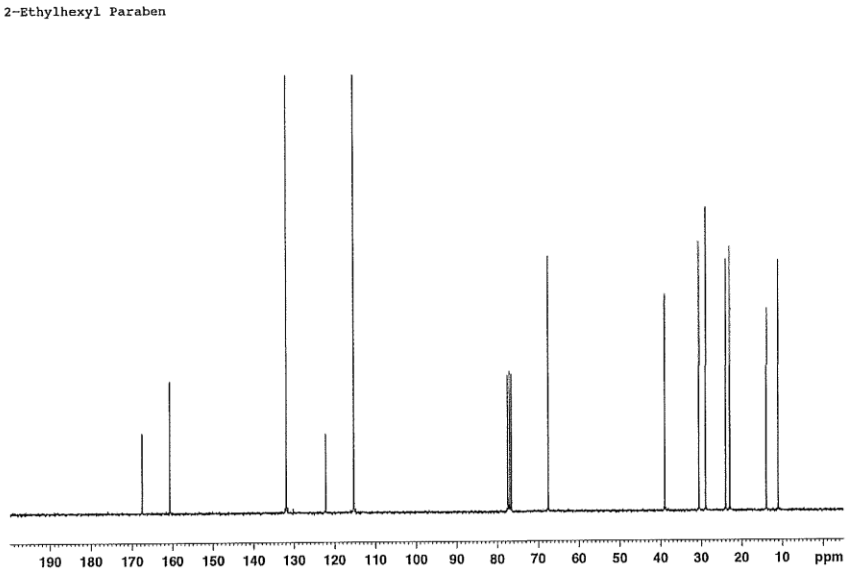
Structure and Assignments:



Assignment	Chemical Shift (ppm)
1	14
2	23
3	29
4	30.6
5	39
6	67.5
13	167.9
7	122
8, 12	132
9, 11	115
10	160.7
14	24
15	11
solvent	77

The observed spectrum (Figure 2) shows chemical shifts consistent with the structure of 2-ethylhexyl paraben and with the predicted spectrum generated using ACD/C + H NMR Predictors and DB (V.10.02).

Figure 2. ^{13}C NMR of 2-Ethylhexyl Paraben (Lot # 7CZZO) in $CDCl_3$



3.0 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

The test sample of 2-ethylhexyl paraben was prepared as 0.5 mg/mL solution in acetonitrile. The solution was analyzed on the HPLC system described below.

3.1 HPLC Parameters

Instrument	Waters Alliance 2695
Column	Phenomenex Prodigy ODS3, 5 µm; 250 x 4.6 mm w/ Phenomenex Prodigy ODS3, 5 µm precolumn
Column Temperature	Room Temperature
Mobile Phase	A: Water 0.1% Formic Acid B: Acetonitrile 0.1% Formic Acid
Gradient Conditions	Hold 70% B for 20 min, 70% B to 95% B for 5 min, hold 95% B for 10 min, 95% B to 70% B for 1 min; total run time 36 min
Flow Rate	1 mL/min
Injection Volume	10 µL
Detector	Waters W2487, UV monitored at 280 nm
Data System	Waters Empower 3; Build 3471

3.2 HPLC Results

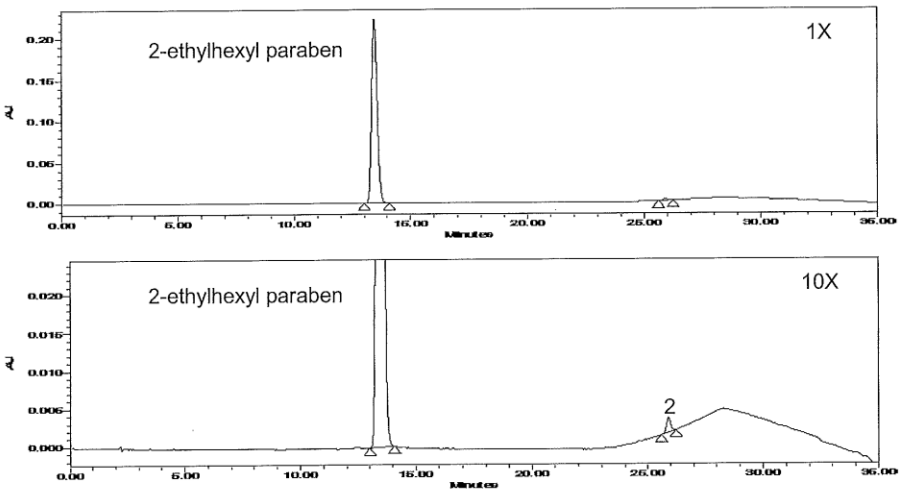
Purity was determined on two days to bracket the period of use for TO13. The chromatograms showed one major peak and one impurity peak. The purity is comparable to the vendor stated purity of 99.3%. Figure 3 shows representative chromatograms for each analysis date and also chromatograms with the Y axis magnified by 10x to show the impurity peak. The purity of 2-ethylhexyl paraben is unchanged between the initial and later analysis (a period of about 3.5 months).

Date of Analysis	Peak #	Peak ID	Retention Time (min)	% of Total Area
11/2/2015	1	2-ethylhexyl paraben	13.4	99.34
	2	unknown	25.9	0.66
3/15/2016	1	2-ethylhexyl paraben	13.8	99.35*
	2	unknown	26.2	0.66*

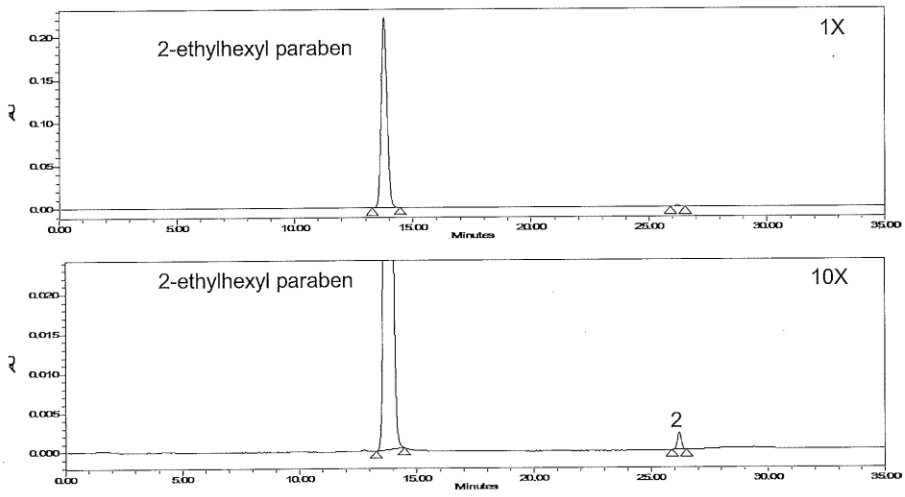
*Average of two determinations.

Figure 3. HPLC Chromatograms of 2-Ethylhexyl Paraben

Analysis date: 11/02/15



Analysis date: 03/15/16



APPENDIX VI: Dose Formulation Stability and Sample Analysis Reports

Study Title

Storage Stability of 2-Ethylhexyl Paraben in Corn Oil

Data Requirement

OCSPP 860.1380

Author

Xianai Wu, Ph.D., DABT

Study Completed On

29 April 2016

Study Sponsor

Integrated Laboratory Systems, Inc.
PO Box 13501
Research Triangle Park, North Carolina 27709

Performing Laboratory

Smithers Viscient
790 Main Street
Wareham, Massachusetts 02571-1037

Laboratory Project ID

Smithers Viscient Study No. 13974.6117
ILS Project/Study No. 10005/0104

Page 1 of 35

STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA sec. 10(d)(1)(A), (B), or (C) and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provisions regarding disclosure to multinational entities under FIFRA 10(g).

Company: _____

Company Agent: _____ Date: _____

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The data and report presented for **"Storage Stability of 2-Ethylhexyl Paraben in Corn Oil"** were produced and compiled in accordance with all pertinent U.S. Environmental Protection Agency (EPA) Good Laboratory Practices as set forth under the Federal Insecticide, Fungicide and Rodenticide Act (40 CFR, Part 160) and as compatible with OECD Principles of Good Laboratory Practice (OECD, 1998) with the following exception:

- The study was conducted using a vendor-supplied test substance with a non-GLP certificate of analysis that did not include an expiration date.

SMITHERS VISCIENT

Xianai Wu, Ph.D., DABT — 29 Apr 16
Study Director — Date

INTEGRATED LABORATORY SYSTEMS, INC.

Study Sponsor — Date

Submitter — Date

QUALITY ASSURANCE STATEMENT

The study conduct, raw data and interim report for **"Storage Stability of 2-Ethylhexyl Paraben in Corn Oil"** were inspected by the Quality Assurance Unit at Smithers Viscient to determine adherence with the study protocol, amendments, laboratory standard operating procedures and the applicable GLP regulations. This report is an accurate representation of the raw data. Dates of study inspections, study inspection types, and dates reported to Study Director and to Management are provided below.

Inspection Date	Inspection Types	Reported to Study Director/Management
10 December 2015	Protocol Review	11 December 2015
21 December 2015	In-Life: Day 10 Sampling	23 December 2015
2 March 2016	Data and Draft Report	4 March 2016
22 April 2016	Final Report	22 April 2016

SMITHERS VISCIENT

Robin Dyer
Quality Assurance Auditor

29 April 2016
Date

KEY STUDY PERSONNEL

The following Smithers Viscient personnel were responsible for the conduct of the work and reporting of the study results.

Xianai Wu, Ph.D., DABT	Study Director
Kristen Bentley	Assistant Chemist
Alexis Zelkan	Chemistry Technician II
Silviane Alves	Chemistry Technician II
Gina Giorgio	Technical Report Writer
Paul Reibach, Ph.D.	Director, Chemistry

TABLE OF CONTENTS

1.0 INTRODUCTION

The objective of this study was to determine the stability of 2-ethylhexyl paraben in corn oil when stored refrigerated (2 to 7 °C) for a period of approximately 20 days. Samples were prepared in corn oil at nominal concentrations of approximately 1.00 and 200 mg/mL (see Protocol Deviation). Three samples of each concentration level were removed and analyzed at days 0, 10 and 20 for stability assessment under refrigerated conditions.

The study was initiated on 2 December 2015, the date the Study Director signed the protocol, and was completed on the day the Study Director signed the final report. The testing was performed from 11 to 31 December 2015 at Smithers Viscient (SMV), located in Wareham, Massachusetts. All raw data, the original protocol and the original final report produced during this study will be transferred to ILS, Inc. at issuance of the final report for archival purposes.

2.0 METHODS AND MATERIALS

2.1 Protocol

This study was conducted according to Smithers Viscient's protocol entitled "Storage Stability of 2-Ethylhexyl Paraben in Corn Oil" (Appendix 1). This study followed OCSPP guideline 860.1380 (U.S. EPA, 1996).

2.2 Test Substances and Standard Reagents

2.2.1 Test Substance

The test substance, 2-ethylhexyl paraben, was received on 11 November 2015 from Research Triangle Institute, Durham, North Carolina. The following information was provided:

Name:	2-ethylhexyl paraben
Synonym:	2-ethylhexyl 4-hydroxybenzoate
Lot No.:	7CZZO

CAS No.:	5153-25-3
Purity:	99.3% (Certificate of Analysis, Appendix 2)
Expiration Date:	Not Available

Upon receipt at Smithers Viscient, the test substance (SMV No. 7945) was stored at room temperature in a dark, ventilated cabinet in the original container. Concentrations were adjusted for the purity of the test substance.

Another sample of the same lot of the test substance was received on 18 December 2015 (SMV No. 7995).

The test substances were used to prepare stability and quality control samples during testing.

Determination of stability and characterization, verification of the test substance identity, maintenance of records on the test substance, and archival of a sample of the test substance are the responsibility of the Study Sponsor.

2.2.2 Standard Reagents

All chemicals used were at least reagent grade from commercial sources.

2.3 Test System

The test system consisted of 2-ethylhexyl paraben dissolved (or suspended) in corn oil and stored refrigerated in foil-covered glass vials for approximately 0, 10 and 20 days. The corn oil vehicle was received from Animal Health International on 25 November 2015 (Lot 16303-100175) and was stored ambient prior to use.

2.4 Test Procedures

2.4.1 Preparation of Stock Solutions

A 5.00 mg/mL primary stock solution was prepared by placing 0.1260 g (0.1251 g as active ingredient) of 2-ethylhexyl paraben in a 25.0-mL volumetric flask and bringing it to volume with acetone. The 5.00 mg/mL primary stock solution was used to prepare the low concentration stability and quality control samples.

A 1.00 mg/mL primary stock solution was prepared by placing 0.05037 g (0.05002 g as active ingredient) of 2-ethylhexyl paraben in a 50.0-mL volumetric flask and bringing it to volume with acetonitrile. This 1.00 mg/mL primary stock solution was used to prepare secondary solutions as follows:

Fortifying Stock ID	Fortifying Stock Concentration (mg/L)	Volume of Fortification (mL)	Final Volume (mL)	Stock Solvent	Stock ID	Stock Concentration (mg/L)	Stock Use
7945A	1000	0.500	50.0	Acetonitrile	7945A-1	10.0	Calibration Standards
		5.00	50.0		7945A-2	100	

The stock solutions were stored in a refrigerator in glass amber bottles fitted with Teflon®-lined caps until use.

2.4.2 Preparation of Calibration Standards

The 2-ethylhexyl paraben calibration standards were prepared in acetonitrile by fortifying with the 10.0 and 100 mg/L secondary stock solutions to yield concentrations of 0.0500, 0.100, 0.250, 0.500, 1.00 and 2.50 mg/L.

2.4.3 Quality Control Sample Preparation and Dilution

The 1.00 mg/mL quality control (QC) samples were prepared by fortifying corn oil with the 5.00 mg/mL primary stock solution of 2-ethylhexyl paraben. The remaining QC sample was prepared by adding the appropriate amount of test material in corn oil at days 10 and 20 as presented in the table below:

Sample ID	Weighing Range of 2-ethylhexyl paraben (g)	Final Volume with Corn Oil (mL)	QC Sample Concentration (mg/mL)
QC #3	0.4019-0.4038	2.00	200

Samples were mixed well prior to dilution by vortexing. To minimize losses of the test material, samples were not sub-sampled prior to dilution. The entire volume of each sample was immediately diluted with 100% hexane by the addition of the hexane to the corn oil sample directly. Samples were subsequently diluted into the calibration standard range first with acetone followed by acetonitrile.

Sample	Nominal Concentration (mg/mL)	Sample Volume (mL)	Final Volume with Hexane (mL)	Sample Volume (mL)	Final Volume with Acetone (mL)	Sample Volume (mL)	Final Volume with Acetonitrile (mL)	Dilution Factor
QC #1	1.00	2.00	50.0	0.500	10.0	2.00	10.0	2500
QC #2	1.00	2.00	50.0	0.500	10.0	2.00	10.0	2500
QC #3	200	2.00	50.0	0.100	20.0	0.100	10.0	500,000

2.4.4 Sample Preparation and Dilution

All test samples were prepared at test initiation (day 0) and analyzed at their appropriate time intervals at either day 10 or 20. The low 2-ethylhexyl paraben stability samples were individually prepared in corn oil at day 0 by placing 0.400 mL of the 5.00 mg/mL 2-ethylhexyl paraben primary stock solution in a 50.0 mL disposable glass vial and bringing it to a final volume of 2.00 mL with corn oil. The high 2-ethylhexyl paraben stability samples were individually prepared at day 0 by weighing test substance directly into a 50.0 mL disposable glass vial and bringing to volume with corn oil as presented in the table below:

Sample ID	Nominal Concentration (mg/mL)	Mass Range of 2-ethylhexyl paraben (g)	Mass Range of 2-ethylhexyl paraben (g as a.i.)	Final Sample Volume (mL)
High Concentration	200	0.4019 - 0.4038[a]	0.3991– 0.4010[a]	2.00

[a] One of the day 20 stability samples was fortified with 0.2025 g of test substance due to limited test material (see Protocol Deviation) yielding a final concentration of 101 mg/mL.

Stability samples were labeled with the sample identification and study number and were mixed well by repetitive vortexing. To minimize losses of the test material, the stability samples were not sub-sampled prior to dilution. Stability samples were immediately diluted with 100% hexane by the addition of the hexane to the corn oil sample directly. Samples were subsequently diluted into the calibration standard range first with acetone followed by acetonitrile. A typical dilution is presented in the table below:

Sample ID	Nominal Concentration (mg/mL)	Sample Volume (mL)	Final Volume with Hexane (mL)	Sample Volume (mL)	Final Volume with Acetone (mL)	Sample Volume (mL)	Final Volume with Acetonitrile (mL)	Dilution Factor
Low Concentration	1.00	2.00	50.0	0.500	10.0	2.00	10.0	2500
High Concentration	200[a]	2.00	50.0	0.100	20.0	0.100	10.0	500,000

[a] One of the day 20 samples had a nominal concentration of 101 mg/mL due to limited test material (see Protocol Deviation).

2.4.5 Test Monitoring

Temperature was monitored daily in the refrigerator during the stability test using a VWR min/max thermometer and recorded over the duration of the study. The temperature over the course of the experiment ranged from 2 to 7 °C.

2.5 Analysis

Samples were analyzed for 2-ethylhexyl paraben by using automated injection on a high performance liquid chromatography equipped with ultraviolet detection (HPLC/UV) based on methodology validated at Smithers Viscient (summarized in Appendix 3). The method validation study was conducted prior to the initiation of the definitive test and established an

average recovery of 105 ± 3.21% from corn oil (Wu, 2016). Defined limits for acceptance of quality control sample performance in subsequent studies were set at 70 to 120%. Conditions and procedures used throughout the analysis of samples during this study were similar to those used in the method validation study.

3.0 RESULTS AND DISCUSSION

The temperature in the refrigerator ranged from 2 to 7 °C over the 20-day period. Analytical results for the 1.00 and 200 mg/mL stability samples are presented in Table 1. Measured concentrations obtained at the day 0, 10 and 20 sampling intervals ranged from 90.3 to 108% of nominal concentration for the low concentration samples. Measured concentration obtained at the day 0, 10 and 20 sampling intervals ranged from 72.5 to 112% of nominal concentration for the high concentration samples. Since subsequent recoveries for each of these samples through the day 20 sampling interval fell within the 70 to 120% acceptance criteria, it was concluded that 2-ethylhexyl paraben was stable under refrigerated storage for a period of 20 days.

Analysis of the QC samples (Table 2) resulted in recoveries ranging from 93.0 to 109% (N = 6) of the nominal fortified concentrations (1.00 to 200 mg/mL). Based on these results, it was demonstrated that satisfactory precision and quality control were maintained during the analysis of the test samples.

Representative chromatograms of stability test samples at test initiation are presented in Figure 1 and Figure 2, respectively. Representative chromatograms of stability test samples, a quality control sample and a calibration standard at test termination are presented in Figure 3 through Figure 6, respectively. A typical regression analysis is presented in Figure 7.

PROTOCOL DEVIATION

The protocol states that the high concentration (200 mg/mL) samples will be prepared in triplicate for each storage interval. Due to limited test material, one of the three high concentration samples was fortified at 101 mg/mL for the day 20 interval. This deviation does not negatively impact the results or interpretation of this study as the duplicate 200 mg/mL concentration samples recovered consistently, ranging from 96.1 to 96.3%, while the 101 mg/mL recovered at 97.3% confirming stability of the test material for 20 days.

REFERENCES

OECD, 1998. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring. Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997). Environment Directorate Chemicals Group and Management Committee. ENV/MC/CHEM(98)17. OECD Paris. France. 41 pp.

U.S. EPA, 1989. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160); FR: 8/17/89; pp. 34052. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA, 1996. OCSPP 860.1380 Storage Stability Data; EPA 712-C-95-177, U.S. Environmental Protection Agency, Washington, D.C.

Wu, Xianai, 2016. Validation of the Analytical Method for the Determination of 2-Ethylhexyl Paraben Technical in Corn Oil. Smithers Viscient, Wareham, MA. Study No. 13974.6116.

Table 1. Analytical results for the 2-ethylhexyl paraben concentration during the 20-day storage stability experiment in corn oil.

Sample ID	Time Interval (Days)	Nominal Concentration (mg/mL)	Measured Concentration (mg/mL)	Percent of Nominal	Mean Measured Concentration (mg/mL)	Standard Deviation	Percent from Day 0 Mean Measured
C12-15-77	0	1.00	1.06	106	1.07	0.0156	NA[a]
C12-15-78		1.00	1.08	108			
C12-15-79		1.00	1.06	106			
C12-15-89		200	189	94.4	171	22.9	
C12-15-90		200	145	72.5			
C12-15-91		200	178	89.0			
C12-15-80	10	1.00	0.946	94.6	1.01	0.0622	-5.05
C12-15-81		1.00	1.02	102			
C12-15-82		1.00	1.07	107			
C12-15-92	20	200	216	108	219	4.28	28.2
C12-15-93		200	217	108			
C12-15-94		200	224	112			
C12-15-83		1.00	0.903	90.3	0.914	0.0115	-14.3
C12-15-84		1.00	0.914	91.4			
C12-15-85		1.00	0.926	92.6			
C12-15-95		200	193	96.3	193[b]	0.707[b]	13.2
C12-15-96		200	192	96.1			
C12-15-97		101[b]	98.3	97.3			

[a] NA = Not Applicable.

[b] Due to limited test material, sample was fortified to achieve a nominal concentration of 101 mg/mL (see Protocol Deviation). Therefore, this sample was not included in mean or standard deviation calculations.

Note: Results were calculated using the actual analytical (unrounded) results and not the rounded values presented in this table.

Table 2. Summary of 2-ethylhexyl paraben quality control samples during the 20-day storage stability experiment in corn oil.

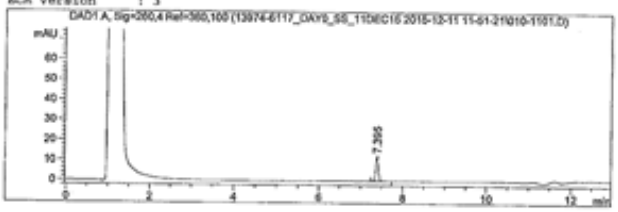
Sample ID	Sample	Time Interval (Days)	Nominal Concentration (mg/mL)	Measured Concentration (mg/mL)	Percent of Nominal
C12-15-140	QC #1		1.00	1.06	106
C12-15-141	QC #2	10	1.00	1.07	107
C12-15-142	QC #3		200	217	109
C12-15-164	QC #1		1.00	0.933	93.3
C12-15-165	QC #2	20	1.00	0.931	93.0
C12-15-166	QC #3		200	193	96.4

Note: Results were calculated using the actual analytical (unrounded) results and not the rounded values presented in this table.

Figure 1. Representative chromatogram of approximately 1.00 mg/mL 2-ethylhexyl paraben sample at initiation of the 20-day storage stability experiment in corn oil.

Data File C:\CHEM32\1\DATA\13974-6117_DAY0_SS_11DEC15 2015-12-11 11-51-21\010-1101.D
Sample Name: C12-15-78

Acq. Operator	: Alexis Zelkan	Seq. Line	: 11
Acq. Instrument	: Instrument 1	Location	: Vial 10
Injection Date	: 12/11/2015 3:04:00 PM	Inj	: 1
		Inj Volume	: 50.0 µl
Acq. Method	: C:\CHEM32\1\DATA\13974-6117_DAY0_SS_11DEC15 2015-12-11 11-51-21\2-ETHYL HEXYL PARABEN_50UL _UV.M		
Last changed	: 12/9/2015 1:50:47 PM by Xianai Wu		
Analysis Method	: C:\CHEM32\1\DATA\13974-6117_DAY0_SS_11DEC15 2015-12-11 11-51-21\2-ETHYL HEXYL PARABEN_50UL _UV.M (Sequence Method)		
Last changed	: 12/14/2015 8:06:09 AM by Alexis Zelkan		
Method Info	: 2-ethyl hexyl paraben, Agilent Zorbax SB-C18 3.5µm 75 x 4.6mm, gradient analysis, Solvent A: 0.05% H3PO4 in PRW, Solvent B: 100% ACN, 1.4 mL/min, 260nm, ambient		
ECM Server	: http://ssl007/SSL		
ECM Operator	: Alexis Zelkan		
ECM Path	: Chemistry\2-ethyl hexyl paraben\13794-6117\Data\13974-6117_DAY0_SS_11DEC15 2015-12-11 11-51-21.SC.SSIzip		
ECM Version	: 3		



Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=260,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.395	BB	0.0650	52.83572	12.57714	100.0000
Totals :				52.83572	12.57714	

*** End of Report ***

Instrument 1 12/14/2015 8:07:14 AM Alexis Zelkan Page 1 of 1

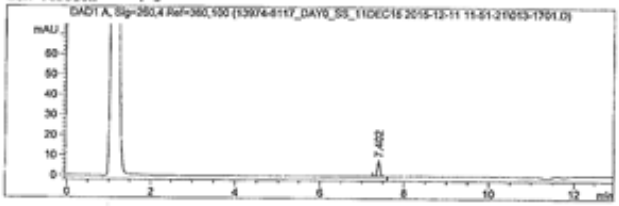
Note: 2-ethylhexyl paraben is ordinarily detected at a retention time of approximately 7.4 minutes.

Figure 2. Representative chromatogram of approximately 200 mg/mL 2-ethylhexyl paraben sample at initiation of the 20-day storage stability experiment in corn oil.

Data File C:\CHEM32\1\DATA\13974-6117_DAY0_SS_11DEC15 2015-12-11 11-51-21\013-1701.D
Sample Name: C12-15-50

Acq. Operator : Alexis Zelkan Seq. Line : 17
Acq. Instrument : Instrument 1 Location : Vial 13
Injection Date : 12/11/2015 4:17:35 PM Inj : 1
Inj Volume : 50.0 µl
Acq. Method : C:\CHEM32\1\DATA\13974-6117_DAY0_SS_11DEC15 2015-12-11 11-51-21\2-ETHYL HEXYL PARABEN_50UL _UV.M
Last changed : 12/9/2015 1:50:47 PM by Xianai Wu
Analysis Method : C:\CHEM32\1\DATA\13974-6117_DAY0_SS_11DEC15 2015-12-11 11-51-21\2-ETHYL HEXYL PARABEN_50UL _UV.M (Sequence Method)
Last changed : 12/14/2015 8:06:09 AM by Alexis Zelkan
Method Info : 2-ethyl hexyl paraben, Agilent Zorbax SB-C18 3.5µm 75 x 4.6mm, gradient analysis, Solvent A: 0.05% H3PO4 in PHW, Solvent B: 100% ACN, 1.4 mL/min, 260nm, ambient

ECM Server : http://ssl007/SSL
ECM Operator : Alexis Zelkan
ECM Path : Chemistry\2-ethyl hexyl paraben\13794-6117\Data\13974-6117_DAY0_SS_11DEC15 2015-12-11 11-51-21.SC.SSIzip
ECM Version : 3



Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=260,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.402	BB	0.0694	35.04162	7.95678	100.0000
Totals :				35.04162	7.95678	

*** End of Report ***

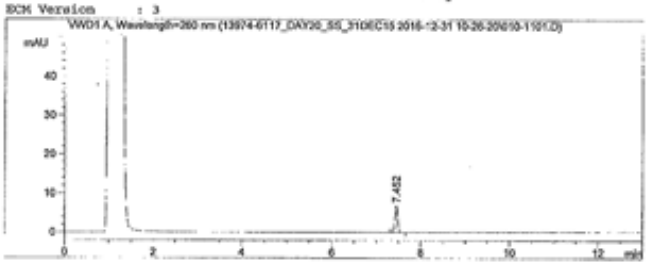
Instrument 1 12/14/2015 8:07:27 AM Alexis Zelkan Page 1 of 1

Note: 2-ethylhexyl paraben is ordinarily detected at a retention time of approximately 7.4 minutes.

Figure 3. Representative chromatogram of approximately 1.00 mg/mL 2-ethylhexyl paraben sample at termination of the 20-day storage stability experiment in corn oil.

Data File D:\CHEM32\2\DATA\13974-6117_DAY20_SS_31DEC15 2015-12-31 10-26-20\010-1101.D
Sample Name: C12-15-84

Acq. Operator	: Kristen Bentley	Seq. Line	: 11
Acq. Instrument	: Instrument 2	Location	: Vial 10
Injection Date	: 12/31/2015 1:30:35 PM	Inj	: 1
		Inj Volume	: 50.0 µl
Acq. Method	: D:\CHEM32\2\DATA\13974-6117_DAY20_SS_31DEC15 2015-12-31 10-26-20\2-ETHYL HEXYL PARABEN_50UL _UV.M		
Last changed	: 12/9/2015 1:50:47 PM by Xianai Wu		
Analysis Method	: D:\CHEM32\2\DATA\13974-6117_DAY20_SS_31DEC15 2015-12-31 10-26-20\2-ETHYL HEXYL PARABEN_50UL _UV.M (Sequence Method)		
Last changed	: 1/4/2016 7:33:34 AM by Kristen Bentley		
Method Info	: 2-ethyl hexyl paraben, Agilent Zorbax SB-C18 3.5µm 75 x 4.6mm, gradient analysis, Solvent A: 0.05% H3PO4 in PRW, Solvent B: 100% ACN, 1.4 mL/min, 260nm, ambient		
ECM Server	: http://asl007/asl		
ECM Operator	: Kristen Bentley		
ECM Path	: Chemistry\2-ethyl hexyl paraben\13794-6117\Data\13974-6117_DAY20_SS_31DEC15 2015-12-31 10-26-20.SC.SSIzip		
ECM Version	: 3		



Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=260 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	7.452	BB	0.0642	28.30827	6.85332	100.0000
Totals :				28.30827	6.85332	

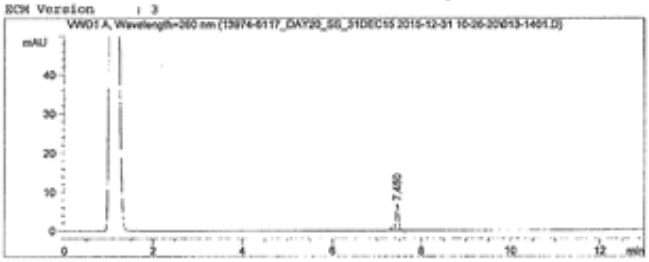
*** End of Report ***

Instrument 2 1/4/2016 7:36:22 AM Kristen Bentley Page 1 of 1

Note: 2-ethylhexyl paraben is ordinarily detected at a retention time of approximately 7.4 minutes.

Figure 4. Representative chromatogram of approximately 200 mg/mL 2-ethylhexyl paraben sample at termination of the 20-day storage stability experiment in corn oil.

```
Data File D:\CHEM32\2\DATA\13974-6117_DAY20_SS_31DEC15 2015-12-31 10-26-20\013-1401.D
Sample Name: C12-15-96

  ======================================================================
  Acq. Operator   : Kristen Bentley                Seq. Line :  14
  Acq. Instrument : Instrument 2                   Location : Vial 13
  Injection Date  : 12/31/2015 2:23:37 PM             Inj :  1
                                                   Inj Volume : 50.0 µl
  Acq. Method     : D:\CHEM32\2\DATA\13974-6117_DAY20_SS_31DEC15 2015-12-31 10-26-20\2-ETHYL
                    HEXYL PARABEN_50UL _UV.M
  Last changed    : 12/9/2015 1:50:47 PM by Xianai Wu
  Analysis Method : D:\CHEM32\2\DATA\13974-6117_DAY20_SS_31DEC15 2015-12-31 10-26-20\2-ETHYL
                    HEXYL PARABEN_50UL _UV.M (Sequence Method)
  Last changed    : 1/4/2016 7:33:34 AM by Kristen Bentley
  Method Info     : 2-ethyl hexyl paraben, Agilent Zorbax SB-C18 3.5µm 75 x 4.6mm, gradient
                    analysis, Solvent A: 0.05% H3PO4 in PRW, Solvent B: 100% ACN, 1.4 mL/
                    min, 260nm, ambient

  ECM Server      : http://ssl007/ssl
  ECM Operator    : Kristen Bentley
  ECM Path        : Chemistry\2-ethyl hexyl paraben\13794-6117\Data\13974-6117_DAY20_SS_
                    31DEC15 2015-12-31 10-26-20.SC.SSIzip
  ECM Version     : 3
```




```
  ======================================================================
                            Area Percent Report
  ======================================================================

  Sorted By             :      Signal
  Multiplier:                   :      1.0000
  Dilution:                     :      1.0000
  Use Multiplier & Dilution Factor with ISTDs


  Signal 1: VWD1 A, Wavelength=260 nm

  Peak RetTime Type  Width     Area      Height     Area
    #   [min]       [min]  mAU   *s    [mAU   ]     %
  ----|-------|----|-------|----------|----------|--------|
     1   7.450 BB    0.0665   29.74638    6.88048 100.0000

  Totals :                   29.74638    6.88048

  ======================================================================
                            *** End of Report ***

Instrument 2 1/4/2016 7:36:30 AM Kristen Bentley                Page  1 of 1
```

Note: 2-ethylhexyl paraben is ordinarily detected at a retention time of approximately 7.4 minutes.

Figure 5. Representative chromatogram of a 1.00 mg/mL quality control sample at termination of the 20-day storage stability experiment with 2-ethylhexyl paraben in corn oil.

Data File D:\CHEM32\2\DATA\13974-6117_DAY20_SS_31DEC15 2015-12-31 10-26-20\016-1701.D
Sample Name: C12-15-165

Acq. Operator	: Kristen Bentley	Seq. Line	: 17
Acq. Instrument	: Instrument 2	Location	: Vial 16
Injection Date	: 12/31/2015 3:16:41 PM	Inj	: 1
		Inj Volume	: 50.0 µl
Acq. Method	: D:\CHEM32\2\DATA\13974-6117_DAY20_SS_31DEC15 2015-12-31 10-26-20\2-ETHYL HEXYL PARABEN_50UL _UV.M		
Last changed	: 12/9/2015 1:50:47 PM by Xianai Wu		
Analysis Method	: D:\CHEM32\2\DATA\13974-6117_DAY20_SS_31DEC15 2015-12-31 10-26-20\2-ETHYL HEXYL PARABEN_50UL _UV.M (Sequence Method)		
Last changed	: 1/4/2016 7:33:34 AM by Kristen Bentley		
Method Info	: 2-ethyl hexyl paraben, Agilent Zorbax SB-C18 3.5µm 75 x 4.6mm, gradient analysis, Solvent A: 0.05% H3PO4 in PRM, Solvent B: 100% ACN, 1.4 mL/min, 260nm, ambient		
ECM Server	: http://sal007/sal		
ECM Operator	: Kristen Bentley		
ECM Path	: Chemistry\2-ethyl hexyl paraben\13794-6117\Data\13974-6117_DAY20_SS_31DEC15 2015-12-31 10-26-20.SC.SSZip		
ECM Version	: 3		

Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=260 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	7.452	BB	0.0641	28.82517	6.99671	100.0000
Totals :				28.82517	6.99671	

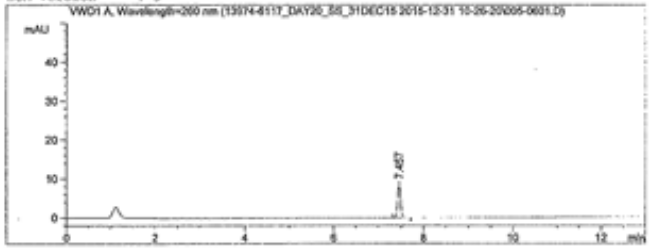
*** End of Report ***

Instrument 2 1/4/2016 7:36:37 AM Kristen Bentley Page 1 of 1

Note: 2-ethylhexyl paraben is ordinarily detected at a retention time of approximately 7.4 minutes.

Figure 6. Representative chromatogram of a 0. 500 mg/L calibration standard during the 20-day storage stability experiment with 2-ethylhexyl paraben.

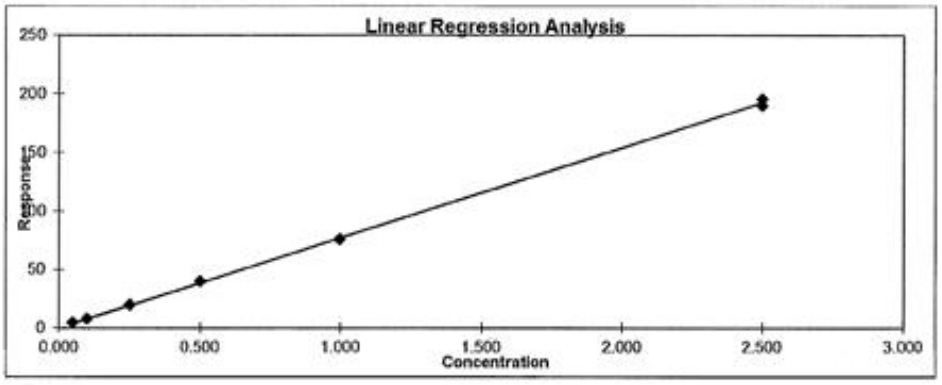
```
Data File D:\CHEM32\2\DATA\13974-6117_DAY20_SS_31DEC15 2015-12-31 10-26-20\005-0601.D
Sample Name: Std 4

  ======================================================================
  Acq. Operator   : Kristen Bentley              Seq. Line :   6
  Acq. Instrument : Instrument 2                Location : Vial 5
  Injection Date  : 12/31/2015 12:02:02 PM          Inj :   1
                                                Inj Volume : 50.0 µl
  Acq. Method     : D:\CHEM32\2\DATA\13974-6117_DAY20_SS_31DEC15 2015-12-31 10-26-20\2-ETHYL
                    HEXYL PARABEN_50UL _UV.M
  Last changed    : 12/9/2015 1:50:47 PM by Xianai Wu
  Analysis Method : D:\CHEM32\2\DATA\13974-6117_DAY20_SS_31DEC15 2015-12-31 10-26-20\2-ETHYL
                    HEXYL PARABEN_50UL _UV.M (Sequence Method)
  Last changed    : 1/4/2016 7:33:34 AM by Kristen Bentley
  Method Info     : 2-ethyl hexyl paraben, Agilent Zorbax SB-C18 3.5µm 75 x 4.6mm, gradient
                    analysis, Solvent A: 0.05% H3PO4 in PRW, Solvent B: 100% ACN, 1.4 mL/
                    min, 260nm, ambient

  ECM Server      : http://ssl007/ssl
  ECM Operator    : Kristen Bentley
  ECM Path        : Chemistry\2-ethyl hexyl paraben\13794-6117\Data\13974-6117_DAY20_SS_
                    31DEC15 2015-12-31 10-26-20.SC.SSIzip
  ECM Version     : 3
```




```
  ======================================================================
                            Area Percent Report
  ======================================================================

  Sorted By             :      Signal
  Multiplier:                   :      1.0000
  Dilution:                     :      1.0000
  Use Multiplier & Dilution Factor with ISTDs


  Signal 1: VWD1 A, Wavelength=260 nm

  Peak RetTime Type  Width     Area      Height     Area
    #   [min]       [min]  mAU   *s    [mAU   ]     %
  ----|-------|----|-------|----------|----------|--------|
     1   7.457 BB    0.0680   39.49229    9.12602 100.0000

  Totals :                   39.49229    9.12602

  ======================================================================
                            *** End of Report ***

Instrument 2 1/4/2016 7:36:07 AM Kristen Bentley                Page   1 of 1
```

Note: 2-ethylhexyl paraben is ordinarily detected at a retention time of approximately 7.4 minutes.

Figure 7. **A typical regression analysis for the calibration standards used to quantitate 2-ethylhexyl paraben during the 20-day storage stability experiment.**



Regression Analysis

$R^2 = 0.99963$
$Y = 76.668x + 0.2897$

Standard Concentration mg/L	Standard Response Area
0.0500	4.09251
0.100	7.82810
0.250	19.64194
0.500	39.49229
1.00	75.55050
2.50	194.88641
0.0500	4.18655
0.100	7.87521
0.250	19.68494
0.500	39.58051
1.00	75.58984
2.50	189.74132

APPENDIX 1 - STUDY PROTOCOL

SMITHERS VISCIENT

TEST PROTOCOL

Title:	Storage Stability of 2-Ethylhexyl Paraben in Corn Oil
Data Requirement(s):	OCSPP 880.1380, 40CFR160, OECD GLP
Test Substance(s): Name:	2-Ethylhexyl Paraben
Purity:	99.3%
Batch or Lot #:	7CZZO
Analytical Standard: Name:	2-Ethylhexyl Paraben
Purity:	99.3%
Batch or Lot #:	7CZZO
Study Sponsor:	Integrated Laboratory Systems, Inc.
Address:	P.O. Box 13501, Research Triangle Park, NC 27709
Study Monitor:	Jeffrey P. Davis, MBA, LATG
Email / Phone Number:	jdavis@ils-inc.com (919) 281-1110 x720
Sponsor Protocol/Project No. (when applicable):	10005.0104
Testing Facility:	Smithers Viscient 790 Main Street Wareham, Massachusetts 02571
Study Director:	Xianai Wu, Ph.D, DABT
Smithers Viscient Study No.:	13974-6117
Test Concentrations:	1.0 & 200 mg/mL
Proposed Experimental Dates	
Start:	December 2015
Termination:	January 2016

Sponsor Approval — 02 December 2015 — Date

Study Director Signature — 02 Dec 2015 — Study Initiation Date

Page 1 of 6

Storage Stability of of 2-Ethylhexyl Paraben in Corn Oil

1.0 INTRODUCTION

The purpose of this project is to verify the stability of 2-ethylhexyl paraben in corn oil over a 20-day refrigerated storage period. Corn oil will be used as a dosing vehicle for Tier 1 EDSP *in vivo* mammalian studies.

2.0 JUSTIFICATION OF THE TEST SYSTEM

Based on the EPA GLP guideline, the stability of the test substance in the vehicle should be determined. If the test substance is stable for the duration of the study, then one starting aliquot of the test substance may be prepared (at each dose level) prior to administration, and the specified dose levels can be dispensed into multiple aliquots to be used daily, taking care to avoid contamination and spoilage of the samples.

3.0 MATERIALS AND METHODS

3.1 Chemical System

3.1.1 Test Substance (and Analytical Standard, if applicable)

Upon arrival at Smithers Viscient (SMV), Massachusetts Research Center (MRC), the test substance (and analytical standard, if applicable) will be received into the Test Material Center. Records will be maintained in accordance with GLP requirements, and a Chain-of-Custody and use record established. The condition of the external packaging of the test substance will be recorded and any damage noted. The shipping packaging will be removed, the primary storage container inspected for leakage or damage, and the condition recorded. Any damage will be reported to the Sponsor and/or manufacturer.

The following information should be provided by the Study Sponsor or otherwise obtained by Smithers Viscient, if applicable: test substance lot or batch number, test substance purity, water solubility (pH and temperature of solubility determination), vapor pressure, storage stability, methods of analysis of the test substance in water, MSDS, and safe handling procedures, and a verified expiration or re-analysis date.

3.1.2 Dose Levels

Two dose levels set at approximately the low dose and the high dose levels to be used in the toxicity testing will be tested.

3.1.3 Preparation

3.1.3.1 Vehicle

The test substance will be mixed into the corn oil matrix in a manner that will ensure even distribution of the test substance throughout the matrix, following procedures provided by the Sponsor or Sponsor designate.

3.1.3.2 Sample preparation

Test samples will be prepared by dosing an aliquot of the matrix with a solvent stock (or raw material if necessary to achieve desired concentration) of the test substance. Individual aliquots will be prepared for each sampling interval with additional aliquots prepared as contingency samples. The entire aliquot will be removed at each interval and processed without sub-sampling to determine the stability of the test substance in the matrix during storage.

3.1.4 Sampling of Dose formulation

3.1.4.1 Stability

Stability of 2-ethylhexyl paraben in corn oil under refrigerated conditions (1-10°C) will be determined. Refrigerated stability samples will be placed under environmental conditions similar to testing conditions. The temperature will be monitored during the stability test. Samples for stability assessment will be taken from corn oil prepared at the approximate low and high concentrations to be tested as indicated above.

The following table summarizes the 2-ethylhexyl paraben storage intervals.

Sample Type		Dose Level Sampled	No. Samples	Analyses per sample
Storage Stability	Day 0	Low (1.00 mg/mL)	3	1
		High (200 mg/mL)	3	1
	Day11 ± 2	Low (1.00 mg/mL)	3	1
		High (200 mg/mL)	3	1
	Day 20 ± 2	Low (1.00 mg/mL)	3	1
		High (200 mg/mL)	3	1

3.1.5 2-Ethylhexyl Paraben Analysis

Quality control samples will be prepared and analyzed at each sampling interval, except Day 0 where the actual sample analysis will determine recoveries. The QC samples will be prepared in corn oil at the treatment level range. Results of these analyses indicate accuracy of the analytical method for measuring test substance concentration at each

sampling interval. In addition, QC samples will be used (as necessary) to adjust or correct for analytical method recoveries of the stability samples. This will be done by dividing the apparent residue level of an analyte after storage by the analytical method recoveries obtained for the QC samples analyzed at the same time for that interval. If storage stability results require correcting, both the uncorrected and corrected stability results will be reported in the final report.

The analytical method used to measure test substance concentrations in corn oil will be validated by Smithers Viscient at the expected nominal test concentration range prior to stability testing.

3.2 Test System

3.2.1 Identification of Test System

Test system consists of 2-ethylhexyl paraben dissolved (or suspended) in corn oil and stored for approximately 0, 11 and 20 days. Samples will be stored in amber or foil-covered glass vials labeled with, at a minimum, study number and sample ID.

3.2.2 Control of Bias

Bias will be controlled by sampling multiple aliquots of the appropriate dose levels indicated, as shown in the table above.

3.2.3 Justification of Test System

Storage stability is required to verify storage conditions for dose formulation preparation of test substance for Tier 1 mammalian in vivo assays. If stability is not demonstrated at day 11 the study may be truncated prior to day 20.

4.0 STATISTICAL ANALYSIS

Statistics will include, but will not be limited to, mean and standard deviation determinations.

5.0 RECORDS TO BE MAINTAINED

Records to be maintained will include, but will not be limited to, correspondence and other documents relating to the interpretation and evaluation of data as well as all raw data and documentation generated as a result of the study. All raw data, original protocol and original final report (with the exception of QA and facility records which will be maintained within the test facility archive) will be transferred to ILS, Inc. at issuance of the final report for archival purposes.

6.0 REPORTING

The raw data generated at Smithers Viscient will be peer-reviewed and the final report will be reviewed by the Study Director. All values will be reported to various levels of significance depending on the accuracy of the measuring devices employed during any one process. The Quality Assurance Unit will inspect the final report to confirm that the methods, procedures, and observations are accurately and completely described, that the reported results accurately and completely reflect the raw data generated at Smithers Viscient and to confirm adherence with the study protocol. A single copy of the draft report will be submitted to the Sponsor for review. The report will be finalized according to Standard Operating Procedures. The final report will meet the formatting requirements of EPA's PR Notice 86-5. All reports will include, but will not be limited to, the following information:

- Name and address of the facility performing the study.
- Dates on which the study was initiated and completed.
- Objectives and procedures stated in the approved protocol, including any changes from the original protocol.
- The test, control and reference substances identified by name, chemical abstract number or code number, strength, purity, and composition or other appropriate characteristics, as provided by the Sponsor.
- Results of all analytical chemistry analyses.
- Stability and, when relevant to the conduct of the study, the solubility of the test substance, control and reference substances under the conditions of administration.
- A description of the methods used.
- A description of the test system used in the study.
- A description of all circumstances that may have affected the quality or integrity of the data.
- The names of the Study Director, Principal Analyst and other key personnel involved in the study.
- A description of the transformations, calculations, and statistical analyses performed on the data, a summary and analysis of the data, and a statement of the conclusions drawn from the analyses.
- Deviations from the protocol not addressed in protocol amendments, together with a discussion of the impact on the study.
- Good Laboratory Practice (GLP) compliance statement signed by the Study Director.
- Date(s) of Quality Assurance reviews, and dates reported to the Study Director and management, signed by the Quality Assurance Unit.
- Location of the raw data and report.
- Any signature from the Study monitor.

7.0 PROTOCOL CHANGES

All amendments to the approved protocol, once signed by the Study Director, must be documented in writing and signed by both the Study Director, and the Sponsor's representative (Study Monitor). Changes made before the Study Director has signed the

protocol will be approved verbally, or in writing, by the Study Sponsor(s). Protocol amendments and deviations must include the reasons for the change and the predicted impact of the change on the results of the study, if any. If necessary, amendments other than the one providing the information required by page one of this protocol, may initially be verbally authorized, followed by Smithers Viscient's written documentation. In such cases, the effective date of the amendment will be the date of verbal authorization.

8.0 GOOD LABORATORY PRACTICES

All test procedures, documentation, records, and reports will comply with the U. S. Environmental Protection Agency's Good Laboratory Practice Standards as set forth under the Federal Insecticide, Fungicide, and Rodenticide Act (40 CFR, Part 160).

9.0 REFERENCES

OECD, 1998. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring. Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997). Environment Directorate Chemicals Group and Management Committee. ENV/MC/CHEM(98)17. OECD Paris. France. 41 pp.

U.S. EPA. 1989. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160); FR: 8/17/89; pp. 34052. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1996. OCSPP 860.1380 Storage Stability Data; EPA 712-C-95-177, U.S. Environmental Protection Agency, Washington, D.C.

APPENDIX 2 - CERTIFICATE OF ANALYSIS

TCI

Certificate of Analysis

Sep 25, 2015 (JST)

TOKYO CHEMICAL INDUSTRY CO.,LTD.
6-10-1 Nihonbashi-honcho, Chuo-ku, Tokyo 103-0023 Japan

Chemical Name: 2-Ethylhexyl 4-Hydroxybenzoate		
Product Number: H0505 CAS: 5153-25-3	Lot: TCZ2O	

Tests	Results	Specifications
Purity(HPLC)	99.3 area%	min. 98.0 area%
Purity(Neutralization titration)	99.6 %	min. 98.0 %
Specific gravity (20/20)	1.0362	1.0360 to 1.0390
Refractive Index n20/D	1.5210	1.5190 to 1.5230

TCI Lot numbers are 4-5 characters in length.
Characters listed after the first 4-5 characters are control numbers for internal purpose only.

Customer service:
TCI AMERICA
Tel: +1-800-423-8616 / +1-503-283-1601
Fax: +1-888-520-1075 / +1-503-283-1987
E-mail: Sales.US@TCIchemicals.com

APPENDIX 3 - ANALYTICAL SUMMARY

This appendix is a summary of the analytical procedures used during this testing. These procedures follow the methodology determined during the method validation (Wu, 2016).

Equipment

Instrument:	Agilent Series 1100 quaternary solvent pump Agilent Series 1100 autosampler Agilent diode array detector Agilent ChemStation ECM Version B.04.02 for data acquisition

Instrumental Conditions

The high performance liquid chromatographic (HPLC/UV) analysis was conducted utilizing the following instrumental conditions:

Column:	Agilent Zorbax SB-C18, 3.5 µm, 75 x 4.6 mm
Mobile Phase (A):	0.05% phosphoric acid in purified reagent water
Mobile Phase (B):	100% acetonitrile

Gradient:

Time (min.)	Solvent A (%)	Solvent B (%)
0.00	60.0	40.0
1.00	60.0	40.0
10.00	0.00	100.0
12.00	0.00	100.0
13.00	60.0	40.0

Run Time:	13.0 minutes
Equilibration Delay:	3.00 minutes
Flow Rate:	1.40 mL/minute
Injection Volume:	50.0 µL
Wavelength:	260 nm
Retention Time:	approximately 7.4 minutes

Study Title

Concentration and Homogeneity Sample Analysis -
The Hershberger Bioassay (OPPTS 890.1400) and
Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats;
2-Ethylhexyl Paraben

Author

Xianai Wu, Ph.D., DABT

Study Completed On

7 June 2016

Study Sponsor

RTI International
3040 Cornwallis Road
Research Triangle Park, North Carolina 27709

Performing Laboratory

Study Director: Jeffrey Davis, B.S., LATG
Integrated Laboratory Systems, Inc. (ILS)
635 Davis Drive, Suite 600
Morrisville, North Carolina 27560

Testing Facility

Smithers Viscient
790 Main Street
Wareham, Massachusetts 02571-1037

Laboratory Project ID

Smithers Viscient Study Number 13974.6118
ILS Projects/Study Numbers 10005.0103 and 10005.0102

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The data and phase report presented for **"Concentration and Homogeneity Sample Analysis - The Hershberger Bioassay (OPPTS 890.1400) and Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben"** were produced and compiled in accordance with all pertinent U.S. Environmental Protection Agency (EPA) Good Laboratory Practices as set forth under the Federal Insecticide, Fungicide and Rodenticide Act (40 CFR, Part 160) and as compatible with OECD Principles of Good Laboratory Practice (OECD, 1998) with the following exception:

- The study was conducted using a vendor-supplied reference substance with a non-GLP certificate of analysis that did not include an expiration date.

SMITHERS VISCIENT

Xianai Wu, Ph.D., DABT
Principal Investigator

7 June 2016
Date

QUALITY ASSURANCE STATEMENT

The study conduct, raw data and phase report for **"Concentration and Homogeneity Sample Analysis - The Hershberger Bioassay (OPPTS 890.1400) and Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben"** were inspected by the Quality Assurance Unit (QAU) at Smithers Viscient to determine adherence with the study protocol and laboratory standard operating procedures. This phase report accurately reflects the raw data. Dates of study inspections, inspection types, and dates reported to the Study Director and to Management are listed below.

Inspection Date	Inspection Type	Reported to Study Director/Management
17 February 2016	In-Life: Sample Dilution	18 February 2016
19-20 April 2016	Data Audit	20 April 2016
23 May 2016	Draft Report	23 May 2016
3, 6 June 2016	Final Report	6 June 2016

SMITHERS VISCIENT

Jenna Chicoine
Quality Assurance Auditor

6 June 2016
Date

KEY STUDY PERSONNEL

The following Smithers Viscient personnel were responsible for the conduct of the work and reporting of the study phase results.

Xianai Wu, Ph.D., DABT	Principal Investigator, Senior Chemist
Silviane Alves	Chemistry Technician II
Daniel P. Benza	Chemistry Technician II
Alexis Zelkan	Chemistry Technician II
Helen Flavin, Ph.D.	Technical Report Writer
Paul Reibach, Ph.D.	Director, Chemistry

TABLE OF CONTENTS

1.0 INTRODUCTION AND STUDY SUMMARY

Dose formulations of 2-ethylhexyl paraben in corn oil were prepared by Integrated Laboratory Systems, Inc. (ILS) and shipped to Smithers Viscient for analysis, where they were received on the following dates at the following nominal concentrations:

- 26 January 2016 (nominal concentrations of 50.0, 100, 150, and 200 mg/mL and control) [ILS 10005.0103]
- 17 February 2016 (nominal concentrations of 50.0, 100, 150, and 200 mg/mL and control) [ILS 10005.0102]
- 4 March 2016 (nominal concentrations of 50.0, 100, 150, and 200 mg/mL and control) [ILS 10005.0102]

The number of samples received and analyzed is described in Section 2.7. Samples were analyzed for verification of concentration and homogeneity in duplicate.

Analytical results (mean percent recovery and coefficient of variation) for the 2-ethylhexyl paraben samples in corn oil are summarized in the following tables:

Samples received on 26 January 2016 [ILS 10005.0103]

Nominal Sample Concentration (mg/mL)	Mean Percent Recovery	Coefficient of Variation
50.0	96.5	1.13
100	95.2	1.26
150	92.4	2.33
200	90.4	6.20

Samples received on 17 February 2016 [ILS 10005.0102]

Nominal Sample Concentration (mg/mL)	Mean Percent Recovery	Coefficient of Variation
50.0	99.5	3.76
100	99.2	4.88
150	99.4	2.27
200	98.5	2.90

Samples received on 4 March 2016 [ILS 10005.0102]

Nominal Sample Concentration (mg/mL)	Mean Percent Recovery	Coefficient of Variation
50.0	90.5	4.82
100	87.9	2.20
150	92.7	2.85
200	91.7	5.36

All control samples were below the limit of quantitation (LOQ) for each sample set analyzed.

2.0 MATERIALS AND METHODS

2.1 Study Phases

This analytical study phase was conducted in support of the ILS protocols entitled "The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben" (ILS study # 10005.0103) and "Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben" (ILS study # 10005.0102).

Study 10005.0103 was initiated on 21 January 2016, while study 10005.0102 was initiated on 6 January 2016, the day the Study Director signed the protocols, and was completed on the day the Study Director signed the final report. The analytical phases of the study were conducted from 26 to 27 January 2016 for study 10005.0103 and 17 February to 4 March 2016 for study 10005.0102 at Smithers Viscient (SMV), located in Wareham, Massachusetts. All raw data and the original final phase report produced during this study will be transferred to ILS, Inc. at issuance of the final report for archival purposes.

2.2 Reference Substance

The reference substance, 2-ethylhexyl paraben, was received on 18 December 2015 from Research Triangle Institute, Durham, North Carolina. The following information was provided:

Name:	2-ethylhexyl paraben
Synonym:	2-ethylhexyl 4-hydroxybenzoate
Lot No.:	7CZZO
CAS No.:	5153-25-3
Purity:	99.3% (Certificate of Analysis, Appendix 1)
Expiration Date:	Not Available

Upon receipt at Smithers Viscient, the reference substance (SMV No. 7995) was stored at room temperature in the original container in a dark ventilated cabinet. Concentrations were adjusted for the purity of the reference substance.

Determination of stability and characterization, verification of the reference substance identity, maintenance of records on the reference substance, and archival of a sample of the reference substance are the responsibility of the Study Sponsor.

2.3 Standard Reagents

All chemicals used were at least reagent grade from commercial sources.

2.4 Preparation of Stock Solutions

2.4.1 ILS 10005.0103

Primary stock solutions were typically prepared as described in the table below:

Primary Stock ID	Amount Weighed (g), Net Weight	Amount Weighed (g), as Active Ingredient	Stock Solvent	Final Volume (mL)	Primary Stock Concentration (mg/L)	Primary Stock Use
7995C	0.05040	0.05005	Acetonitrile	50.0	1000	Secondary stock solutions
7995D	0.1260	0.1251	Acetone	25.0	5000	Quality Control Samples

Secondary stock solutions were typically prepared as described in the table below:

Fortifying Stock ID	Fortifying Stock Concentration (mg/L)	Volume of Fortification (mL)	Final Volume (mL)	Stock Solvent	Stock ID	Stock Concentration (mg/L)	Stock Use
7995C	1000	0.500	50.0	Acetonitrile	7995C-1	10.0	Calibration Standards
7995C	1000	5.00	50.0		7995C -2	100	Calibration Standards

Stock solutions were stored in a refrigerator in amber glass bottles fitted with Teflon®-lined caps until use.

2.4.2 ILS 10005.0102

Primary stock solutions were typically prepared as described in the table below:

Primary Stock ID	Amount Weighed (g), Net Weight	Amount Weighed (g), as Active Ingredient	Stock Solvent	Final Volume (mL)	Primary Stock Concentration (mg/L)	Primary Stock Use
7995C	0.05040	0.05005	Acetonitrile	50.0	1000	Secondary stock solutions
7995D	0.1260	0.1251	Acetone	25.0	5000	Quality Control Samples
7995K	0.05035	0.5000	Acetonitrile	50.0	1000	Secondary stock solutions
7995L	0.1259	0.1250	Acetone	25.0	5000	Quality Control Samples

Secondary stock solutions were typically prepared as described in the table below:

Fortifying Stock ID	Fortifying Stock Concentration (mg/L)	Volume of Fortification (mL)	Final Volume (mL)	Stock Solvent	Stock ID	Stock Concentration (mg/L)	Stock Use
7995C	1000	0.500	50.0	Acetonitrile	7995C-1	10.0	Calibration Standards
7995C	1000	5.00	50.0		7995C-2	100	Calibration Standards
7995K	1000	0.500	50.0	Acetonitrile	7995K-1	10.0	Calibration Standards
7995K	1000	5.00	50.0		7995K -2	100	Calibration Standards

Stock solutions were stored in a refrigerator in amber glass bottles fitted with Teflon®-lined caps until use.

2.5 Preparation of Calibration Standards

The calibration standards were prepared in acetonitrile using the 10.0 mg/L secondary stock solution to yield concentrations of 0.0500, 0.100, 0.250, 0.500 mg/L and the 100 mg/L secondary stock solution to yield concentrations of 1.00 and 2.50 mg/L.

2.6 Quality Control Sample Fortification

2.6.1 ILS 10005.0103

Three quality control (QC) samples were individually prepared in corn oil. The 1.00 mg/mL QC samples were prepared by fortifying corn oil with the 5.00 mg/mL primary stock solution of 2-ethylhexyl paraben. The remaining QC samples were prepared by adding the appropriate amount of test material to corn oil as described in the table below:

Sample ID	Mass of 2-ethylhexyl paraben (g)	Mass of 2-ethylhexyl paraben (g as a.i.)	Corn Oil Final Volume (mL)	QC Sample Concentration (mg/mL)
QC #2	0.2022	0.2008	2.00	100
QC #3	0.4028	0.4000	2.00	200

2.6.2 ILS 10005.0102

Three quality control (QC) samples were individually prepared in corn oil. The 1.00 mg/mL QC samples were prepared by fortifying corn oil with the 5.00 mg/mL primary stock solution of 2-ethylhexyl paraben. The remaining QC samples were prepared by adding the appropriate amount of test material to corn oil as described in the table below:

Sample ID	Mass Range of 2-ethylhexyl paraben (g)	Mass Range of 2-ethylhexyl paraben (g as a.i.)	Corn Oil Final Volume (mL)	QC Sample Concentration (mg/mL)
QC #2	0.2018 – 0.2021	0.2004 – 0.2007	2.00	100
QC #3	0.4033 – 0.4037	0.4005 – 0.4009	2.00	200

2.7 Sample Receipt and Dilution

2.7.1 ILS 10005.0103

On 26 January 2016, more than 2.0-mL aliquots of two control samples (0.0 mg/mL) and four dose formulation concentrations at 50.0, 100, 150 and 200 mg/mL (sampled from the top, middle and bottom for a total of three samples at each concentration) were received cold on ice packs by Smithers Viscient from ILS. Upon arrival, samples were opened, inspected, and checked against the enclosed shipping form. All dose formulation samples were processed and analyzed in duplicate for verification of concentration and homogeneity within 24 hours of receipt.

Samples were mixed well prior to analysis. A 2.0-mL aliquot of each sample was quantitatively transferred to separate 50.0-mL disposable glass vials using disposable glass pipets. Samples were diluted in duplicate, first with hexane, followed by acetone, and finally with acetonitrile. These duplicate aliquots were distinguished using the notations "-1" and "-2" to create unique sample identifiers. A representative dilution scheme based on nominal concentration of the dose formulations received is presented in the table below:

Nominal Sample Concentration (mg/mL)	Sample Volume (mL)	Final Volume with Hexane (mL)	Sample Volume (mL)	Final Volume with Acetone (mL)	Sample Volume (mL)	Final Volume with Acetonitrile (mL)	Dilution Factor
0.00	2.00	50.0	0.500	10.0	2.00	10.0	2,500
50.0	2.00	50.0	0.200	10.0	0.100	10.0	125,000
			0.200	10.0	0.100	10.0	125,000
100	2.00	50.0	0.100	10.0	0.100	10.0	250,000
			0.100	10.0	0.100	10.0	250,000
150	2.00	50.0	0.150	20.0	0.100	10.0	333,333
			0.150	20.0	0.100	10.0	333,333
200	2.00	50.0	0.100	20.0	0.100	10.0	500,000
			0.100	20.0	0.100	10.0	500,000
QC#1 (1.00)	2.00	50.0	0.500	10.0	2.00	10.0	2,500
QC#2 (100)	2.00	50.0	0.100	10.0	0.100	10.0	250,000
QC#3 (200)	2.00	50.0	0.100	20.0	0.100	10.0	500,000

2.7.2 ILS 10005.0102

On 17 February and 4 March 2016, more than 2.0-mL aliquots of two control samples (0.0 mg/mL) and four dose formulation concentrations at 50.0, 100, 150 and 200 mg/mL (sampled from the top, middle and bottom for a total of three samples at each concentration) were received cold on ice packs by Smithers Viscient from ILS. Upon arrival, samples were opened, inspected, and checked against the enclosed shipping form. All dose formulation samples were processed and analyzed in duplicate for verification of concentration and homogeneity within 24 hours of receipt.

Samples were mixed well prior to analysis. Duplicate 1.0-mL aliquots of each sample were quantitatively transferred to separate 50.0-mL disposable glass vials using disposable glass pipets. These duplicate aliquots were distinguished using the notations "-1" and "-2" to create unique sample identifiers. Samples were diluted first with hexane, followed by acetone, and finally with acetonitrile. A representative dilution scheme based on nominal concentration of the dose formulations received is presented in the table below:

Nominal Sample Concentration (mg/mL)	Sample Volume (mL)	Final Volume with Hexane (mL)	Sample Volume (mL)	Final Volume with Acetone (mL)	Sample Volume (mL)	Final Volume with Acetonitrile (mL)	Dilution Factor
0.00	1.00	25.0	0.500	10.0	2.00	10.0	2500
50.0	1.00	25.0	0.200	10.0	0.100	10.0	125,000
100	1.00	25.0	0.100	10.0	0.100	10.0	250,000
150	1.00	25.0	0.150	20.0	0.100	10.0	333,333
200	1.00	25.0	0.100	20.0	0.100	10.0	500,000
QC#1 (1.00)	2.00	50.0	0.500	10.0	2.00	10.0	2500
QC#2 (100)	2.00	50.0	0.100	10.0	0.100	10.0	250,000
QC#3 (200)	2.00	50.0	0.100	20.0	0.100	10.0	500,000

2.8 Analysis

Samples were analyzed for 2-ethylhexyl paraben using high performance liquid chromatographic (HPLC/UV) analysis based on methodology validated at Smithers Viscient. The method validation study was conducted prior to the initiation of the definitive test and established an average recovery of 105% ± 3.21% from corn oil (Wu, 2016). Defined limits for acceptance of

quality control sample performance in subsequent studies were set at 70 to 120%. Conditions and procedures used throughout the analysis of samples during this study were similar to those used in the method validation study.

2.8.1 Equipment

2.8.1.1 ILS 10005.0103

Instrument:	Agilent Series 1100/G1311A quaternary solvent pump Agilent Series 1100/G1313A autosampler Agilent Series 1100/G1314A variable wavelength detector Agilent Series 1100/G1322A vacuum degasser Agilent ChemStation ECM Version B.04.02 software for data acquisition

2.8.1.2 ILS 10005.0102

Samples analyzed 17 February 2016:

Instrument:	Agilent Series 1260/G1311B quaternary solvent pump with integrated degasser Agilent Series 1260/G1329B autosampler Agilent Series 1260/G4212 variable wavelength detector Agilent ChemStation ECM Version B.04.03 software for data acquisition

Samples analyzed 4 March 2016:

Instrument:	Agilent Series 1100/G1310A quaternary solvent pump Agilent Series 1100/G1313A autosampler Agilent Series 1100/G1314A variable wavelength detector Agilent Series 1200/G1379B vacuum degasser Agilent ChemStation ECM Version B.04.02 software for data acquisition

2.8.2 Instrumental Conditions

The high performance liquid chromatographic (HPLC/UV) analysis was conducted utilizing the following instrumental conditions:

Column:	Agilent Zorbax SB-C18, 3.5 µm, 75 x 4.6 mm		
Mobile Phase (A):	0.05% phosphoric acid in purified reagent water		
Mobile Phase (B):	100% acetonitrile		
Gradient:	Time (min.)	Solvent A (%)	Solvent B (%)
	0.00	60.0	40.0
	1.00	60.0	40.0
	10.00	0.00	100.0
	12.00	0.00	100.0
	13.00	60.0	40.0
Run Time:	13.0 minutes		
Equilibration Delay:	3.00 minutes		
Flow Rate:	1.40 mL/minute		
Injection Volume:	50.0 µL		
Wavelength:	260 nm		
Retention Time:	approximately 7.4 minutes		

Note: The HPLC systems used for the sample analyses are comparable to one another, with analogous software and hardware.

3.0 RESULTS AND DISCUSSION

3.1 ILS 10005.0103

Analytical results for the dose formulation samples received on 26 January 2016 are presented in Table 1. The mean measured concentrations were 48.3, 95.2, 139, and 181 mg/mL, for the homogeneity samples (50.0, 100, 150, and 200 mg/mL nominal), respectively. Dose formulation samples recovered with an average and coefficient of variation of 96.5 ± 1.13% for 50.0 mg/mL, 95.2 ± 1.26% for 100 mg/mL, 92.4 ± 2.33% for 150 mg/mL, and 90.4 ± 6.20% for 200 mg/mL with all controls below the limit of quantitation (LOQ).

Analysis of QC samples resulted in recoveries ranging from 99.3 to 102% (N = 3) of the nominal fortified concentrations (1.00, 100 and 200 mg/mL). Since all dose formulation mean concentrations recovered within 15% of the target concentration, with a coefficient of variation less than 15%, it can be determined that the appropriate dose concentration verification and homogeneity was achieved for each dose formulation sample received on 26 January 2016.

Representative chromatograms from the analysis of a calibration standard, a control, a mid-concentration dose formulation sample, a high-concentration dose formulation sample and a QC sample are presented in Figure 1 through Figure 5. A typical regression analysis for 2-ethylhexyl paraben is presented in Figure 6.

3.2 ILS 10005.0102

Analytical results for the dose formulation samples received on 17 February 2016 are presented in Table 2. The mean measured concentrations were 49.8, 99.2, 149, and 197 mg/mL, for the homogeneity samples (50.0, 100, 150, and 200 mg/mL nominal), respectively. Dose formulation samples recovered with an average and coefficient of variation of 99.5 ± 3.76% for 50.0 mg/mL, 99.2 ± 4.88% for 100 mg/mL, 99.4 ± 2.27% for 150 mg/mL, and 98.5 ± 2.90% for 200 mg/mL with all controls below the limit of quantitation (LOQ).

Analysis of QC samples resulted in recoveries ranging from 98.8 to 100% (N = 3) of the nominal fortified concentrations (1.00, 100 and 200 mg/mL). Since all dose formulation mean concentrations recovered within 15% of the target concentration, with a coefficient of variation less than 15%, it can be determined that the appropriate dose concentration verification and homogeneity was achieved for each dose formulation sample received on 17 February 2016.

Representative chromatograms from the 17 February 2016 analysis of a calibration standard, a control, a mid-concentration dose formulation sample, a high-concentration dose formulation

sample and a QC sample are presented in Figure 7 through Figure 11. A typical regression analysis for 2-ethylhexyl paraben is presented in Figure 12.

Analytical results for the dose formulation samples received on 4 March 2016 are presented in Table 3. The mean measured concentrations were 45.2, 87.9, 139, and 183 mg/mL, for the homogeneity samples (50.0, 100, 150, and 200 mg/mL nominal), respectively. Dose formulation samples recovered with an average and coefficient of variation of 90.5 ± 4.82% for 50.0 mg/mL, 87.9 ± 2.20% for 100 mg/mL, 92.7 ± 2.85% for 150 mg/mL, and 91.7 ± 5.36% for 200 mg/mL with all controls below the limit of quantitation (LOQ).

Analysis of QC samples resulted in recoveries ranging from 95.6 to 102% (N = 3) of the nominal fortified concentrations (1.00, 100 and 200 mg/mL). Since all dose formulation mean concentrations recovered within 15% of the target concentration, with a coefficient of variation less than 15%, it can be determined that the appropriate dose concentration verification and homogeneity was achieved for each dose formulation sample received on 4 March 2016.

Representative chromatograms from the 4 March 2016 analysis of a calibration standard, a control, a mid-concentration dose formulation sample, a high-concentration dose formulation sample and a QC sample are presented in Figure 13 through Figure 17. A typical regression analysis for 2-ethylhexyl paraben is presented in Figure 18.

REFERENCES

OECD, 1998. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring. Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997). Environment Directorate Chemicals Group and Management Committee. ENV/MC/CHEM(98)17. OECD Paris, France. 41 pp.

U.S. EPA, 1989. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160); FR: 8/17/89; pp. 34052. U.S. Environmental Protection Agency, Washington, D.C.

Wu, Xianai, 2016. Validation of the Analytical Method for the Determination of 2-Ethylhexyl Paraben Technical in Corn Oil. Smithers Viscient, Wareham, MA. Study No. 13974.6116.

Table 1. ILS 10005.0103: Analytical results for measuring the concentration of 2-ethylhexyl paraben in dose formulation samples received on 26 January 2016.

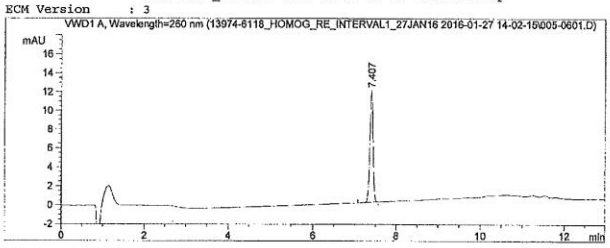
SMV Sample ID	Sample Type	Nominal Concentration (mg/mL)	Measured Concentration (mg/mL)	Percent of Nominal
15-206-14T-1[a]	Homogeneity - Control	0.00	<0.122[b]	NA[c]
15-206-14T-2[a]	Homogeneity - Control	0.00	<0.122	NA
15-206-14B-1[a]	Homogeneity - Control	0.00	<0.122	NA
15-206-14B-2[a]	Homogeneity - Control	0.00	<0.122	NA
15-172-71T-1	Homogeneity - Top	50.0	49.2	98.4
15-172-71T-2	Homogeneity - Top	50.0	48.0	96.1
15-172-71M-1	Homogeneity - Middle	50.0	48.3	96.5
15-172-71M-2	Homogeneity - Middle	50.0	48.4	96.8
15-172-71B-1	Homogeneity - Bottom	50.0	48.1	96.2
15-172-71B-2	Homogeneity - Bottom	50.0	47.6	95.1
		Mean	**48.3**	**96.5**
		Std Dev	**0.547**	**1.09**
		%CV	**1.13**	**1.13**
15-172-72T-1	Homogeneity - Top	100	94.1	94.1
15-172-72T-2	Homogeneity - Top	100	94.9	94.9
15-172-72M-1	Homogeneity - Middle	100	96.4	96.4
15-172-72M-2	Homogeneity - Middle	100	96.5	96.5
15-172-72B-1	Homogeneity - Bottom	100	95.6	95.6
15-172-72B-2	Homogeneity - Bottom	100	93.5	93.5
		Mean	**95.2**	**95.2**
		Std Dev	**1.20**	**1.20**
		%CV	**1.26**	**1.26**
15-172-73T-1	Homogeneity - Top	150	138	91.9
15-172-73T-2	Homogeneity - Top	150	140	93.2
15-172-73M-1	Homogeneity - Middle	150	134	89.5
15-172-73M-2	Homogeneity - Middle	150	136	90.5
15-172-73B-1	Homogeneity - Bottom	150	141	93.7
15-172-73B-2	Homogeneity - Bottom	150	143	95.3
		Mean	**139**	**92.4**
		Std Dev	**3.23**	**2.15**
		%CV	**2.33**	**2.33**
15-172-74T-1	Homogeneity - Top	200	170	84.9
15-172-74T-2	Homogeneity - Top	200	170	84.8
15-172-74M-1	Homogeneity - Middle	200	179	89.7
15-172-74M-2	Homogeneity - Middle	200	177	88.7
15-172-74B-1	Homogeneity - Bottom	200	193	96.6
15-172-74B-2	Homogeneity - Bottom	200	195	97.7
		Mean	**181**	**90.4**
		Std Dev	**11.2**	**5.60**
		%CV	**6.20**	**6.20**
C01-16-92	QC #1	1.00	0.993	99.3[d]
C01-16-95	QC #2	100	101	101[e]
C01-16-96	QC #3	200	203	102[e]

[a] Two control samples (ILS code 15-206-14) were received. SMV assigned unique sample identifiers.
[b] Concentrations expressed as less than values were below the limit of quantitation (LOQ). The LOQ for each analysis is dependent upon the regression, the area of the low standards and the dilution factor of the controls.
[c] NA = Not Applicable.
[d] This quality control sample was prepared on 26 January 2016 and reinjected to demonstrate storage stability of the homogeneity samples.
[e] These quality control samples were prepared on 27 January 2016.
Notes: Results were calculated using the actual analytical (unrounded) results and not the rounded values presented in this table.

Figure 1. ILS 10005.0103: Representative chromatogram of a 0.500 mg/L calibration standard used for quantification of 2-ethylhexyl paraben during the analysis of dose formulation samples.

Data File D:\CHEM32\...3974-6118_HOMOG_RE_INTERVAL1_27JAN16 2016-01-27 14-02-15\005-0601.D
Sample Name: Standard 4-sys

Acq. Operator	: Daniel Benza	Seq. Line :	6
Acq. Instrument	: Instrument 1	Location :	Vial 5
Injection Date	: 1/27/2016 3:32:25 PM	Inj :	1
		Inj Volume :	50.0 µl
Acq. Method	: D:\CHEM32\1\DATA\13974-6118_HOMOG_RE_INTERVAL1_27JAN16 2016-01-27 14-02-15\2-ETHYL HEXYL PARABEN_50UL _UV.M		
Last changed	: 12/9/2015 1:50:47 PM by Xianai Wu		
Analysis Method	: D:\CHEM32\1\DATA\13974-6118_HOMOG_RE_INTERVAL1_27JAN16 2016-01-27 14-02-15\2-ETHYL HEXYL PARABEN_50UL _UV.M (Sequence Method)		
Last changed	: 1/28/2016 8:01:02 AM by Daniel Benza		
Method Info	: 2-ethyl hexyl paraben, Agilent Zorbax SB-C18 3.5µm 75 x 4.6mm, gradient analysis, Solvent A: 0.05% H3PO4 in PRW, Solvent B: 100% ACN, 1.4 mL/min, 260nm, ambient		
ECM Server	: http://ssl007/sal		
ECM Operator	: Daniel Benza		
ECM Path	: Chemistry\2-ethyl hexyl paraben\13974-6118\Data\13974-6118_HOMOG_RE_INTERVAL1_27JAN16 2016-01-27 14-02-15.SC.SSIzip		
ECM Version	: 3		



Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=260 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	7.407	BB	0.0822	62.99947	11.89482	100.0000
Totals :				62.99947	11.89482	

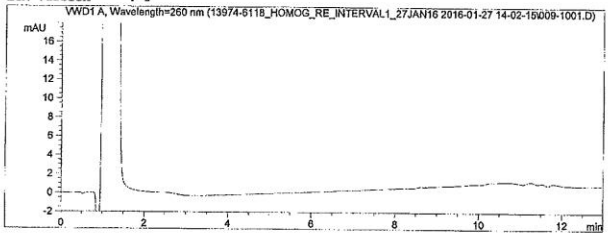
*** End of Report ***

Instrument 1 1/28/2016 8:01:51 AM Daniel Benza Page 1 of 1

Figure 2. ILS 10005.0103: Representative chromatogram of a control sample used for quantification of 2-ethylhexyl paraben during the analysis of dose formulation samples.

Data File D:\CHEM32\...3974-6118_HOMOG_RE_INTERVAL1_27JAN16 2016-01-27 14-02-15\009-1001.D
Sample Name: 15-206-14T-1-sys

Acq. Operator	: Daniel Benza	Seq. Line	: 10
Acq. Instrument	: Instrument 1	Location	: Vial 9
Injection Date	: 1/27/2016 4:43:12 PM	Inj	: 1
		Inj Volume	: 50.0 µl
Acq. Method	: D:\CHEM32\1\DATA\13974-6118_HOMOG_RE_INTERVAL1_27JAN16 2016-01-27 14-02-15\2-ETHYL HEXYL PARABEN_50UL _UV.M		
Last changed	: 12/9/2015 1:50:47 PM by Xianai Wu		
Analysis Method	: D:\CHEM32\1\DATA\13974-6118_HOMOG_RE_INTERVAL1_27JAN16 2016-01-27 14-02-15\2-ETHYL HEXYL PARABEN_50UL _UV.M (Sequence Method)		
Last changed	: 1/28/2016 8:01:02 AM by Daniel Benza		
Method Info	: 2-ethyl hexyl paraben, Agilent Zorbax SB-C18 3.5µm 75 x 4.6mm, gradient analysis, Solvent A: 0.05% H3PO4 in PRW, Solvent B: 100% ACN, 1.4 mL/min, 260nm, ambient		
ECM Server	: http://ssl007/ssl		
ECM Operator	: Daniel Benza		
ECM Path	: Chemistry\2-ethyl hexyl paraben\13974-6118\Data\13974-6118_HOMOG_RE_INTERVAL1_27JAN16 2016-01-27 14-02-15.SC.SSIzip		
ECM Version	: 3		



Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

No peaks found

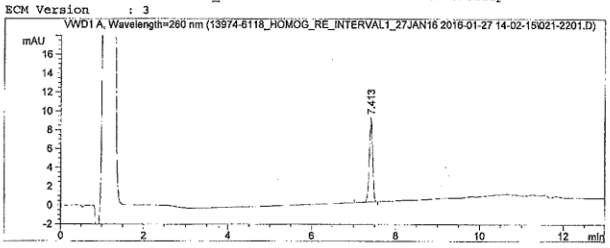
*** End of Report ***

Instrument 1 1/28/2016 8:02:04 AM Daniel Benza Page 1 of 1

Figure 3. ILS 10005.0103: Representative chromatogram of a 100 mg/mL, nominal dose formulation sample fortified with 2-ethylhexyl paraben.

Data File D:\CHEM32\...3974-6118_HOMOG_RE_INTERVAL1_27JAN16 2016-01-27 14-02-15\021-2201.D
Sample Name: 15-172-72M-1-sys

Acq. Operator	: Daniel Benza	Seq. Line	: 22
Acq. Instrument	: Instrument 1	Location	: Vial 21
Injection Date	: 1/27/2016 8:15:35 PM	Inj	: 1
		Inj Volume	: 50.0 µl
Acq. Method	: D:\CHEM32\1\DATA\13974-6118_HOMOG_RE_INTERVAL1_27JAN16 2016-01-27 14-02-15\2-ETHYL HEXYL PARABEN_50UL _UV.M		
Last changed	: 12/9/2015 1:50:47 PM by Xianai Wu		
Analysis Method	: D:\CHEM32\1\DATA\13974-6118_HOMOG_RE_INTERVAL1_27JAN16 2016-01-27 14-02-15\2-ETHYL HEXYL PARABEN_50UL _UV.M (Sequence Method)		
Last changed	: 1/28/2016 8:01:02 AM by Daniel Benza		
Method Info	: 2-ethyl hexyl paraben, Agilent Zorbax SB-C18 3.5µm 75 x 4.6mm, gradient analysis, Solvent A: 0.05% H3PO4 in PRW, Solvent B: 100% ACN, 1.4 mL/min, 260nm, ambient		
ECM Server	: http://ssl007/ssl		
ECM Operator	: Daniel Benza		
ECM Path	: Chemistry\2-ethyl hexyl paraben\13974-6118\Data\13974-6118_HOMOG_RE_INTERVAL1_27JAN16 2016-01-27 14-02-15.SC.SSIzip		
ECM Version	: 3		



Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=260 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	7.413	BB	0.0824	47.89554	9.00971	100.0000
Totals :				47.89554	9.00971	

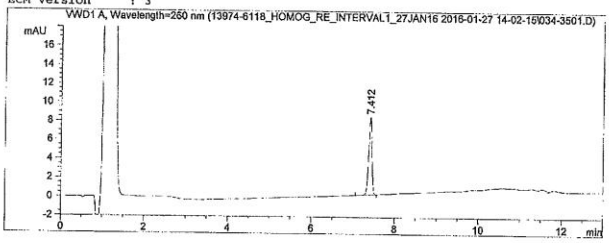
*** End of Report ***

Instrument 1 1/28/2016 8:02:45 AM Daniel Benza Page 1 of 1

Figure 4. ILS 10005.0103: Representative chromatogram of a 200 mg/mL, nominal dose formulation sample fortified with 2-ethylhexyl paraben.

Data File D:\CHEM32\...3974-6118_HOMOG_RE_INTERVAL1_27JAN16 2016-01-27 14-02-15\034-3501.D
Sample Name: 15-172-74M-2-sys

Acq. Operator	: Daniel Benza	Seq. Line	: 35
Acq. Instrument	: Instrument 1	Location	: Vial 34
Injection Date	: 1/28/2016 12:05:35 AM	Inj	: 1
		Inj Volume	: 50.0 µl
Acq. Method	: D:\CHEM32\1\DATA\13974-6118_HOMOG_RE_INTERVAL1_27JAN16 2016-01-27 14-02-15\2-ETHYL HEXYL PARABEN_50UL _UV.M		
Last changed	: 12/9/2015 1:50:47 PM by Xianai Wu		
Analysis Method	: D:\CHEM32\1\DATA\13974-6118_HOMOG_RE_INTERVAL1_27JAN16 2016-01-27 14-02-15\2-ETHYL HEXYL PARABEN_50UL _UV.M (Sequence Method)		
Last changed	: 1/28/2016 8:01:02 AM by Daniel Benza		
Method Info	: 2-ethyl hexyl paraben, Agilent Zorbax SB-C18 3.5µm 75 x 4.6mm, gradient analysis, Solvent A: 0.05% H3PO4 in PRW, Solvent B: 100% ACN, 1.4 mL/min, 260nm, ambient		
ECM Server	: http://ssl007/ssl		
ECM Operator	: Daniel Benza		
ECM Path	: Chemistry\2-ethyl hexyl paraben\13974-6118\Data\13974-6118_HOMOG_RE_INTERVAL1_27JAN16 2016-01-27 14-02-15.SC.SSIzip		
ECM Version	: 3		



Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=260 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	7.412	BB	0.0827	44.09586	8.25457	100.0000
Totals :				44.09586	8.25457	

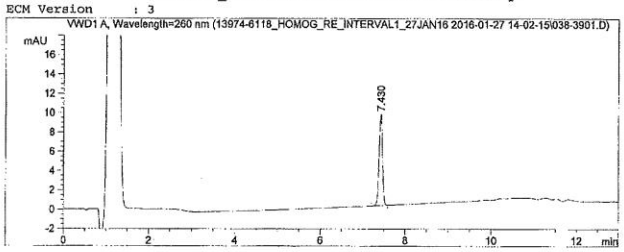
*** End of Report ***

Instrument 1 1/28/2016 8:03:29 AM Daniel Benza Page 1 of 1

Figure 5. ILS 10005.0103: Representative chromatogram of a 100 mg/mL QC sample in corn oil fortified with 2-ethylhexyl paraben.

Data File D:\CHEM32\...3974-6118_HOMOG_RE_INTERVAL1_27JAN16 2016-01-27 14-02-15\038-3901.D
Sample Name: C01-16-95

Acq. Operator	: Daniel Benza	Seq. Line	: 39
Acq. Instrument	: Instrument 1	Location	: Vial 38
Injection Date	: 1/28/2016 1:16:20 AM	Inj	: 1
		Inj Volume	: 50.0 µl
Acq. Method	: D:\CHEM32\1\DATA\13974-6118_HOMOG_RE_INTERVAL1_27JAN16 2016-01-27 14-02-15\2-ETHYL HEXYL PARABEN_50UL _UV.M		
Last changed	: 12/9/2015 1:50:47 PM by Xianai Wu		
Analysis Method	: D:\CHEM32\1\DATA\13974-6118_HOMOG_RE_INTERVAL1_27JAN16 2016-01-27 14-02-15\2-ETHYL HEXYL PARABEN_50UL _UV.M (Sequence Method)		
Last changed	: 1/28/2016 8:01:02 AM by Daniel Benza		
Method Info	: 2-ethyl hexyl paraben, Agilent Zorbax SB-C18 3.5µm 75 x 4.6mm, gradient analysis, Solvent A: 0.05% H3PO4 in PRW, Solvent B: 100% ACN, 1.4 mL/min, 260nm, ambient		
ECM Server	: http://ssl007/ssl		
ECM Operator	: Daniel Benza		
ECM Path	: Chemistry\2-ethyl hexyl paraben\13974-6118\Data\13974-6118_HOMOG_RE_INTERVAL1_27JAN16 2016-01-27 14-02-15.SC.SSIzip		
ECM Version	: 3		



Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

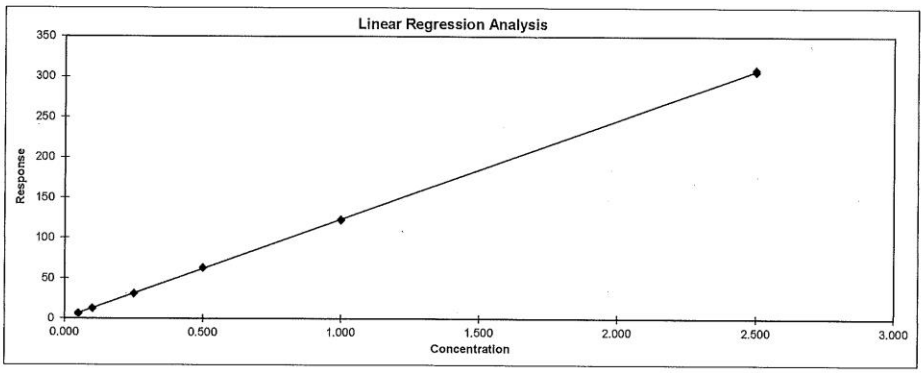
Signal 1: VWD1 A, Wavelength=260 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	7.430	BB	0.0809	50.34643	9.47194	100.0000
Totals :				50.34643	9.47194	

*** End of Report ***

Instrument 1 1/28/2016 8:03:43 AM Daniel Benza Page 1 of 1

Figure 6. **ILS 10005.0103: A typical regression analysis for the calibration standards used to quantitate 2-ethylhexyl paraben during analysis of dose formulation samples.**



$R^2 = 0.99994$
$y = 122.833x + 0.5304$

Standard Concentration mg/L	Standard Response Area
0.0500	6.56745
0.100	12.62591
0.250	31.00886
0.500	62.99947
1.00	122.08902
2.50	306.63745
0.0500	6.45024
0.100	12.75475
0.250	31.12635
0.500	63.36967
1.00	122.77820
2.50	308.88821

Table 2. ILS 10005.0102: Analytical results for measuring the concentration of 2-ethylhexyl paraben in dose formulation samples received on 17 February 2016.

SMV Sample ID	Sample Type	Nominal Concentration (mg/mL)	Measured Concentration (mg/mL)	Percent of Nominal
15-206-20A-1	Homogeneity - Control	0.00	< 0.113[a]	NA[b]
15-206-20A-2	Homogeneity - Control	0.00	< 0.113	NA
15-206-20B-1	Homogeneity - Control	0.00	< 0.113	NA
15-206-20B-2	Homogeneity - Control	0.00	< 0.113	NA
15-172-75T-1	Homogeneity - Top	50.0	52.0	104
15-172-75T-2	Homogeneity - Top	50.0	52.0	104
15-172-75M-1	Homogeneity - Middle	50.0	48.0	96.1
15-172-75M-2	Homogeneity - Middle	50.0	48.8	97.7
15-172-75B-1	Homogeneity - Bottom	50.0	49.7	99.4
15-172-75B-2	Homogeneity - Bottom	50.0	47.9	95.8
		Mean	**49.8**	**99.5**
		Std Dev	**1.87**	**3.74**
		%CV	**3.76**	**3.76**
15-172-76T-1	Homogeneity - Top	100	99.7	99.7
15-172-76T-2	Homogeneity - Top	100	92.0	92.0
15-172-76M-1	Homogeneity - Middle	100	105	105
15-172-76M-2	Homogeneity - Middle	100	98.9	98.9
15-172-76B-1	Homogeneity - Bottom	100	104	104
15-172-76B-2	Homogeneity - Bottom	100	95.8	95.8
		Mean	**99.2**	**99.2**
		Std Dev	**4.84**	**4.84**
		%CV	**4.88**	**4.88**
15-172-77T-1	Homogeneity - Top	150	152	102
15-172-77T-2	Homogeneity - Top	150	154	103
15-172-77M-1	Homogeneity - Middle	150	147	98.2
15-172-77M-2	Homogeneity - Middle	150	146	97.0
15-172-77B-1	Homogeneity - Bottom	150	149	99.2
15-172-77B-2	Homogeneity - Bottom	150	147	97.8
		Mean	**149**	**99.4**
		Std Dev	**3.39**	**2.26**
		%CV	**2.27**	**2.27**
15-172-78T-1	Homogeneity - Top	200	199	99.6
15-172-78T-2	Homogeneity - Top	200	195	97.4
15-172-78M-1	Homogeneity - Middle	200	208	104
15-172-78M-2	Homogeneity - Middle	200	193	96.6
15-172-78B-1	Homogeneity - Bottom	200	192	96.1
15-172-78B-2	Homogeneity - Bottom	200	195	97.6
		Mean	**197**	**98.5**
		Std Dev	**5.72**	**2.86**
		%CV	**2.90**	**2.90**
C02-16-110	QC #1	1.00	0.988	98.8
C02-16-111	QC #2	100	99.2	99.2
C02-16-112	QC #3	200	200	100

[a] Concentrations expressed as less than values were below the limit of quantitation (LOQ). The LOQ for each analysis is dependent upon the regression, the area of the low standards and the dilution factor of the controls.

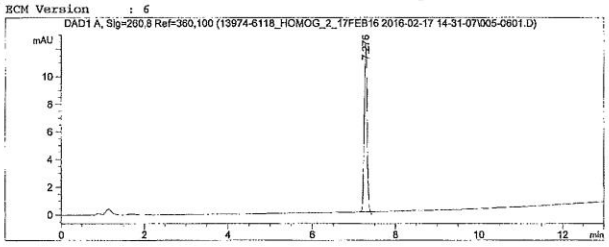
[b] NA = Not Applicable.

Notes: Results were calculated using the actual analytical (unrounded) results and not the rounded values presented in this table

Figure 7. **ILS 10005.0102 (17 February 2016): Representative chromatogram of a 0.500 mg/L calibration standard used for quantification of 2-ethylhexyl paraben during the analysis of dose formulation samples.**

Data File C:\CHEM32\1\DATA\13974-6118_HOMOG_2_17FEB16 2016-02-17 14-31-07\005-0601.D
Sample Name: Standard 4

```
Acq. Operator   : Daniel Benza                    Seq. Line :   6
Acq. Instrument : HPLC 2-1                        Location : Vial 5
Injection Date  : 2/17/2016 4:02:55 PM              Inj :   1
                                                  Inj Volume : 50.000 µl
Acq. Method     : C:\CHEM32\1\DATA\13974-6118_HOMOG_2_17FEB16 2016-02-17 14-31-07\2-ETHYL
                  HEXYL PARABEN_DAD_50UL_2-1.M
Last changed    : 12/9/2015 1:35:40 PM by System
Analysis Method : C:\CHEM32\1\DATA\13974-6118_HOMOG_2_17FEB16 2016-02-17 14-31-07\2-ETHYL
                  HEXYL PARABEN_DAD_50UL_2-1.M (Sequence Method)
Last changed    : 2/25/2016 2:31:38 PM by Daniel Benza
Method Info     : SMV#7945, Agilent Zorbax SB-C18 3.5µm 75 x 4.6mm, gradient analysis,
                  Solvent A: 0.05% H3PO4 in PRW, Solvent B: 100% ACN, 1.4 mL/min, 260nm,
                  ambient

ECM Server      : http://ssl007/ssl
ECM Operator    : Daniel Benza
ECM Path        : Chemistry\2-ethyl hexyl paraben\13974-6118\Data\13974-6118_HOMOG_2_
                  17FEB16 2016-02-17 14-31-07.SC.SSIzip
ECM Version     : 6
```

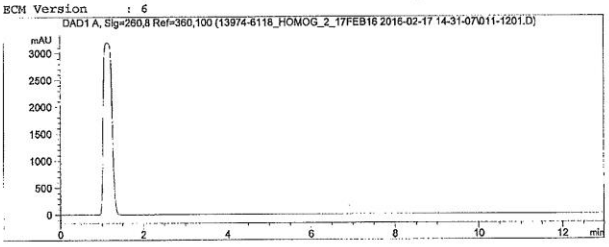



```
                          Area Percent Report

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=260,8 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]    [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   7.276 BB    0.0775   60.38728   12.24258 100.0000

Totals :                    60.38728   12.24258


                          *** End of Report ***

HPLC 3-1 2/25/2016 2:33:14 PM Daniel Benza                    Page   1 of 1
```

Figure 8. ILS 10005.0102 (17 February 2016): Representative chromatogram of a control sample used for quantification of 2-ethylhexyl paraben during the analysis of dose formulation samples.

Data File C:\CHEM32\1\DATA\13974-6118_HOMOG_2_17FEB16 2016-02-17 14-31-07\011-1201.D
Sample Name: 15-206-20B-1

Acq. Operator	: Daniel Benza	Seq. Line	: 12
Acq. Instrument	: HPLC 2-1	Location	: Vial 11
Injection Date	: 2/17/2016 5:50:15 PM	Inj	: 1
		Inj Volume	: 50.000 µl
Acq. Method	: C:\CHEM32\1\DATA\13974-6118_HOMOG_2_17FEB16 2016-02-17 14-31-07\2-ETHYL HEXYL PARABEN_DAD_50UL_2-1.M		
Last changed	: 12/9/2015 1:35:40 PM by System		
Analysis Method	: C:\CHEM32\1\DATA\13974-6118_HOMOG_2_17FEB16 2016-02-17 14-31-07\2-ETHYL HEXYL PARABEN_DAD_50UL_2-1.M (Sequence Method)		
Last changed	: 2/25/2016 2:31:38 PM by Daniel Benza		
Method Info	: SMV#7945, Agilent Zorbax SB-C18 3.5µm 75 x 4.6mm, gradient analysis, Solvent A: 0.05% H3PO4 in PRW, Solvent B: 100% ACN, 1.4 mL/min, 260nm, ambient		
ECM Server	: http://ssl007/ssl		
ECM Operator	: Daniel Benza		
ECM Path	: Chemistry\2-ethyl hexyl paraben\13974-6118\Data\13974-6118_HOMOG_2_17FEB16 2016-02-17 14-31-07.SC.SSIzip		
ECM Version	: 6		



Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

No peaks found

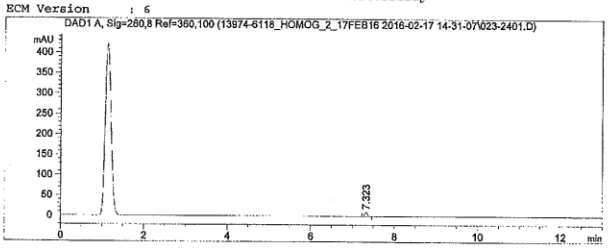
*** End of Report ***

HPLC 3-1 2/25/2016 2:34:05 PM Daniel Benza Page 1 of 1

Figure 9. **ILS 10005.0102 (17 February 2016): Representative chromatogram of a 100 mg/mL, nominal dose formulation sample fortified with 2-ethylhexyl paraben.**

Data File C:\CHEM32\1\DATA\13974-6118_HOMOG_2_17FEB16 2016-02-17 14-31-07\023-2401.D
Sample Name: 15-172-76B-1

Acq. Operator	: Daniel Benza	Seq. Line	: 24
Acq. Instrument	: HPLC 2-1	Location	: Vial 23
Injection Date	: 2/17/2016 9:24:58 PM	Inj	: 1
		Inj Volume	: 50.000 µl
Acq. Method	: C:\CHEM32\1\DATA\13974-6118_HOMOG_2_17FEB16 2016-02-17 14-31-07\2-ETHYL HEXYL PARABEN_DAD_50UL_2-1.M		
Last changed	: 12/9/2015 1:35:40 PM by System		
Analysis Method	: C:\CHEM32\1\DATA\13974-6118_HOMOG_2_17FEB16 2016-02-17 14-31-07\2-ETHYL HEXYL PARABEN_DAD_50UL_2-1.M (Sequence Method)		
Last changed	: 2/25/2016 2:31:38 PM by Daniel Benza		
Method Info	: SMV#7945, Agilent Zorbax SB-C18 3.5µm 75 x 4.6mm, gradient analysis, Solvent A: 0.05% H3PO4 in PRW, Solvent B: 100% ACN, 1.4 mL/min, 260nm, ambient		
ECM Server	: http://ssl007/ssl		
ECM Operator	: Daniel Benza		
ECM Path	: Chemistry\2-ethyl hexyl paraben\13974-6118\Data\13974-6118_HOMOG_2_17FEB16 2016-02-17 14-31-07.SC.SSIzip		
ECM Version	: 6		



Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=260,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.323	BB	0.0775	49.49842	10.04403	100.0000
Totals :				49.49842	10.04403	

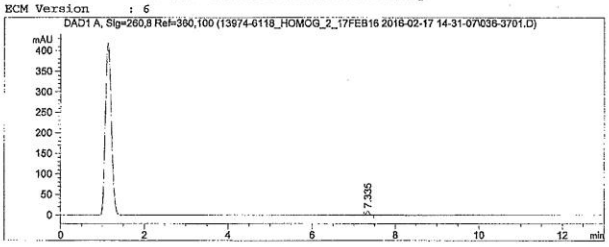
*** End of Report ***

HPLC 3-1 2/25/2016 2:35:48 PM Daniel Benza Page 1 of 1

Figure 10. ILS 10005.0102 (17 February 2016): Representative chromatogram of a 200 mg/mL, nominal dose formulation sample fortified with 2-ethylhexyl paraben.

Data File C:\CHEM32\1\DATA\13974-6118_HOMOG_2_17FEB16 2016-02-17 14-31-07\036-3701.D
Sample Name: 15-172-78B-2

```
Acq. Operator   : Daniel Benza                    Seq. Line :  37
Acq. Instrument : HPLC 2-1                        Location : Vial 36
Injection Date  : 2/18/2016 1:17:37 AM               Inj :  1
                                                  Inj Volume : 50.000 µl
Acq. Method     : C:\CHEM32\1\DATA\13974-6118_HOMOG_2_17FEB16 2016-02-17 14-31-07\2-ETHYL
                  HEXYL PARABEN_DAD_50UL_2-1.M
Last changed    : 12/9/2015 1:35:40 PM by System
Analysis Method : C:\CHEM32\1\DATA\13974-6118_HOMOG_2_17FEB16 2016-02-17 14-31-07\2-ETHYL
                  HEXYL PARABEN_DAD_50UL_2-1.M (Sequence Method)
Last changed    : 2/25/2016 2:31:38 PM by Daniel Benza
Method Info     : SMV#7945, Agilent Zorbax SB-C18 3.5µm 75 x 4.6mm, gradient analysis,
                  Solvent A: 0.05% H3PO4 in PRW, Solvent B: 100% ACN, 1.4 mL/min, 260nm,
                  ambient

ECM Server      : http://ssl007/ssl
ECM Operator    : Daniel Benza
ECM Path        : Chemistry\2-ethyl hexyl paraben\13974-6118\Data\13974-6118_HOMOG_2_
                  17FEB16 2016-02-17 14-31-07.SC.SSIzip
ECM Version     : 6
```

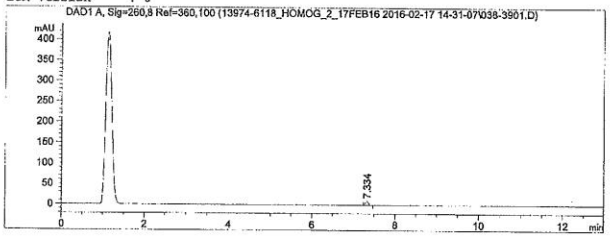



```
                          Area Percent Report

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=260,8 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]    [mAU*s]    [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   7.335 BB    0.0777   46.71900    9.45236 100.0000

Totals :                    46.71900    9.45236

                          *** End of Report ***

HPLC 3-1 2/25/2016 2:37:39 PM Daniel Benza                    Page  1 of 1
```

Figure 11. ILS 10005.0102 (17 February 2016): Representative chromatogram of a 100 mg/mL QC sample in corn oil fortified with 2-ethylhexyl paraben.

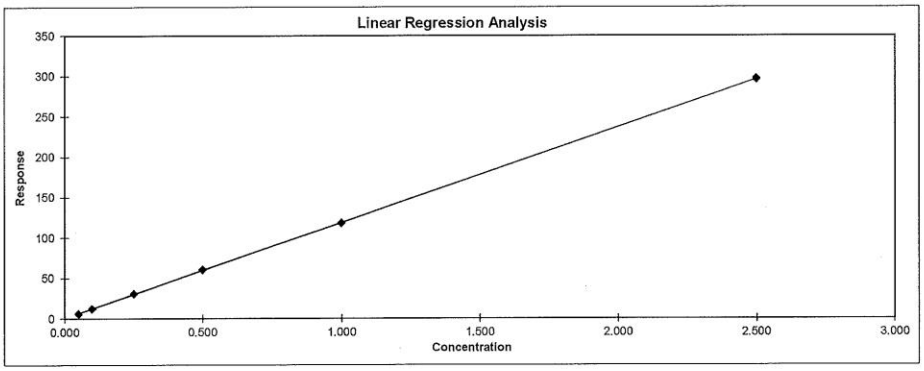
Data File C:\CHEM32\1\DATA\13974-6118_HOMOG_2_17FEB16 2016-02-17 14-31-07\038-3901.D
Sample Name: C02-16-111

```
Acq. Operator    : Daniel Benza                    Seq. Line :  39
Acq. Instrument  : HPLC 2-1                         Location : Vial 38
Injection Date   : 2/18/2016 1:53:24 AM                Inj :  1
                                                  Inj Volume : 50.000 µl
Acq. Method      : C:\CHEM32\1\DATA\13974-6118_HOMOG_2_17FEB16 2016-02-17 14-31-07\2-ETHYL
                   HEXYL PARABEN_DAD_50UL_2-1.M
Last changed     : 12/9/2015 1:35:40 PM by System
Analysis Method  : C:\CHEM32\1\DATA\13974-6118_HOMOG_2_17FEB16 2016-02-17 14-31-07\2-ETHYL
                   HEXYL PARABEN_DAD_50UL_2-1.M (Sequence Method)
Last changed     : 2/25/2016 2:31:38 PM by Daniel Benza
Method Info      : SMV#7945, Agilent Zorbax SB-C18 3.5µm 75 x 4.6mm, gradient analysis,
                   Solvent A: 0.05% H3PO4 in PRW, Solvent B: 100% ACN, 1.4 mL/min, 260nm,
                   ambient

ECM Server       : http://ssl007/ssl
ECM Operator     : Daniel Benza
ECM Path         : Chemistry\2-ethyl hexyl paraben\13974-6118\Data\13974-6118_HOMOG_2_
                   17FEB16 2016-02-17 14-31-07.SC.SSIzip
ECM Version      : 6
```




```
                          Area Percent Report

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=260,8 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]      %
----|-------|----|-------|----------|----------|--------|
   1   7.334 BB    0.0776   47.47314    9.61025 100.0000

Totals :                    47.47314    9.61025

                          *** End of Report ***

HPLC 3-1 2/25/2016 2:37:57 PM Daniel Benza                Page  1 of 1
```

Figure 12. ILS 10005.0102 (17 February 2016): A typical regression analysis for the calibration standards used to quantitate 2-ethylhexyl paraben during analysis of dose formulation samples.



$R^2 = 0.99997$
$y = 118.230x + 0.5499$

Standard Concentration mg/L	Standard Response Area
0.0500	5.94767
0.100	12.18842
0.250	30.47505
0.500	60.38728
1.00	117.97561
2.50	295.61786
0.0500	5.85503
0.100	12.23228
0.250	30.57564
0.500	60.61165
1.00	118.45434
2.50	296.70117

Table 3. ILS 10005.0102: Analytical results for measuring the concentration of 2-ethylhexyl paraben in dose formulation samples received on 4 March 2016.

SMV Sample ID	Sample Type	Nominal Concentration (mg/mL)	Measured Concentration (mg/mL)	Percent of Nominal
15-206-22A-1	Homogeneity - Control	0.00	< 0.138[a]	NA[b]
15-206-22A-2	Homogeneity - Control	0.00	< 0.138	NA
15-206-22B-1	Homogeneity - Control	0.00	< 0.138	NA
15-206-22B-2	Homogeneity - Control	0.00	< 0.138	NA
15-172-79T-1	Homogeneity - Top	50.0	46.1	92.2
15-172-79T-2	Homogeneity - Top	50.0	48.3	96.5
15-172-79M-1	Homogeneity - Middle	50.0	42.5	84.9
15-172-79M-2	Homogeneity - Middle	50.0	44.1	88.2
15-172-79B-1	Homogeneity - Bottom	50.0	45.2	90.4
15-172-79B-2	Homogeneity - Bottom	50.0	< 6.92[c]	NA
		Mean	**45.2**	**90.5**
		Std Dev	**2.18**	**4.36**
		%CV	**4.82**	**4.82**
15-172-80T-1	Homogeneity - Top	100	87.5	87.5
15-172-80T-2	Homogeneity - Top	100	86.1	86.1
15-172-80M-1	Homogeneity - Middle	100	87.9	87.9
15-172-80M-2	Homogeneity - Middle	100	85.6	85.6
15-172-80B-1	Homogeneity - Bottom	100	90.7	90.7
15-172-80B-2	Homogeneity - Bottom	100	89.5	89.5
		Mean	**87.9**	**87.9**
		Std Dev	**1.94**	**1.94**
		%CV	**2.20**	**2.20**
15-172-81T-1	Homogeneity - Top	150	138	92.2
15-172-81T-2	Homogeneity - Top	150	140	93.3
15-172-81M-1	Homogeneity - Middle	150	140	93.7
15-172-81M-2	Homogeneity - Middle	150	134	89.5
15-172-81B-1	Homogeneity - Bottom	150	146	97.0
15-172-81B-2	Homogeneity - Bottom	150	136	90.7
		Mean	**139**	**92.7**
		Std Dev	**3.96**	**2.64**
		%CV	**2.85**	**2.85**
15-172-82T-1	Homogeneity - Top	200	169	84.3
15-172-82T-2	Homogeneity - Top	200	179	89.4
15-172-82M-1	Homogeneity - Middle	200	184	92.0
15-172-82M-2	Homogeneity - Middle	200	183	91.5
15-172-82B-1	Homogeneity - Bottom	200	188	93.8
15-172-82B-2	Homogeneity - Bottom	200	198	99.2
		Mean	**183**	**91.7**
		Std Dev	**9.83**	**4.91**
		%CV	**5.36**	**5.36**
C3-16-33	QC #1	1.00	0.956	95.6
C3-16-34	QC #2	100	99.8	99.8
C3-16-35	QC #3	200	204	102

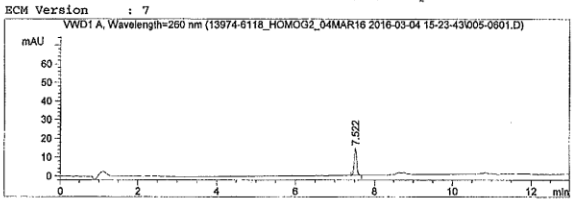
[a] Concentrations expressed as less than values were below the limit of quantitation (LOQ). The LOQ for each analysis is dependent upon the regression, the area of the low standards and the dilution factor of the controls.
[b] NA = Not Applicable.
[c] This duplicate sample showed no peak in the chromatogram, likely due to a processing error.

Notes: Results were calculated using the actual analytical (unrounded) results and not the rounded values presented in this table

Figure 13. ILS 10005.0102 (4 March 2016): Representative chromatogram of a 0.500 mg/L calibration standard used for quantification of 2-ethylhexyl paraben during the analysis of dose formulation samples.

Data File C:\CHEM32\2\DATA\13974-6118_HOMOG2_04MAR16 2016-03-04 15-23-43\005-0601.D
Sample Name: Standard 4

Acq. Operator	: Daniel Benza	Seq. Line	: 6
Acq. Instrument	: HPLC 4-2	Location	: Vial 5
Injection Date	: 3/4/2016 4:53:33 PM	Inj	: 1
		Inj Volume	: 50.0 µl
Acq. Method	: C:\CHEM32\2\DATA\13974-6118_HOMOG2_04MAR16 2016-03-04 15-23-43\2-ETHYL HEXYL PARABEN_50UL _UV.M		
Last changed	: 12/9/2015 1:50:47 PM by Xianai Wu		
Analysis Method	: C:\CHEM32\2\DATA\13974-6118_HOMOG2_04MAR16 2016-03-04 15-23-43\2-ETHYL HEXYL PARABEN_50UL _UV.M (Sequence Method)		
Last changed	: 3/7/2016 8:39:17 AM by Alexis Zelkan		
Method Info	: 2-ethyl hexyl paraben, Agilent Zorbax SB-C18 3.5µm 75 x 4.6mm, gradient analysis, Solvent A: 0.05% H3PO4 in PRW, Solvent B: 100% ACN, 1.4 mL/min, 260nm, ambient		
ECM Server	: http://ssl007/SSL		
ECM Operator	: Alexis Zelkan		
ECM Path	: Chemistry\2-ethyl hexyl paraben\13974-6118\Data\13974-6118_HOMOG2_04MAR16 2016-03-04 15-23-43.SC.SSIzip		
ECM Version	: 7		



Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=260 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	7.522	BBA	0.0685	63.06995	14.42940	100.0000
Totals :				63.06995	14.42940	

*** End of Report ***

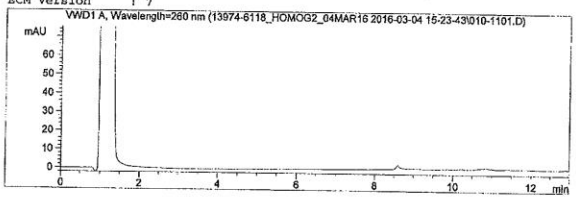
HPLC 4-2 3/7/2016 8:40:03 AM Alexis Zelkan Page 1 of 1

Figure 14. ILS 10005.0102 (4 March 2016): Representative chromatogram of a control sample used for quantification of 2-ethylhexyl paraben during the analysis of dose formulation samples.

Data File C:\CHEM32\2\DATA\13974-6118_HOMOG2_04MAR16 2016-03-04 15-23-43\010-1101.D
Sample Name: 15-206-22A-2

Acq. Operator	: Daniel Benza	Seq. Line	: 11
Acq. Instrument	: HPLC 4-2	Location	: Vial 10
Injection Date	: 3/4/2016 6:22:11 PM	Inj	: 1
		Inj Volume	: 50.0 µl
Acq. Method	: C:\CHEM32\2\DATA\13974-6118_HOMOG2_04MAR16 2016-03-04 15-23-43\2-ETHYL HEXYL PARABEN_50UL _UV.M		
Last changed	: 12/9/2015 1:50:47 PM by Xianai Wu		
Analysis Method	: C:\CHEM32\2\DATA\13974-6118_HOMOG2_04MAR16 2016-03-04 15-23-43\2-ETHYL HEXYL PARABEN_50UL _UV.M (Sequence Method)		
Last changed	: 3/7/2016 8:39:17 AM by Alexis Zelkan		
Method Info	: 2-ethyl hexyl paraben, Agilent Zorbax SB-C18 3.5µm 75 x 4.6mm, gradient analysis, Solvent A: 0.05% H3PO4 in PRW, Solvent B: 100% ACN, 1.4 mL/min, 260nm, ambient		

ECM Server : http://ssl007/SSL
ECM Operator : Alexis Zelkan
ECM Path : Chemistry\2-ethyl hexyl paraben\13974-6118\Data\13974-6118_HOMOG2_04MAR16 2016-03-04 15-23-43.SC.SSIzip
ECM Version : 7



Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

No peaks found

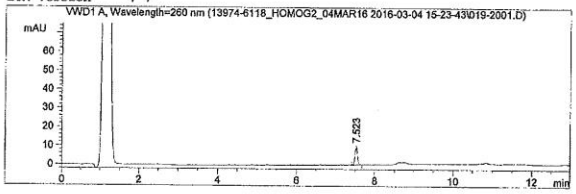
*** End of Report ***

HPLC 4-2 3/7/2016 8:40:30 AM Alexis Zelkan Page 1 of 1

Figure 15. ILS 10005.0102 (4 March 2016): Representative chromatogram of a 100 mg/mL, nominal dose formulation sample fortified with 2-ethylhexyl paraben.

Data File C:\CHEM32\2\DATA\13974-6118_HOMOG2_04MAR16 2016-03-04 15-23-43\019-2001.D
Sample Name: 15-172-80T-1

Acq. Operator	: Daniel Benza	Seq. Line	: 20
Acq. Instrument	: HPLC 4-2	Location	: Vial 19
Injection Date	: 3/4/2016 9:01:40 PM	Inj	: 1
		Inj Volume	: 50.0 µl
Acq. Method	: C:\CHEM32\2\DATA\13974-6118_HOMOG2_04MAR16 2016-03-04 15-23-43\2-ETHYL HEXYL PARABEN_50UL _UV.M		
Last changed	: 12/9/2015 1:50:47 PM by Xianai Wu		
Analysis Method	: C:\CHEM32\2\DATA\13974-6118_HOMOG2_04MAR16 2016-03-04 15-23-43\2-ETHYL HEXYL PARABEN_50UL _UV.M (Sequence Method)		
Last changed	: 3/7/2016 8:39:17 AM by Alexis Zelkan		
Method Info	: 2-ethyl hexyl paraben, Agilent Zorbax SB-C18 3.5µm 75 x 4.6mm, gradient analysis, Solvent A: 0.05% H3PO4 in PRW, Solvent B: 100% ACN, 1.4 mL/min, 260nm, ambient		
ECM Server	: http://ssl007/SSL		
ECM Operator	: Alexis Zelkan		
ECM Path	: Chemistry\2-ethyl hexyl paraben\13974-6118\Data\13974-6118_HOMOG2_04MAR16 2016-03-04 15-23-43.SC.SSIzip		
ECM Version	: 7		



Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=260 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	7.523	BBA	0.0667	44.26055	10.17978	100.0000
Totals :				44.26055	10.17978	

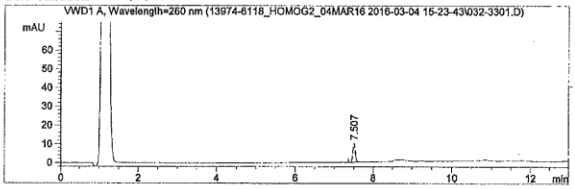
*** End of Report ***

HPLC 4-2 3/7/2016 8:50:34 AM Alexis Zelkan Page 1 of 1

Figure 16. ILS 10005.0102 (4 March 2016): Representative chromatogram of a 200 mg/mL, nominal dose formulation sample fortified with 2-ethylhexyl paraben.

Data File C:\CHEM32\2\DATA\13974-6118_HOMOG2_04MAR16 2016-03-04 15-23-43\032-3301.D
Sample Name: 15-172-82T-2

```
Acq. Operator    : Daniel Benza                    Seq. Line :  33
Acq. Instrument  : HPLC 4-2                         Location : Vial 32
Injection Date   : 3/5/2016 12:51:57 AM                Inj :  1
                                                  Inj Volume : 50.0 µl
Acq. Method      : C:\CHEM32\2\DATA\13974-6118_HOMOG2_04MAR16 2016-03-04 15-23-43\2-ETHYL
                   HEXYL PARABEN_50UL _UV.M
Last changed     : 12/9/2015 1:50:47 PM by Xianai Wu
Analysis Method  : C:\CHEM32\2\DATA\13974-6118_HOMOG2_04MAR16 2016-03-04 15-23-43\2-ETHYL
                   HEXYL PARABEN_50UL _UV.M (Sequence Method)
Last changed     : 3/7/2016 8:39:17 AM by Alexis Zelkan
Method Info      : 2-ethyl hexyl paraben, Agilent Zorbax SB-C18 3.5µm 75 x 4.6mm, gradient
                   analysis, Solvent A: 0.05% H3PO4 in PRW, Solvent B: 100% ACN, 1.4 mL/
                   min, 260nm, ambient

ECM Server       : http://ssl007/SSL
ECM Operator     : Alexis Zelkan
ECM Path         : Chemistry\2-ethyl hexyl paraben\13974-6118\Data\13974-6118_HOMOG2_
                   04MAR16 2016-03-04 15-23-43.SC.SSIzip
ECM Version      : 7
```




```
                          Area Percent Report

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=260 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]       [min]  mAU   *s    [mAU   ]      %
----|-------|----|-------|----------|----------|--------|
   1   7.507 BBA   0.0686   45.23090   10.32425 100.0000

Totals :                   45.23090   10.32425


                          *** End of Report ***
```

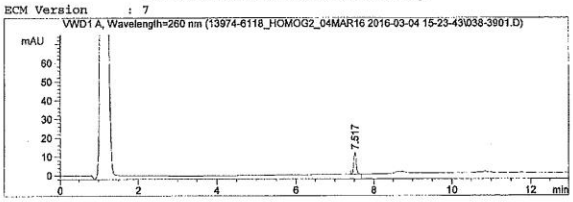
HPLC 4-2 3/7/2016 8:41:26 AM Alexis Zelkan Page 1 of 1

Figure 17. ILS 10005.0102 (4 March 2016): Representative chromatogram of a 100 mg/mL QC sample in corn oil fortified with 2-ethylhexyl paraben.

Data File C:\CHEM32\2\DATA\13974-6118_HOMOG2_04MAR16 2016-03-04 15-23-43\038-3901.D
Sample Name: C03-16-34

Acq. Operator : Daniel Benza Seq. Line : 39
Acq. Instrument : HPLC 4-2 Location : Vial 38
Injection Date : 3/5/2016 2:38:15 AM Inj : 1
Inj Volume : 50.0 µl
Acq. Method : C:\CHEM32\2\DATA\13974-6118_HOMOG2_04MAR16 2016-03-04 15-23-43\2-ETHYL HEXYL PARABEN_50UL _UV.M
Last changed : 12/9/2015 1:50:47 PM by Xianai Wu
Analysis Method : C:\CHEM32\2\DATA\13974-6118_HOMOG2_04MAR16 2016-03-04 15-23-43\2-ETHYL HEXYL PARABEN_50UL _UV.M (Sequence Method)
Last changed : 3/7/2016 8:39:17 AM by Alexis Zelkan
Method Info : 2-ethyl hexyl paraben, Agilent Zorbax SB-C18 3.5µm 75 x 4.6mm, gradient analysis, Solvent A: 0.05% H3PO4 in PRW, Solvent B: 100% ACN, 1.4 mL/min, 260nm, ambient

ECM Server : http://ssl007/SSL
ECM Operator : Alexis Zelkan
ECM Path : Chemistry\2-ethyl hexyl paraben\13974-6118\Data\13974-6118_HOMOG2_04MAR16 2016-03-04 15-23-43.SC.SSIzip
ECM Version : 7



Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

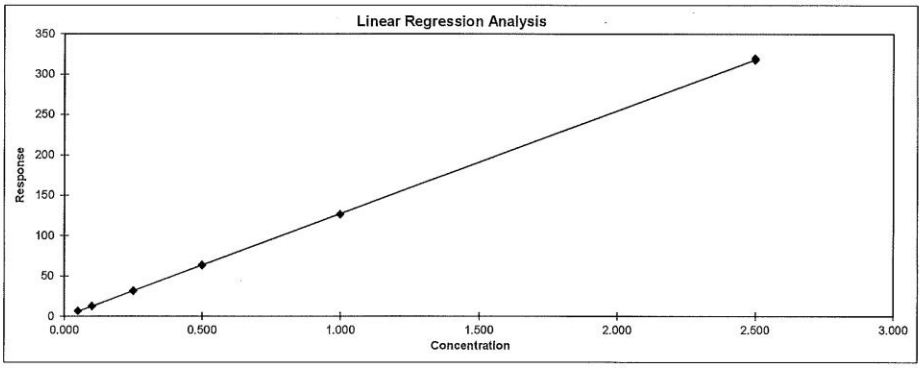
Signal 1: VWD1 A, Wavelength=260 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	7.517	BBA	0.0684	50.57186	11.59040	100.0000
Totals :				50.57186	11.59040	

*** End of Report ***

HPLC 4-2 3/7/2016 8:41:42 AM Alexis Zelkan Page 1 of 1

Figure 18. ILS 10005.0102 (4 March 2016): A typical regression analysis for the calibration standards used to quantitate 2-ethylhexyl paraben during analysis of dose formulation samples.



$R^2 = 0.99996$
$y = 127.392x - 0.3067$

Standard Concentration mg/L	Standard Response Area
0.0500	6.68371
0.100	12.31548
0.250	31.28907
0.500	63.06995
1.00	126.52518
2.50	317.41107
0.0500	6.80826
0.100	12.58458
0.250	31.58118
0.500	63.44545
1.00	126.02801
2.50	319.62909

APPENDIX 1 – CERTIFICATE OF ANALYSIS

Certificate of Analysis

Sep 29, 2016 (JST)

TOKYO CHEMICAL INDUSTRY CO., LTD.
4-10-1 Nihonbashi-Honcho, Chuo-ku, Tokyo 103-0023 Japan

Chemical Name: 2-Ethylhexyl 4-Hydroxybenzoate		
Product Number: H0506 CAS: 5153-23-3	Lot: TCZFO	

Tests	Results	Specifications
Purity(HPLC)	99.3 area%	min. 98.0 area%
Purity(Neutralization titration)	99.8 %	min. 98.0 %
Specific gravity (20/20)	1.0382	1.0360 to 1.0550
Refractive Index n20/D	1.5210	1.5190 to 1.5220

TCI Lot numbers are 4-5 characters in length.
Characters listed after the first 4-5 characters are control numbers for internal purposes only.

Customer service:
TCI AMERICA
Tel: +1-800-423-8616 / +1-503-283-1681
Fax: +1-888-520-1075 / +1-503-283-1937
E-mail: Sales-US@TCIchemicals.com

APPENDIX VII: Study Protocol and Protocol Deviation

Study Title
The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Guideline Numbers
OPPTS 890.1400
OECD 441

ILS Project-Study Number
10005.0103

Performing Laboratory
Integrated Laboratory Systems, Inc.
635 Davis Drive, Suite 600
Morrisville, NC 27560 USA

Sponsor
RTI International
3040 Cornwallis Road
Research Triangle Park, NC 27709 USA

ILS Project No. – Study No.: 10005.0103; The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Study Protocol Approval

Sherry Black, B.S.
Sponsor Representative

1/20/2016
Date

Jeffrey P. Davis, B.S., LATG
Study Director
Integrated Laboratory Systems, Inc.

21 January 2016
Date

Reviewed by:

Cheryl Hobbs, Ph.D.
Director of Toxicology
Integrated Laboratory Systems, Inc.

20 Jan 2016
Date

Leslie Recio, Ph.D., DABT
Vice President, Research and Development
Integrated Laboratory Systems, Inc.

20 Jan 2016
Date

ILS Project No. – Study No.: 10005.0103; The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

TABLE OF CONTENTS

ILS Project No. – Study No.: 10005.0103; The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

INTRODUCTION

1.1 Background

Endocrine Disruptor Screening Program (EDSP) Tier 1 screening assays will be used to identify substances that have the potential to interact with the estrogen, androgen, or thyroid hormone systems (test guidelines in the OPPTS 890 series). The determination of a chemical's ability to interact with hormone systems will be made on a weight-of-evidence basis, taking into account data from the Tier 1 assays and other scientifically relevant information available. If a substance interacts with a hormone system, it does not imply that when used it will cause adverse effects in humans or ecological systems.

EPA has requested *in vivo* mammalian studies to bridge data gaps regarding a chemical's potential endocrine effects; the data from these studies may also be used to evaluate and refine computational models that predict *in vivo* responses from *in vitro* assays.

1.2 Purpose

The purpose of the Hershberger assay is to screen 2-ethylhexyl paraben for androgen agonist/antagonist activity and 5α-reductase inhibition properties using a castrated rat model (U.S. EPA, 2009; OECD, 2009).

1.3 Regulatory Compliance

This study will be conducted in accordance with Good Laboratory Practice regulations as promulgated by the United States Environmental Protection Agency's (U.S. EPA) Good Laboratory Practice (GLP) Regulations (40 CFR Part 160), the Endocrine Disruptor Screening Program Test Guideline OPPTS 890.1400: Hershberger Bioassay (U.S. EPA, 2009), OECD Guideline 441: Hershberger Bioassay in Rats (OECD, 2009), and ILS Standard Operating Procedures (SOPs). The study protocol will be reviewed by the ILS Quality Assurance (QA) Unit before final approval by the Sponsor. All changes to the study protocol will be approved by the Sponsor.

All staff are signed off on the appropriate SOPs. Protocol amendments, if necessary, will be prepared to document changes to the study protocol and will be approved by the Sponsor. Deviations to the study protocol, any SOP, or the Quality Assurance Project Plan (QAPP) will be communicated to the Sponsor and properly documented including section of the protocol, SOP, or QAPP deviated from, nature of deviation, reason for deviation, corrective action, and impact on the study. The animal facility in which the study will occur is AAALAC accredited (2015).

ILS Project No. – Study No.: 10005.0103; The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Flutamide and testosterone propionate will not be analyzed as stated in 40 CFR 160.113(a) of the U.S. EPA GLP requirements. A positive response in the test system following administration will be evident following statistical analysis of the tissue weights.

A QA inspection of critical study phases will be conducted by ILS to assure the quality and integrity of the study results and conformance to the study protocol. An audit of the final report will be conducted to determine consistency between reported information and raw data. An appropriate QA statement will be included in the final report.

QA functions related to the analytical procedures of the dose formulations will be the responsibility of the QA Unit at Smithers Viscient, LLC. All findings will be reported to the Study Director, and the QA Unit at Smithers Viscient, LLC will provide a QA inspection statement to the Study Director to be included in the final report.

1.4 Sponsor

RTI International
3040 Cornwallis Road
Research Triangle Park, NC 27709 USA

Sponsor Representative

Sherry Black, B.S.
Telephone No.: (919) 541-7353
E-mail: sherryb@rti.org

1.5 Testing Facility

Integrated Laboratory Systems, Inc. (ILS)

Shipping Address: 635 Davis Drive, Suite 600
Morrisville, NC 27560 USA

Mailing Address: P.O. Box 13501
Research Triangle Park, NC 27709 USA

Study Director

Jeffrey P. Davis, B.S., LATG
Telephone No.: (919) 281-1110 ext. 720
Facsimile No.: (919) 281-1118
E-mail: jdavis@ils-inc.com

ILS Project No. – Study No.: 10005.0103; The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

1.6 Proposed Study Dates

Animal Arrival Date:	21 January 2016
Experimental Start Date:	29 January 2016
Experimental In-Life Termination Date:	09 February 2016
Experimental Termination Date:	09 February 2016

TEST SUBSTANCE, REFERENCE SUBSTANCE, VEHICLE

2.1	**Test Substance:**	**2-Ethylhexyl Paraben**
	CAS No.:	5153-25-3
	Source:	Tokyo Chemistry Industry Co., Ltd. (Tokyo, Japan)
	Lot/Batch No.:	7CZZO
	ILS Repository No.:	15-172
	Formula:	$C_{15}H_{22}O_3$
	Description:	Colorless, clear liquid
	Purity:	99.8%
	Expiration Date:	None given on Certificate of Analysis
	Dose Formulation:	2-Ethylhexyl paraben will be prepared at ILS in corn oil once at dose concentrations of 50, 100, 150, and 200 mg/mL and aliquoted into vials to be used daily during the study.
	Storage:	
	Test Substance:	Room temperature and protected from light
	Dose Formulation:	Between 1 and 10°C
	Stability:	
	Dose Formulation:	Concentrations of 1 and 200 mg/mL stored between 1 and 10°C were shown to be stable for 20 days (report in progress).

ILS Project No. – Study No.: 10005.0103; The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

2.2	**Reference Substance:**	**Testosterone Propionate (androgen agonist)**
	CAS No.:	57-85-2
	Source: Lot/Batch No.:	Spectrum Chemicals (New Brunswick, NJ) 1EL0103
	ILS Repository No.:	16-18
	Formula:	$C_{22}H_{32}O_3$
	Description:	White to off-white powder
	Purity:	99.7%
	Expiration Date:	31 July 2020
	Dose Formulation:	Testosterone propionate (TP) will be prepared at ILS in corn oil once at a dose level of 0.8 mg/mL and aliquoted into vials to be used daily during the study.
	Storage:	
	Reference Substance:	Room temperature, away from light
	Dose Formulation:	1-10°C
	Stability:	
	Dose Formulation:	TP in corn oil stored between 1 and 10°C was shown to be stable for 14 days (Smith, 2011).
2.3	**Reference Substance:**	**Flutamide (androgen antagonist)**
	CAS No.:	13311-84-7
	Source:	Spectrum Chemicals (New Brunswick, NJ)
	Lot/Batch No.:	2EL0006
	ILS Repository No.:	15-212
	Formula:	$C_{11}H_{11}F_3N_2O_3$
	Description:	Yellow powder

ILS Project No. – Study No.: 10005.0103; The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Purity:	99.9%
Expiration Date:	None given on Certificate of Analysis
Dose Formulation:	Flutamide (FT) will be prepared at ILS in corn oil once at a dose level of 0.6 mg/mL and aliquoted into vials to be used daily during the study.
Storage:	
Reference Substance:	Room temperature, away from light
Dose Formulation:	1-10°C
Stability:	
Dose Formulation:	FT in corn oil stored between 1 and 10°C was shown to be stable for 42 days (Graves, 2001).

2.4 Vehicle **Corn oil**

To be used in dose formulations of 2-ethylhexyl paraben and flutamide.

CAS No.	8001-30-7
Source:	Animal Health International (Greeley, CO)
Lot/Batch No.:	16303-100175
Formula:	$C_{27}H_{50}O_6$
Description:	Yellow oil
Storage:	Room temperature and protected from light

2.5 Vehicle **Corn oil**

To be used in dose formulation of testosterone propionate. National Formulary grade for use in injections.

CAS No.	8001-30-7
Source:	Spectrum Chemicals (New Brunswick, NJ)
Lot/Batch No.:	2EJ0098

ILS Project No. – Study No.: 10005.0103; The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Formula:	$C_{27}H_{50}O_6$
Description:	Yellow oil
Storage:	Room temperature and protected from light

2.6 Archival Samples

Approximately a 1 mg sample of the test and reference substances, and 1 mL of the vehicle and dose formulations will be stored between 0 and -30°C. After acceptance of the study report by the Sponsor, archival dose formulation samples will be discarded. Test and reference substances will be maintained by ILS for five years following finalization of the study report.

2.7 Dose Formulation Analysis

Dose formulations will be prepared at ILS and analyzed at Smithers Viscient, LLC (Wareham, MA) in accordance with GLP regulations as promulgated by the U.S. EPA GLP Regulations (40 CFR Part 160). Three samples (top, middle, and bottom) of the test substance formulations will be analyzed in duplicate for concentration and homogeneity. Concentration results will be acceptable if the mean concentration is within 15% of the target concentration. Homogeneity results will be acceptable if the coefficient of variation is less than 15%.

Principal Investigator- Xianai Wu, Ph.D., DABT
Smithers Viscient, LLC
790 Main Street
Wareham, MA 02571-1075

EXPERIMENTAL DESIGN

Eighty eight castrated male Sprague Dawley rats will be allocated to one of 11 designated dose groups (8 animals/test group). Agonist and antagonist modes will be run concurrently. To evaluate the test substance for agonist properties, animals will be administered one of four dose levels of 2-ethylhexyl paraben, the vehicle control (corn oil), or an agonist reference substance (testosterone propionate, 0.4 mg/kg/day). To evaluate the test substance for antagonist properties, animals will be administered one of four dose levels of 2-ethylhexyl paraben and co-administered testosterone propionate (0.4 mg/kg/day, agonist). The antagonist, flutamide (3 mg/kg/day, will also be co-administered with 0.4 mg/kg/day testosterone propionate as a positive control. Animals will be dosed for 10 consecutive days, staggered start on two days, via oral gavage (2-ethylhexyl paraben and flutamide) and subcutaneous injection (testosterone propionate) based upon daily body weight. Approximately 24 hours following the final dose administration, the animals will be humanely euthanized; the glans penis, ventral prostate, levator ani plus bulbocavernous muscle, Cowper's glands, and seminal vesicle

ILS Project No. – Study No.: 10005.0103; The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

with coagulating gland with fluid will be excised and weights recorded. Changes in androgen dependent tissue weights will be evaluated to determine the ability of 2-ethylhexyl paraben to act as an androgen agonist/antagonist or inhibitor of 5α-reductase.

3.1 Test System

Species:	Rat, *Rattus norvegicus*
Strain:	Sprague Dawley Crl:CD®(SD) IGS
Source:	Charles River Laboratories International, Inc. (Raleigh, NC)
Number/Sex:	88/Castrated male. Surgical manipulation on PND 49 males will be performed by Charles River Laboratories International, Inc. Note: PND 0 is the day of birth
Acclimation:	Animals will be allowed to recover from the surgical manipulation for seven days at Charles River Laboratories International, Inc. The animals will then be acclimated to ILS for at least seven days in the room in which the study will occur.
Age at Initial Dose Administration:	Postnatal Day (PND) 59/60
Weight at Initial Dose Administration:	200-350 grams
Identification:	Each animal will be uniquely identified by ear punch prior to the start of the study. Until the animals are ear punched, they will be identified by the temporary numbers located on the animals' cages.
Justification:	The animal model used is in accordance with the test guidelines (U.S. EPA, 2009; OECD, 2009).

3.2 Animal Husbandry

All procedures are in compliance with the Animal Welfare Act Regulations, 9 CFR 1-4 and animals will be handled and treated according to the *Guide for the Care and Use of Laboratory Animals* (ILAR, 2011).

ILS Project No. – Study No.: 10005.0103; The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Housing (pre-allocation):	Two per cage
Housing (post-allocation):	Two per cage
Cage Type:	Polycarbonate with micro-isolator tops
Cage Size:	23 cm wide by 44 cm long (1012 cm^2 area) and 21 cm high
Bedding:	Absorbent heat-treated hardwood bedding (Northeastern Products Corp., Warrensburg, NY)
Cage Changes:	Twice per week
Diet:	Teklad Global 16% Protein Rodent Diet (Teklad Diets, Madison WI) *ad libitum* Prior to shipment, rats are fed autoclaved Purina 5L79 Rat and Mouse diet *ad libitum* at Charles River Laboratories International, Inc. A copy of the diet composition will be included in the raw data.
Analysis:	The manufacturer's analytical results will be included in the raw data and reviewed prior to animal arrival to ensure that the genistein equivalent content of genistein plus daidzein (as described by Owens et al., 2003) does not exceed 300 µg/g. The same batch of diet will be used during the acclimatization and treatment phases of the study.
Archival:	A sample of the diet (~200 g) will be retained and stored between 0 and -30°C until acceptance of the final report.
Water:	Reverse osmosis treated tap water (City of Durham, NC) *ad libitum*
Supplied:	Glass water bottles with stainless steel sipper tubes
Analysis:	The results of the current annual comprehensive chemical analyses of water from National Testing Laboratories, Inc. (Cleveland, OH) will be reviewed prior to initiation of the study and will be included in the raw data.

ILS Project No. – Study No.: 10005.0103; The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Water Bottle Changes:	At least once per week
Animal Room Conditions:	
Temperature:	20-25°C
Humidity	30-70%
Lighting:	12/12 hour light/dark cycle
Enrichment:	None

3.3 Allocation

The animals will be assigned to a dose group within Provantis® (electronic data capture software) one day prior to initial dose administration using a procedure that stratifies animals across groups by body weight into randomized blocks (e.g. dose groups) such that mean body weight of each group is not statistically different from any other group using analysis of variance [ANOVA Statistical Analysis System (SAS) version 9.2, SAS Institute, Cary, NC]. Only clinically healthy animals that have undergone complete preputial separation (PPS) will be used for allocation.

ILS Project No. – Study No.: 10005.0103; The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

3.4 Group Designation

Table 1. Androgen Agonist- Group Designation, Animal Identification, and Dose Levels

Group Number[a]	Animal Identification	Dose Group	Test Substance Dose Level (mg/kg/day)
1[b]	01-08	2-Ethylhexyl Paraben	0
2	09-16	2-Ethylhexyl Paraben	250
3	17-24	2-Ethylhexyl Paraben	500
4	25-32	2-Ethylhexyl Paraben	750
5	33-40	2-Ethylhexyl Paraben	1000

[a]Positive control for this assay is Group 6 (see Table 2)
[b]Negative control group

ILS Project No. – Study No.: 10005.0103; The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Table 2. Androgen Antagonist- Group Designation, Animal Identification, Dose Group Levels

Group Number	Animal Identification	Dose Group	Test or Reference Substance Dose Level (mg/kg/day)
6[a]	41-48	2-Ethylhexyl Paraben + Testosterone Propionate	0 + 0.4
7	49-56	2-Ethylhexyl Paraben + Testosterone Propionate	250 + 0.4
8	57-64	2-Ethylhexyl Paraben + Testosterone Propionate	500 + 0.4
9	65-72	2-Ethylhexyl Paraben + Testosterone Propionate	750 + 0.4
10	73-80	2-Ethylhexyl Paraben + Testosterone Propionate	1000 + 0.4
11[b]	81-88	Flutamide + Testosterone Propionate	3.0 + 0.4

[a]Negative control
[b]Positive control

3.5 Dose Administration

The test substance, FT, and vehicle control dose formulations will be administered by oral gavage at a dosing volume of 5 mL/kg body weight. The TP dose formulation will be administered by subcutaneous injection into the dorsoscapular region at a dosing volume of 0.5 mL/kg body weight.

In co-administered animals, oral gavage will precede subcutaneous injections. The dose formulations will be administered on a staggered start for 10 consecutive days (PND 59/60 through PND 68/69). The first four animals from each group will be dosed beginning on PND 59, and the second four animals from each group will begin on PND 60.

Dosing will occur 24 hours (± 2 hours) from the previous dose. Dose volume will be determined on the basis of individual animal daily body weight. Dose formulations will be placed on a stir plate at least 30 minutes prior to dosing and

ILS Project No. – Study No.: 10005.0103; The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

continuously stirred. The dosing sequence will be stratified across dosing groups; one animal from each group and then repeated until all animals are dosed.

3.5.1 Justification of Route of Administration

The route of administration is in accordance with the test guidelines (U.S. EPA, 2009; OECD, 2009).

3.5.2 Justification of Dose Levels

A dose range finding study was conducted to select a maximum tolerated dose (MTD) for this assay. Male rats were administered 0, 200, 400, 600, 800, or 1000 mg/kg/day 2-ethylhexyl paraben from PND 36 through 49. All rats survived to the scheduled termination. Two animals administered 1000 mg/kg/day 2-ethylhexyl paraben were lethargic three hours following dosing on Study Day 1, but were normal thereafter. One animal administered 800 mg/kg/day 2-ethylhexyl paraben was observed with an ungroomed appearance on Study Day 7 both before and after dose administration which coincided with a 21.6 g body weight loss from the previous day. Final body weight and body weight gain were assessed following ten days of dose administration and were not significantly different in rats administered 2-ethylhexyl paraben as compared to the vehicle control group. Body weights following ten days of dosing averaged 91.8%, 95.1%, 101.5%, 90.7% (94.2% if animal with marked decrease is excluded), and 93.4% of controls in animals administered 200, 400, 600, 800, or 1000 mg/kg/day 2-ethylhexyl paraben, respectively.

Based on the results described above and the current test guideline recommendations, the limit dose of 1000 mg/kg/day was selected as the top dose to be evaluated.

TP and FT dose levels are in accordance with the test guidelines (U.S. EPA, 2009; OECD, 2009).

3.5.3 Disposal of Dose Formulations

Dose formulations will be disposed of as hazardous material following dosing each day.

3.6 In-Life Animal Observations

Mortality/Moribundity:	Twice daily on weekdays, once daily on weekends/holidays

ILS Project No. – Study No.: 10005.0103; The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Clinical Observations:	Observed within two days of arrival, again for allocation of animals to study groups, daily prior to dose administration, and prior to euthanasia. If adverse clinical signs are seen, additional observations may be recorded.
Cage-Side Observations:	Observations will be performed 1 hour (± 30 minutes) following dosing each day.
Body Weights:	Collected within two days of arrival, again for allocation of animals to study groups, daily prior to dose administration, and prior to euthanasia. Weights will be recorded to the nearest 0.1 g.

3.7 Termination

Moribunds/Unscheduled:	Tissue collection will not be performed on accidental deaths, moribund, or animals found dead during the acclimation period. Beginning on the first day of dose administration, any animals found moribund or dead will be necropsied, and cause of death will be determined and recorded, if possible. Moribund animals will be euthanized by carbon dioxide (CO_2) inhalation and death confirmed by cervical dislocation.
Scheduled:	Twenty-four hours (± two hours) after the final dose administration, animals will be humanely euthanized by CO_2 asphyxiation with death confirmed by cervical dislocation in the same order as they were dosed.
Tissue Collection:	Gross observations of the tissues that are being excised for tissue weights will be recorded.
Tissue Weights:	The following tissues will be excised, trimmed of excess adhering tissue and fat, and weighed to the nearest 0.1 mg: 1. Ventral prostate 2. Seminal vesicle with coagulating gland with fluid 3. Levator ani plus bulbocavernous muscle complex

ILS Project No. – Study No.: 10005.0103; The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

4. Cowper's glands (weighed as a pair)
5. Glans penis

3.8 Statistical Analysis

Descriptive statistics (mean, standard deviation, coefficient of variance, and sample size) will be calculated using Provantis version 9.3.1 (Instem, Philadelphia, PA). Each data set will be analyzed using Statistical Analysis System version 9.2 (SAS Institute, Cary, NC). In the androgen agonist phase, data collected from animals administered 2-ethylhexyl paraben will be compared to the negative control group (0 mg/kg/day 2-ethylhexyl paraben). In the androgen antagonist phase, data collected from animal's co-administered 2-ethylhexyl paraben and TP will be compared to the animal's co-administered 0 mg/kg/day 2-ethylhexyl paraben and TP.

Studentized residual plots will be used to detect possible outliers and Levene's test will be used to assess homogeneity of variance. If the data are heterogeneous, then appropriate transformation will be performed (logarithm, square root, or multiplicative inverse) and the data will be re-analyzed to assess homogeneity.

Final body weight, body weight gain, and tissue weights will be analyzed by an ANOVA test followed by pairwise comparisons using a Dunnett's t test (tissue weights [one-tailed] and final body weight and body weight gain [two-tailed]). Statistically significant effects will be reported when $p < 0.05$. Positive controls will be analyzed by appropriate t tests. TP will be utilized as the positive control group for androgen agonist activity. TP co-administered with FT will be the positive control group for androgen antagonist activity.

If PPS has not occurred in any of the groups, the incidence will be compared to the control group using a Fisher Exact test.

3.9 Performance Criteria

The study will be considered valid if two or less of the ten possible individual coefficient of variances in the negative control and high dose groups exceed the maximum allowable CV's designated for androgenic and anti-androgenic effects listed in Table 3 and if one or fewer of the target tissue p values falls between 0.05 and 0.10 when compared to the negative control.

Table 3. Maximum Allowable Coefficient of Variation

Tissue	Androgen Agonist	Androgen Antagonist
Glans Penis	22%	17%

ILS Project No. – Study No.: 10005.0103; The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Tissue	Androgen Agonist	Androgen Antagonist
Cowper's Glands	55%	35%
LABC	30%	20%
Ventral Prostate	45%	40%
Seminal Vesicles	40%	40%

Source: U.S. EPA (2009) and OECD (2009)

REPORT

One electronic copy of the draft summary report in MS Word file format will be sent to the Sponsor. The report will include all items listed in the protocol and in the OECD guidance as well as a presentation of all data collected in the study, including an executive summary. Data will be summarized in tables. Subcontract reports (i.e., dose formulation analysis) will be provided in the report. The final report will be submitted to the Sponsor as an Adobe Acrobat (.pdf) file. Data Entry Spreadsheet Templates will be prepared and submitted for the reporting of raw data in electronic format.

The final signed report will be maintained in the archives at ILS.

RECORD RETENTION

All original data (including the original signed study protocol and all amendments [if any], test substance information, animal receipt records, animal caretaker records, body weight records, clinical observations, etc.) and the original final report will be maintained by ILS for five years following finalization of the study report. Transfer of study records may be requested by the Sponsor prior to the end of the five-year archival period. At the end of the five-year archival period, the Sponsor will be notified for direction of appropriate disposition of study records remaining at ILS.

REFERENCES

Institute of Laboratory Animal Resources. (2011). *Guide for the Care and Use of Laboratory Animals.* National Academy Press, Washington, DC.

Graves, S. (2001). Dose Formulation Development Study Report Flutamide. Study Project Number: G004110-AXG. Unpublished study report prepared by Battelle.

OECD (Organisation for Economic Co-operation and Development). (2009). Hershberger Bioassay in Rats: A Short-term Screening Assay for (Anti)Androgenic Properties. OECD Guideline for the Testing of Chemicals 441.

ILS Project No. – Study No.: 10005.0103; The Hershberger Bioassay (OPPTS 890.1400); 2-Ethylhexyl Paraben

Owens, W., Ashby, J., Odum, J., and Onyon, L. (2003). The OECD Program to Validate the Rat Uterotrophic Bioassay. Phase 2: Dietary Phytoestrogen Analyses. 111: 1559-1567.

Smith, R. (2011). Storage Stability of Testosterone Propionate in Corn Oil. Unpublished study report prepared by Smithers Viscient, LLC. Study No. 13974.6106.

U.S. EPA (Environmental Protection Agency). 2009. Endocrine Disruptor Screening Program Test Guidelines. OPPTS 890.1400: Hershberger Bioassay. EPA 740-C-09-008. Office of Prevention, Pesticides and Toxic Substances, U.S. EPA, Washington, DC.

KEY PERSONNEL

Study Director:	Jeffrey P. Davis, B.S., LATG
Vice President, Research and Development:	Leslie Recio, Ph.D., DABT
Director of Toxicology:	Cheryl Hobbs, Ph.D.
Toxicology Study Coordinator:	Eileen Phillips, B.S.
Formulations Manager:	Carol Swartz, Ph.D., DVM
Necropsy Manager:	John Pope, B.S.
Attending Veterinarian:	Alyssa McIntyre, D.V.M., DACLAM
Health and Safety Officer:	Michael Streicker, B.S., LATG

ILS-A-066
Last Revised: 06/08/12

Integrated Laboratory Systems, Inc.
Protocol Deviation

ILS Project No.-Study No.:	10005.0103
Sponsor Study No.:	NA
Protocol Deviation No.:	1
Protocol Section Deviated from:	3.2
Nature of Deviation:	The results of the annual water testing were not reviewed by the Study Director prior to the initiation of the study.
Reason for Deviation:	The water testing results were not available until one week following receipt of study animals.
Corrective Action:	None.
Impact on Study:	None. Review of the results by the Attending Veterinarian and Study Director found no contaminants that would interfere with the health of the animals or the study outcome.

Jeffrey P. Davis, B.S., LATG
Study Director
Integrated Laboratory Systems, Inc.

18 March 2016
Date

Page **1** of **1**

ILS-A-066
Last Revised: 06/08/12

Integrated Laboratory Systems, Inc.
Protocol Deviation

ILS Project No.-Study No.: 10005.0103

Sponsor Study No.: NA

Protocol Deviation No.: 2

Protocol Section Deviated from: 3.2

Nature of Deviation: The temperature and humidity were outside of the protocol defined ranges (20-25°C and 30-70%, respectively) during the course of the study. The excursions are summarized in the table below.

Maximum Temp °C	25.1		Maximum Humidity %	71.2
Minimum Temp °C	20.0		Minimum Humidity %	29.9
Mean Temp °C	21.3		Mean Humidity %	46.8
No. of Points Out of Range	5		No. of Points Out of Range	5
Time out of Range (min)	75		Time out of Range (min)	75
% Out of Range	0.3%		% Out of Range	0.3%
Total Days Out	1		Total Days Out	3

Reason for Deviation: The temperature and humidity were slightly out of range transiently.

Corrective Action: Technical malfunctions in the HVAC system are fixed immediately by professional contractors however; some deviations cannot be avoided due to the limitations of the system design.

Impact on Study: Minimal. Excursions from the defined temperature and humidity ranges were short and did not affect the health of the animals.

Jeffrey P. Davis, B.S., LATG
Study Director
Integrated Laboratory Systems, Inc.

31 March 2016
Date