



## **FINAL REPORT**

### **Study Title**

**Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal  
Male Rats; 2-Ethylhexyl Paraben**

### **Test Guideline**

**OPPTS 890.1500**

### **ILS Project-Study Number**

**10005.0102**

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### **Performing Laboratory**

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### **Sponsor**

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### **Date of Completion**

**16 March 2017**

**STATEMENT OF NO DATA CLAIM OF CONFIDENTIALITY**

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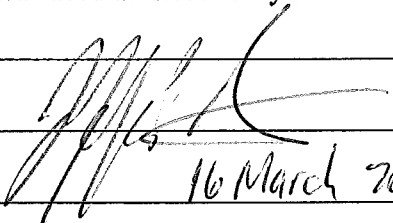
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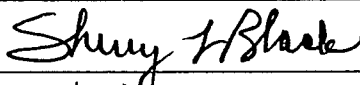


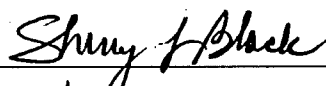
### GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in accordance with U.S. EPA Good Laboratory Practice Standards, 40 CFR Part 160.

The following exceptions to the Endocrine Disruptor Screening Program Test Guidelines OPPTS 890.1500: Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats (U.S. EPA, 2009) were noted. Animals were not transported to a holding room the night before euthanasia as stated in Section j of the male pubertal test guideline, but were maintained and dosed in the study room and taken to a room adjacent to the necropsy room at least two hours prior to euthanasia. Reverse osmosis treated water was supplied to the animals in lieu of deionized water specified in Section d of the male pubertal test guideline. A 3 mL syringe was used in place of a 1 mL (disposable) tuberculin syringe when the dose volume was greater than 1 mL for dose administration as stated in Section h of the male pubertal test guidelines. In addition, a 2 inch length gavage needle was used as dictated by the size of the animal.

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### QUALITY ASSURANCE INSPECTION STATEMENT

ILS Project - Study No.: 10005.0102  
Test Article: 2-Ethylhexyl Paraben  
ILS Repository No.: 15-172  
Study Title: Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 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	11 Dec 2015	11 Dec 2015 / 11 Dec 2015
Animal Receipt	14 Jan 2015	14 Jan 2016 / 19 Jan 2016
Dose Administration	23 Feb 2016	23 Feb 2016 / 26 Feb 2016
Clinical Observations	23 Feb 2016	23 Feb 2016 / 26 Feb 2016
Euthanasia/Necropsy	21 Mar 2016	21 Mar 2016 / 23 Mar 2016
Pathology Report	27 July 2016	27 July 2016 / 01 Aug 2016
Study Data/Draft Report	15-16 Aug 2016	17 Aug 2016 / 22 Aug 2016
Final Report	10 Mar 2017	10 Mar 2017 / 10 Mar 2017

  
Jeanne deWard, B.S., LATG, RQAP-GLP  
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16 Mar 2017  
Date

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## **EXECUTIVE SUMMARY**

In a Male Pubertal assay, 16 male Sprague Dawley rats/dose group were treated daily via oral gavage (5 mL/kg) with 2-ethylhexyl paraben (99.8% a.i., Lot #: 7CZZO) in corn oil at doses of 0, 250, 500, 750, or 1000 mg/kg/day from post-natal day (PND) 23 to 53/54. Animals were examined for preputial separation (PPS) daily beginning on PND 30, and age and body weight at day of attainment were recorded. Following humane euthanasia on PND 53 or 54, blood was collected for clinical chemistry and hormone analyses. Total serum testosterone, thyroxine (T<sub>4</sub>), and thyroid stimulating hormone (TSH) levels were determined using radioimmunoassays. Urogenital and thyroid organ weights were recorded and microscopic examination of the left testis, left epididymis, mammary glands, thyroid, and left kidney were performed

No treatment-related effects were observed on survival, clinical signs, gross pathology, or microscopic histology (including follicular cell height and colloid area in the thyroid glands). No treatment-related effects were observed on age at PPS; a slight decrease ( $p<0.05$ ) of 8% in body weight at PPS was noted in the 1000 mg/kg/day group. Several differences were noted in clinical chemistry parameters, but these changes were minor in magnitude and within the range (as mean  $\pm$  2 SD) of historical controls.

At 1000 mg/kg/day, final body weights and body weight gain were decreased ( $p<0.01$ ) by 12% and 14%, respectively. Decreases ( $p<0.05$ ) in TSH ( $\downarrow$ 51%) and testosterone ( $\downarrow$ 52%) were noted. Organ weights (adjusted for PND 21 body weights) were decreased ( $p<0.05$ ) by 12-20% in liver, pituitary, adrenals, SV coagulating gland without fluid, ventral prostate, LABC, and thyroid (in some cases, unadjusted body weights also differed from controls).

The Guideline (U.S. EPA, 2009) performance criteria were met, with the following exceptions: (i) coefficient of variation (CV)=8.3% for body weight at PPS (top of acceptable range: 7.57%); (ii) CV=10.8% for final body weight (top of acceptable range: 7.47%); (iii) mean kidney weight of 2.10 g (acceptable range: 2.242-3.050 g); (iv) CV=17.3% for liver weight (top of acceptable range: 14.93%); (v) mean serum TSH level was 3.12 ng/mL (acceptable range: 4.212-24.112 ng/mL); and (vi) CV=18.7% for pituitary (top acceptable range: 15.98%).

In this study at the limit dose (1000 mg/kg/day), there was evidence to support an effect of 2-ethylhexyl paraben on the endocrine system (decreased serum TSH and testosterone and decreased urogenital organ and thyroid weights), where general systemic toxicity (decreased final body weight and body weight gain) was also noted.

## 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 (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 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 of the Study

The purpose of this assay was to assess the potential of 2-ethylhexyl paraben to interact with the endocrine system by identifying effects on pubertal development and thyroid function in the intact juvenile/peripubertal male rat (OPPTS 890.1500).

### 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  
Email: [sherry@rti.org](mailto:sherry@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

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E-mail: jdavis@ils-inc.com

## 1.5 Study Dates

Study Initiation Date: 06 January 2016

Animal Arrival Date: 14 January 2016

Experimental Start Date: 20 February 2016

Experimental In-Life Termination Date: 22 March 2016

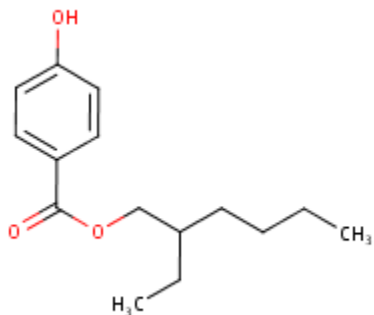
Experimental End Date: 28 July 2016

## TEST SUBSTANCE / VEHICLE

### 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<sub>15</sub>H<sub>22</sub>O<sub>3</sub>

Description: Colorless, clear liquid

Purity: 99.8%

Expiration Date:	Not provided on Certificate of Analysis
Dose Formulation:	2-Ethylhexyl paraben were prepared at ILS in corn oil at dose concentrations of 0, 50, 100, 150 and 200 mg/mL. Prepared dose formulations were dispensed 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:	Dose formulations in corn oil held between 1-10°C for 20 days were considered stable (Appendix VIII).

**2.2 Vehicle: Corn oil**

CAS No.:	8001-30-7
Source:	Animal Health International (Greeley, CO)
Lot/Batch No.:	16303-100175
ILS Repository No.:	12-206
Formula:	C <sub>27</sub> H <sub>50</sub> O <sub>6</sub>
Description:	Yellow Oil
Storage:	Room temperature and protected from light

**2.3 Archival Samples**

An approximate 1 mg sample of the test substance and 1 mL of the vehicle and dose formulations for each preparation are stored between 0 and -30°C. After acceptance of the final report by the Sponsor archival dose formulation samples will be discarded. The test substance sample will be maintained by ILS in the archive laboratory freezer for five years following finalization of the study report.



## 2.4 Dose Formulation Analysis

Dose formulations were prepared at ILS, 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 prepared dose formulations prepared on 16 February and 03 March 2016 were collected from the top, middle, and bottom of each formulation and sent to Smithers Viscient for analysis. Smithers Viscient analyzed samples for concentration and homogeneity in duplicate.

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, <i>Rattus norvegicus</i>
Strain:	Sprague Dawley Crl:CD <sup>®</sup> (SD) IGS
Source:	Charles River Laboratories International, Inc. (Raleigh, NC)
Number/Sex F <sub>0</sub> :	50 timed pregnant nulliparous dams at gestation day (GD) 8 to generate the F <sub>1</sub> animals

Note: GD 0 is the day of sperm positive identification.

Culling/Standardized  
Litters:

Between PND 3 and 5, litters with the same date of birth were standardized to 8 pups with equal numbers of males and females, with the exception of four litters: the litter from dam #25 was culled to three male and five female pups, while dams #30, 34, and 40 were culled to five male and three female pups. The four litters with unequal gender differences were maintained on study to ensure enough litters were available for allocation while avoiding littermates in the same group.

Note: PND 0 is the day of birth (day when pups were first seen during morning room check)

Number/Sex F <sub>1</sub> :	80 males
Acclimation:	Animals were acclimated in the study room from PND 0 to PND 22.
Age at Initial Dose Administration:	PND 23
Weight at Initial Administration:	56.5 – 67.4 grams
Identification:	F <sub>0</sub> animals were identified by cage cards placed on cages. F <sub>1</sub> animals were identified by markings on the tail for randomization purposes and ear punched when assigned to treatment groups.
Justification:	Test system and treatment are in accordance with the OPPTS 890.1500: Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats (U.S. EPA, 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 F <sub>0</sub> :	One per cage (with litter)
Housing F <sub>1</sub> :	Two per cage following allocation
Cage Type:	Polycarbonate with micro-isolator filter lids
Cage Size:	23 cm wide by 44 cm long (1012 cm <sup>2</sup> area) and 21 cm high
Bedding:	Absorbent heat-treated hardwood bedding (Northeastern Products Corp., Warrensburg, NY)
Cage Changes F <sub>0</sub> :	Once per week without litter, twice per week with litter
Cage Changes F <sub>1</sub> :	Twice per week

Diet:	<p>Teklad Global 16% Protein Rodent Diet (Teklad Diets, Madison WI), <i>ad libitum</i></p> <p>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) was determined to be 5.4 µg/g of feed.</p> <p>Prior to arrival at the test site, rats were fed autoclaved Purina 5L79 Rat and Mouse diet <i>ad libitum</i> at Charles River Laboratories International, Inc. A copy of the diet composition is included in the study records.</p>
Archival:	<p>A sample of the diet (~200 g) is retained and stored between 0 and -30°C until acceptance of the final report; after acceptance of the final report by the Sponsor archival diet samples will be discarded.</p>
Water:	<p>Reverse osmosis treated tap water (City of Durham, NC), <i>ad libitum</i></p>
Supplied:	<p>Glass water bottles with stainless steel sipper tube</p>
Analysis:	<p>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.</p>
Water Bottle Changes:	<p>At least once per week</p>
Animal Room Conditions:	
Temperature:	<p>19.1 – 24.8°C</p>
Humidity	<p>24.9 – 85.6%</p>
	<p>The excursions from the defined temperature and humidity range did not affect animal health.</p>
Lighting:	<p>14/10 hour light/dark cycle (lights on: lights off; 0500-1900)</p>
Enrichment:	<p>None</p>

## STUDY DESIGN

### 4.1 Allocation

The animals were assigned to a dose group using a procedure that stratified animals across groups by body weight 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]. Littermates were not placed in the same dose group.

### 4.2 Group Designation

**Table 1. Group Designation, Animal Identification, and Dose Levels**

Group Number	Animal Identification	Test Substance	Test Substance Dose Level (mg/kg/day)
1	01-16	2-Ethylhexyl Paraben	0
2	17-32	2-Ethylhexyl Paraben	250
3	33-48	2-Ethylhexyl Paraben	500
4	49-64	2-Ethylhexyl Paraben	750
5	65-80	2-Ethylhexyl Paraben	1000

### 4.3 Dose Administration

The test substance or corn oil (vehicle control) dose formulations were administered by oral gavage at a dose volume of 5.0 mL/kg body weight. The dose formulations were administered for 31/32 (PND 23 through 53 or 54) consecutive days. Dose formulations were administered daily between 0700 and 0900 using an 18 gauge appropriate length stainless steel gavage needle and a 1 or 3 mL syringe. Dose volume was determined on the basis of individual animal daily body weight. Dose formulations were placed on a stir plate at least 30 minutes prior to dosing and continuously stirred. The dosing sequence

was stratified across all dose groups; one animal from each group and then repeated until all animals were dosed.

#### **4.3.1 Justification of Route of Administration**

Selection of the route of administration was in accordance with the Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats guideline (U.S. EPA, 2009).

#### **4.3.2 Justification of Dose Levels**

The highest dose level should be at or just below the MTD level but need not exceed the limit dose of 1 g/kg/day. A dose level will generally be considered to be at or just below the MTD level if it causes a statistically significant reduction in terminal body weight gain in animals administered test substance versus controls, the reduction is no greater than approximately 10% of the mean terminal body weight for the controls, and no clinical signs of toxicity associated with the dose level are observed throughout the study. In addition, abnormal blood chemistry values at termination, particularly creatinine and blood urea nitrogen (BUN), may indicate that MTD was exceeded, even in the absence of a reduction in terminal body weight compared to controls. Histopathology of the kidney (or any other organ where gross observations indicate damage) may be used as evidence that MTD was exceeded.

In a dose range finding study conducted at ILS, all immature male rats administered 2-ethylhexyl paraben for 14 days survived to study termination. No clinical signs of toxicity were observed prior to or following dose administration for animals administered 0, 200, 400, 600, or 800 mg/kg/day 2-ethylhexyl paraben. Lethargy was noted in two animals administered 1000 mg/kg/day 2-ethylhexyl paraben post-dose on Study Day 1; however, there were no further signs of toxicity during the dose administration period.

There was not a significant change in final body weight or body weight gain following 14 days of 2-ethylhexyl paraben dose administration. Final body weights averaged 93.0%, 94.0%, 97.4%, 89.7% (93.5% if an animal with a marked overnight decrease in body weight is excluded), and 91.4% of controls in animals administered 200, 400, 600, 800, or 1000 mg/kg/day 2-ethylhexyl paraben, respectively. Mean liver, kidney, and ventral prostate weights (absolute and relative) in all dose groups were not significantly different from the control group.

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

#### **4.3.3 Disposal of Dose Formulations**

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

#### **4.4 In-Life Animal Observations**

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

Clinical Observations: F<sub>0</sub> animals were observed within two days of arrival. F<sub>1</sub> animals were observed for allocation of animals to dose groups, daily prior to dose administration, and prior to humane euthanasia.

Cage-Side Observations: Observations were performed 1 hour ( $\pm$  30 minutes) following dosing each day.

Body Weights: Body weights were collected on F<sub>0</sub> dams within two days of arrival. Body weights were collected on F<sub>1</sub> animals weekly following birth (litter weights), for allocation of animals to dose groups, daily prior to dose administration, and prior to humane euthanasia.

Preputial Separation: Following dosing beginning on PND 30, males were examined for PPS. Progression not initiated, the appearance of partial separation, a persistent thread of tissue between the glans and prepuce, or complete PPS, were recorded each day.

#### **4.5 Termination**

Scheduled: Animals were moved to the necropsy holding room at least two hours before euthanasia. Animals were euthanized at least two hours following that day's dose administration and between the hours of 0900 and 1300, with the exception of two rats euthanized as late as 1321 on the first day of necropsy (one rat each from the 750 and 1000 mg/kg/day dose groups). Animals were humanely euthanized by decapitation, in the same order as they were dosed.

Blood Collection: Prior to tissue collection, trunk blood was collected. Blood was collected in a glass serum separator tube and stored on wet ice until centrifugation. Blood was centrifuged at 3,000 g for 30 minutes at 4°C. If lipemia was observed, serum was re-centrifuged at 10,000 g for 10 minutes at 4°C

(see Protocol Deviation 4). Serum was dispensed into siliconized microcentrifuge tubes. All serum samples were stored at or below -70°C.

**Tissue Collection:** Gross observations of the tissues that were excised for tissue weights, along with the left and right 4<sup>th</sup> and 5<sup>th</sup> mammary glands, were recorded.

**Tissue Weights:** The following tissues were excised, trimmed of excess adhering tissue and fat, and weights recorded to the nearest 0.1 mg:

1. Adrenals (paired)
2. Kidneys (paired)
3. Liver
4. Thyroid, post fixation\*
5. Ventral prostate
6. Dorsolateral prostate
7. Seminal vesicles with coagulating gland (with and without fluid)
8. LABC
9. Epididymides (left and right separately)
10. Testes (left and right separately)
11. Pituitary

\*The thyroid with attached trachea was fixed in 10% neutral buffered formalin (NBF) for at least 24 hours. The thyroid was then dissected from the trachea, blotted, weighed to the nearest 0.01 mg, and transferred to 80% histology grade alcohol.

To reduce variability, dissections of specific tissues were performed by a specific, sole individual.

#### Hormone and Clinical

**Chemistry Concentrations:** T<sub>4</sub>, TSH, and T concentrations were measured in sera using radioimmunoassays (RIAs). Samples were assayed in duplicate and in conjunction with multiple quality control samples. Serum samples were shipped on dry ice to:

Principal Investigator- Seena Polivy  
AniLytics Inc.  
20 Girard Street  
Suite 200  
Gaithersburg, MD 20877

Serum samples were analyzed for creatinine, blood urea nitrogen, sorbitol dehydrogenase, sodium, potassium, chloride, calcium, phosphorous, total protein, albumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyltransferase, and total bilirubin. Serum samples were shipped on dry ice to:

Principal Investigator- Charles Walker  
Antech GLP  
600 Airport Blvd  
Suite 500  
Morrisville, NC 27560

**Histopathology:**

The left kidney, left testis, left epididymis, thyroid, and right 4th and 5th mammary glands were fixed in 10% NBF for at least 24 hours, transferred to 80% histology grade alcohol opposed to 70% histology grade alcohol, histologically processed, embedded in paraffin, 5 µm sections prepared, and stained with hematoxylin and eosin (H&E). Sections of the thyroid included 2 serial sections approximately halfway into the tissue.

Left testis and left epididymis were fixed in 10% NBF rather than Bouin's fixative (see protocol deviation 3).

Histologically processed tissues were microscopically evaluated by the study pathologist. A minimum of 2 sections of each of the 2 lobes of the thyroid were examined and evaluated for follicular cell height and colloid area using a 5-point grading scale; other lesions or abnormalities were noted.

## **4.6 Statistical Analysis**

Descriptive statistics (mean, standard deviation, coefficient of variance, and sample size) were calculated using Provantis version 9.3.1 (Instem, Philadelphia, PA). Data sets listed below were analyzed using Statistical Analysis System version 9.2 (SAS Institute, Cary, NC). Studentized residual plots were used to detect possible outliers in the data and Levene's test was used to assess homogeneity of variance. Heterogeneous data were transformed (logarithm, multiplicative inverse and square root) and were re-analyzed for homogeneity.

Homogenous data sets listed below were analyzed using a one-way analysis of variance (ANOVA) followed by pair wise comparisons performed using Dunnett's two tailed t- tests.



Initial body weight  
Final body weight (last day all body weights collected)  
Final body weight gain (last day all body weights collected)  
Age and body weight at PPS  
Age at partial PPS

Data sets listed below were analyzed using a two-way ANOVA with treatment and necropsy day as main effects. Pair wise comparisons were performed using Dunnett's two tailed t tests.

Tissue weights  
Relative tissue weights (liver, kidneys, pituitary and adrenals)  
Hormone levels  
Clinical chemistry levels

The data sets listed below were analyzed using an analysis of covariance (ANCOVA) with PND 21 body weight (allocation body weight) as the covariable (adjusted weights). Pair wise comparisons were performed using Dunnett's two-tailed t-tests

Initial body weight  
Final body weight (last day all body weights collected)  
Final body weight gain (last day all body weights collected)  
Age and body weight at PPS  
Age at partial PPS  
Tissue weights

If PPS, body weight, and tissue weight data were not statistically significant, dose-dependent changes were evaluated using a linear regression model for both adjusted and unadjusted values.

Statistical analyses of thyroid scoring (colloid area and follicular cell height) were performed by Fisher's Exact test, and when statistically significant, followed by Kruskal-Wallis and Dunn's test.

For all data sets, statistically significant effects were reported when  $p < 0.05$ .

## RESULTS

### 5.1 Dose Formulation Analysis

Actual concentration and homogeneity results of each dose formulation administered to rats on study and reported in the tables below (Tables 2-3) were within acceptance criteria (Appendix VIII).

**Table 2. Dose Formulation and Homogeneity Results;  
Preparation Date: 16 February 2016**

<b>Dose Group</b>	<b>Nominal Dose Concentration (mg/mL)</b>	<b>Actual Dose Concentration* (mg/mL) [Percent of Nominal]</b>	<b>Percent CV* (Homogeneity)</b>	<b>Nominal Dose Level (mg/kg/day)</b>
2-Ethylhexyl Paraben	50	49.8 [99.5]	3.76	250
2-Ethylhexyl Paraben	100	99.2 [99.2]	4.88	500
2-Ethylhexyl Paraben	150	149 [99.4]	2.27	750
2-Ethylhexyl Paraben	200	197 [98.5]	2.90	1000

\*See Appendix VIII

Abbreviation: CV – coefficient of variation

**Table 3. Dose Formulation and Homogeneity Results;  
Preparation Date: 03 March 2016**

<b>Dose Group</b>	<b>Nominal Dose Concentration (mg/mL)</b>	<b>Actual Dose Concentration* (mg/mL) [Percent of Nominal]</b>	<b>Percent CV* (Homogeneity)</b>	<b>Nominal Dose Level (mg/kg/day)</b>
2-Ethylhexyl Paraben	50	45.2 [90.5]	4.82	250
2-Ethylhexyl Paraben	100	87.9 [87.9]	2.20	500
2-Ethylhexyl Paraben	150	139 [92.7]	2.85	750
2-Ethylhexyl Paraben	200	183 [91.7]	5.36	1000

\*See Appendix VIII

Abbreviation: CV – coefficient of variation

## 5.2 In-Life Animal Observations

### Mortality/Moribundity

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

### Cage-Side and Clinical Observations

Cage-side (1 hour  $\pm$  30 minutes) and clinical observations (~24 hours) were performed post dose administration. Individual animal data are listed in Appendix I.

There were no abnormal clinical observations noted in animals administered 0 or 250 mg/kg/day 2-ethylhexyl paraben. Mild salivation was observed post-dose in rats administered 500 (#38), 750 (#51, #52, #53, #55, #59, #60) or 1000 mg/kg/day (#67, #68, #70) 2-ethylhexyl paraben on one to three occasions per animal, with one exception. Animal #70 was observed with mild salivation post-dose on Study Day 9 and again prior to dose administration on Study Day 10. There were no other abnormal observations recorded during the dosing period.

## **Body Weights**

Group mean initial and final body weights and body weight gain data for males euthanized following 31/32 consecutive days of 2-ethylhexyl paraben administration are presented in Table 4. Group mean daily body weights are shown in Figure 1. Individual animal data are listed in Appendix II.

There were no statistically significant changes in body weights following administration of 0, 250, 500, or 750 mg/kg/day 2-ethylhexyl paraben compared to the control group (Group 1). Final body weight and body weight gain (adjusted and unadjusted) were statistically decreased following 1000 mg/kg/day 2-ethylhexyl paraben administration compared to the control group (Group 1) with final body weight measuring 88.4% of mean control weight; a decrease in 12% ( $p < 0.01$ ) from control.

## **Preputial Separation**

Mean age and body weight of males at complete or partial PPS are presented in Table 4. Individual animal data are listed in Appendix III.

Age at PPS or partial PPS was not statistically affected by administration of 2-ethylhexyl paraben compared to the control group (Group 1). Following administration of 1000 mg/kg/day 2-ethylhexyl paraben, but not lower dose levels, body weight at PPS was statistically decreased (8%,  $p < 0.05$ ) compared to the control group (Group 1).

## **5.3 Termination**

### **Gross Observations**

The following gross observations were made at necropsy in surviving rats:

Animal 09 (0 mg/kg/day):	Thyroid, right, small, marked
Animal 66 (1000 mg/kg/day):	Adrenal, left, not present during dissection. Weight was excluded from statistical analysis.

No other gross observations were recorded at necropsy.

### **Tissue Weights**

Group mean tissue weights for animals euthanized following 31/32 consecutive days of 2-ethylhexyl paraben administration are presented in Table 5. Individual animal tissue weight data are listed in Appendix IV.

Weights of seminal vesicles with fluid, dorso-lateral prostate, epididymides, and testes were not statistically different compared to the control group (Group 1). Liver (adjusted only) and ventral prostate (unadjusted and adjusted) weights were statistically decreased

following 500 or 1000 mg/kg/day 2-ethylhexyl paraben administration. A significant dose-dependent decreasing trend was found in unadjusted liver weights without a significant pair wise comparison. Unadjusted and adjusted pituitary, seminal vesicles without fluid, LABC, and thyroid (adjusted only) weights were statistically decreased following administration of 1000 mg/kg/day 2-ethylhexyl paraben compared to the control group (Group 1). A statistically significant decrease in unadjusted and adjusted adrenal gland weights was found following administration of 750 or 1000 mg/kg/day 2-ethylhexyl paraben. Administration of 1000 mg/kg/day 2-ethylhexyl paraben resulted in a statistically significant increase in relative kidney weights.

### **Hormone and Clinical Chemistry Analysis**

Mean group serum hormone and clinical chemistry concentration results are shown in Tables 6 and 7. Individual animal data are listed in Appendix V.

There was no statistically significant change in serum T4 levels following administration of 2-ethylhexyl paraben compared to the control group (Group 1). TSH and testosterone levels were statistically decreased (51% and 52%, respectively;  $p < 0.05$ ) following administration of 1000 mg/kg/day 2-ethylhexyl paraben compared to the control group (Group 1).

There were no statistically significant changes in serum aspartate aminotransferase, gamma glutamyltransferase, alkaline phosphatase, creatinine, total bilirubin, total protein, and albumin levels compared to the control group (Group 1). Sodium levels were statistically increased, while potassium, phosphorous, and sorbitol dehydrogenase were statistically decreased following administration of 1000 mg/kg/day 2-ethylhexyl paraben. Chloride and alanine aminotransferase levels were statistically increased following  $\geq 500$  mg/kg/day 2-ethylhexyl paraben administration. Administration of  $\geq 500$  mg/kg/day 2-ethylhexyl paraben resulted in a statistically significant decrease in calcium levels. BUN levels were statistically decreased following 2-ethylhexyl paraben administration at all dose levels compared to the control group (Group 1).

Statistical analysis with and without data from the two rats that were euthanized after 1300 hours identified one instance in which the results were not identical. Calcium levels were not statistically decreased following administration of 500 mg/kg/day 2-ethylhexyl paraben. However, this change does not impact the ability to interpret the results.

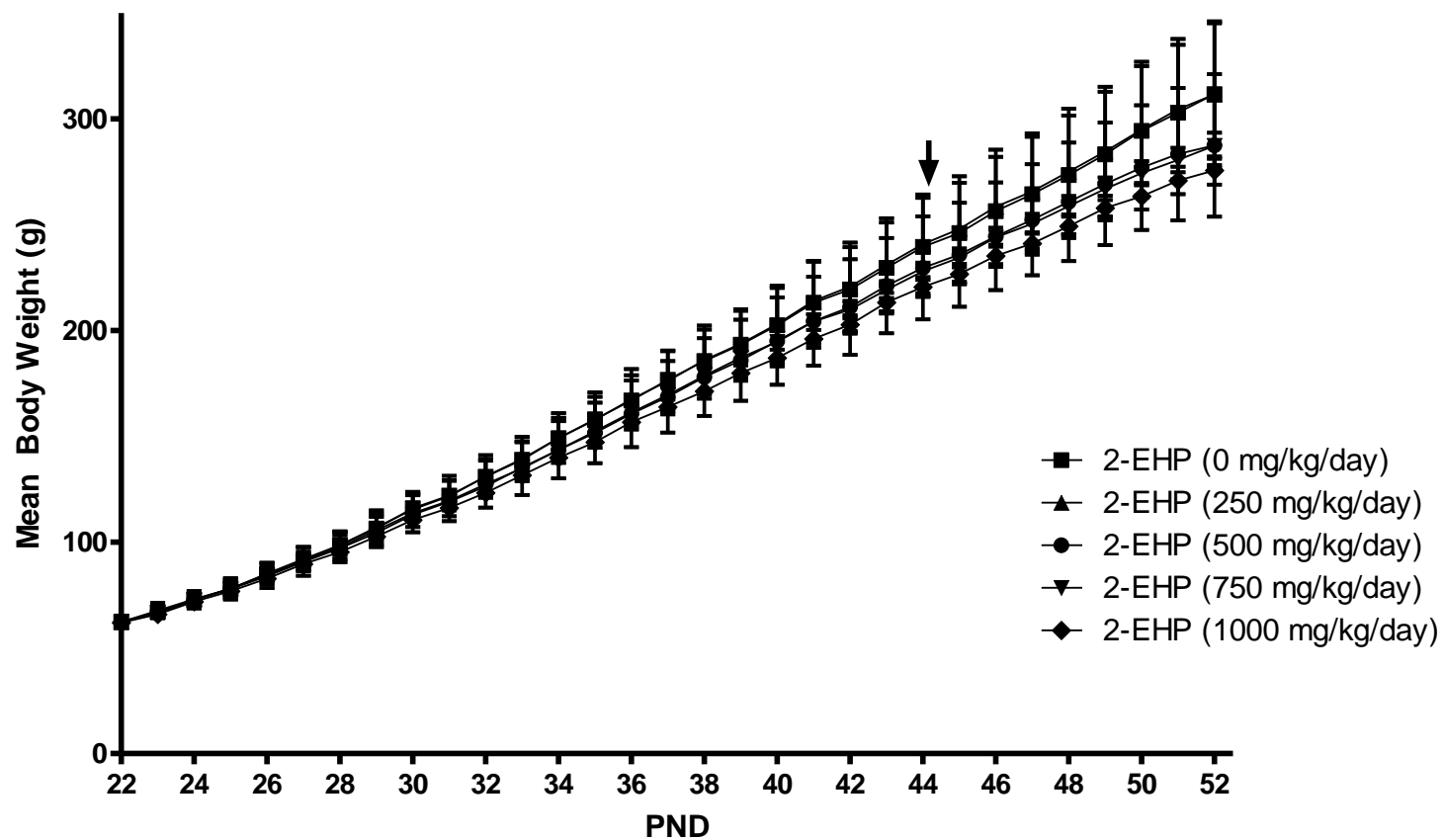
### **Histopathology**

Summary tables of histologic findings in the thyroid gland are shown in Table 9. The histopathology report, in its entirety, is located in Appendix VI.

Administration of 2-ethylhexyl paraben to male rats was not associated with histopathologic changes in the testis, epididymis, kidney, mammary gland, or thyroid gland. There were no apparent or statistical differences in follicular cell height and

colloid area in the thyroid glands of 2-ethylhexyl paraben administered animals compared to vehicle controls (Group 1).

**Figure 1. Daily Group Mean Body Weights**



Note: Arrow indicates the mean age of the vehicle control group at PPS.

**Table 4. Male Preputial Separation and General Growth**

Test Substance/Control		Effect	Log Transformed	Pairwise Test	2-Ethylhexyl Paraben (0 mg/kg/day)				2-Ethylhexyl Paraben (250 mg/kg/day)					2-Ethylhexyl Paraben (500 mg/kg/day)				
					Mean	SD	CV	n	Mean	SD	CV	n	p-value	Mean	SD	CV	n	p-value
Age at PPS (PND)	U			D	43.8	1.3	2.9	16	43.6	1.4	3.2	16	0.9866	43.8	1.7	4.0	16	0.9998
	A			D	43.8	-			43.6	-			0.9924	43.8	-			1.0000
Age at Partial PPS (PND)	U			D	41.6	1.2	2.8	16	41.4	1.0	2.5	16	0.9954	41.6	1.7	4.0	16	0.9997
	A			D	41.5	-			41.4	-			0.9976	41.6	-			0.9991
Body weight at PPS (g)	U	*		D	227.6	18.8	8.3	16	226.7	18.2	8.0	16	0.9998	217.9	20.0	9.2	16	0.4335
	A	*		D	228.5	-			226.4	-			0.9932	217.6	-			0.2919
Initial body weight (PND 23, g)	U			D	62.2	3.4	5.4	16	62.2	3.1	4.9	16	1.0000	62.0	2.6	4.2	16	0.9996
	A			D	62.6	-			62.1	-			0.6057	61.9	-			0.2613
Final body weight (g)	U	*		D	311.8	33.6	10.8	16	311.5	34.6	11.1	16	1.0000	287.5	33.7	11.7	16	0.0939
	A	*		D	313.1	-			311.1	-			0.9989	287.0	-			0.0502
Final body weight (% of control)	U			-	-				99.9	-				92.2	-			
	A			-					-					-				
Body weight gain (final minus initial body weight) (g)	U	*		D	249.6	34.1	13.7	16	249.3	33.8	13.6	16	1.0000	225.5	32.3	14.3	16	0.0840
	A	*		D	250.6	-			249.0	-			0.9996	225.1	-			0.0570

Proportion of rats not completing PPS (#/n)

0/16

0/16

0/16

Abbreviations/Symbols: SD - standard deviation, CV - coefficient of variation, n - number of animals, PPS - preputial separation, PND - postnatal day, U - unadjusted, A - adjusted for PND 21 body weight, D - Dunnett's test

\*Endpoint exhibits a statistical difference compared to vehicle control.

Shaded/bolded cells are statistically different compared to the vehicle control mean.



**Table 4. Male Preputial Separation and General Growth (Continued)**

Test Substance/Control		Effect	Log Transformed	Pairwise Test	2-Ethylhexyl Paraben (750 mg/kg/day)					2-Ethylhexyl Paraben (1000 mg/kg/day)					
					Mean	SD	CV	n	p-value	Mean	SD	CV	n	p-value	
Age at PPS (PND)	U			D	43.2	0.8	1.7	16	0.5225	43.6	1.6	3.6	16	0.9866	
	A			D	43.2	-			0.5585	43.6	-			0.9917	
Age at Partial PPS (PND)	U			D	41.1	0.9	2.2	16	0.7072	41.4	1.3	3.0	16	0.9954	
	A			D	41.1	-			0.7375	41.4	-			0.9973	
Body weight at PPS (g)	U	*		D	212.4	16.1	7.6	16	0.0956	208.8	23.4	11.2	16	0.0267	
	A	*		D	212.2	-			0.0526	208.7	-			0.0128	
Initial body weight (PND 23, g)	U			D	62.4	1.7	2.8	16	0.9973	61.9	3.4	5.5	16	0.9975	
	A			D	62.4	-			0.9583	61.9	-			0.2791	
Final body weight (g)	U	*		D	287.3	24.5	8.5	16	0.0904	275.6	26.4	9.6	16	0.0052	
	A	*		D	287.1	-			0.0510	275.5	-			0.0022	
Final body weight (% of control)	U			-	92.1	-				88.4	-				
	A			-	-					-					
Body weight gain (final minus initial body weight) (g)	U	*		D	224.9	23.9	10.6	16	0.0739	213.7	23.8	11.1	16	0.0042	
	A	*		D	224.7	-			0.0517	213.6	-			0.0025	
Proportion of rats not completing PPS (#/n)								0/16						0/16	

Abbreviations/Symbols: SD - standard deviation, CV - coefficient of variation, n - number of animals, PPS - preputial separation, PND - postnatal day, U - unadjusted, A - adjusted for PND 21 body weight, D - Dunnett's test

\*Endpoint exhibits a statistical difference compared to vehicle control.

Shaded/bolded cells are statistically different compared to the vehicle control mean.

**Table 5. Tissue Weights at Necropsy**

Test Substance/Control					2-Ethylhexyl Paraben (0 mg/kg/day)				2-Ethylhexyl Paraben (250 mg/kg/day)					2-Ethylhexyl Paraben (500 mg/kg/day)				
Tissue Weights	Effect	Log Transformed	Pairwise Test		Mean	SD	CV	n	Mean	SD	CV	n	p-value	Mean	SD	CV	n	p-value
Liver (g)	U			D	14.73	2.55	17.3	16	14.17	1.82	12.8	16	0.8481	13.04	2.12	16.3	16	0.0686
	A	*		D	14.82	-	-	16	14.14	-	-	16	0.7216	<b>13.01</b>	<b>-</b>	<b>-</b>	<b>16</b>	<b>0.0347</b>
	R			D	4.70	0.38	8.0	16	4.55	0.33	7.2	16	0.4769	4.52	0.30	6.7	16	0.3038
Kidneys (g)	U			D	2.10	0.22	10.7	16	2.05	0.26	12.9	16	0.9450	1.98	0.25	12.9	16	0.4292
	A			D	2.10	-	-	16	2.05	-	-	16	0.8834	1.98	-	-	16	0.3240
	R	*		D	0.67	0.04	6.5	16	0.66	0.03	5.2	16	0.7185	0.69	0.05	7.4	16	0.6591
Pituitary (mg)	U	*		D	9.6	1.8	18.7	16	9.2	1.8	19.2	16	0.8602	9.1	1.2	13.2	16	0.7375
	A	*		D	9.7	-	-	-	9.2	-	-	16	0.8456	9.1	-	-	16	0.7188
	R			D	0.003	0.001	16.1	16	0.003	0.001	20.0	16	0.8952	0.003	0.000	12.5	16	0.9670
Adrenals (mg)	U	*		D	40.8	7.4	18.1	15 <sup>a</sup>	38.6	5.95	15.4	16	0.7503	37.3	8.4	22.6	16	0.3857
	A	*		D	40.9	-	-	15 <sup>a</sup>	38.5	-	-	16	0.7258	37.2	-	-	16	0.3633
	R			D	0.013	0.002	16.2	15 <sup>a</sup>	0.012	0.002	13.7	16	0.6813	0.013	0.002	17.8	16	0.9968
SV + coagulating gland, with fluid (mg)	U			D	513.9	103.2	20.1	16	511.2	98.5	19.3	16	1.0000	479.2	78.8	16.4	16	0.6785
	A			D	513.8	-	-	16	511.2	-	-	16	1.0000	479.2	-	-	16	0.6817
SV + coagulating gland, without fluid (mg)	U	*		D	320.8	42.3	13.2	16	308.5	32.3	10.5	16	0.8972	307.0	69.5	22.6	16	0.8537
	A	*		D	322.2	-	-	16	308.1	-	-	16	0.8349	306.4	-	-	16	0.7774
Ventral prostate (mg)	U	*		D	260.4	37.4	14.4	16	254.2	46.1	18.1	16	0.9686	<b>226.5</b>	<b>32.3</b>	<b>14.3</b>	<b>16</b>	<b>0.0374</b>
	A	*		D	260.7	-	-	16	254.1	-	-	16	0.9643	<b>226.3</b>	<b>-</b>	<b>-</b>	<b>16</b>	<b>0.0418</b>

Test Substance/Control					2-Ethylhexyl Paraben (0 mg/kg/day)				2-Ethylhexyl Paraben (250 mg/kg/day)					2-Ethylhexyl Paraben (500 mg/kg/day)				
Tissue Weights		Effect	Log Transformed	Pairwise Test	Mean	SD	CV	n	Mean	SD	CV	n	p-value	Mean	SD	CV	n	p-value
Dorsolateral prostate (mg)	U			D	87.8	19.2	21.8	16	93.7	19.9	21.2	16	0.8449	90.0	23.4	26.0	16	0.9950
	A			D	88.0	-	-	16	93.7	-	-	16	0.8641	89.9	-	-	16	0.9971
LABC (mg)	U	*		D	497.2	58.3	11.7	16	483.9	71.3	14.7	16	0.9375	444.6	69.8	15.7	16	0.0731
	A	*		D	499.2	-	-	16	483.3	-	-	16	0.8846	443.8	-	-	16	0.0511
Epididymis (left, mg)	U			D	201.0	25.6	12.7	16	214.7	25.9	12.1	16	0.3204	186.0	21.7	11.7	16	0.2422
	A			D	200.3	-	-	16	214.9	-	-	16	0.3137	186.2	-	-	16	0.3395
Epididymis (right, mg)	U			D	204.3	21.3	10.4	16	206.8	26.2	12.7	16	0.9955	193.0	11.7	6.1	16	0.5373
	A			D	203.6	-	-	16	207.1	-	-	16	0.9863	193.2	-	-	16	0.6196
Testis (left, mg)	U			D	1439.6	109.2	7.6	16	1499.5	119.7	8.0	16	0.3373	1429.4	118.5	8.3	16	0.9965
	A			D	1442.0	-	-	16	1498.8	-	-	16	0.3787	1428.5	-	-	16	0.9894
Testis (right, mg)	U			D	1434.2	102.7	7.2	16	1485.9	124.0	8.3	16	0.5052	1423.3	118.6	8.3	16	0.9961
	A			D	1436.8	-	-	16	1485.1	-	-	16	0.5695	1422.3	-	-	16	0.9889
Thyroid (mg)	U			D	18.5	2.9	15.5	16	18.0	3.4	18.8	16	0.9623	17.8	2.3	13.0	16	0.8738
	A	*		D	18.6	-	-	16	18.0	-	-	16	0.9012	17.7	-	-	16	0.7623

Abbreviations/Symbols: SD - standard deviation, CV - coefficient of variation, n - number of animals, U - unadjusted, A - adjusted for PND 21 body weight, R - relative tissue weight (absolute tissue weight/final body weight\*100), D - Dunnett's test, LABC – levator ani plus bulbocavernous muscle

<sup>a</sup>One adrenal gland from animal 12 was not located and weighed at necropsy.

\*Endpoint exhibits a statistical difference compared to vehicle control.

Shaded/bolded cells are statistically different compared to the vehicle control mean.

**Table 5. Tissue Weights at Necropsy (Continued)**

Test Substance/Control					2-Ethylhexyl Paraben (750 mg/kg/day)					2-Ethylhexyl Paraben (1000 mg/kg/day)				
Tissue Weights		Effect	Log Transformed	Pairwise Test	Mean	SD	CV	n	p-value	Mean	SD	CV	N	p-value
Liver (g)	U			D	13.17	1.41	10.7	16	0.1042	13.09	2.00	15.0	16	0.0807
	A			D	13.16	-	-	16	0.0593	<b>13.08</b>	<b>-</b>	<b>-</b>	<b>16</b>	<b>0.0454</b>
	R			D	4.58	0.28	6.2	16	0.6782	4.73	0.31	6.6	16	0.9969
Kidneys (g)	U			D	2.02	0.16	7.9	16	0.8070	2.01	0.20	10.1	16	0.6428
	A			D	2.02	-	-	16	0.7109	2.00	-	-	16	0.5413
	R	*		D	0.71	0.06	8.2	16	0.1118	<b>0.73</b>	<b>0.05</b>	<b>7.5</b>	<b>16</b>	<b>0.0029</b>
Pituitary (mg)	U	*		D	8.4	1.7	20.1	15 <sup>b</sup>	0.1061	<b>7.8</b>	<b>1.4</b>	<b>17.5</b>	<b>15<sup>b</sup></b>	<b>0.0083</b>
	A	*		D	8.4	-	-	15 <sup>b</sup>	0.1063	<b>7.8</b>	<b>-</b>	<b>-</b>	<b>15<sup>b</sup></b>	<b>0.0085</b>
	R			D	0.003	0.001	18.7	15 <sup>b</sup>	0.7582	0.003	0.001	16.6	15 <sup>b</sup>	0.5883
Adrenals (mg)	U	*		D	<b>33.4</b>	<b>5.7</b>	<b>17.1</b>	<b>16</b>	<b>0.0106</b>	<b>34.1</b>	<b>5.4</b>	<b>16.0</b>	<b>15<sup>a</sup></b>	<b>0.0252</b>
	A	*		D	<b>33.4</b>	<b>-</b>	<b>-</b>	<b>16</b>	<b>0.0097</b>	<b>34.1</b>	<b>-</b>	<b>-</b>	<b>15<sup>a</sup></b>	<b>0.0251</b>
	R			D	0.012	0.002	14.7	16	0.0953	0.012	0.001	11.9	15	0.6196
SV + coagulating gland, with fluid (mg)	U			D	476.3	79.6	16.7	16	0.6175	491.4	102.7	20.9	16	0.9018
	A			D	476.3	-	-	16	0.6202	491.4	-	-	16	0.9030
SV + coagulating gland, without fluid (mg)	U	*		D	292.6	40.0	13.7	16	0.3250	<b>272.2</b>	<b>56.8</b>	<b>20.9</b>	<b>16</b>	<b>0.0272</b>
	A	*		D	292.3	-	-	16	0.2626	<b>272.0</b>	<b>-</b>	<b>-</b>	<b>16</b>	<b>0.0188</b>
Ventral prostate (mg)	U	*		D	230.5	35.2	15.3	16	0.0789	<b>222.5</b>	<b>35.9</b>	<b>16.1</b>	<b>16</b>	<b>0.0166</b>
	A	*		D	230.4	-	-	16	0.0859	<b>222.4</b>	<b>-</b>	<b>-</b>	<b>16</b>	<b>0.0194</b>

Test Substance/Control					2-Ethylhexyl Paraben (750 mg/kg/day)					2-Ethylhexyl Paraben (1000 mg/kg/day)				
Tissue Weights		Effect	Log Transformed	Pairwise Test	Mean	SD	CV	n	p-value	Mean	SD	CV	N	p-value
Dorsolateral prostate (mg)	U			D	82.2	21.1	25.7	16	0.8663	86.3	21.5	24.9	16	0.9990
	A			D	82.1	-	-	16	0.8485	86.3	-	-	16	0.9982
LABC (mg)	U	*		D	455.5	69.5	15.2	16	0.2037	<b>413.7</b>	<b>47.6</b>	<b>11.5</b>	<b>16</b>	<b>0.0015</b>
	A	*		D	455.1	-	-	16	0.1582	<b>413.5</b>	-	-	<b>16</b>	<b>0.0009</b>
Epididymis (left, mg)	U			D	198.7	30.5	15.3	16	0.9966	200.1	24.3	12.1	16	0.9999
	A			D	198.9	-	-	16	0.9995	200.2	-	-	16	1.0000
Epididymis (right, mg)	U			D	198.9	22.8	11.5	16	0.9369	204.2	40.0	19.5	16	1.0000
	A			D	199.0	-	-	16	0.9655	204.3	-	-	16	1.0000
Testis (left, mg)	U			D	1447.4	109.1	7.5	16	0.9988	1420.7	77.0	5.4	16	0.9659
	A			D	1446.9	-	-	16	0.9998	1420.5	-	-	16	0.9450
Testis (right, mg)	U			D	1437.2	108.2	7.5	16	1.0000	1452.6	115.7	8.0	16	0.9737
	A			D	1436.7	-	-	16	1.0000	1452.3	-	-	16	0.9861
Thyroid (mg)	U			D	16.9	2.9	17.4	16	0.2769	16.1	2.2	13.7	16	0.0587
	A	*		D	16.8	-	-	16	0.1950	<b>16.1</b>	-	-	<b>16</b>	<b>0.0356</b>

Abbreviations/Symbols: SD - standard deviation, CV - coefficient of variation, n - number of animals, U - unadjusted, A - adjusted for PND 21 body weight, R - relative tissue weight (absolute tissue weight/final body weight\*100), D - Dunnett's test,

<sup>a</sup>One adrenal gland from animal 66 was not located and weighed at necropsy and therefore not included in the statistical analysis.

<sup>b</sup>Pituitary weights for animals 49 and 70 were not biologically plausible and therefore not included in the statistical analysis.

\*Endpoint exhibits a statistical difference compared to vehicle control.

Shaded/bolded cells are statistically different compared to the vehicle control mean.

**Table 6. Serum Hormone Concentrations**

Test Substance/Control				2-Ethylhexyl Paraben (0 mg/kg/day)				2-Ethylhexyl Paraben (250 mg/kg/day)					2-Ethylhexyl Paraben (500 mg/kg/day)				
	Effect	Log Transformed	Pairwise test	Mean	SD	CV	n	Mean	SD	CV	n	p-value	Mean	SD	CV	n	p-value
Serum T <sub>4</sub> (µg/dL)			D	4.71	0.93	19.8	15	5.16	1.10	21.3	15	0.5577	4.49	1.01	22.6	15	0.9197
Serum TSH (ng/mL)	*		D	3.12	1.46	46.7	15	2.50	1.26	50.5	15	0.5942	2.45	1.98	80.6	15	0.5341
Testosterone, total (ng/mL)	*		D	1.29	0.79	61.0	15	0.80	0.52	64.7	15	0.1361	0.98	0.91	92.3	15	0.5267

Abbreviations: CV - coefficient of variation, D - Dunnett's test, n - number of animals, SD - standard deviation, T<sub>4</sub> - thyroxine, TSH - thyroid-stimulating hormone

\*Endpoint exhibits a statistical difference compared to vehicle control. Shaded/bolded cells are statistically different compared to the vehicle control mean.

**Table 6. Serum Hormone Concentrations (Continued)**

Test Substance/Control				2-Ethylhexyl Paraben (750 mg/kg/day)					2-Ethylhexyl Paraben (1000 mg/kg/day)				
	Effect	Log Transformed	Pairwise test	Mean	SD	CV	n	p-value	Mean	SD	CV	n	p-value
Serum T <sub>4</sub> (µg/dL)			D	4.33	1.00	23.0	16	0.6294	4.23	0.75	17.6	15	0.4345
Serum TSH (ng/mL)	*		D	2.23	1.43	64.2	16	0.2666	<b>1.52</b>	<b>0.85</b>	<b>56.2</b>	<b>15</b>	<b>0.0131</b>
Testosterone, total (ng/mL)	*		D	0.84	0.67	79.8	16	0.1787	<b>0.62</b>	<b>0.34</b>	<b>54.8</b>	<b>15</b>	<b>0.0248</b>

Abbreviations: CV - coefficient of variation, D - Dunnett's test, n - number of animals, SD - standard deviation, T<sub>4</sub> - thyroxine, TSH - thyroid-stimulating hormone

\*Endpoint exhibits a statistical difference compared to vehicle control. Shaded/bolded cells are statistically different compared to the vehicle control mean.

**Table 7. Serum Clinical Chemistry Levels**

Test Substance/Control				2-Ethylhexyl Paraben (0 mg/kg/day)				2-Ethylhexyl Paraben (250 mg/kg/day)					2-Ethylhexyl Paraben (500 mg/kg/day)				
	Effect	Log Transformed	Pairwise test	Mean	SD	CV	n	Mean	SD	CV	n	p-value	Mean	SD	CV	n	p-value
Na <sup>+</sup> (MEQ/L)	*		D	142	2	1	15	143	2	1	15	0.4939	143	2	1	15	0.3557
K <sup>+</sup> (MEQ/L)	*		D	9.0	0.6	7.2	15	8.8	0.7	7.6	15	0.7249	8.6	0.9	10.3	15	0.4611
Cl <sup>-</sup> (MEQ/L)	*		D	103	2	2	15	104	2	2	15	0.1272	<b>105</b>	<b>1</b>	<b>1</b>	<b>15</b>	<b>0.0001</b>
Calcium (mg/dL)	*	✓	D	10.9	0.4	4.1	15	10.9	0.2	2.1	15	0.1212	<b>10.7</b>	<b>0.3</b>	<b>3.0</b>	<b>15</b>	<b>0.0001</b>
Phosphorous (mg/dL)	*		D	10.0	1.0	10.1	15	9.7	0.7	7.5	15	0.5662	9.4	0.8	8.1	15	0.0910
AST (U/L)			D	187	42	22	15	175	39	23	15	0.7351	175	45	26	15	0.8073
ALT (U/L)	*		D	67	9	14	15	68	10	14	15	0.9833	<b>79</b>	<b>12</b>	<b>15</b>	<b>15</b>	<b>0.0082</b>
GGT (U/L)			D	0	0	-	15	0	0	-	15	1.0000	0	0	-	15	1.0000
ALP (U/L)			D	507	114	23	15	507	108	21	15	1.0000	525	124	24	15	0.9745
BUN (mg/dL)	*		D	13	3	20	15	<b>11</b>	<b>2</b>	<b>16</b>	<b>15</b>	<b>0.0259</b>	<b>9</b>	<b>3</b>	<b>28</b>	<b>15</b>	<b>&lt;0.0001</b>
Creatinine (mg/dL)			D	0.2	0.0	12.5	15	0.2	0.0	16.5	15	0.9587	0.2	0.0	16.5	15	0.9396
Total Bilirubin (mg/dL)			D	0.2	0.0	12.5	15	0.2	0.0	12.5	15	1.0000	0.2	0.0	16.5	15	0.8822
SDH (U/L)	*		D	12.0	3.9	32.4	15	9.7	3.0	30.8	15	0.1425	9.8	2.6	27.1	15	0.1870
Total Protein (g/dL)			D	5.6	0.4	6.3	15	5.6	0.3	5.2	15	0.9994	5.5	0.2	4.3	15	0.5154
Albumin (g/dL)			D	2.9	0.2	5.6	15	3.0	0.2	5.7	15	0.9598	2.9	0.1	4.6	15	1.0000

Abbreviations: CV - coefficient of variation, D - Dunnett's test, n - number of animals, SD - standard deviation, ✓ - log transformed, Na<sup>+</sup> - sodium, K<sup>+</sup> - potassium, Cl<sup>-</sup> - chloride, AST - aspartate aminotransferase, ALT - alanine aminotransferase, GGT - gamma glutamyltransferase, ALP - alkaline phosphatase, BUN - blood urea nitrogen, SDH - sorbitol dehydrogenase

\*Endpoint exhibits a statistical difference compared to vehicle control.

Shaded/bolded cells are statistically different compared to the vehicle control mean.

**Table 7. Serum Clinical Chemistry Levels (Continued)**

Test Substance/Control				2-Ethylhexyl Paraben (750 mg/kg/day)					2-Ethylhexyl Paraben (1000 mg/kg/day)				
	Effect	Log Transformed	Pairwise test	Mean	SD	CV	n	p-value	Mean	SD	CV	n	p-value
Na <sup>+</sup> (MEQ/L)	*		D	142	2	1	16	0.9693	<b>144</b>	<b>2</b>	<b>1</b>	<b>15</b>	<b>0.0088</b>
K <sup>+</sup> (MEQ/L)	*		D	8.6	0.7	8.5	16	0.3641	<b>8.3</b>	<b>0.6</b>	<b>7.3</b>	<b>15</b>	<b>0.0447</b>
Cl <sup>-</sup> (MEQ/L)	*		D	<b>104</b>	<b>2</b>	<b>2</b>	<b>16</b>	<b>0.0072</b>	<b>106</b>	<b>1</b>	<b>1</b>	<b>15</b>	<b>&lt;0.0001</b>
Calcium (mg/dL)	*	✓	D	<b>10.5</b>	<b>0.4</b>	<b>3.4</b>	<b>16</b>	<b>0.0070</b>	<b>10.4</b>	<b>0.3</b>	<b>2.7</b>	<b>15</b>	<b>&lt;0.0001</b>
Phosphorous (mg/dL)	*		D	9.5	0.6	6.3	16	0.0761	<b>9.3</b>	<b>0.7</b>	<b>7.3</b>	<b>15</b>	<b>0.0124</b>
AST (U/L)			D	189	41	22	16	1.0000	170	35	21	15	0.5036
ALT (U/L)	*		D	<b>80</b>	<b>9</b>	<b>11</b>	<b>16</b>	<b>0.0057</b>	<b>86</b>	<b>14</b>	<b>16</b>	<b>15</b>	<b>&lt;0.0001</b>
GGT (U/L)			D	0	0	-	16	1.0000	0	0	-	15	1.0000
ALP (U/L)			D	474	93	20	16	0.8180	456	96	21	15	0.5291
BUN (mg/dL)	*		D	<b>8</b>	<b>2</b>	<b>26</b>	<b>16</b>	<b>&lt;0.0001</b>	<b>8</b>	<b>2</b>	<b>28</b>	<b>15</b>	<b>&lt;0.0001</b>
Creatinine (mg/dL)			D	0.2	0.0	16.1	16	0.9674	0.2	0.0	12.5	15	1.0000
Total Bilirubin (mg/dL)			D	0.2	0.0	0.0	16	0.8706	0.2	0.0	13.4	15	0.3985
SDH (U/L)	*		D	11.4	4.2	37.0	16	0.9383	<b>8.2</b>	<b>2.3</b>	<b>27.6</b>	<b>15</b>	<b>0.0048</b>
Total Protein (g/dL)			D	5.5	0.3	5.8	16	0.8371	5.4	0.2	3.7	15	0.1464
Albumin (g/dL)			D	2.9	0.1	3.7	16	0.9987	2.9	0.1	4.2	15	0.8567

Abbreviations: CV - coefficient of variation, D - Dunnett's test, n - number of animals, SD - standard deviation, ✓ - log transformed, Na<sup>+</sup> - sodium, K<sup>+</sup> - potassium, Cl<sup>-</sup> - chloride, AST - aspartate aminotransferase, ALT - alanine aminotransferase, GGT - gamma glutamyltransferase, ALP - alkaline phosphatase, BUN - blood urea nitrogen, SDH - sorbitol dehydrogenase

\*Endpoint exhibits a statistical difference compared to vehicle control.

Shaded/bolded cells are statistically different compared to the vehicle control mean.



**Table 8. ILS Laboratory Historical Male Hormone and Clinical Chemistry Ranges**

Hormone/Clinical Chemistry	n	Range (Min-Max)	Range (Mean $\pm$ 2 SD)
Serum T <sub>4</sub> (µg/dL)	158	1.91 – 6.53	2.90 – 6.04
Serum TSH (ng/mL)	157	0.34 – 12.64	0 – 6.60
Serum Testosterone (ng/mL)	158	0.27 – 7.56	0 – 4.41
Na <sup>+</sup> (MEQ/L)	157	132 – 164	132 – 156
K <sup>+</sup> (MEQ/L)	157	6.2 – 11.8	6.3 – 10.2
Cl <sup>-</sup> (MEQ/L)	157	99 – 118	98 – 116
Calcium (mg/dL)	157	9.0 – 11.3	9.4 – 11.4
Phosphorous (mg/dL)	157	7.0 – 13.9	7.9 – 12.7
AST (U/L)	157	119 – 709	84 – 392
ALT (U/L)	157	21.0 – 218	32 – 149
GGT (U/L)	157	0 – 7	0 – 2
ALP (U/L)	157	217 – 821	211 – 728
BUN (mg/dL)	157	7 – 22	7 – 18
Creatinine (mg/dL)	157	0.2 – 0.8	0.2 – 0.6
Total Bilirubin (mg/dL)	157	0.0 – 0.4	0 – 0.2
SDH (U/L)	157	1.0 – 38.0	0 – 39.1
Total Protein (g/dL)	157	5.0 – 7.1	5.0 – 6.8
Albumin (g/dL)	157	3.8 – 5.6	3.9 – 5.2

Data collected 2011 - present

Abbreviations: n - number of animals, SD - standard deviation, T<sub>4</sub> - thyroxine, TSH - thyroid stimulating hormone, Na<sup>+</sup> - sodium, K<sup>+</sup> - potassium, Cl<sup>-</sup> - chloride, AST - aspartate aminotransferase, ALT - alanine aminotransferase, GGT - gamma glutamyltransferase, ALP - alkaline phosphatase, BUN - blood urea nitrogen, SDH - sorbitol dehydrogenase

**Table 9. Thyroid Gland: Follicular Cell Height and Colloid Area**

	2-Ethylhexyl Paraben (0 mg/kg/day)	2-Ethylhexyl Paraben (250 mg/kg/day)	2-Ethylhexyl Paraben (500 mg/kg/day)	2-Ethylhexyl Paraben (750 mg/kg/day)	2-Ethylhexyl Paraben (1000 mg/kg/day)
n	16	16	16	16	16
<b>Follicular cell (height)<sup>1</sup></b>					
1	7 (44%)	6 (38%)	12 (75%)	10 (63%)	9 (56%)
2	6 (38%)	9 (56%)	4 (25%)	5 (31%)	6 (38%)
3	3 (19%)	1 (6%)	0	1 (6%)	1 (6%)
4	0	0	0	0	0
5	0	0	0	0	0
<b>Follicular colloid (area)<sup>1</sup></b>					
1	0	0	0	0	0
2	0	0	0	0	0
3	3 (19%)	1 (6%)	0	1 (6%)	1 (6%)
4	6 (38%)	9 (56%)	4 (25%)	5 (31%)	6 (38%)
5	7 (44%)	6 (38%)	12 (75%)	10 (63%)	9 (56%)

Abbreviation: n - number of animals.

<sup>1</sup>Thyroid gland subjectively assessed for follicular cell height and colloid area using a five-point grading scale (1 = shortest / smallest; 5 = tallest / largest) was used and the thyroids were evaluated relative to U.S. EPA photomicrographs (U.S. EPA, 2009; Capen and Martin, 1989).

## 5.4 Performance Criteria

Data generated in this study from the vehicle control (corn oil) rats were within the U.S. EPA performance criteria with the following exceptions: mean kidney weights and TSH concentrations were slightly below the acceptable range; coefficient of variation (CV) for body weight at PPS, final body weight, liver and pituitary weights was above the top EPA acceptable CV limit (Table 10). These excursions did not affect the integrity of the study.

**Table 10. Performance Criteria for Male Sprague Dawley Rats**

Endpoint	EPA Acceptable Mean Range*	Vehicle Control Mean	EPA Top of Acceptable CV Range*	Vehicle Control CV
Age at PPS (PND)	37.781 – 46.513	43.8	5.67	2.9
Weight at PPS (g)	188.277 – 256.169	227.6	7.57	<b>8.3</b>
Weaning weight (g)	45.472 – 59.812	55.0	10.25	4.7
Final body weight (g)	259.235 – 332.059	311.8	7.47	<b>10.8</b>
Thyroid (mg)	14 - 26	18.5	23.63	15.5
Adrenals (mg)	31.842 – 61.114	40.8	NA	18.1
Kidneys (g)	2.242 – 3.050	<b>2.10</b>	14.76	10.7
Liver (g)	9.990 – 15.350	14.73	14.93	<b>17.3</b>
Pituitary (mg)	7.810 – 12.898	9.6	15.98	<b>18.7</b>
Ventral prostate (g)	0.160 – 0.332	0.2604	22.32	14.4
LABC (g)	0.447 – 0.855	0.4972	27.100	11.7
Epididymides (g)	0.364 – 0.528	0.405	16.39	10.0
Seminal Vesicles (g)	0.295 – 0.719	With fluid: 0.5139	21.06	With fluid: 20.1
		Without fluid: 0.3208		Without fluid: 13.2
Testis (g)	NA	2.87	17.62	7.2
T <sub>4</sub> , total (µg/dL)	4.056 – 7.376	4.71	27.46	19.8
TSH (ng/mL)	4.212 – 24.112	<b>3.12</b>	58.29	46.7
Testosterone, total (ng/mL)	0.260 – 3.960	1.29	89.70	61.0

\*Source: U.S. EPA (2009)

Abbreviations: CV - coefficient of variation, PPS – preputial separation, PND - postnatal day, LABC - levator ani plus bulbocavernous muscle, TSH – thyroid stimulating hormone, T<sub>4</sub> - thyroxine

## 5.5 Summary and Discussion

The purpose of this assay was to assess the potential for 2-ethylhexyl paraben to interact with the endocrine system by identifying effects on pubertal development and thyroid gland function in intact juvenile/peripubertal male rats (US EPA, 2009). Male rats were orally administered 2-ethylhexyl paraben (250, 500, 750, or 1000 mg/kg/day) or corn oil (vehicle control) for 31/32 days. At least 2 hours following the final dose administration, the animals were humanely euthanized.

A significant decrease in final body weight (12 decrease from control) and body weight gain was observed following administration of 1000 mg/kg/day 2-ethylhexyl paraben. Instances of salivation were present in animals administered  $\geq 500$  mg/kg/day 2-ethylhexyl paraben with no apparent dose-related increase. No other abnormal clinical observations were noted during the course of the study. Therefore, 1000 mg/kg/day may have slightly exceeded the MTD.

Adjusted liver weights, but not absolute or relative weights, were statistically decreased following administration of 500 or 1000 mg/kg/day 2-ethylhexyl paraben. Alanine aminotransferase levels were statistically increased following administration  $\geq 500$  mg/kg/day; however, other liver enzymes did not exhibit changes (albumin, alkaline phosphatase, aspartate aminotransferase, bilirubin, and gamma glutamyl transferase). Relative kidney weights were statistically increased while absolute or adjusted kidney weights were not altered after 1000 mg/kg/day 2-ethylhexyl paraben administration. Blood urea nitrogen levels were decreased at all dose levels; however, creatinine levels were not altered and there were no microscopic changes evident in the kidney.

Administration of 2-ethylhexyl paraben to male rats did not significantly delay pubertal development as indicated by changes in age at preputial separation (PPS). Body weight at PPS was statistically decreased coinciding with a statistically significant decrease in final body weight.

There were significant decreases in weights of a number of androgen-dependent tissues: levator ani plus bulbocavernous muscle and seminal vesicles without fluid (but not with fluid) were statistically decreased at a dose level of 1000 mg/kg/day and ventral prostate weights were statistically decreased following dose administration of 500 or 1000 mg/kg/day. Epididymides weights were not statistically different from controls. Serum testosterone levels were significantly decreased (52%) following administration of 1000 mg/kg/day 2-ethylhexyl paraben.

After 31/32 days of 2-ethylhexyl paraben administration, there was a significant decrease in adjusted, but not absolute, thyroid gland weights in animals administered 1000 mg/kg/day. There was also a significant decrease in TSH levels (51%) at 1000 mg/kg/day. No changes in T<sub>4</sub> serum hormone levels and no histopathological findings were noted for the thyroid glands. As noted by O'Connor et al. (1999), potential thyroid toxicants induce one or more of the following responses: elevation in TSH, decrease in T<sub>3</sub>/T<sub>4</sub> concentrations, increased thyroid weight and/or altered thyroid histology. In

general, organ weight measurements are useful endpoints for detecting thyroid toxicants. An increase in relative liver weight, which was observed in this study, is typically observed with hepatic-enzyme inducers, a secondary mechanism for peripheral metabolism of thyroid hormones (McClain et al., 1989). If a thyroid toxicant induced enzymes involved in thyroid metabolism, changes in serum hormones would be reflected as a decrease in T<sub>4</sub> and a concomitant increase in TSH, neither of which were observed in this study. The weight changes that occurred in the thyroid gland in the limit (1000 mg/kg/day) dose group likely have no biological significance as no effects were observed in other thyroid endpoints to suggest thyroid gland modulation.

Overall, administration of 2-ethylhexyl paraben to intact juvenile/peripubertal male rats results in changes in endpoints that suggest steroidogenesis inhibition or hypothalamic–pituitary–gonadal axis suppression, but no effect on pubertal development and thyroid function. However, the test guideline (US EPA, 2009) recommends that when changes are measured that suggest the chemical interacts with the endocrine system at a dose level that causes more than approximately a 10% body weight loss compared to controls, it will require information from other Tier 1 assays for a complete interpretation of the significant findings.

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## KEY PERSONNEL

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

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					1	1	2	2	3	3
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		1-Hour Post-Dose			1-Hour Post-Dose
ff										
1	m	01	No Abnormalities Detected		X	X	X	X	X	X
		02	No Abnormalities Detected		X	X	X	X	X	X
		03	No Abnormalities Detected		X	X	X	X	X	X
		04	No Abnormalities Detected		X	X	X	X	X	X
		05	No Abnormalities Detected		X	X	X	X	X	X
		06	No Abnormalities Detected		X	X	X	X	X	X
		07	No Abnormalities Detected		X	X	X	X	X	X
		08	No Abnormalities Detected		X	X	X	X	X	X
		09	No Abnormalities Detected		X	X	X	X	X	X
		10	No Abnormalities Detected		X	X	X	X	X	X
		11	No Abnormalities Detected		X	X	X	X	X	X
		12	No Abnormalities Detected		X	X	X	X	X	X
		13	No Abnormalities Detected		X	X	X	X	X	X
		14	No Abnormalities Detected		X	X	X	X	X	X
		15	No Abnormalities Detected		X	X	X	X	X	X
		16	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP



Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					4	4	5	5	6	6
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose	
ff										
1	m	01	No Abnormalities Detected		X	X	X	X	X	X
		02	No Abnormalities Detected		X	X	X	X	X	X
		03	No Abnormalities Detected		X	X	X	X	X	X
		04	No Abnormalities Detected		X	X	X	X	X	X
		05	No Abnormalities Detected		X	X	X	X	X	X
		06	No Abnormalities Detected		X	X	X	X	X	X
		07	No Abnormalities Detected		X	X	X	X	X	X
		08	No Abnormalities Detected		X	X	X	X	X	X
		09	No Abnormalities Detected		X	X	X	X	X	X
		10	No Abnormalities Detected		X	X	X	X	X	X
		11	No Abnormalities Detected		X	X	X	X	X	X
		12	No Abnormalities Detected		X	X	X	X	X	X
		13	No Abnormalities Detected		X	X	X	X	X	X
		14	No Abnormalities Detected		X	X	X	X	X	X
		15	No Abnormalities Detected		X	X	X	X	X	X
		16	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					7	7	8	8	9	9
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose	
ff										
1	m	01	No Abnormalities Detected		X	X	X	X	X	X
		02	No Abnormalities Detected		X	X	X	X	X	X
		03	No Abnormalities Detected		X	X	X	X	X	X
		04	No Abnormalities Detected		X	X	X	X	X	X
		05	No Abnormalities Detected		X	X	X	X	X	X
		06	No Abnormalities Detected		X	X	X	X	X	X
		07	No Abnormalities Detected		X	X	X	X	X	X
		08	No Abnormalities Detected		X	X	X	X	X	X
		09	No Abnormalities Detected		X	X	X	X	X	X
		10	No Abnormalities Detected		X	X	X	X	X	X
		11	No Abnormalities Detected		X	X	X	X	X	X
		12	No Abnormalities Detected		X	X	X	X	X	X
		13	No Abnormalities Detected		X	X	X	X	X	X
		14	No Abnormalities Detected		X	X	X	X	X	X
		15	No Abnormalities Detected		X	X	X	X	X	X
		16	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					10	10	11	11	12	12
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose	
ff										
1	m	01	No Abnormalities Detected		X	X	X	X	X	X
		02	No Abnormalities Detected		X	X	X	X	X	X
		03	No Abnormalities Detected		X	X	X	X	X	X
		04	No Abnormalities Detected		X	X	X	X	X	X
		05	No Abnormalities Detected		X	X	X	X	X	X
		06	No Abnormalities Detected		X	X	X	X	X	X
		07	No Abnormalities Detected		X	X	X	X	X	X
		08	No Abnormalities Detected		X	X	X	X	X	X
		09	No Abnormalities Detected		X	X	X	X	X	X
		10	No Abnormalities Detected		X	X	X	X	X	X
		11	No Abnormalities Detected		X	X	X	X	X	X
		12	No Abnormalities Detected		X	X	X	X	X	X
		13	No Abnormalities Detected		X	X	X	X	X	X
		14	No Abnormalities Detected		X	X	X	X	X	X
		15	No Abnormalities Detected		X	X	X	X	X	X
		16	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					13	13	14	14	15	15
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose	
ff										
1	m	01	No Abnormalities Detected		X	X	X	X	X	X
		02	No Abnormalities Detected		X	X	X	X	X	X
		03	No Abnormalities Detected		X	X	X	X	X	X
		04	No Abnormalities Detected		X	X	X	X	X	X
		05	No Abnormalities Detected		X	X	X	X	X	X
		06	No Abnormalities Detected		X	X	X	X	X	X
		07	No Abnormalities Detected		X	X	X	X	X	X
		08	No Abnormalities Detected		X	X	X	X	X	X
		09	No Abnormalities Detected		X	X	X	X	X	X
		10	No Abnormalities Detected		X	X	X	X	X	X
		11	No Abnormalities Detected		X	X	X	X	X	X
		12	No Abnormalities Detected		X	X	X	X	X	X
		13	No Abnormalities Detected		X	X	X	X	X	X
		14	No Abnormalities Detected		X	X	X	X	X	X
		15	No Abnormalities Detected		X	X	X	X	X	X
		16	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					16	16	17	17	18	18
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose	
ff										
1	m	01	No Abnormalities Detected		X	X	X	X	X	X
		02	No Abnormalities Detected		X	X	X	X	X	X
		03	No Abnormalities Detected		X	X	X	X	X	X
		04	No Abnormalities Detected		X	X	X	X	X	X
		05	No Abnormalities Detected		X	X	X	X	X	X
		06	No Abnormalities Detected		X	X	X	X	X	X
		07	No Abnormalities Detected		X	X	X	X	X	X
		08	No Abnormalities Detected		X	X	X	X	X	X
		09	No Abnormalities Detected		X	X	X	X	X	X
		10	No Abnormalities Detected		X	X	X	X	X	X
		11	No Abnormalities Detected		X	X	X	X	X	X
		12	No Abnormalities Detected		X	X	X	X	X	X
		13	No Abnormalities Detected		X	X	X	X	X	X
		14	No Abnormalities Detected		X	X	X	X	X	X
		15	No Abnormalities Detected		X	X	X	X	X	X
		16	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					19	19	20	20	21	21
Group	Sex	Animal	Clinical Sign	Site		1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose
ff										
1	m	01	No Abnormalities Detected		X	X	X	X	X	X
		02	No Abnormalities Detected		X	X	X	X	X	X
		03	No Abnormalities Detected		X	X	X	X	X	X
		04	No Abnormalities Detected		X	X	X	X	X	X
		05	No Abnormalities Detected		X	X	X	X	X	X
		06	No Abnormalities Detected		X	X	X	X	X	X
		07	No Abnormalities Detected		X	X	X	X	X	X
		08	No Abnormalities Detected		X	X	X	X	X	X
		09	No Abnormalities Detected		X	X	X	X	X	X
		10	No Abnormalities Detected		X	X	X	X	X	X
		11	No Abnormalities Detected		X	X	X	X	X	X
		12	No Abnormalities Detected		X	X	X	X	X	X
		13	No Abnormalities Detected		X	X	X	X	X	X
		14	No Abnormalities Detected		X	X	X	X	X	X
		15	No Abnormalities Detected		X	X	X	X	X	X
		16	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					22	22	23	23	24	24
Group	Sex	Animal	Clinical Sign	Site		1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose
ff										
1	m	01	No Abnormalities Detected		X	X	X	X	X	X
		02	No Abnormalities Detected		X	X	X	X	X	X
		03	No Abnormalities Detected		X	X	X	X	X	X
		04	No Abnormalities Detected		X	X	X	X	X	X
		05	No Abnormalities Detected		X	X	X	X	X	X
		06	No Abnormalities Detected		X	X	X	X	X	X
		07	No Abnormalities Detected		X	X	X	X	X	X
		08	No Abnormalities Detected		X	X	X	X	X	X
		09	No Abnormalities Detected		X	X	X	X	X	X
		10	No Abnormalities Detected		X	X	X	X	X	X
		11	No Abnormalities Detected		X	X	X	X	X	X
		12	No Abnormalities Detected		X	X	X	X	X	X
		13	No Abnormalities Detected		X	X	X	X	X	X
		14	No Abnormalities Detected		X	X	X	X	X	X
		15	No Abnormalities Detected		X	X	X	X	X	X
		16	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					25	25	26	26	27	27
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose	
ff										
1	m	01	No Abnormalities Detected		X	X	X	X	X	X
		02	No Abnormalities Detected		X	X	X	X	X	X
		03	No Abnormalities Detected		X	X	X	X	X	X
		04	No Abnormalities Detected		X	X	X	X	X	X
		05	No Abnormalities Detected		X	X	X	X	X	X
		06	No Abnormalities Detected		X	X	X	X	X	X
		07	No Abnormalities Detected		X	X	X	X	X	X
		08	No Abnormalities Detected		X	X	X	X	X	X
		09	No Abnormalities Detected		X	X	X	X	X	X
		10	No Abnormalities Detected		X	X	X	X	X	X
		11	No Abnormalities Detected		X	X	X	X	X	X
		12	No Abnormalities Detected		X	X	X	X	X	X
		13	No Abnormalities Detected		X	X	X	X	X	X
		14	No Abnormalities Detected		X	X	X	X	X	X
		15	No Abnormalities Detected		X	X	X	X	X	X
		16	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP



Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					28	28	29	29	30	30
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose	
ff										
1	m	01	No Abnormalities Detected		X	X	X	X	X	X
		02	No Abnormalities Detected		X	X	X	X	X	X
		03	No Abnormalities Detected		X	X	X	X	X	X
		04	No Abnormalities Detected		X	X	X	X	X	X
		05	No Abnormalities Detected		X	X	X	X	X	X
		06	No Abnormalities Detected		X	X	X	X	X	X
		07	No Abnormalities Detected		X	X	X	X	X	X
		08	No Abnormalities Detected		X	X	X	X	X	X
		09	No Abnormalities Detected		X	X	X	X	X	X
		10	No Abnormalities Detected		X	X	X	X	X	X
		11	No Abnormalities Detected		X	X	X	X	X	X
		12	No Abnormalities Detected		X	X	X	X	X	X
		13	No Abnormalities Detected		X	X	X	X	X	X
		14	No Abnormalities Detected		X	X	X	X	X	X
		15	No Abnormalities Detected		X	X	X	X	X	X
		16	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					31	31	32	
Group	Sex	Animal	Clinical Sign		Site	1-Hour Post-Dose		
ff								
1	m	01	No Abnormalities	Detected		X	.	.
		02	No Abnormalities	Detected		X	.	.
		03	No Abnormalities	Detected		X	.	.
		04	No Abnormalities	Detected		X	.	.
		05	No Abnormalities	Detected		X	.	.
		06	No Abnormalities	Detected		X	.	.
		07	No Abnormalities	Detected		X	.	.
		08	No Abnormalities	Detected		X	.	.
		09	No Abnormalities	Detected		X	X	X
		10	No Abnormalities	Detected		X	X	X
		11	No Abnormalities	Detected		X	X	X
		12	No Abnormalities	Detected		X	X	X
		13	No Abnormalities	Detected		X	X	X
		14	No Abnormalities	Detected		X	X	X
		15	No Abnormalities	Detected		X	X	X
		16	No Abnormalities	Detected		X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					1	1	2	2	3	3
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		1-Hour Post-Dose			1-Hour Post-Dose
ff										
2	m	17	No Abnormalities Detected		X	X	X	X	X	X
		18	No Abnormalities Detected		X	X	X	X	X	X
		19	No Abnormalities Detected		X	X	X	X	X	X
		20	No Abnormalities Detected		X	X	X	X	X	X
		21	No Abnormalities Detected		X	X	X	X	X	X
		22	No Abnormalities Detected		X	X	X	X	X	X
		23	No Abnormalities Detected		X	X	X	X	X	X
		24	No Abnormalities Detected		X	X	X	X	X	X
		25	No Abnormalities Detected		X	X	X	X	X	X
		26	No Abnormalities Detected		X	X	X	X	X	X
		27	No Abnormalities Detected		X	X	X	X	X	X
		28	No Abnormalities Detected		X	X	X	X	X	X
		29	No Abnormalities Detected		X	X	X	X	X	X
		30	No Abnormalities Detected		X	X	X	X	X	X
		31	No Abnormalities Detected		X	X	X	X	X	X
		32	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					4	4	5	5	6	6
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose	
%%%										
2	m	17	No Abnormalities Detected		X	X	X	X	X	X
		18	No Abnormalities Detected		X	X	X	X	X	X
		19	No Abnormalities Detected		X	X	X	X	X	X
		20	No Abnormalities Detected		X	X	X	X	X	X
		21	No Abnormalities Detected		X	X	X	X	X	X
		22	No Abnormalities Detected		X	X	X	X	X	X
		23	No Abnormalities Detected		X	X	X	X	X	X
		24	No Abnormalities Detected		X	X	X	X	X	X
		25	No Abnormalities Detected		X	X	X	X	X	X
		26	No Abnormalities Detected		X	X	X	X	X	X
		27	No Abnormalities Detected		X	X	X	X	X	X
		28	No Abnormalities Detected		X	X	X	X	X	X
		29	No Abnormalities Detected		X	X	X	X	X	X
		30	No Abnormalities Detected		X	X	X	X	X	X
		31	No Abnormalities Detected		X	X	X	X	X	X
		32	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					7	7	8	8	9	9
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose	
ff										
2	m	17	No Abnormalities Detected		X	X	X	X	X	X
		18	No Abnormalities Detected		X	X	X	X	X	X
		19	No Abnormalities Detected		X	X	X	X	X	X
		20	No Abnormalities Detected		X	X	X	X	X	X
		21	No Abnormalities Detected		X	X	X	X	X	X
		22	No Abnormalities Detected		X	X	X	X	X	X
		23	No Abnormalities Detected		X	X	X	X	X	X
		24	No Abnormalities Detected		X	X	X	X	X	X
		25	No Abnormalities Detected		X	X	X	X	X	X
		26	No Abnormalities Detected		X	X	X	X	X	X
		27	No Abnormalities Detected		X	X	X	X	X	X
		28	No Abnormalities Detected		X	X	X	X	X	X
		29	No Abnormalities Detected		X	X	X	X	X	X
		30	No Abnormalities Detected		X	X	X	X	X	X
		31	No Abnormalities Detected		X	X	X	X	X	X
		32	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					10	10	11	11	12	12
Group	Sex	Animal	Clinical Sign	Site		1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose
ff										
2	m	17	No Abnormalities Detected		X	X	X	X	X	X
		18	No Abnormalities Detected		X	X	X	X	X	X
		19	No Abnormalities Detected		X	X	X	X	X	X
		20	No Abnormalities Detected		X	X	X	X	X	X
		21	No Abnormalities Detected		X	X	X	X	X	X
		22	No Abnormalities Detected		X	X	X	X	X	X
		23	No Abnormalities Detected		X	X	X	X	X	X
		24	No Abnormalities Detected		X	X	X	X	X	X
		25	No Abnormalities Detected		X	X	X	X	X	X
		26	No Abnormalities Detected		X	X	X	X	X	X
		27	No Abnormalities Detected		X	X	X	X	X	X
		28	No Abnormalities Detected		X	X	X	X	X	X
		29	No Abnormalities Detected		X	X	X	X	X	X
		30	No Abnormalities Detected		X	X	X	X	X	X
		31	No Abnormalities Detected		X	X	X	X	X	X
		32	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					13	13	14	14	15	15
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose	
ff										
2	m	17	No Abnormalities Detected		X	X	X	X	X	X
		18	No Abnormalities Detected		X	X	X	X	X	X
		19	No Abnormalities Detected		X	X	X	X	X	X
		20	No Abnormalities Detected		X	X	X	X	X	X
		21	No Abnormalities Detected		X	X	X	X	X	X
		22	No Abnormalities Detected		X	X	X	X	X	X
		23	No Abnormalities Detected		X	X	X	X	X	X
		24	No Abnormalities Detected		X	X	X	X	X	X
		25	No Abnormalities Detected		X	X	X	X	X	X
		26	No Abnormalities Detected		X	X	X	X	X	X
		27	No Abnormalities Detected		X	X	X	X	X	X
		28	No Abnormalities Detected		X	X	X	X	X	X
		29	No Abnormalities Detected		X	X	X	X	X	X
		30	No Abnormalities Detected		X	X	X	X	X	X
		31	No Abnormalities Detected		X	X	X	X	X	X
		32	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					16	16	17	17	18	18
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose	
ff										
2	m	17	No Abnormalities Detected		X	X	X	X	X	X
		18	No Abnormalities Detected		X	X	X	X	X	X
		19	No Abnormalities Detected		X	X	X	X	X	X
		20	No Abnormalities Detected		X	X	X	X	X	X
		21	No Abnormalities Detected		X	X	X	X	X	X
		22	No Abnormalities Detected		X	X	X	X	X	X
		23	No Abnormalities Detected		X	X	X	X	X	X
		24	No Abnormalities Detected		X	X	X	X	X	X
		25	No Abnormalities Detected		X	X	X	X	X	X
		26	No Abnormalities Detected		X	X	X	X	X	X
		27	No Abnormalities Detected		X	X	X	X	X	X
		28	No Abnormalities Detected		X	X	X	X	X	X
		29	No Abnormalities Detected		X	X	X	X	X	X
		30	No Abnormalities Detected		X	X	X	X	X	X
		31	No Abnormalities Detected		X	X	X	X	X	X
		32	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP



Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					19	19	20	20	21	21
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose	
ff										
2	m	17	No Abnormalities Detected		X	X	X	X	X	X
		18	No Abnormalities Detected		X	X	X	X	X	X
		19	No Abnormalities Detected		X	X	X	X	X	X
		20	No Abnormalities Detected		X	X	X	X	X	X
		21	No Abnormalities Detected		X	X	X	X	X	X
		22	No Abnormalities Detected		X	X	X	X	X	X
		23	No Abnormalities Detected		X	X	X	X	X	X
		24	No Abnormalities Detected		X	X	X	X	X	X
		25	No Abnormalities Detected		X	X	X	X	X	X
		26	No Abnormalities Detected		X	X	X	X	X	X
		27	No Abnormalities Detected		X	X	X	X	X	X
		28	No Abnormalities Detected		X	X	X	X	X	X
		29	No Abnormalities Detected		X	X	X	X	X	X
		30	No Abnormalities Detected		X	X	X	X	X	X
		31	No Abnormalities Detected		X	X	X	X	X	X
		32	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					22	22	23	23	24	24
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose	
ff										
2	m	17	No Abnormalities Detected		X	X	X	X	X	X
		18	No Abnormalities Detected		X	X	X	X	X	X
		19	No Abnormalities Detected		X	X	X	X	X	X
		20	No Abnormalities Detected		X	X	X	X	X	X
		21	No Abnormalities Detected		X	X	X	X	X	X
		22	No Abnormalities Detected		X	X	X	X	X	X
		23	No Abnormalities Detected		X	X	X	X	X	X
		24	No Abnormalities Detected		X	X	X	X	X	X
		25	No Abnormalities Detected		X	X	X	X	X	X
		26	No Abnormalities Detected		X	X	X	X	X	X
		27	No Abnormalities Detected		X	X	X	X	X	X
		28	No Abnormalities Detected		X	X	X	X	X	X
		29	No Abnormalities Detected		X	X	X	X	X	X
		30	No Abnormalities Detected		X	X	X	X	X	X
		31	No Abnormalities Detected		X	X	X	X	X	X
		32	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					25	25	26	26	27	27
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose	
ff										
2	m	17	No Abnormalities Detected		X	X	X	X	X	X
		18	No Abnormalities Detected		X	X	X	X	X	X
		19	No Abnormalities Detected		X	X	X	X	X	X
		20	No Abnormalities Detected		X	X	X	X	X	X
		21	No Abnormalities Detected		X	X	X	X	X	X
		22	No Abnormalities Detected		X	X	X	X	X	X
		23	No Abnormalities Detected		X	X	X	X	X	X
		24	No Abnormalities Detected		X	X	X	X	X	X
		25	No Abnormalities Detected		X	X	X	X	X	X
		26	No Abnormalities Detected		X	X	X	X	X	X
		27	No Abnormalities Detected		X	X	X	X	X	X
		28	No Abnormalities Detected		X	X	X	X	X	X
		29	No Abnormalities Detected		X	X	X	X	X	X
		30	No Abnormalities Detected		X	X	X	X	X	X
		31	No Abnormalities Detected		X	X	X	X	X	X
		32	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					28	28	29	29	30	30
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose	
ff										
2	m	17	No Abnormalities Detected		X	X	X	X	X	X
		18	No Abnormalities Detected		X	X	X	X	X	X
		19	No Abnormalities Detected		X	X	X	X	X	X
		20	No Abnormalities Detected		X	X	X	X	X	X
		21	No Abnormalities Detected		X	X	X	X	X	X
		22	No Abnormalities Detected		X	X	X	X	X	X
		23	No Abnormalities Detected		X	X	X	X	X	X
		24	No Abnormalities Detected		X	X	X	X	X	X
		25	No Abnormalities Detected		X	X	X	X	X	X
		26	No Abnormalities Detected		X	X	X	X	X	X
		27	No Abnormalities Detected		X	X	X	X	X	X
		28	No Abnormalities Detected		X	X	X	X	X	X
		29	No Abnormalities Detected		X	X	X	X	X	X
		30	No Abnormalities Detected		X	X	X	X	X	X
		31	No Abnormalities Detected		X	X	X	X	X	X
		32	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					31	31	32
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		
ff							
2	m	17	No Abnormalities Detected		X	.	.
		18	No Abnormalities Detected		X	.	.
		19	No Abnormalities Detected		X	.	.
		20	No Abnormalities Detected		X	.	.
		21	No Abnormalities Detected		X	.	.
		22	No Abnormalities Detected		X	.	.
		23	No Abnormalities Detected		X	.	.
		24	No Abnormalities Detected		X	.	.
		25	No Abnormalities Detected		X	X	X
		26	No Abnormalities Detected		X	X	X
		27	No Abnormalities Detected		X	X	X
		28	No Abnormalities Detected		X	X	X
		29	No Abnormalities Detected		X	X	X
		30	No Abnormalities Detected		X	X	X
		31	No Abnormalities Detected		X	X	X
		32	No Abnormalities Detected		X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					1	1	2	2	3	3
Group	Sex	Animal	Clinical Sign	Site		1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose
ff										
3	m	33	No Abnormalities Detected		X	X	X	X	X	X
		34	No Abnormalities Detected		X	X	X	X	X	X
		35	No Abnormalities Detected		X	X	X	X	X	X
		36	No Abnormalities Detected		X	X	X	X	X	X
		37	No Abnormalities Detected		X	X	X	X	X	X
		38	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		39	No Abnormalities Detected		X	X	X	X	X	X
		40	No Abnormalities Detected		X	X	X	X	X	X
		41	No Abnormalities Detected		X	X	X	X	X	X
		42	No Abnormalities Detected		X	X	X	X	X	X
		43	No Abnormalities Detected		X	X	X	X	X	X
		44	No Abnormalities Detected		X	X	X	X	X	X
		45	No Abnormalities Detected		X	X	X	X	X	X
		46	No Abnormalities Detected		X	X	X	X	X	X
		47	No Abnormalities Detected		X	X	X	X	X	X
48	No Abnormalities Detected		X	X	X	X	X	X		

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					4	4	5	5	6	6
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose	
ff										
3	m	33	No Abnormalities Detected		X	X	X	X	X	X
		34	No Abnormalities Detected		X	X	X	X	X	X
		35	No Abnormalities Detected		X	X	X	X	X	X
		36	No Abnormalities Detected		X	X	X	X	X	X
		37	No Abnormalities Detected		X	X	X	X	X	X
		38	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		39	No Abnormalities Detected		X	X	X	X	X	X
		40	No Abnormalities Detected		X	X	X	X	X	X
		41	No Abnormalities Detected		X	X	X	X	X	X
		42	No Abnormalities Detected		X	X	X	X	X	X
		43	No Abnormalities Detected		X	X	X	X	X	X
		44	No Abnormalities Detected		X	X	X	X	X	X
		45	No Abnormalities Detected		X	X	X	X	X	X
		46	No Abnormalities Detected		X	X	X	X	X	X
		47	No Abnormalities Detected		X	X	X	X	X	X
48	No Abnormalities Detected		X	X	X	X	X	X		

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					7	7	8	8	9	9
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose	
ff										
3	m	33	No Abnormalities Detected		X	X	X	X	X	X
		34	No Abnormalities Detected		X	X	X	X	X	X
		35	No Abnormalities Detected		X	X	X	X	X	X
		36	No Abnormalities Detected		X	X	X	X	X	X
		37	No Abnormalities Detected		X	X	X	X	X	X
		38	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		39	No Abnormalities Detected		X	X	X	X	X	X
		40	No Abnormalities Detected		X	X	X	X	X	X
		41	No Abnormalities Detected		X	X	X	X	X	X
		42	No Abnormalities Detected		X	X	X	X	X	X
		43	No Abnormalities Detected		X	X	X	X	X	X
		44	No Abnormalities Detected		X	X	X	X	X	X
		45	No Abnormalities Detected		X	X	X	X	X	X
		46	No Abnormalities Detected		X	X	X	X	X	X
		47	No Abnormalities Detected		X	X	X	X	X	X
48	No Abnormalities Detected		X	X	X	X	X	X		

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP



Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					10	10	11	11	12	12
Group	Sex	Animal	Clinical Sign	Site		1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose
ff										
3	m	33	No Abnormalities Detected		X	X	X	X	X	X
		34	No Abnormalities Detected		X	X	X	X	X	X
		35	No Abnormalities Detected		X	X	X	X	X	X
		36	No Abnormalities Detected		X	X	X	X	X	X
		37	No Abnormalities Detected		X	X	X	X	X	X
		38	No Abnormalities Detected		X	X	X	X	X	.
			Salivation		.	.	.	.	.	1
		39	No Abnormalities Detected		X	X	X	X	X	X
		40	No Abnormalities Detected		X	X	X	X	X	X
		41	No Abnormalities Detected		X	X	X	X	X	X
		42	No Abnormalities Detected		X	X	X	X	X	X
		43	No Abnormalities Detected		X	X	X	X	X	X
		44	No Abnormalities Detected		X	X	X	X	X	X
		45	No Abnormalities Detected		X	X	X	X	X	X
		46	No Abnormalities Detected		X	X	X	X	X	X
		47	No Abnormalities Detected		X	X	X	X	X	X
		48	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

				13	13	14	14	15	15	
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose	1-Hour Post-Dose	1-Hour Post-Dose	1-Hour Post-Dose	1-Hour Post-Dose	
ff										
3	m	33	No Abnormalities Detected		X	X	X	X	X	X
		34	No Abnormalities Detected		X	X	X	X	X	X
		35	No Abnormalities Detected		X	X	X	X	X	X
		36	No Abnormalities Detected		X	X	X	X	X	X
		37	No Abnormalities Detected		X	X	X	X	X	X
		38	No Abnormalities Detected		X	.	X	X	X	X
			Salivation		.	1	.	.	.	.
		39	No Abnormalities Detected		X	X	X	X	X	X
		40	No Abnormalities Detected		X	X	X	X	X	X
		41	No Abnormalities Detected		X	X	X	X	X	X
		42	No Abnormalities Detected		X	X	X	X	X	X
		43	No Abnormalities Detected		X	X	X	X	X	X
		44	No Abnormalities Detected		X	X	X	X	X	X
		45	No Abnormalities Detected		X	X	X	X	X	X
		46	No Abnormalities Detected		X	X	X	X	X	X
		47	No Abnormalities Detected		X	X	X	X	X	X
		48	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

				16	16	17	17	18	18	
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose	
ff										
3	m	33	No Abnormalities Detected		X	X	X	X	X	X
		34	No Abnormalities Detected		X	X	X	X	X	X
		35	No Abnormalities Detected		X	X	X	X	X	X
		36	No Abnormalities Detected		X	X	X	X	X	X
		37	No Abnormalities Detected		X	X	X	X	X	X
		38	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		39	No Abnormalities Detected		X	X	X	X	X	X
		40	No Abnormalities Detected		X	X	X	X	X	X
		41	No Abnormalities Detected		X	X	X	X	X	X
		42	No Abnormalities Detected		X	X	X	X	X	X
		43	No Abnormalities Detected		X	X	X	X	X	X
		44	No Abnormalities Detected		X	X	X	X	X	X
		45	No Abnormalities Detected		X	X	X	X	X	X
		46	No Abnormalities Detected		X	X	X	X	X	X
		47	No Abnormalities Detected		X	X	X	X	X	X
		48	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					19	19	20	20	21	21
Group	Sex	Animal	Clinical Sign	Site		1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose
ff										
3	m	33	No Abnormalities Detected		X	X	X	X	X	X
		34	No Abnormalities Detected		X	X	X	X	X	X
		35	No Abnormalities Detected		X	X	X	X	X	X
		36	No Abnormalities Detected		X	X	X	X	X	X
		37	No Abnormalities Detected		X	X	X	X	X	X
		38	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		39	No Abnormalities Detected		X	X	X	X	X	X
		40	No Abnormalities Detected		X	X	X	X	X	X
		41	No Abnormalities Detected		X	X	X	X	X	X
		42	No Abnormalities Detected		X	X	X	X	X	X
		43	No Abnormalities Detected		X	X	X	X	X	X
		44	No Abnormalities Detected		X	X	X	X	X	X
		45	No Abnormalities Detected		X	X	X	X	X	X
		46	No Abnormalities Detected		X	X	X	X	X	X
		47	No Abnormalities Detected		X	X	X	X	X	X
		48	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

				22	22	23	23	24	24	
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose	1-Hour Post-Dose	1-Hour Post-Dose	1-Hour Post-Dose	1-Hour Post-Dose	
ff										
3	m	33	No Abnormalities Detected		X	X	X	X	X	X
		34	No Abnormalities Detected		X	X	X	X	X	X
		35	No Abnormalities Detected		X	X	X	X	X	X
		36	No Abnormalities Detected		X	X	X	X	X	X
		37	No Abnormalities Detected		X	X	X	X	X	X
		38	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		39	No Abnormalities Detected		X	X	X	X	X	X
		40	No Abnormalities Detected		X	X	X	X	X	X
		41	No Abnormalities Detected		X	X	X	X	X	X
		42	No Abnormalities Detected		X	X	X	X	X	X
		43	No Abnormalities Detected		X	X	X	X	X	X
		44	No Abnormalities Detected		X	X	X	X	X	X
		45	No Abnormalities Detected		X	X	X	X	X	X
		46	No Abnormalities Detected		X	X	X	X	X	X
		47	No Abnormalities Detected		X	X	X	X	X	X
		48	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

				25	25	26	26	27	27	
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose	1-Hour Post-Dose	1-Hour Post-Dose	1-Hour Post-Dose	1-Hour Post-Dose	
ff										
3	m	33	No Abnormalities Detected		X	X	X	X	X	X
		34	No Abnormalities Detected		X	X	X	X	X	X
		35	No Abnormalities Detected		X	X	X	X	X	X
		36	No Abnormalities Detected		X	X	X	X	X	X
		37	No Abnormalities Detected		X	X	X	X	X	X
		38	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		39	No Abnormalities Detected		X	X	X	X	X	X
		40	No Abnormalities Detected		X	X	X	X	X	X
		41	No Abnormalities Detected		X	X	X	X	X	X
		42	No Abnormalities Detected		X	X	X	X	X	X
		43	No Abnormalities Detected		X	X	X	X	X	X
		44	No Abnormalities Detected		X	X	X	X	X	X
		45	No Abnormalities Detected		X	X	X	X	X	X
		46	No Abnormalities Detected		X	X	X	X	X	X
		47	No Abnormalities Detected		X	X	X	X	X	X
		48	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					28	28	29	29	30	30
Group	Sex	Animal	Clinical Sign	Site		1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose
ff										
3	m	33	No Abnormalities Detected		X	X	X	X	X	X
		34	No Abnormalities Detected		X	X	X	X	X	X
		35	No Abnormalities Detected		X	X	X	X	X	X
		36	No Abnormalities Detected		X	X	X	X	X	X
		37	No Abnormalities Detected		X	X	X	X	X	X
		38	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		39	No Abnormalities Detected		X	X	X	X	X	X
		40	No Abnormalities Detected		X	X	X	X	X	X
		41	No Abnormalities Detected		X	X	X	X	X	X
		42	No Abnormalities Detected		X	X	X	X	X	X
		43	No Abnormalities Detected		X	X	X	X	X	X
		44	No Abnormalities Detected		X	X	X	X	X	X
		45	No Abnormalities Detected		X	X	X	X	X	X
		46	No Abnormalities Detected		X	X	X	X	X	X
		47	No Abnormalities Detected		X	X	X	X	X	X
		48	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

				31	31	32	
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		
ff							
3	m	33	No Abnormalities Detected		X	.	.
		34	No Abnormalities Detected		X	.	.
		35	No Abnormalities Detected		X	.	.
		36	No Abnormalities Detected		X	.	.
		37	No Abnormalities Detected		X	.	.
		38	No Abnormalities Detected		X	.	.
			Salivation		.	.	.
		39	No Abnormalities Detected		X	.	.
		40	No Abnormalities Detected		X	.	.
		41	No Abnormalities Detected		X	X	X
		42	No Abnormalities Detected		X	X	X
		43	No Abnormalities Detected		X	X	X
		44	No Abnormalities Detected		X	X	X
		45	No Abnormalities Detected		X	X	X
		46	No Abnormalities Detected		X	X	X
		47	No Abnormalities Detected		X	X	X
		48	No Abnormalities Detected		X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP



Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					1	1	2	2	3	3
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose	
ff										
4	m	49	No Abnormalities Detected		X	X	X	X	X	X
		50	No Abnormalities Detected		X	X	X	X	X	X
		51	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		52	No Abnormalities Detected		X	X	X	X	X	.
			Salivation		.	.	.	.	.	1
		53	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		54	No Abnormalities Detected		X	X	X	X	X	X
		55	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		56	No Abnormalities Detected		X	X	X	X	X	X
		57	No Abnormalities Detected		X	X	X	X	X	X
		58	No Abnormalities Detected		X	X	X	X	X	X
		59	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
60	No Abnormalities Detected		X	X	X	X	X	X		
	Salivation		.	.	.	.	.	.		
61	No Abnormalities Detected		X	X	X	X	X	X		
62	No Abnormalities Detected		X	X	X	X	X	X		
63	No Abnormalities Detected		X	X	X	X	X	X		
64	No Abnormalities Detected		X	X	X	X	X	X		

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					4	4	5	5	6	6
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose	
ff										
4	m	49	No Abnormalities Detected		X	X	X	X	X	X
		50	No Abnormalities Detected		X	X	X	X	X	X
		51	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		52	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		53	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		54	No Abnormalities Detected		X	X	X	X	X	X
		55	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		56	No Abnormalities Detected		X	X	X	X	X	X
		57	No Abnormalities Detected		X	X	X	X	X	X
		58	No Abnormalities Detected		X	X	X	X	X	X
		59	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
60	No Abnormalities Detected		X	X	X	X	X	X		
	Salivation		.	.	.	.	.	.		
61	No Abnormalities Detected		X	X	X	X	X	X		
62	No Abnormalities Detected		X	X	X	X	X	X		
63	No Abnormalities Detected		X	X	X	X	X	X		
64	No Abnormalities Detected		X	X	X	X	X	X		

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					7	7	8	8	9	9
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose	
ff										
4	m	49	No Abnormalities Detected		X	X	X	X	X	X
		50	No Abnormalities Detected		X	X	X	X	X	X
		51	No Abnormalities Detected		X	.	X	X	X	X
			Salivation		.	1	.	.	.	.
		52	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		53	No Abnormalities Detected		X	.	X	X	X	X
			Salivation		.	1	.	.	.	.
		54	No Abnormalities Detected		X	X	X	X	X	X
		55	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		56	No Abnormalities Detected		X	X	X	X	X	X
		57	No Abnormalities Detected		X	X	X	X	X	X
		58	No Abnormalities Detected		X	X	X	X	X	X
		59	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
60	No Abnormalities Detected		X	X	X	X	X	X		
	Salivation		.	.	.	.	.	.		
61	No Abnormalities Detected		X	X	X	X	X	X		
62	No Abnormalities Detected		X	X	X	X	X	X		
63	No Abnormalities Detected		X	X	X	X	X	X		
64	No Abnormalities Detected		X	X	X	X	X	X		

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

				10	10	11	11	12	12	
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose	
ff										
4	m	49	No Abnormalities Detected		X	X	X	X	X	X
		50	No Abnormalities Detected		X	X	X	X	X	X
		51	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		52	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		53	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		54	No Abnormalities Detected		X	X	X	X	X	X
		55	No Abnormalities Detected		X	X	X	.	X	X
			Salivation		.	.	.	1	.	.
		56	No Abnormalities Detected		X	X	X	X	X	X
		57	No Abnormalities Detected		X	X	X	X	X	X
		58	No Abnormalities Detected		X	X	X	X	X	X
		59	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
60	No Abnormalities Detected		X	X	X	X	X	X		
	Salivation		.	.	.	.	.	.		
61	No Abnormalities Detected		X	X	X	X	X	X		
62	No Abnormalities Detected		X	X	X	X	X	X		
63	No Abnormalities Detected		X	X	X	X	X	X		
64	No Abnormalities Detected		X	X	X	X	X	X		

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

				13	13	14	14	15	15	
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose	
ff										
4	m	49	No Abnormalities Detected		X	X	X	X	X	X
		50	No Abnormalities Detected		X	X	X	X	X	X
		51	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		52	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		53	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		54	No Abnormalities Detected		X	X	X	X	X	X
		55	No Abnormalities Detected		X	.	X	X	X	X
			Salivation		.	1	.	.	.	.
		56	No Abnormalities Detected		X	X	X	X	X	X
		57	No Abnormalities Detected		X	X	X	X	X	X
		58	No Abnormalities Detected		X	X	X	X	X	X
		59	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
60	No Abnormalities Detected		X	X	X	X	X	X		
	Salivation		.	.	.	.	.	.		
61	No Abnormalities Detected		X	X	X	X	X	X		
62	No Abnormalities Detected		X	X	X	X	X	X		
63	No Abnormalities Detected		X	X	X	X	X	X		
64	No Abnormalities Detected		X	X	X	X	X	X		

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					16	16	17	17	18	18
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose	
ff										
4	m	49	No Abnormalities Detected		X	X	X	X	X	X
		50	No Abnormalities Detected		X	X	X	X	X	X
		51	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		52	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		53	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		54	No Abnormalities Detected		X	X	X	X	X	X
		55	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		56	No Abnormalities Detected		X	X	X	X	X	X
		57	No Abnormalities Detected		X	X	X	X	X	X
		58	No Abnormalities Detected		X	X	X	X	X	X
		59	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
60	No Abnormalities Detected		X	X	X	X	X	X		
	Salivation		.	.	.	.	.	.		
61	No Abnormalities Detected		X	X	X	X	X	X		
62	No Abnormalities Detected		X	X	X	X	X	X		
63	No Abnormalities Detected		X	X	X	X	X	X		
64	No Abnormalities Detected		X	X	X	X	X	X		

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					19	19	20	20	21	21
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose	
ff										
4	m	49	No Abnormalities Detected		X	X	X	X	X	X
		50	No Abnormalities Detected		X	X	X	X	X	X
		51	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		52	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		53	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		54	No Abnormalities Detected		X	X	X	X	X	X
		55	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		56	No Abnormalities Detected		X	X	X	X	X	X
		57	No Abnormalities Detected		X	X	X	X	X	X
		58	No Abnormalities Detected		X	X	X	X	X	X
		59	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
60	No Abnormalities Detected		X	.	X	X	X	X		
	Salivation		.	1	.	.	.	.		
61	No Abnormalities Detected		X	X	X	X	X	X		
62	No Abnormalities Detected		X	X	X	X	X	X		
63	No Abnormalities Detected		X	X	X	X	X	X		
64	No Abnormalities Detected		X	X	X	X	X	X		

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					22	22	23	23	24	24
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose	
ff										
4	m	49	No Abnormalities Detected		X	X	X	X	X	X
		50	No Abnormalities Detected		X	X	X	X	X	X
		51	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		52	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		53	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		54	No Abnormalities Detected		X	X	X	X	X	X
		55	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		56	No Abnormalities Detected		X	X	X	X	X	X
		57	No Abnormalities Detected		X	X	X	X	X	X
		58	No Abnormalities Detected		X	X	X	X	X	X
		59	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
60	No Abnormalities Detected		X	X	X	X	X	X		
	Salivation		.	.	.	.	.	.		
61	No Abnormalities Detected		X	X	X	X	X	X		
62	No Abnormalities Detected		X	X	X	X	X	X		
63	No Abnormalities Detected		X	X	X	X	X	X		
64	No Abnormalities Detected		X	X	X	X	X	X		

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP



Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					25	25	26	26	27	27
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose	
ff										
4	m	49	No Abnormalities Detected		X	X	X	X	X	X
		50	No Abnormalities Detected		X	X	X	X	X	X
		51	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		52	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		53	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		54	No Abnormalities Detected		X	X	X	X	X	X
		55	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		56	No Abnormalities Detected		X	X	X	X	X	X
		57	No Abnormalities Detected		X	X	X	X	X	X
		58	No Abnormalities Detected		X	X	X	X	X	X
		59	No Abnormalities Detected		X	X	X	.	X	X
			Salivation		.	.	.	1	.	.
60	No Abnormalities Detected		X	X	X	X	X	X		
	Salivation		.	.	.	.	.	.		
61	No Abnormalities Detected		X	X	X	X	X	X		
62	No Abnormalities Detected		X	X	X	X	X	X		
63	No Abnormalities Detected		X	X	X	X	X	X		
64	No Abnormalities Detected		X	X	X	X	X	X		

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					28	28	29	29	30	30
Group	Sex	Animal	Clinical Sign	Site		1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose
ff										
4	m	49	No Abnormalities Detected		X	X	X	X	X	X
		50	No Abnormalities Detected		X	X	X	X	X	X
		51	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		52	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		53	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		54	No Abnormalities Detected		X	X	X	X	X	X
		55	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		56	No Abnormalities Detected		X	X	X	X	X	X
		57	No Abnormalities Detected		X	X	X	X	X	X
		58	No Abnormalities Detected		X	X	X	X	X	X
		59	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
60	No Abnormalities Detected		X	X	X	X	X	X		
	Salivation		.	.	.	.	.	.		
61	No Abnormalities Detected		X	X	X	X	X	X		
62	No Abnormalities Detected		X	X	X	X	X	X		
63	No Abnormalities Detected		X	X	X	X	X	X		
64	No Abnormalities Detected		X	X	X	X	X	X		

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					31	31	32
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		
ff							
4	m	49	No Abnormalities Detected		X	.	.
		50	No Abnormalities Detected		X	.	.
		51	No Abnormalities Detected		X	.	.
			Salivation		.	.	.
		52	No Abnormalities Detected		X	.	.
			Salivation		.	.	.
		53	No Abnormalities Detected		X	.	.
			Salivation		.	.	.
		54	No Abnormalities Detected		X	.	.
		55	No Abnormalities Detected		X	.	.
			Salivation		.	.	.
		56	No Abnormalities Detected		X	.	.
		57	No Abnormalities Detected		X	X	X
		58	No Abnormalities Detected		X	X	X
		59	No Abnormalities Detected		X	X	X
			Salivation		.	.	.
		60	No Abnormalities Detected		X	X	X
			Salivation		.	.	.
		61	No Abnormalities Detected		X	X	X
		62	No Abnormalities Detected		X	X	X
		63	No Abnormalities Detected		X	X	X
		64	No Abnormalities Detected		X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					1	1	2	2	3	3
Group	Sex	Animal	Clinical Sign	Site		1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose
ff										
5	m	65	No Abnormalities Detected		X	X	X	X	X	X
		66	No Abnormalities Detected		X	X	X	X	X	X
		67	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		68	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		69	No Abnormalities Detected		X	X	X	X	X	X
		70	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		71	No Abnormalities Detected		X	X	X	X	X	X
		72	No Abnormalities Detected		X	X	X	X	X	X
		73	No Abnormalities Detected		X	X	X	X	X	X
		74	No Abnormalities Detected		X	X	X	X	X	X
		75	No Abnormalities Detected		X	X	X	X	X	X
		76	No Abnormalities Detected		X	X	X	X	X	X
		77	No Abnormalities Detected		X	X	X	X	X	X
		78	No Abnormalities Detected		X	X	X	X	X	X
		79	No Abnormalities Detected		X	X	X	X	X	X
		80	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					4	4	5	5	6	6
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose	
ff										
5	m	65	No Abnormalities Detected		X	X	X	X	X	X
		66	No Abnormalities Detected		X	X	X	X	X	X
		67	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		68	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		69	No Abnormalities Detected		X	X	X	X	X	X
		70	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		71	No Abnormalities Detected		X	X	X	X	X	X
		72	No Abnormalities Detected		X	X	X	X	X	X
		73	No Abnormalities Detected		X	X	X	X	X	X
		74	No Abnormalities Detected		X	X	X	X	X	X
		75	No Abnormalities Detected		X	X	X	X	X	X
		76	No Abnormalities Detected		X	X	X	X	X	X
		77	No Abnormalities Detected		X	X	X	X	X	X
		78	No Abnormalities Detected		X	X	X	X	X	X
		79	No Abnormalities Detected		X	X	X	X	X	X
		80	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					7	7	8	8	9	9
Group	Sex	Animal	Clinical Sign	Site		1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose
ff										
5	m	65	No Abnormalities Detected		X	X	X	X	X	X
		66	No Abnormalities Detected		X	X	X	X	X	X
		67	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		68	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		69	No Abnormalities Detected		X	X	X	X	X	X
		70	No Abnormalities Detected		X	X	X	X	X	.
			Salivation		.	.	.	.	.	1
		71	No Abnormalities Detected		X	X	X	X	X	X
		72	No Abnormalities Detected		X	X	X	X	X	X
		73	No Abnormalities Detected		X	X	X	X	X	X
		74	No Abnormalities Detected		X	X	X	X	X	X
		75	No Abnormalities Detected		X	X	X	X	X	X
		76	No Abnormalities Detected		X	X	X	X	X	X
		77	No Abnormalities Detected		X	X	X	X	X	X
		78	No Abnormalities Detected		X	X	X	X	X	X
		79	No Abnormalities Detected		X	X	X	X	X	X
		80	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					10	10	11	11	12	12
Group	Sex	Animal	Clinical Sign	Site		1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose
ff										
5	m	65	No Abnormalities Detected		X	X	X	X	X	X
		66	No Abnormalities Detected		X	X	X	X	X	X
		67	No Abnormalities Detected		X	X	X	X	X	.
			Salivation		.	.	.	.	.	1
		68	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		69	No Abnormalities Detected		X	X	X	X	X	X
		70	No Abnormalities Detected		.	X	X	X	X	X
			Salivation		1	.	.	.	.	.
		71	No Abnormalities Detected		X	X	X	X	X	X
		72	No Abnormalities Detected		X	X	X	X	X	X
		73	No Abnormalities Detected		X	X	X	X	X	X
		74	No Abnormalities Detected		X	X	X	X	X	X
		75	No Abnormalities Detected		X	X	X	X	X	X
		76	No Abnormalities Detected		X	X	X	X	X	X
		77	No Abnormalities Detected		X	X	X	X	X	X
		78	No Abnormalities Detected		X	X	X	X	X	X
		79	No Abnormalities Detected		X	X	X	X	X	X
		80	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					13	13	14	14	15	15
Group	Sex	Animal	Clinical Sign	Site		1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose
ff										
5	m	65	No Abnormalities Detected		X	X	X	X	X	X
		66	No Abnormalities Detected		X	X	X	X	X	X
		67	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		68	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		69	No Abnormalities Detected		X	X	X	X	X	X
		70	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		71	No Abnormalities Detected		X	X	X	X	X	X
		72	No Abnormalities Detected		X	X	X	X	X	X
		73	No Abnormalities Detected		X	X	X	X	X	X
		74	No Abnormalities Detected		X	X	X	X	X	X
		75	No Abnormalities Detected		X	X	X	X	X	X
		76	No Abnormalities Detected		X	X	X	X	X	X
		77	No Abnormalities Detected		X	X	X	X	X	X
		78	No Abnormalities Detected		X	X	X	X	X	X
		79	No Abnormalities Detected		X	X	X	X	X	X
		80	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP



Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					16	16	17	17	18	18
Group	Sex	Animal	Clinical Sign	Site		1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose
ff										
5	m	65	No Abnormalities Detected		X	X	X	X	X	X
		66	No Abnormalities Detected		X	X	X	X	X	X
		67	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		68	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		69	No Abnormalities Detected		X	X	X	X	X	X
		70	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		71	No Abnormalities Detected		X	X	X	X	X	X
		72	No Abnormalities Detected		X	X	X	X	X	X
		73	No Abnormalities Detected		X	X	X	X	X	X
		74	No Abnormalities Detected		X	X	X	X	X	X
		75	No Abnormalities Detected		X	X	X	X	X	X
		76	No Abnormalities Detected		X	X	X	X	X	X
		77	No Abnormalities Detected		X	X	X	X	X	X
		78	No Abnormalities Detected		X	X	X	X	X	X
		79	No Abnormalities Detected		X	X	X	X	X	X
		80	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					19	19	20	20	21	21
Group	Sex	Animal	Clinical Sign	Site		1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose
ff										
5	m	65	No Abnormalities Detected		X	X	X	X	X	X
		66	No Abnormalities Detected		X	X	X	X	X	X
		67	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		68	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		69	No Abnormalities Detected		X	X	X	X	X	X
		70	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		71	No Abnormalities Detected		X	X	X	X	X	X
		72	No Abnormalities Detected		X	X	X	X	X	X
		73	No Abnormalities Detected		X	X	X	X	X	X
		74	No Abnormalities Detected		X	X	X	X	X	X
		75	No Abnormalities Detected		X	X	X	X	X	X
		76	No Abnormalities Detected		X	X	X	X	X	X
		77	No Abnormalities Detected		X	X	X	X	X	X
		78	No Abnormalities Detected		X	X	X	X	X	X
		79	No Abnormalities Detected		X	X	X	X	X	X
		80	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					22	22	23	23	24	24
Group	Sex	Animal	Clinical Sign	Site		1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose
ff										
5	m	65	No Abnormalities Detected		X	X	X	X	X	X
		66	No Abnormalities Detected		X	X	X	X	X	X
		67	No Abnormalities Detected		X	X	X	X	X	.
			Salivation		.	.	.	.	.	1
		68	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		69	No Abnormalities Detected		X	X	X	X	X	X
		70	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		71	No Abnormalities Detected		X	X	X	X	X	X
		72	No Abnormalities Detected		X	X	X	X	X	X
		73	No Abnormalities Detected		X	X	X	X	X	X
		74	No Abnormalities Detected		X	X	X	X	X	X
		75	No Abnormalities Detected		X	X	X	X	X	X
		76	No Abnormalities Detected		X	X	X	X	X	X
		77	No Abnormalities Detected		X	X	X	X	X	X
		78	No Abnormalities Detected		X	X	X	X	X	X
		79	No Abnormalities Detected		X	X	X	X	X	X
		80	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					25	25	26	26	27	27
Group	Sex	Animal	Clinical Sign	Site		1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose
ff										
5	m	65	No Abnormalities Detected		X	X	X	X	X	X
		66	No Abnormalities Detected		X	X	X	X	X	X
		67	No Abnormalities Detected		X	.	X	X	X	X
			Salivation		.	1	.	.	.	.
		68	No Abnormalities Detected		X	.	X	X	X	X
			Salivation		.	1	.	.	.	.
		69	No Abnormalities Detected		X	X	X	X	X	X
		70	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		71	No Abnormalities Detected		X	X	X	X	X	X
		72	No Abnormalities Detected		X	X	X	X	X	X
		73	No Abnormalities Detected		X	X	X	X	X	X
		74	No Abnormalities Detected		X	X	X	X	X	X
		75	No Abnormalities Detected		X	X	X	X	X	X
		76	No Abnormalities Detected		X	X	X	X	X	X
		77	No Abnormalities Detected		X	X	X	X	X	X
		78	No Abnormalities Detected		X	X	X	X	X	X
		79	No Abnormalities Detected		X	X	X	X	X	X
		80	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					28	28	29	29	30	30
Group	Sex	Animal	Clinical Sign	Site		1-Hour Post-Dose		1-Hour Post-Dose		1-Hour Post-Dose
ff										
5	m	65	No Abnormalities Detected		X	X	X	X	X	X
		66	No Abnormalities Detected		X	X	X	X	X	X
		67	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		68	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		69	No Abnormalities Detected		X	X	X	X	X	X
		70	No Abnormalities Detected		X	X	X	X	X	X
			Salivation		.	.	.	.	.	.
		71	No Abnormalities Detected		X	X	X	X	X	X
		72	No Abnormalities Detected		X	X	X	X	X	X
		73	No Abnormalities Detected		X	X	X	X	X	X
		74	No Abnormalities Detected		X	X	X	X	X	X
		75	No Abnormalities Detected		X	X	X	X	X	X
		76	No Abnormalities Detected		X	X	X	X	X	X
		77	No Abnormalities Detected		X	X	X	X	X	X
		78	No Abnormalities Detected		X	X	X	X	X	X
		79	No Abnormalities Detected		X	X	X	X	X	X
		80	No Abnormalities Detected		X	X	X	X	X	X

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

ff

Day numbers relative to Start Date

					31	31	32
Group	Sex	Animal	Clinical Sign	Site	1-Hour Post-Dose		
ff							
5	m	65	No Abnormalities Detected		X	.	.
		66	No Abnormalities Detected		X	.	.
		67	No Abnormalities Detected		X	.	.
			Salivation		.	.	.
		68	No Abnormalities Detected		X	.	.
			Salivation		.	.	.
		69	No Abnormalities Detected		X	.	.
		70	No Abnormalities Detected		X	.	.
			Salivation		.	.	.
		71	No Abnormalities Detected		X	.	.
		72	No Abnormalities Detected		X	.	.
		73	No Abnormalities Detected		X	X	X
		74	No Abnormalities Detected		X	X	X
		75	No Abnormalities Detected		X	X	X
		76	No Abnormalities Detected		X	X	X
		77	No Abnormalities Detected		X	X	X
78	No Abnormalities Detected		X	X	X		
79	No Abnormalities Detected		X	X	X		
80	No Abnormalities Detected		X	X	X		

ff

Severity Codes: X = Present; 1 = Mild

Group 1 - 0 mg/kg/day 2-EHP      Group 2 - 250 mg/kg/day 2-EHP      Group 3 - 500 mg/kg/day 2-EHP  
Group 4 - 750 mg/kg/day 2-EHP      Group 5 - 1000 mg/kg/day 2-EHP

Clinical Observations - Clinical Signs with Site by Animal

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

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# APPENDIX II: Individual Animal and Group Mean Body Weight Data



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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 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	60.0	65.7	70.9	75.4	83.4	90.4	97.5
02	58.1	61.8	66.7	71.3	79.3	84.7	91.7
03	67.4	72.1	77.2	81.4	88.0	93.7	100.9
04	59.3	63.4	69.4	73.5	80.9	86.1	92.4
05	65.2	70.2	76.8	82.0	88.4	93.8	102.0
06	59.6	64.1	71.0	76.2	83.7	89.1	97.5
07	66.1	70.7	77.5	82.8	88.4	96.4	104.1
08	66.2	69.9	76.2	79.4	87.2	93.4	101.8
09	61.4	66.5	72.1	77.0	84.8	90.6	96.9
10	63.4	65.8	70.6	75.9	82.5	88.8	94.4
11	58.3	64.9	72.3	79.8	87.2	98.1	106.5
12	61.6	66.0	72.5	79.4	83.6	89.6	96.7

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 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)
	8	9	10	11	12	13	14
01	106.5	115.8	120.6	130.2	137.8	148.1	156.1
02	98.0	108.6	116.2	121.9	132.2	139.4	149.2
03	109.0	118.2	125.9	132.4	138.7	148.4	158.7
04	100.6	108.3	113.4	119.6	128.3	140.2	147.5
05	109.1	118.8	124.0	134.7	140.4	152.0	160.5
06	106.4	115.9	120.6	130.8	141.0	151.3	162.5
07	112.4	121.1	129.6	137.1	144.9	155.2	162.9
08	107.8	118.3	125.2	136.3	141.9	154.9	161.8
09	103.7	113.0	118.9	126.3	132.6	143.6	151.7
10	100.7	110.2	115.1	121.8	128.8	137.2	145.2
11	116.3	129.2	137.6	149.7	160.2	171.8	184.2
12	106.5	115.7	119.8	129.0	135.4	146.5	154.9

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 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)
	15	16	17	18	19	20	21
01	165.3	173.7	185.7	191.7	201.0	212.1	217.4
02	156.6	166.5	175.4	184.7	192.4	201.2	208.9
03	168.0	177.6	186.3	192.8	202.8	213.0	219.5
04	155.3	163.9	174.7	180.5	192.6	199.3	203.8
05	167.8	176.5	184.9	194.5	201.4	214.9	219.5
06	168.9	179.3	188.3	195.5	202.3	215.6	224.2
07	171.9	184.8	192.8	201.1	210.1	222.1	228.7
08	174.3	182.3	191.2	201.8	210.7	223.4	230.8
09	160.4	167.3	174.6	183.0	187.7	194.3	200.9
10	152.5	161.0	167.2	172.4	182.3	192.6	197.0
11	196.5	210.1	223.2	235.8	252.2	263.5	271.7
12	163.8	173.9	182.9	190.2	198.5	207.6	213.4

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 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)
	22	23	24	25	26	27	28
01	231.0	238.0	247.5	256.7	263.1	278.0	287.3
02	218.8	229.4	235.8	243.5	251.2	263.0	269.6
03	227.6	239.1	245.4	255.1	260.7	269.6	281.9
04	215.8	226.5	231.8	239.5	248.6	258.5	270.5
05	232.8	239.5	247.9	260.4	270.1	279.7	286.5
06	232.6	240.5	245.5	260.7	264.8	273.3	284.3
07	239.3	248.0	257.5	267.0	275.9	285.7	294.2
08	241.0	252.9	259.5	271.5	277.6	288.1	297.7
09	208.4	217.9	223.0	238.1	244.4	250.2	259.8
10	206.2	214.4	221.1	229.5	236.1	241.5	250.1
11	284.3	297.9	304.8	317.3	327.5	336.4	349.0
12	222.0	227.7	235.5	242.9	252.7	259.0	267.0

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

0 mg/kg/day 2-EHP					Bodyweight Gain (g)
	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	
	29	30	31	32	1 → 31
01	300.0	309.0	314.6	-	254.6
02	281.5	290.3	295.8	-	237.7
03	289.5	297.6	306.2	-	238.8
04	280.2	288.7	300.3	-	241.0
05	297.4	306.9	317.6	-	252.4
06	293.0	300.5	307.2	-	247.6
07	308.4	315.9	324.3	-	258.2
08	314.9	318.9	326.9	-	260.7
09	270.8	278.3	285.8	295.1	224.4
10	257.5	264.2	271.9	279.1	208.5
11	362.1	373.2	387.4	393.2	329.1
12	278.0	285.6	296.6	301.1	235.0

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 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
13	57.8	63.8	69.2	74.4	81.0	88.0	96.9
14	63.6	68.5	74.1	80.4	87.7	93.2	100.3
15	60.0	65.4	68.9	74.1	79.9	85.3	92.1
16	66.9	70.9	77.3	83.5	91.5	98.0	106.2
Mean	62.18	66.86	72.67	77.91	84.84	91.20	98.62
SD	3.37	3.11	3.47	3.67	3.60	4.24	4.75
N	16	16	16	16	16	16	16

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 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)
	8	9	10	11	12	13	14
13	105.6	116.8	124.6	136.4	146.4	158.5	170.3
14	106.9	115.8	120.2	129.1	137.2	143.8	151.5
15	97.6	105.8	110.2	118.6	126.4	134.7	141.9
16	115.9	124.8	130.9	140.4	150.1	160.2	168.6
Mean	106.44	116.02	122.05	130.89	138.89	149.11	157.97
SD	5.55	6.09	6.99	8.28	8.79	9.66	10.71
N	16	16	16	16	16	16	16

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 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)
	15	16	17	18	19	20	21
13	181.5	194.6	204.8	210.9	222.5	234.8	242.5
14	160.5	169.2	176.3	182.0	191.0	198.1	203.5
15	149.5	157.8	163.4	171.1	178.1	185.8	191.9
16	178.4	189.2	198.3	205.6	213.4	231.4	236.3
Mean	166.95	176.73	185.63	193.35	202.44	213.11	219.38
SD	11.98	13.49	14.84	15.96	17.68	19.41	20.07
N	16	16	16	16	16	16	16



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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 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)
	22	23	24	25	26	27	28
13	258.4	272.0	281.5	295.4	312.5	322.7	336.5
14	210.7	220.2	224.7	230.6	238.3	244.8	249.4
15	199.6	207.7	212.9	217.4	224.9	232.6	241.7
16	244.8	260.9	263.9	277.1	283.3	293.4	306.9
Mean	229.58	239.54	246.14	256.42	264.48	273.53	283.28
SD	21.48	23.20	23.77	25.71	27.09	28.07	29.68
N	16	16	16	16	16	16	16

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

0 mg/kg/day 2-EHP					Bodyweight Gain (g)
	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	
	29	30	31	32	1 → 31
13	347.1	361.1	372.5	386.9	314.7
14	261.9	265.5	275.1	279.9	211.5
15	251.5	261.0	266.8	273.9	206.8
16	316.8	330.4	339.2	346.7	272.3
Mean	294.41	302.94	311.76	319.49	249.58
SD	30.55	32.16	33.55	49.18	34.12
N	16	16	16	8	16

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

250 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
17	59.0	64.8	71.2	76.4	83.8	92.3	98.2
18	67.3	73.0	78.9	85.0	94.1	100.7	107.8
19	60.4	67.1	73.9	77.8	84.9	92.7	99.2
20	61.6	69.5	73.8	80.4	87.7	94.3	100.5
21	64.3	70.7	77.4	83.5	92.3	100.6	111.8
22	58.1	62.1	68.0	71.0	80.0	86.0	91.7
23	64.0	69.1	72.7	76.4	80.7	87.0	91.7
24	62.4	67.3	72.1	76.3	83.0	90.0	95.9
25	61.1	64.4	69.7	73.2	79.7	85.0	91.1
26	61.6	66.8	73.3	77.8	84.6	92.0	99.8
27	62.5	67.1	72.4	75.9	82.0	88.3	94.4
28	61.0	65.4	71.2	76.7	84.4	90.2	96.1

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

250 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)
	8	9	10	11	12	13	14
17	109.8	118.5	125.6	133.2	144.0	155.8	163.3
18	118.1	127.4	137.1	147.2	154.3	162.0	173.5
19	110.1	117.4	125.2	135.4	142.6	155.2	162.1
20	109.1	117.6	124.5	131.2	139.0	149.6	156.4
21	122.3	133.3	141.0	150.9	161.3	176.3	188.6
22	100.5	109.4	114.8	121.6	129.8	138.4	145.6
23	96.8	103.8	109.5	115.1	123.3	130.9	138.0
24	103.7	112.4	118.2	126.8	135.1	146.4	154.2
25	97.7	105.2	111.7	120.6	126.9	135.2	143.1
26	109.5	119.1	112.3	136.0	147.6	156.1	165.7
27	101.4	110.1	115.0	124.6	132.3	141.3	147.1
28	103.3	111.2	120.3	126.5	136.3	146.7	154.3

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

250 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)
	15	16	17	18	19	20	21
17	175.4	183.3	198.9	204.0	213.7	225.6	235.2
18	183.3	190.0	201.5	210.8	218.6	230.7	236.7
19	176.3	184.9	197.4	203.9	217.3	228.9	240.4
20	166.8	174.3	180.4	189.0	196.0	210.0	211.1
21	201.0	211.7	225.9	231.8	245.8	259.9	270.0
22	153.6	162.4	170.9	176.4	181.1	192.5	197.3
23	143.1	154.0	159.5	166.9	174.3	185.2	187.6
24	162.9	171.6	179.4	187.5	196.4	205.2	212.0
25	152.7	160.2	170.7	178.7	184.6	195.6	199.0
26	176.3	186.2	194.1	205.6	218.4	228.2	235.5
27	156.6	165.9	174.1	181.1	192.0	200.0	205.4
28	164.8	176.6	184.4	193.5	202.7	211.1	220.7

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

250 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)
	22	23	24	25	26	27	28
17	246.5	257.7	267.0	283.6	284.9	295.8	306.4
18	245.5	253.6	263.1	275.0	282.1	290.2	301.6
19	250.7	261.4	273.9	282.5	293.7	305.3	315.1
20	222.8	231.5	239.0	246.8	254.7	263.2	272.3
21	283.3	298.9	309.4	323.2	330.3	347.1	355.6
22	206.2	213.8	218.3	228.1	230.5	238.7	245.0
23	196.9	205.7	212.4	219.4	225.3	231.7	239.7
24	219.7	230.7	235.9	245.0	250.2	259.8	268.5
25	205.8	214.7	220.3	229.3	237.6	242.9	253.4
26	248.8	257.2	263.7	278.2	287.4	297.2	305.4
27	215.9	225.1	230.4	237.9	246.5	252.3	257.6
28	230.6	241.9	247.8	258.1	264.9	275.4	283.9

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

250 mg/kg/day 2-EHP					Bodyweight Gain (g)
	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	
	29	30	31	32	1 → 31
17	320.5	328.4	335.9	-	276.9
18	309.0	321.8	327.4	-	260.1
19	329.9	343.1	350.0	-	289.6
20	286.2	292.9	299.8	-	238.2
21	370.1	378.4	390.7	-	326.4
22	255.6	262.4	268.5	-	210.4
23	247.2	253.4	258.0	-	194.0
24	277.1	288.9	294.8	-	232.4
25	262.0	267.0	276.6	282.1	215.5
26	318.8	328.9	336.6	347.0	275.0
27	268.0	279.3	281.4	286.9	218.9
28	291.0	303.4	310.8	315.0	249.8

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

250 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
29	66.3	71.4	76.8	82.3	90.3	95.2	101.7
30	56.8	61.8	66.2	70.8	77.6	81.9	88.2
31	67.4	74.4	79.4	86.2	92.6	101.7	107.4
32	61.8	67.5	71.1	77.0	84.8	93.3	99.9
Mean	62.23	67.65	73.01	77.92	85.16	91.95	98.46
SD	3.06	3.59	3.69	4.54	4.97	5.74	6.55
N	16	16	16	16	16	16	16
%Diff	0.1	1.2	0.5	0.0	0.4	0.8	-0.2



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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

250 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)
	8	9	10	11	12	13	14
29	109.1	118.1	125.5	134.7	142.1	151.9	160.4
30	94.6	103.9	110.2	118.6	126.9	134.0	144.1
31	117.5	123.9	133.5	141.9	148.2	158.2	169.8
32	108.4	115.8	123.4	132.0	139.4	148.5	156.5
Mean	106.99	115.44	121.74	131.02	139.32	149.16	157.67
SD	7.94	8.32	9.58	10.08	10.42	11.75	13.03
N	16	16	16	16	16	16	16
%Diff	0.5	-0.5	-0.3	0.1	0.3	0.0	-0.2

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

250 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)
	15	16	17	18	19	20	21
29	169.7	176.5	186.0	196.2	205.4	215.0	220.3
30	152.5	160.1	170.6	176.6	186.5	196.9	203.5
31	177.6	185.7	199.1	206.3	215.0	227.7	236.8
32	167.4	174.5	182.4	191.7	202.2	212.7	219.6
Mean	167.50	176.12	185.96	193.75	203.13	214.08	220.69
SD	14.32	14.37	16.33	16.37	18.09	18.89	20.94
N	16	16	16	16	16	16	16
%Diff	0.3	-0.3	0.2	0.2	0.3	0.5	0.6

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

250 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)
	22	23	24	25	26	27	28
29	232.2	239.4	247.9	255.7	265.3	274.7	287.7
30	213.6	224.0	231.0	238.6	250.0	258.4	268.4
31	246.5	258.4	263.7	280.2	288.5	299.5	313.9
32	232.1	240.4	243.9	253.1	261.7	269.1	278.8
Mean	231.07	240.90	247.98	258.42	265.85	275.08	284.58
SD	22.02	23.37	24.88	27.09	27.37	29.77	30.58
N	16	16	16	16	16	16	16
%Diff	0.6	0.6	0.7	0.8	0.5	0.6	0.5

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

250 mg/kg/day 2-EHP					Bodyweight Gain (g)
	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	
	29	30	31	32	1 → 31
29	291.6	302.3	308.3	317.9	242.0
30	280.1	287.0	294.4	297.8	237.6
31	323.3	336.3	344.7	355.9	277.3
32	288.9	300.7	306.1	311.4	244.3
Mean	294.96	304.64	311.50	314.25	249.28
SD	32.05	33.22	34.56	26.41	33.82
N	16	16	16	8	16
%Diff	0.2	0.6	-0.1	-1.6	.

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

500 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
33	66.1	73.1	80.0	88.5	95.4	104.0	107.2
34	57.7	62.7	67.6	72.1	79.1	87.0	93.8
35	62.5	66.7	72.2	78.3	84.2	90.6	97.9
36	63.0	68.7	75.9	80.5	88.2	95.5	104.4
37	63.1	69.0	73.4	78.0	82.8	88.5	94.4
38	57.4	61.2	65.0	70.4	75.1	81.8	88.0
39	63.3	70.8	75.7	82.0	87.0	94.2	101.7
40	65.8	71.7	77.4	84.1	92.5	100.2	108.4
41	57.8	62.6	66.8	70.7	74.5	78.0	84.2
42	62.2	68.0	75.0	80.4	87.2	93.8	103.3
43	60.4	64.7	69.8	74.7	80.0	85.5	93.3
44	62.9	68.6	74.9	82.0	90.8	95.8	104.0

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

500 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)
	8	9	10	11	12	13	14
33	115.6	127.7	135.2	143.9	154.6	165.4	174.2
34	101.5	109.8	119.6	126.5	132.5	139.8	148.0
35	104.6	114.2	123.8	134.5	141.3	149.9	158.0
36	113.2	120.5	129.4	136.9	146.0	154.1	164.2
37	101.0	108.0	115.2	121.1	128.1	133.5	142.5
38	96.2	104.1	108.6	115.7	123.6	127.3	132.4
39	110.0	118.1	121.7	132.9	138.3	148.7	155.6
40	118.2	128.2	132.6	142.4	152.3	163.5	172.2
41	89.9	95.3	99.6	107.7	111.3	119.5	126.4
42	111.5	114.8	120.4	130.7	138.0	149.0	155.1
43	100.2	107.6	114.5	114.6	123.6	131.4	140.7
44	112.2	123.2	129.0	137.5	147.9	156.3	166.6

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

500 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)
	15	16	17	18	19	20	21
33	186.6	196.2	210.4	217.6	230.2	239.3	250.3
34	157.1	167.8	175.8	184.1	192.6	202.1	210.0
35	168.7	179.6	190.5	199.5	212.1	222.6	229.7
36	171.3	180.0	190.7	198.5	210.1	217.5	224.1
37	150.1	158.0	166.8	172.0	180.6	186.8	193.6
38	139.5	149.7	155.6	164.2	173.9	181.1	188.4
39	165.8	172.0	181.5	186.0	195.0	202.8	208.9
40	182.6	194.2	202.1	212.0	222.7	229.4	238.3
41	131.8	137.4	143.7	150.1	157.1	164.9	168.8
42	167.5	174.2	184.9	194.3	204.7	215.5	223.5
43	148.8	156.9	164.0	173.2	176.7	188.3	193.5
44	176.7	183.4	192.7	201.1	210.0	220.9	227.8

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

500 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)
	22	23	24	25	26	27	28
33	260.6	270.5	279.9	289.5	296.8	312.5	322.0
34	220.2	225.4	231.1	242.0	247.3	257.9	264.5
35	242.1	252.1	256.8	262.9	261.6	283.0	291.0
36	232.8	243.2	244.3	252.6	271.4	271.0	276.9
37	204.3	208.9	214.4	221.5	228.4	237.5	245.8
38	197.2	203.3	210.1	218.5	227.2	234.2	244.1
39	217.2	224.6	229.5	235.5	245.5	248.3	256.2
40	245.9	258.7	264.0	273.6	283.7	292.2	302.0
41	179.1	186.3	190.1	196.0	202.5	208.4	216.0
42	230.3	241.6	249.3	255.2	268.6	277.3	286.4
43	204.7	213.5	222.9	231.6	240.3	248.5	258.9
44	237.2	246.1	254.5	264.1	267.9	274.5	285.8



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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

500 mg/kg/day 2-EHP					Bodyweight Gain (g)
	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	
	29	30	31	32	1 → 31
33	323.0	332.5	340.2	-	274.1
34	273.4	281.1	287.4	-	229.7
35	302.9	308.4	312.1	-	249.6
36	287.7	294.9	300.6	-	237.6
37	250.8	262.2	257.2	-	194.1
38	248.0	255.4	262.3	-	204.9
39	262.4	266.9	274.7	-	211.4
40	310.6	314.5	320.1	-	254.3
41	223.7	227.8	233.0	238.5	175.2
42	297.7	306.9	311.2	324.6	249.0
43	269.3	282.0	289.6	296.2	229.2
44	298.4	297.8	303.0	305.4	240.1

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

500 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
45	61.4	65.2	69.5	73.6	79.0	83.9	89.9
46	63.8	67.9	74.6	78.0	87.4	95.0	101.7
47	62.2	67.7	73.2	77.0	83.3	91.6	97.6
48	62.8	66.3	71.9	76.0	81.7	88.3	92.6
Mean	62.03	67.18	72.68	77.89	84.26	90.86	97.65
SD	2.59	3.32	4.07	4.98	5.99	6.81	7.12
N	16	16	16	16	16	16	16
%Diff	-0.3	0.5	0.0	0.0	-0.7	-0.4	-1.0

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

500 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)
	8	9	10	11	12	13	14
45	97.4	104.2	109.1	118.2	122.2	131.3	135.9
46	109.7	119.4	126.3	137.1	144.4	155.1	163.3
47	106.0	114.6	113.5	121.4	128.0	138.7	146.7
48	100.4	109.1	114.0	117.3	124.1	134.8	143.3
Mean	105.48	113.68	119.53	127.40	134.76	143.64	151.57
SD	7.87	9.03	9.69	11.04	12.45	13.50	14.34
N	16	16	16	16	16	16	16
%Diff	-0.9	-2.0	-2.1	-2.7	-3.0	-3.7	-4.1

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

500 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)
	15	16	17	18	19	20	21
45	145.0	150.4	158.4	165.6	174.5	185.3	188.7
46	171.9	182.0	193.6	204.5	212.5	226.0	233.1
47	156.1	163.5	172.6	183.6	191.3	201.0	209.6
48	150.4	153.3	165.2	168.6	176.2	185.7	188.8
Mean	160.62	168.66	178.03	185.93	195.01	204.33	211.07
SD	15.72	16.89	18.35	19.16	20.61	21.00	22.56
N	16	16	16	16	16	16	16
%Diff	-3.8	-4.6	-4.1	-3.8	-3.7	-4.1	-3.8

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

500 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)
	22	23	24	25	26	27	28
45	198.5	204.3	212.4	223.2	226.1	233.4	237.9
46	246.5	257.4	263.0	276.7	287.0	296.2	307.7
47	222.6	231.1	238.6	249.3	255.8	264.0	273.2
48	200.0	206.4	212.5	220.6	227.2	233.6	240.5
Mean	221.20	229.59	235.84	244.55	252.33	260.78	269.31
SD	22.51	24.33	24.57	25.39	26.28	28.03	28.96
N	16	16	16	16	16	16	16
%Diff	-3.7	-4.2	-4.2	-4.6	-4.6	-4.7	-4.9

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

500 mg/kg/day 2-EHP					Bodyweight Gain (g)
	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	
	29	30	31	32	1 → 31
45	246.4	250.9	256.3	263.6	194.9
46	316.7	330.5	337.5	344.4	273.7
47	274.8	281.9	286.0	298.1	223.8
48	246.1	240.2	229.0	236.3	166.2
Mean	276.99	283.37	287.51	288.39	225.49
SD	29.49	31.24	33.67	39.11	32.28
N	16	16	16	8	16
%Diff	-5.9	-6.5	-7.8	-9.7	.

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

750 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
49	64.5	69.7	75.7	81.4	87.4	94.6	102.4
50	61.0	64.5	71.9	77.4	84.7	90.8	98.9
51	62.5	67.1	74.2	79.0	85.9	94.9	100.1
52	60.4	65.1	71.2	74.8	79.0	86.2	93.8
53	62.8	67.8	74.9	81.2	88.4	94.8	100.1
54	64.5	71.8	77.4	83.5	91.0	98.6	105.6
55	63.7	66.7	72.8	79.3	81.1	86.5	93.9
56	62.2	65.3	70.2	75.5	81.4	88.1	94.3
57	60.5	65.9	72.0	77.0	84.6	90.3	97.9
58	61.6	64.8	71.4	75.5	81.1	86.9	92.5
59	61.5	65.0	69.4	73.4	79.1	83.7	88.3
60	64.6	69.0	74.8	79.6	86.7	93.0	98.0

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

750 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)
	8	9	10	11	12	13	14
49	107.6	114.8	119.6	126.3	136.3	143.8	150.6
50	106.1	113.2	119.4	126.3	134.5	144.3	153.6
51	101.9	110.0	115.9	124.6	134.9	143.2	151.4
52	103.3	112.6	118.6	127.6	136.9	146.5	157.7
53	106.8	116.5	123.3	131.6	143.6	151.7	159.9
54	116.3	124.3	132.7	134.0	143.7	154.3	163.4
55	102.3	108.7	115.5	120.6	128.8	136.9	146.7
56	101.3	112.2	117.7	125.0	133.4	139.7	149.4
57	107.5	116.4	124.6	135.3	144.2	151.2	161.9
58	102.2	109.3	116.9	122.7	131.3	137.1	143.8
59	95.9	104.5	107.3	115.4	123.6	129.1	137.7
60	105.6	114.9	121.0	127.9	136.2	145.7	154.6



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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

750 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)
	15	16	17	18	19	20	21
49	163.7	172.9	181.5	190.4	199.7	208.9	214.9
50	161.8	174.0	182.2	190.6	200.0	208.4	217.6
51	163.1	169.0	179.8	188.3	194.3	205.5	210.8
52	168.3	174.8	184.7	194.5	202.6	215.1	222.8
53	169.0	176.0	185.9	192.4	203.8	209.6	214.9
54	170.6	180.2	190.7	199.7	208.6	219.6	225.5
55	154.5	164.7	172.8	184.0	189.9	200.9	209.8
56	155.6	163.3	170.6	180.6	181.1	192.3	200.7
57	168.7	176.8	188.6	198.5	207.3	219.9	229.3
58	151.5	160.9	167.3	176.8	180.5	190.7	192.8
59	143.4	151.0	158.1	167.1	174.1	181.6	188.7
60	164.8	169.7	178.5	186.8	194.4	198.1	203.9

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

750 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)
	22	23	24	25	26	27	28
49	223.6	234.6	242.2	249.0	256.5	262.2	269.9
50	226.9	237.4	247.1	253.7	263.3	270.8	281.4
51	221.0	229.0	234.9	243.1	250.3	258.4	266.7
52	231.9	240.6	247.4	257.1	263.5	269.4	279.9
53	228.7	233.2	240.7	248.0	253.6	264.0	270.4
54	235.0	245.1	252.0	259.3	266.8	273.7	287.5
55	218.2	226.2	232.8	243.9	251.7	260.8	266.5
56	209.5	217.7	223.5	234.1	242.3	249.6	256.5
57	234.0	241.5	249.0	260.4	267.9	281.7	289.5
58	203.9	210.6	212.8	228.0	228.8	236.8	245.3
59	198.0	206.1	211.3	221.9	229.0	236.7	240.4
60	214.9	223.9	228.6	241.3	246.3	254.4	261.4

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

750 mg/kg/day 2-EHP					Bodyweight Gain (g)
	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	
	29	30	31	32	1 → 31
49	276.5	285.4	297.4	-	232.9
50	291.9	293.8	304.4	-	243.4
51	270.5	276.8	280.5	-	218.0
52	282.4	289.1	298.2	-	237.8
53	278.6	288.8	294.9	-	232.1
54	287.8	287.9	298.3	-	233.8
55	281.7	285.9	286.7	-	223.0
56	265.8	271.9	278.6	-	216.4
57	303.7	308.1	304.5	312.6	244.0
58	245.6	252.0	256.6	262.8	195.0
59	246.9	253.1	258.0	267.7	196.5
60	268.7	273.5	284.7	289.4	220.1

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

750 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
61	58.9	61.9	66.8	71.9	77.6	83.9	90.6
62	62.8	66.0	70.8	76.1	82.7	90.3	95.4
63	64.8	68.6	76.1	81.5	88.9	97.7	104.9
64	62.7	66.8	71.3	77.2	83.1	89.4	95.9
Mean	62.44	66.63	72.56	77.77	83.92	90.61	97.04
SD	1.73	2.38	2.79	3.22	3.93	4.64	4.89
N	16	16	16	16	16	16	16
%Diff	0.4	-0.3	-0.2	-0.2	-1.1	-0.7	-1.6

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

750 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)
	8	9	10	11	12	13	14
61	96.2	104.9	110.2	119.1	125.7	133.4	142.0
62	101.5	110.5	116.3	122.6	130.6	138.6	143.8
63	115.1	122.4	129.5	138.9	149.5	161.4	172.5
64	100.8	111.1	116.3	124.3	131.9	139.3	148.3
Mean	104.40	112.89	119.05	126.39	135.32	143.51	152.33
SD	5.62	5.39	6.37	6.16	7.07	8.28	9.09
N	16	16	16	16	16	16	16
%Diff	-1.9	-2.7	-2.5	-3.4	-2.6	-3.8	-3.6

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

750 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)
	15	16	17	18	19	20	21
61	153.7	161.6	170.5	177.8	188.2	196.1	186.1
62	152.6	160.7	172.0	178.3	183.8	196.1	197.1
63	182.3	193.3	202.3	212.9	224.0	234.7	248.5
64	154.8	164.0	172.1	176.5	182.5	191.2	194.5
Mean	161.15	169.56	178.60	187.20	194.68	204.29	209.87
SD	9.60	9.95	10.70	11.24	13.05	13.63	16.77
N	16	16	16	16	16	16	16
%Diff	-3.5	-4.1	-3.8	-3.2	-3.8	-4.1	-4.3

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

750 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)
	22	23	24	25	26	27	28
61	196.9	207.5	212.0	222.3	227.2	235.1	245.1
62	208.1	221.5	228.3	233.8	240.9	249.1	258.1
63	256.1	266.1	274.9	289.0	294.3	306.1	317.7
64	202.2	207.4	212.9	220.6	225.4	230.6	235.1
Mean	219.31	228.03	234.40	244.09	250.49	258.71	266.96
SD	16.06	16.41	17.80	17.90	18.53	19.68	21.27
N	16	16	16	16	16	16	16
%Diff	-4.5	-4.8	-4.8	-4.8	-5.3	-5.4	-5.8

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

750 mg/kg/day 2-EHP					Bodyweight Gain (g)
	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	
	29	30	31	32	1 → 31
61	252.3	260.3	265.9	274.4	207.0
62	267.7	277.3	285.1	289.2	222.3
63	329.5	339.5	352.6	361.7	287.8
64	240.5	246.5	250.9	259.5	188.2
Mean	274.38	280.62	287.33	289.66	224.89
SD	22.96	23.01	24.45	33.94	23.87
N	16	16	16	8	16
%Diff	-6.8	-7.4	-7.8	-9.3	.



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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

1000 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
65	60.6	65.7	71.0	77.0	84.0	91.5	97.1
66	64.0	67.0	71.7	78.5	83.7	92.0	98.7
67	65.1	70.7	78.0	85.0	93.5	101.0	108.8
68	63.2	67.4	73.5	76.4	82.3	88.8	93.8
69	60.1	64.0	69.5	75.2	80.1	85.6	92.1
70	56.5	59.2	64.0	68.6	73.4	79.5	84.3
71	65.4	68.6	72.6	75.6	81.8	86.7	95.0
72	65.7	70.2	75.9	82.4	89.2	97.8	106.0
73	67.4	72.0	78.6	83.2	89.7	98.1	104.6
74	57.6	60.3	65.6	69.9	74.2	80.2	83.8
75	63.1	68.3	76.4	82.2	84.8	91.5	97.7
76	58.2	60.0	66.9	72.7	78.8	87.7	89.6

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

1000 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)
	8	9	10	11	12	13	14
65	105.4	113.8	116.7	127.0	133.9	142.6	151.8
66	105.8	113.2	116.9	126.7	132.3	144.5	152.1
67	118.5	125.6	133.0	140.6	153.8	159.8	166.7
68	100.3	109.1	114.9	120.0	127.0	137.5	144.7
69	92.6	100.4	106.7	112.9	121.4	129.2	136.4
70	90.3	96.7	101.7	109.3	114.3	122.3	127.3
71	103.2	113.3	120.2	126.1	137.8	146.9	157.4
72	115.6	124.2	128.9	138.1	149.3	158.8	171.0
73	110.0	118.2	124.4	131.0	136.6	145.5	153.1
74	91.1	98.4	103.7	108.8	116.3	124.8	131.0
75	107.3	116.6	123.7	132.0	139.2	145.8	155.7
76	98.8	106.6	113.4	121.0	130.3	136.0	144.5

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

1000 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)
	15	16	17	18	19	20	21
65	159.4	168.1	178.8	185.4	197.5	207.1	214.3
66	157.3	168.6	173.4	184.9	189.8	199.7	206.5
67	180.1	187.5	195.7	204.4	212.7	219.2	228.4
68	153.1	160.4	168.1	174.2	182.4	189.1	198.2
69	144.2	152.8	160.5	167.3	175.5	184.1	190.5
70	135.8	143.0	146.7	155.5	162.5	170.8	176.1
71	172.3	179.7	191.5	199.4	206.9	221.2	228.5
72	180.1	190.3	189.7	203.8	212.3	223.1	228.9
73	163.0	168.5	176.0	184.7	193.5	203.4	208.1
74	142.2	147.4	155.5	163.8	168.8	177.0	180.3
75	165.6	174.2	181.8	190.7	199.2	209.5	216.5
76	154.8	158.8	168.5	175.2	182.3	187.3	196.7

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

1000 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)
	22	23	24	25	26	27	28
65	226.1	235.3	240.5	249.3	255.6	262.6	274.2
66	219.1	222.7	232.1	239.9	243.4	248.2	258.7
67	238.1	246.1	252.4	259.1	268.9	278.3	288.1
68	207.0	215.9	219.6	230.0	227.4	230.1	238.5
69	201.2	207.3	214.7	221.3	228.3	236.1	243.4
70	184.0	190.0	197.1	203.0	207.2	213.1	219.6
71	242.4	250.7	260.3	275.4	280.6	290.7	302.9
72	240.5	252.0	256.0	267.7	269.8	279.3	286.1
73	217.7	223.7	232.0	240.2	248.5	262.0	269.0
74	190.6	196.4	200.8	209.8	213.7	223.0	227.4
75	226.0	231.6	239.3	248.5	256.4	266.5	275.8
76	205.5	212.5	217.7	227.0	233.6	239.5	248.0

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

1000 mg/kg/day 2-EHP					Bodyweight Gain (g)
	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	
	29	30	31	32	1 → 31
65	283.5	288.4	296.3	-	235.7
66	264.5	272.7	278.9	-	214.9
67	288.5	294.6	301.6	-	236.5
68	243.0	252.8	260.8	-	197.6
69	242.4	253.1	257.3	-	197.2
70	224.9	231.0	233.8	-	177.3
71	311.3	324.2	330.2	-	264.8
72	295.9	305.2	310.8	-	245.1
73	277.8	283.6	292.2	298.0	224.8
74	234.7	239.9	245.2	250.0	187.6
75	283.6	296.2	292.5	298.9	229.4
76	237.0	244.2	247.6	253.5	189.4

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

1000 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
77	63.6	67.5	73.3	78.2	83.7	89.3	91.7
78	56.7	60.5	67.3	72.1	77.1	82.8	88.5
79	60.6	67.3	70.4	73.9	81.2	87.8	94.2
80	63.1	64.7	73.4	77.9	85.7	92.6	99.8
Mean	61.93	65.84	71.76	76.80	82.70	89.56	95.36
SD	3.41	4.04	4.34	4.77	5.42	6.11	7.22
N	16	16	16	16	16	16	16
%Diff	-0.4	-1.5	-1.3	-1.4	-2.5	-1.8	-3.3

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

1000 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)
	8	9	10	11	12	13	14
77	98.3	104.0	111.2	115.2	123.6	130.1	135.4
78	95.8	104.2	111.5	119.5	127.5	133.3	137.6
79	100.6	109.2	116.7	123.1	131.7	142.3	145.6
80	107.0	114.9	115.0	120.8	131.9	139.9	146.1
Mean	102.54	110.53	116.16	123.26	131.68	139.96	147.28
SD	8.20	8.55	8.56	9.32	10.56	10.69	12.18
N	16	16	16	16	16	16	16
%Diff	-3.7	-4.7	-4.8	-5.8	-5.2	-6.1	-6.8

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

1000 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)
	15	16	17	18	19	20	21
77	141.2	147.1	155.5	161.9	169.7	178.2	182.8
78	146.6	152.6	159.8	168.6	170.2	176.9	187.3
79	156.2	163.3	169.2	177.2	184.0	193.5	200.2
80	155.8	160.1	168.8	180.9	186.1	197.2	201.4
Mean	156.73	163.90	171.22	179.87	187.09	196.08	202.79
SD	13.24	14.06	13.94	14.78	15.71	16.80	17.20
N	16	16	16	16	16	16	16
%Diff	-6.1	-7.3	-7.8	-7.0	-7.6	-8.0	-7.6



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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

1000 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)
	22	23	24	25	26	27	28
77	194.2	205.5	209.9	216.4	224.0	232.6	245.0
78	197.6	199.7	203.6	211.9	220.1	228.7	236.5
79	209.7	218.2	223.4	230.5	236.3	244.6	251.6
80	213.5	221.3	227.8	235.3	244.2	253.2	261.1
Mean	213.33	220.56	226.70	235.33	241.13	249.28	257.87
SD	17.93	18.89	19.50	20.90	21.19	22.32	23.38
N	16	16	16	16	16	16	16
%Diff	-7.1	-7.9	-7.9	-8.2	-8.8	-8.9	-9.0

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

1000 mg/kg/day 2-EHP					Bodyweight Gain (g)
	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	
	29	30	31	32	1 → 31
77	251.9	257.2	263.3	270.6	199.7
78	244.9	253.0	257.0	265.7	200.3
79	261.3	268.5	269.9	277.3	209.3
80	270.2	270.5	272.7	281.6	209.6
Mean	263.46	270.94	275.63	274.45	213.70
SD	25.02	25.97	26.39	18.28	23.82
N	16	16	16	8	16
%Diff	-10.5	-10.6	-11.6	-14.1	.

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

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Key Page

**Measurement Descriptions**

<u>Headings Used</u>	<u>Description</u>
Body Weight	Bodyweight
Bodyweight Gain	Bodyweight Gain

**Unit Descriptions**

<u>Headings Used</u>	<u>Description</u>
g	g

**Measurement/Statistics**

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

**Group Information**

<u>Short Name</u>	<u>Long Name</u>	<u>Report Headings 1-4</u>		
1	1	0	mg/kg/day	2-EHP
2	2	250	mg/kg/day	2-EHP
3	3	500	mg/kg/day	2-EHP
4	4	750	mg/kg/day	2-EHP

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

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Key Page

**Group Information (Continued)**

Short Name

Long Name

Report Headings 1-4

5

5

1000

mg/kg/day

2-EHP



# APPENDIX III: Individual Animal Preputial Separation Data

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

0 mg/kg/day 2-EHP							
	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation
	8	9	10	11	12	13	14
01	NI	NI	NI	NI	NI	NI	NI
02	NI	NI	NI	NI	NI	NI	NI
03	NI	NI	NI	NI	NI	NI	NI
04	NI	NI	NI	NI	NI	NI	NI
05	NI	NI	NI	NI	NI	NI	NI
06	NI	NI	NI	NI	NI	NI	NI
07	NI	NI	NI	NI	NI	NI	NI
08	NI	NI	NI	NI	NI	NI	NI
09	NI	NI	NI	NI	NI	NI	NI
10	NI	NI	NI	NI	NI	NI	NI
11	NI	NI	NI	NI	NI	NI	NI
12	NI	NI	NI	NI	NI	NI	NI

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

0 mg/kg/day 2-EHP							
	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation
	15	16	17	18	19	20	21
01	NI	NI	NI	NI	Partial	Thread	Thread
02	NI	NI	NI	Partial	Thread	Thread	Complete
03	NI	NI	NI	NI	NI	Partial	Thread
04	NI	NI	NI	NI	NI	NI	Partial
05	NI	NI	NI	Partial	Thread	Complete	.
06	NI	NI	NI	Partial	Thread	Complete	.
07	NI	NI	NI	NI	NI	Partial	Thread
08	NI	NI	NI	NI	Partial	Thread	Complete
09	NI	NI	NI	NI	NI	Partial	Thread
10	NI	NI	NI	NI	NI	NI	Partial
11	NI	NI	NI	Partial	Thread	Complete	.
12	NI	NI	NI	NI	NI	Partial	Thread



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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

0 mg/kg/day 2-EHP						Bodywt on day of PPS
	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	
	22	23	24	25	26	Day of PPS
01	Complete	.	.	.	.	231.0
02	.	.	.	.	.	208.9
03	Complete	.	.	.	.	227.6
04	Thread	Thread	Complete	.	.	231.8
05	.	.	.	.	.	214.9
06	.	.	.	.	.	215.6
07	Complete	.	.	.	.	239.3
08	.	.	.	.	.	230.8
09	Complete	.	.	.	.	208.4
10	Thread	Thread	Complete	.	.	221.1
11	.	.	.	.	.	263.5
12	Complete	.	.	.	.	222.0

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

0 mg/kg/day 2-EHP	Preputial Separation						
	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation
	8	9	10	11	12	13	14
13	NI	NI	NI	NI	NI	NI	NI
14	NI	NI	NI	NI	NI	NI	NI
15	NI	NI	NI	NI	NI	NI	NI
16	NI	NI	NI	NI	NI	NI	NI
Mean	.	.	.	.	.	.	.
SD	.	.	.	.	.	.	.
N	.	.	.	.	.	.	.

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

0 mg/kg/day 2-EHP	Preputial Separation						
	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation
	15	16	17	18	19	20	21
13	NI	NI	NI	NI	NI	NI	Partial
14	NI	NI	NI	NI	NI	Partial	Thread
15	NI	NI	NI	NI	NI	NI	Partial
16	NI	NI	NI	NI	Partial	Thread	Complete
Mean	.	.	.	.	.	.	.
SD	.	.	.	.	.	.	.
N	.	.	.	.	.	.	.

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

0 mg/kg/day 2-EHP						
	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Bodywt on day of PPS
	22	23	24	25	26	Day of PPS
13	Thread	Complete	.	.	.	272.0
14	Complete	.	.	.	.	210.7
15	Thread	Complete	.	.	.	207.7
16	.	.	.	.	.	236.3
Mean	.	.	.	.	.	227.60
SD	.	.	.	.	.	18.78
N	.	.	.	.	.	16

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

250 mg/kg/day 2-EHP							
	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation
	8	9	10	11	12	13	14
17	NI	NI	NI	NI	NI	NI	NI
18	NI	NI	NI	NI	NI	NI	NI
19	NI	NI	NI	NI	NI	NI	NI
20	NI	NI	NI	NI	NI	NI	NI
21	NI	NI	NI	NI	NI	NI	NI
22	NI	NI	NI	NI	NI	NI	NI
23	NI	NI	NI	NI	NI	NI	NI
24	NI	NI	NI	NI	NI	NI	NI
25	NI	NI	NI	NI	NI	NI	NI
26	NI	NI	NI	NI	NI	NI	NI
27	NI	NI	NI	NI	NI	NI	NI
28	NI	NI	NI	NI	NI	NI	NI

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

250 mg/kg/day 2-EHP							
	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation
	15	16	17	18	19	20	21
17	NI	NI	NI	NI	Partial	Thread	Complete
18	NI	NI	NI	NI	Partial	Thread	Complete
19	NI	NI	NI	NI	Partial	Thread	Complete
20	NI	NI	NI	NI	NI	Partial	Thread
21	NI	NI	NI	Partial	Thread	Complete	.
22	NI	NI	NI	NI	Partial	Thread	Complete
23	NI	NI	NI	NI	NI	NI	Partial
24	NI	NI	NI	NI	NI	Partial	Thread
25	NI	NI	NI	Partial	Thread	Complete	.
26	NI	NI	NI	NI	Partial	Thread	Complete
27	NI	NI	NI	Partial	Thread	Complete	.
28	NI	NI	NI	NI	NI	Partial	Thread

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

250 mg/kg/day 2-EHP						Bodywt on day of PPS
	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	
	22	23	24	25	26	Day of PPS
17	.	.	.	.	.	235.2
18	.	.	.	.	.	236.7
19	.	.	.	.	.	240.4
20	Complete	.	.	.	.	222.8
21	.	.	.	.	.	259.9
22	.	.	.	.	.	197.3
23	Thread	Thread	Thread	Complete	.	219.4
24	Complete	.	.	.	.	219.7
25	.	.	.	.	.	195.6
26	.	.	.	.	.	235.5
27	.	.	.	.	.	200.0
28	Complete	.	.	.	.	230.6

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

250 mg/kg/day 2-EHP	Preputial Separation						
	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation
	8	9	10	11	12	13	14
29	NI	NI	NI	NI	NI	NI	NI
30	NI	NI	NI	NI	NI	NI	NI
31	NI	NI	NI	NI	NI	NI	NI
32	NI	NI	NI	NI	NI	NI	NI
Mean	.	.	.	.	.	.	.
SD	.	.	.	.	.	.	.
N	.	.	.	.	.	.	.



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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

250 mg/kg/day 2-EHP	Preputial Separation						
	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation
	15	16	17	18	19	20	21
29	NI	NI	NI	NI	NI	NI	Partial
30	NI	NI	NI	NI	NI	Partial	Thread
31	NI	NI	NI	NI	Partial	Thread	Complete
32	NI	NI	NI	NI	NI	NI	Partial
Mean	.	.	.	.	.	.	.
SD	.	.	.	.	.	.	.
N	.	.	.	.	.	.	.

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

250 mg/kg/day 2-EHP						Bodywt on day of PPS
	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	
	22	23	24	25	26	Day of PPS
29	Thread	Complete	.	.	.	239.4
30	Complete	.	.	.	.	213.6
31	.	.	.	.	.	236.8
32	Thread	Thread	Complete	.	.	243.9
Mean	.	.	.	.	.	226.68
SD	.	.	.	.	.	18.16
N	.	.	.	.	.	16

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

500 mg/kg/day 2-EHP							
	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation
	8	9	10	11	12	13	14
33	NI	NI	NI	NI	NI	NI	NI
34	NI	NI	NI	NI	NI	NI	NI
35	NI	NI	NI	NI	NI	NI	NI
36	NI	NI	NI	NI	NI	NI	NI
37	NI	NI	NI	NI	NI	NI	NI
38	NI	NI	NI	NI	NI	NI	NI
39	NI	NI	NI	NI	NI	NI	NI
40	NI	NI	NI	NI	NI	NI	NI
41	NI	NI	NI	NI	NI	NI	NI
42	NI	NI	NI	NI	NI	NI	NI
43	NI	NI	NI	NI	NI	NI	NI
44	NI	NI	NI	NI	NI	NI	NI

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

500 mg/kg/day 2-EHP							
	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation
	15	16	17	18	19	20	21
33	NI	NI	NI	Partial	Thread	Complete	.
34	NI	NI	NI	NI	NI	Partial	Thread
35	NI	NI	NI	NI	NI	Partial	Thread
36	NI	NI	NI	Partial	Thread	Complete	.
37	NI	NI	NI	NI	NI	Partial	Thread
38	NI	NI	NI	NI	Partial	Thread	Complete
39	NI	NI	Partial	Partial	Thread	Complete	.
40	NI	NI	NI	NI	Partial	Thread	Complete
41	NI	NI	NI	NI	NI	NI	NI
42	NI	NI	NI	NI	Partial	Thread	Complete
43	NI	NI	NI	NI	NI	NI	Partial
44	NI	NI	Partial	Partial	Thread	Complete	.

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

500 mg/kg/day 2-EHP						Bodywt on day of PPS
	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	
	22	23	24	25	26	Day of PPS
33	.	.	.	.	.	239.3
34	Complete	.	.	.	.	220.2
35	Complete	.	.	.	.	242.1
36	.	.	.	.	.	217.5
37	Complete	.	.	.	.	204.3
38	.	.	.	.	.	188.4
39	.	.	.	.	.	202.8
40	.	.	.	.	.	238.3
41	NI	Partial	Thread	Thread	Complete	202.5
42	.	.	.	.	.	223.5
43	Thread	Complete	.	.	.	213.5
44	.	.	.	.	.	220.9

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

500 mg/kg/day 2-EHP							
	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation
	8	9	10	11	12	13	14
45	NI	NI	NI	NI	NI	NI	NI
46	NI	NI	NI	NI	NI	NI	NI
47	NI	NI	NI	NI	NI	NI	NI
48	NI	NI	NI	NI	NI	NI	NI
Mean	.	.	.	.	.	.	.
SD	.	.	.	.	.	.	.
N	.	.	.	.	.	.	.

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

500 mg/kg/day 2-EHP	Preputial Separation						
	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation
	15	16	17	18	19	20	21
45	NI	NI	NI	NI	NI	NI	NI
46	NI	NI	NI	NI	NI	NI	NI
47	NI	NI	NI	NI	Partial	Thread	Complete
48	NI	NI	NI	NI	Partial	Thread	Complete
Mean	.	.	.	.	.	.	.
SD	.	.	.	.	.	.	.
N	.	.	.	.	.	.	.

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

500 mg/kg/day 2-EHP						Bodywt on day of PPS
	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	
	22	23	24	25	26	Day of PPS
45	Partial	Thread	Complete	.	.	212.4
46	Partial	Thread	Complete	.	.	263.0
47	.	.	.	.	.	209.6
48	.	.	.	.	.	188.8
Mean	.	.	.	.	.	217.94
SD	.	.	.	.	.	20.04
N	.	.	.	.	.	16



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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

750 mg/kg/day 2-EHP							
	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation
	8	9	10	11	12	13	14
49	NI	NI	NI	NI	NI	NI	NI
50	NI	NI	NI	NI	NI	NI	NI
51	NI	NI	NI	NI	NI	NI	NI
52	NI	NI	NI	NI	NI	NI	NI
53	NI	NI	NI	NI	NI	NI	NI
54	NI	NI	NI	NI	NI	NI	NI
55	NI	NI	NI	NI	NI	NI	NI
56	NI	NI	NI	NI	NI	NI	NI
57	NI	NI	NI	NI	NI	NI	NI
58	NI	NI	NI	NI	NI	NI	NI
59	NI	NI	NI	NI	NI	NI	NI
60	NI	NI	NI	NI	NI	NI	NI

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

750 mg/kg/day 2-EHP							
	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation
	15	16	17	18	19	20	21
49	NI	NI	NI	NI	Partial	Thread	Complete
50	NI	NI	NI	NI	Partial	Thread	Complete
51	NI	NI	NI	NI	Partial	Thread	Complete
52	NI	NI	NI	NI	NI	Partial	Thread
53	NI	NI	NI	Partial	Thread	Complete	.
54	NI	NI	NI	NI	Partial	Thread	Complete
55	NI	NI	Partial	Partial	Thread	Complete	.
56	NI	NI	NI	NI	Partial	Thread	Complete
57	NI	NI	NI	NI	Partial	Thread	Complete
58	NI	NI	NI	NI	NI	Partial	Thread
59	NI	NI	NI	NI	NI	Partial	Thread
60	NI	NI	NI	NI	Partial	Thread	Complete

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

750 mg/kg/day 2-EHP						
	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Bodywt on day of PPS
	22	23	24	25	26	Day of PPS
49	.	.	.	.	.	214.9
50	.	.	.	.	.	217.6
51	.	.	.	.	.	210.8
52	Complete	.	.	.	.	231.9
53	.	.	.	.	.	209.6
54	.	.	.	.	.	225.5
55	.	.	.	.	.	200.9
56	.	.	.	.	.	200.7
57	.	.	.	.	.	229.3
58	Complete	.	.	.	.	203.9
59	Complete	.	.	.	.	198.0
60	.	.	.	.	.	203.9

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

750 mg/kg/day 2-EHP							
	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation
	8	9	10	11	12	13	14
61	NI	NI	NI	NI	NI	NI	NI
62	NI	NI	NI	NI	NI	NI	NI
63	NI	NI	NI	NI	NI	NI	NI
64	NI	NI	NI	NI	NI	NI	NI
Mean	.	.	.	.	.	.	.
SD	.	.	.	.	.	.	.
N	.	.	.	.	.	.	.

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

750 mg/kg/day 2-EHP	Preputial Separation						
	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation
	15	16	17	18	19	20	21
61	NI	NI	NI	NI	Partial	Thread	Complete
62	NI	NI	NI	NI	NI	NI	Partial
63	NI	NI	NI	NI	Partial	Thread	Complete
64	NI	NI	NI	NI	Partial	Thread	Complete
Mean	.	.	.	.	.	.	.
SD	.	.	.	.	.	.	.
N	.	.	.	.	.	.	.

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

750 mg/kg/day 2-EHP						Bodywt on day of PPS
	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	
	22	23	24	25	26	Day of PPS
61	.	.	.	.	.	186.1
62	Thread	Complete	.	.	.	221.5
63	.	.	.	.	.	248.5
64	.	.	.	.	.	194.5
Mean	.	.	.	.	.	212.35
SD	.	.	.	.	.	16.13
N	.	.	.	.	.	16

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

1000 mg/kg/day 2-EHP							
	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation
	8	9	10	11	12	13	14
65	NI	NI	NI	NI	NI	NI	NI
66	NI	NI	NI	NI	NI	NI	NI
67	NI	NI	NI	NI	NI	NI	NI
68	NI	NI	NI	NI	NI	NI	NI
69	NI	NI	NI	NI	NI	NI	NI
70	NI	NI	NI	NI	NI	NI	NI
71	NI	NI	NI	NI	NI	NI	NI
72	NI	NI	NI	NI	NI	NI	NI
73	NI	NI	NI	NI	NI	NI	NI
74	NI	NI	NI	NI	NI	NI	NI
75	NI	NI	NI	NI	NI	NI	NI
76	NI	NI	NI	NI	NI	NI	NI

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

1000 mg/kg/day 2-EHP							
	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation
	15	16	17	18	19	20	21
65	NI	NI	NI	NI	Partial	Thread	Complete
66	NI	NI	NI	Partial	Thread	Complete	.
67	NI	NI	NI	NI	NI	Partial	Thread
68	NI	NI	NI	NI	Partial	Thread	Complete
69	NI	NI	NI	NI	Partial	Thread	Complete
70	NI	NI	NI	NI	NI	Partial	Thread
71	NI	NI	NI	NI	NI	NI	Partial
72	NI	NI	NI	NI	Partial	Thread	Complete
73	NI	NI	NI	Partial	Thread	Complete	.
74	NI	NI	NI	NI	Partial	Thread	Complete
75	NI	NI	NI	NI	NI	NI	NI
76	NI	NI	NI	Partial	Thread	Complete	.



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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

1000 mg/kg/day 2-EHP						Bodywt on day of PPS
	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	
	22	23	24	25	26	Day of PPS
65	.	.	.	.	.	214.3
66	.	.	.	.	.	199.7
67	Complete	.	.	.	.	238.1
68	.	.	.	.	.	198.2
69	.	.	.	.	.	190.5
70	Complete	.	.	.	.	184.0
71	Thread	Thread	Complete	.	.	260.3
72	.	.	.	.	.	228.9
73	.	.	.	.	.	203.4
74	.	.	.	.	.	180.3
75	Partial	Thread	Thread	Complete	.	248.5
76	.	.	.	.	.	187.3

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

1000 mg/kg/day 2-EHP	Preputial Separation						
	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation
	8	9	10	11	12	13	14
77	NI	NI	NI	NI	NI	NI	NI
78	NI	NI	NI	NI	NI	NI	NI
79	NI	NI	NI	NI	NI	NI	NI
80	NI	NI	NI	NI	NI	NI	NI
Mean	.	.	.	.	.	.	.
SD	.	.	.	.	.	.	.
N	.	.	.	.	.	.	.

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

1000 mg/kg/day 2-EHP	Preputial Separation						
	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation
	15	16	17	18	19	20	21
77	NI	NI	NI	NI	NI	NI	Partial
78	NI	NI	NI	NI	NI	NI	Partial
79	NI	NI	NI	NI	Partial	Thread	Complete
80	NI	NI	NI	Partial	Thread	Complete	.
Mean	.	.	.	.	.	.	.
SD	.	.	.	.	.	.	.
N	.	.	.	.	.	.	.

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

1000 mg/kg/day 2-EHP						Bodywt on day of PPS
	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	Preputial Separation	
	22	23	24	25	26	Day of PPS
77	Thread	Complete	.	.	.	205.5
78	Thread	Thread	Complete	.	.	203.6
79	.	.	.	.	.	200.2
80	.	.	.	.	.	197.2
Mean	.	.	.	.	.	208.75
SD	.	.	.	.	.	23.40
N	.	.	.	.	.	16

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

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Key Page

**Measurement Descriptions**

<u>Headings Used</u>	<u>Description</u>
Preputial Separation	Preputial Separation
Bodywt on day of PPS	Bodyweight on day of PPS(new)

**Time-Points/Ranges**

<u>Measurement</u>	<u>From</u>	<u>To</u>	<u>Report As</u>
Bodywt on day of PPS	-9,999	9,999	Day of PPS

**Measurement/Statistics**

<u>Measurement</u>	<u>Descriptive</u>
Bodywt on day of PPS	Mean
	Standard Deviation
	Count (N)

**Group Information**

<u>Short Name</u>	<u>Long Name</u>	<u>Report Headings 1-4</u>		
1	1	0	mg/kg/day	2-EHP
2	2	250	mg/kg/day	2-EHP
3	3	500	mg/kg/day	2-EHP
4	4	750	mg/kg/day	2-EHP
5	5	1000	mg/kg/day	2-EHP

# APPENDIX IV: Individual Animal Tissue Weight Data

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

0 mg/kg/day 2-EHP							
	Adrenal Gland Weight (g)	Epididymis Right Weight (g)	Epididymis Left Weight (g)	Kidneys Weight (g)	LABC Weight (g)	Liver Weight (g)	Pituitary Gland Weight (g)
	Termination	Termination	Termination	Termination	Termination	Termination	Termination
01	0.0423	0.2095	0.2167	1.9621	0.5268	16.2293	0.0133
02	0.0392	0.1896	0.2036	1.9680	0.5397	11.9854	0.0109
03	0.0453	0.1881	0.1988	2.1471	0.4335	15.0748	0.0065 <
04	0.0303	0.1786	0.1428	2.0811	0.4381	13.1370	0.0087
05	0.0384	0.2096	0.1639	2.1635	0.4835	14.7828	0.0101
06	0.0404	0.1912	0.1823	2.0399	0.4934	13.7871	0.0110
07	0.0348	0.2036	0.1827	2.0328	0.4650	14.4797	0.0107
08	0.0316	0.1691	0.1927	2.1318	0.4666	17.4349	0.0086
09	0.0425	0.2319	0.2378	1.9152	0.4072	13.4017	0.0085
10	0.0404	0.1872	0.2207	1.8986	0.5057	12.7858	0.0073
11	0.0497	0.2520	0.2247	2.4333	0.5618	19.9642	0.0098
12	0.0233 <	0.2080	0.2019	1.9707	0.5771	13.2783	0.0095

General Footnote: [Adrenal weights for animals 12 and 66 were not used in statistical analysis || Pituitary weights from animals 49 and 71 were not used for statistical analysis]

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

0 mg/kg/day 2-EHP							
	DLP Gland Weight (g)	VP Gland Weight (g)	SV Weight (g)	SV - Blotted Weight (g)	Right Testis Weight (g)	Left Testis Weight (g)	Thyroid Weight (EDSP (g)
	Termination	Termination	Termination	Termination	Termination	Termination	Termination
01	0.1136	0.2796	0.6413	0.3835 >	1.3894	1.4487	0.01764
02	0.0953 <	0.2302	0.4682	0.3214	1.3773	1.3997	0.01311
03	0.1215	0.2875	0.5220	0.3407	1.4447	1.4791	0.01792
04	0.0943 <	0.2174	0.3926	0.2732 <	1.2231	1.2402	0.02546 >>! <sup>1</sup>
05	0.0530 <	0.1919	0.4632	0.2985	1.4946	1.4667	0.01927
06	0.0626 <	0.3003 >	0.3758	0.2592 <	1.4775	1.4334	0.01953
07	0.0771 <	0.2195	0.5180	0.3340	1.4564	1.5656	0.02057
08	0.0858 <	0.2652	0.4659	0.2854	1.3477	1.3214	0.02015
09	0.1034	0.2202	0.4267	0.3237	1.4642	1.4769	0.01594
10	0.0761 <	0.2215	0.6059	0.3794 >	1.5611	1.5543	0.01818
11	0.0759 <	0.2996 >	0.6737	0.3497	1.4504	1.3561	0.01803
12	0.0911 <	0.2966 >	0.3736	0.2916	1.5298	1.5409	0.02001

General Footnote: [Adrenal weights for animals 12 and 66 were not used in statistical analysis || Pituitary weights from animals 49 and 71 were not used for statistical analysis]

1 [ >>, RC:weight verified]



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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

0 mg/kg/day 2-EHP				
	Adrenals Wt Ratio (%)	Kidney Wt Ratio (%)	Liver Wt Ratio (%)	Pituitary Wt Ratio (%)
	Termination	Termination	Termination	Termination
01	0.013	0.62	5.16	0.004
02	0.013	0.67	4.05	0.004
03	0.015	0.70	4.92	0.002
04	0.010	0.69	4.37	0.003
05	0.012	0.68	4.65	0.003
06	0.013	0.66	4.49	0.004
07	0.011	0.63	4.46	0.003
08	0.010	0.65	5.33	0.003
09	0.015	0.67	4.69	0.003
10	0.015	0.70	4.70	0.003
11	0.013	0.63	5.15	0.003
12	0.008	0.66	4.48	0.003

General Footnote: [Adrenal weights for animals 12 and 66 were not used in statistical analysis || Pituitary weights from animals 49 and 71 were not used for statistical analysis]

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

0 mg/kg/day 2-EHP							
	Adrenal Gland Weight (g)	Epididymis Right Weight (g)	Epididymis Left Weight (g)	Kidneys Weight (g)	LABC Weight (g)	Liver Weight (g)	Pituitary Gland Weight (g)
	Termination	Termination	Termination	Termination	Termination	Termination	Termination
13	0.0516	0.2259	0.1998	2.2917	0.4696	19.9345	0.0101
14	0.0466	0.2035	0.2411	1.8733	0.4934	12.3223	0.0092
15	0.0278 <	0.1970	0.1956	1.8756	0.4626	11.8055	0.0077
16	0.0509	0.2234	0.2109	2.6980	0.6306 >	15.2674	0.0124
Mean	0.03969	0.20426	0.20100	2.09267	0.49716	14.72942	0.00964
SD	0.00837	0.02134	0.02558	0.22360	0.05826	2.54928	0.00180
N	16	16	16	16	16	16	16

General Footnote: [Adrenal weights for animals 12 and 66 were not used in statistical analysis || Pituitary weights from animals 49 and 71 were not used for statistical analysis]

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

0 mg/kg/day 2-EHP							
	DLP Gland Weight (g)	VP Gland Weight (g)	SV Weight (g)	SV - Blotted Weight (g)	Right Testis Weight (g)	Left Testis Weight (g)	Thyroid Weight (EDSP (g)
	Termination	Termination	Termination	Termination	Termination	Termination	Termination
13	0.0943 <	0.2820	0.5599	0.3086	1.4313	1.4408	0.02154
14	0.0670 <	0.2663	0.5757	0.3150	1.3879	1.3620	0.01543
15	0.0813 <	0.2820	0.4646	0.2678 <	1.2754	1.2939	0.01627
16	0.1123	0.3072 >	0.6952	0.4006 >	1.6369	1.6545	0.01685
Mean	0.08779	0.26044	0.51389	0.32077	1.43423	1.43964	0.018494
SD	0.01917	0.03740	0.10315	0.04227	0.10272	0.10919	0.002861
N	16	16	16	16	16	16	16

General Footnote: [Adrenal weights for animals 12 and 66 were not used in statistical analysis || Pituitary weights from animals 49 and 71 were not used for statistical analysis]

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

0 mg/kg/day 2-EHP				
	Adrenals Wt Ratio (%)	Kidney Wt Ratio (%)	Liver Wt Ratio (%)	Pituitary Wt Ratio (%)
	Termination	Termination	Termination	Termination
13	0.014	0.62	5.35	0.003
14	0.017	0.68	4.48	0.003
15	0.010	0.70	4.42	0.003
16	0.015	0.80	4.50	0.004
Mean	0.0127	0.673	4.702	0.0031
SD	0.0024	0.044	0.377	0.0005
N	16	16	16	16

General Footnote: [Adrenal weights for animals 12 and 66 were not used in statistical analysis || Pituitary weights from animals 49 and 71 were not used for statistical analysis]

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

250 mg/kg/day 2-EHP							
	Adrenal Gland Weight (g)	Epididymis Right Weight (g)	Epididymis Left Weight (g)	Kidneys Weight (g)	LABC Weight (g)	Liver Weight (g)	Pituitary Gland Weight (g)
	Termination	Termination	Termination	Termination	Termination	Termination	Termination
17	0.0427	0.2220	0.2055	2.4233	0.4939	15.9762	0.0092
18	0.0451	0.2113	0.2303	2.1165	0.6204 >	13.0371	0.0107
19	0.0411	0.2370	0.1969	2.2270	0.5444	14.8698	0.0052 <
20	0.0404	0.1914	0.1906	1.8227	0.4410	14.7418	0.0077
21	0.0424	0.1708	0.2032	2.4786	0.5243	17.6191	0.0114
22	0.0276 <	0.1789	0.2021	1.7709	0.3865	11.7159	0.0097
23	0.0259 <	0.1727	0.1671	1.6625	0.3228	12.8695	0.0076
24	0.0383	0.1846	0.2319	1.9955	0.4730	13.0487	0.0098
25	0.0379	0.2579	0.2772	1.8179	0.5205	13.2542	0.0075
26	0.0422	0.2517	0.2469	2.3375	0.5291	16.2133	0.0110
27	0.0404	0.2095	0.2044	1.9167	0.4639	11.6121	0.0097
28	0.0383	0.2160	0.2378	1.9965	0.5362	13.3089	0.0118

General Footnote: [Adrenal weights for animals 12 and 66 were not used in statistical analysis || Pituitary weights from animals 49 and 71 were not used for statistical analysis]

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

250 mg/kg/day 2-EHP							
	DLP Gland Weight (g)	VP Gland Weight (g)	SV Weight (g)	SV - Blotted Weight (g)	Right Testis Weight (g)	Left Testis Weight (g)	Thyroid Weight (EDSP (g)
	Termination	Termination	Termination	Termination	Termination	Termination	Termination
17	0.1332	0.2449	0.5575	0.3557	1.6502	1.6476	0.01914
18	0.1040	0.2969 >	0.4040	0.3085	1.5815	1.5945	0.01879
19	0.1136	0.3124 >	0.6606	0.3417	1.4385	1.4468	0.01804
20	0.0624 <	0.2493	0.4367	0.2906	1.3991	1.4747	0.01821
21	0.0893 <	0.2618	0.6205	0.3259	1.5315	1.5913	0.01864
22	0.0726 <	0.1485	0.3970	0.2486 <	1.4482	1.4886	0.01850
23	0.0700 <	0.1926	0.3492	0.2453 <	1.2014	1.1837	0.01380
24	0.1041	0.2141	0.5628	0.3466	1.5147	1.5272	0.01893
25	0.0829 <	0.2353	0.5312	0.3299	1.3646	1.4436	0.01433
26	0.1121	0.3148 >	0.6316	0.3298	1.5864	1.5153	0.01599
27	0.1045	0.2736	0.5051	0.2881	1.5713	1.5399	0.02338 >
28	0.0715 <	0.3032 >	0.6661	0.3345	1.4917	1.4781	0.01423

General Footnote: [Adrenal weights for animals 12 and 66 were not used in statistical analysis || Pituitary weights from animals 49 and 71 were not used for statistical analysis]

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

250 mg/kg/day 2-EHP				
	Adrenals Wt Ratio (%)	Kidney Wt Ratio (%)	Liver Wt Ratio (%)	Pituitary Wt Ratio (%)
	Termination	Termination	Termination	Termination
17	0.013	0.72	4.76	0.003
18	0.014	0.65	3.98	0.003
19	0.012	0.64	4.25	0.001
20	0.013	0.61	4.92	0.003
21	0.011	0.63	4.51	0.003
22	0.010	0.66	4.36	0.004
23	0.010	0.64	4.99	0.003
24	0.013	0.68	4.43	0.003
25	0.014	0.66	4.79	0.003
26	0.013	0.69	4.82	0.003
27	0.014	0.68	4.13	0.003
28	0.012	0.64	4.28	0.004

General Footnote: [Adrenal weights for animals 12 and 66 were not used in statistical analysis || Pituitary weights from animals 49 and 71 were not used for statistical analysis]

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

250 mg/kg/day 2-EHP							
	Adrenal Gland Weight (g)	Epididymis Right Weight (g)	Epididymis Left Weight (g)	Kidneys Weight (g)	LABC Weight (g)	Liver Weight (g)	Pituitary Gland Weight (g)
	Termination	Termination	Termination	Termination	Termination	Termination	Termination
29	0.0288 <	0.1995	0.1986	1.9561	0.4873	15.4163	0.0074
30	0.0454	0.1873	0.2116	1.8819	0.4759	12.9099	0.0099
31	0.0396	0.2133	0.2238	2.4779	0.5216	16.8264	0.0102
32	0.0408	0.2056	0.2068	1.8784	0.4012	13.2798	0.0086
Mean	0.03856	0.20684	0.21467	2.04749	0.48388	14.16869	0.00921
SD	0.00595	0.02624	0.02592	0.26434	0.07131	1.81692	0.00177
N	16	16	16	16	16	16	16

General Footnote: [Adrenal weights for animals 12 and 66 were not used in statistical analysis || Pituitary weights from animals 49 and 71 were not used for statistical analysis]



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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

250 mg/kg/day 2-EHP							
	DLP Gland Weight (g)	VP Gland Weight (g)	SV Weight (g)	SV - Blotted Weight (g)	Right Testis Weight (g)	Left Testis Weight (g)	Thyroid Weight (EDSP (g)
	Termination	Termination	Termination	Termination	Termination	Termination	Termination
29	0.0905 <	0.2264	0.4289	0.3005	1.4025	1.4594	0.01686
30	0.0855 <	0.2664	0.4548	0.3073	1.5762	1.5608	0.01909
31	0.0859 <	0.2946 >	0.4882	0.2936	1.6686	1.6936	0.02630 >>¹
32	0.1173	0.2317	0.4853	0.2899	1.3485	1.3474	0.01374
Mean	0.09371	0.25416	0.51122	0.30853	1.48593	1.49953	0.017998
SD	0.01989	0.04606	0.09848	0.03227	0.12401	0.11973	0.003380
N	16	16	16	16	16	16	16

General Footnote: [Adrenal weights for animals 12 and 66 were not used in statistical analysis || Pituitary weights from animals 49 and 71 were not used for statistical analysis]

1 [ >>, RC:weight verified]

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

250 mg/kg/day 2-EHP				
	Adrenals Wt Ratio (%)	Kidney Wt Ratio (%)	Liver Wt Ratio (%)	Pituitary Wt Ratio (%)
	Termination	Termination	Termination	Termination
29	0.009	0.63	5.00	0.002
30	0.015	0.64	4.39	0.003
31	0.011	0.72	4.88	0.003
32	0.013	0.61	4.34	0.003
Mean	0.0124	0.657	4.551	0.0030
SD	0.0017	0.034	0.327	0.0006
N	16	16	16	16

General Footnote: [Adrenal weights for animals 12 and 66 were not used in statistical analysis || Pituitary weights from animals 49 and 71 were not used for statistical analysis]

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

500 mg/kg/day 2-EHP							
	Adrenal Gland Weight (g)	Epididymis Right Weight (g)	Epididymis Left Weight (g)	Kidneys Weight (g)	LABC Weight (g)	Liver Weight (g)	Pituitary Gland Weight (g)
	Termination	Termination	Termination	Termination	Termination	Termination	Termination
33	0.0423	0.2052	0.1788	2.1523	0.5445	15.9075	0.0091
34	0.0368	0.1978	0.1984	1.8647	0.4039	12.7034	0.0085
35	0.0531	0.1930	0.1958	2.0307	0.4012	14.6136	0.0090
36	0.0374	0.1918	0.1638	2.0301	0.4877	12.6736	0.0119
37	0.0267 <	0.1772	0.1472	1.6934	0.4022	12.3678	0.0078
38	0.0398	0.1907	0.1700	1.6691	0.3402	10.7160	0.0082
39	0.0266 <	0.1875	0.2102	1.8928	0.4627	11.0607	0.0080
40	0.0417	0.1800	0.1711	2.1222	0.4891	15.6919	0.0102
41	0.0222 <	0.1751	0.1880	1.7663	0.3400	10.6133	0.0080
42	0.0535	0.1897	0.1685	2.5209	0.4527	15.1811	0.0097
43	0.0361	0.1874	0.1740	2.0129	0.5099	13.6385	0.0101
44	0.0412	0.2138	0.2323	2.0182	0.5698	13.2970	0.0098

General Footnote: [Adrenal weights for animals 12 and 66 were not used in statistical analysis || Pituitary weights from animals 49 and 71 were not used for statistical analysis]

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

500 mg/kg/day 2-EHP							
	DLP Gland Weight (g)	VP Gland Weight (g)	SV Weight (g)	SV - Blotted Weight (g)	Right Testis Weight (g)	Left Testis Weight (g)	Thyroid Weight (EDSP (g)
	Termination	Termination	Termination	Termination	Termination	Termination	Termination
33	0.1238	0.1742	0.5329	0.3209	1.3961	1.3962	0.01873
34	0.1021	0.2813	0.4571	0.2579 <	1.3256	1.3064	0.01898
35	0.0600 <	0.2474	0.5020	0.4931 >	1.4697	1.5017	0.01636
36	0.1353	0.1852	0.5292	0.3230	1.3861	1.3891	0.01898
37	0.0866 <	0.2193	0.4637	0.2748 <	1.2491	1.2987	0.01855
38	0.0490 <	0.1853	0.3129	0.2056 <	1.3432	1.3416	0.01890
39	0.0969 <	0.2399	0.5083	0.3250	1.4024	1.4414	0.01552
40	0.0895 <	0.1808	0.4688	0.3406	1.4033	1.3901	0.01791
41	0.0613 <	0.2144	0.3842	0.2383 <	1.2820	1.3140	0.01460
42	0.0811 <	0.2668	0.5221	0.3798 >	1.5335	1.5424	0.01800
43	0.1211	0.2615	0.4690	0.2642 <	1.3451	1.3342	0.01491
44	0.0800 <	0.2343	0.6479	0.3442	1.5336	1.5357	0.02262 >

General Footnote: [Adrenal weights for animals 12 and 66 were not used in statistical analysis || Pituitary weights from animals 49 and 71 were not used for statistical analysis]

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

500 mg/kg/day 2-EHP				
	Adrenals Wt Ratio (%)	Kidney Wt Ratio (%)	Liver Wt Ratio (%)	Pituitary Wt Ratio (%)
	Termination	Termination	Termination	Termination
33	0.012	0.63	4.68	0.003
34	0.013	0.65	4.42	0.003
35	0.017	0.65	4.68	0.003
36	0.012	0.68	4.22	0.004
37	0.010	0.66	4.81	0.003
38	0.015	0.64	4.09	0.003
39	0.010	0.69	4.03	0.003
40	0.013	0.66	4.90	0.003
41	0.010	0.76	4.56	0.003
42	0.017	0.81	4.88	0.003
43	0.012	0.70	4.71	0.003
44	0.014	0.67	4.39	0.003

General Footnote: [Adrenal weights for animals 12 and 66 were not used in statistical analysis || Pituitary weights from animals 49 and 71 were not used for statistical analysis]

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

500 mg/kg/day 2-EHP							
	Adrenal Gland Weight (g)	Epididymis Right Weight (g)	Epididymis Left Weight (g)	Kidneys Weight (g)	LABC Weight (g)	Liver Weight (g)	Pituitary Gland Weight (g)
	Termination	Termination	Termination	Termination	Termination	Termination	Termination
45	0.0328	0.1875	0.1808	1.6918	0.3669	11.2829	0.0083
46	0.0358	0.2052	0.2176	2.4682	0.4883	16.3858	0.0077
47	0.0351	0.2145	0.1939	2.0092	0.4664	13.3088	0.0107
48	0.0350	0.1908	0.1849	1.7437	0.3875	9.2257	0.0086
Mean	0.03726	0.19295	0.18596	1.98041	0.44456	13.04173	0.00910
SD	0.00843	0.01174	0.02168	0.25476	0.06977	2.12210	0.00120
N	16	16	16	16	16	16	16

General Footnote: [Adrenal weights for animals 12 and 66 were not used in statistical analysis || Pituitary weights from animals 49 and 71 were not used for statistical analysis]

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

500 mg/kg/day 2-EHP							
	DLP Gland Weight (g)	VP Gland Weight (g)	SV Weight (g)	SV - Blotted Weight (g)	Right Testis Weight (g)	Left Testis Weight (g)	Thyroid Weight (EDSP (g)
	Termination	Termination	Termination	Termination	Termination	Termination	Termination
45	0.0782 <	0.2274	0.3664	0.2316 <	1.3121	1.2983	0.01783
46	0.0976 <	0.2432	0.5038	0.3308	1.6442	1.6817	0.02154
47	0.0852 <	0.2174	0.5455	0.3236	1.6122	1.5998	0.01609
48	0.0920 <	0.2448	0.4527	0.2587 <	1.5341	1.4989	0.01488
Mean	0.08998	0.22645	0.47916	0.30701	1.42327	1.42939	0.017775
SD	0.02335	0.03230	0.07878	0.06946	0.11858	0.11852	0.002309
N	16	16	16	16	16	16	16

General Footnote: [Adrenal weights for animals 12 and 66 were not used in statistical analysis || Pituitary weights from animals 49 and 71 were not used for statistical analysis]

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

500 mg/kg/day 2-EHP				
	Adrenals Wt Ratio (%)	Kidney Wt Ratio (%)	Liver Wt Ratio (%)	Pituitary Wt Ratio (%)
	Termination	Termination	Termination	Termination
45	0.013	0.66	4.40	0.003
46	0.011	0.73	4.86	0.002
47	0.012	0.70	4.65	0.004
48	0.015	0.76	4.03	0.004
Mean	0.0129	0.690	4.518	0.0032
SD	0.0023	0.051	0.304	0.0004
N	16	16	16	16

General Footnote: [Adrenal weights for animals 12 and 66 were not used in statistical analysis || Pituitary weights from animals 49 and 71 were not used for statistical analysis]



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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

750 mg/kg/day 2-EHP							
	Adrenal Gland Weight (g)	Epididymis Right Weight (g)	Epididymis Left Weight (g)	Kidneys Weight (g)	LABC Weight (g)	Liver Weight (g)	Pituitary Gland Weight (g)
	Termination	Termination	Termination	Termination	Termination	Termination	Termination
49	0.0400	0.2165	0.1926	2.0622	0.5505	14.0106	0.0217 >
50	0.0371	0.2369	0.2418	2.2242	0.5097	13.9974	0.0069 <
51	0.0368	0.1918	0.1942	1.7517	0.3276	10.9147	0.0043 <
52	0.0360	0.2134	0.2234	1.9752	0.4739	13.1918	0.0099
53	0.0297 <	0.2299	0.2409	1.9216	0.4436	12.6481	0.0082
54	0.0337	0.1495	0.1434	2.0871	0.3385	13.7257	0.0093
55	0.0262 <	0.1954	0.1585	1.8520	0.4473	12.6273	0.0085
56	0.0299 <	0.1929	0.1714	2.0656	0.4631	13.7224	0.0101
57	0.0412	0.2108	0.2408	2.3054	0.5372	15.2913	0.0116
58	0.0323	0.2089	0.2149	2.1722	0.5190	11.5569	0.0069 <
59	0.0350	0.1706	0.1888	1.9607	0.3921	12.6447	0.0084
60	0.0255 <	0.2109	0.2082	1.9297	0.4776	13.1668	0.0085

General Footnote: [Adrenal weights for animals 12 and 66 were not used in statistical analysis || Pituitary weights from animals 49 and 71 were not used for statistical analysis]

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

750 mg/kg/day 2-EHP							
	DLP Gland Weight (g)	VP Gland Weight (g)	SV Weight (g)	SV - Blotted Weight (g)	Right Testis Weight (g)	Left Testis Weight (g)	Thyroid Weight (EDSP (g)
	Termination	Termination	Termination	Termination	Termination	Termination	Termination
49	0.0708 <	0.2513	0.5087	0.2991	1.5368	1.5942	0.02167
50	0.0806 <	0.2931 >	0.5168	0.2846	1.5506	1.5869	0.01690
51	0.1050	0.2162	0.4215	0.2996	1.3460	1.3694	0.01763
52	0.0621 <	0.2151	0.4340	0.2814	1.5174	1.5123	0.01759
53	0.1176	0.2463	0.5998	0.3048	1.4528	1.4781	0.01749
54	0.0890 <	0.1951	0.3186	0.2210 <	1.3980	1.4008	0.01746
55	0.0484 <	0.1991	0.5192	0.3390	1.3566	1.4045	0.01770
56	0.1099	0.2174	0.5750	0.3395	1.5504	1.5061	0.01627
57	0.0929 <	0.2483	0.4298	0.2607 <	1.5303	1.6235	0.01427
58	0.1055	0.2132	0.4551	0.2732 <	1.3074	1.3328	0.01379
59	0.0897 <	0.1960	0.3640	0.2456 <	1.3753	1.3882	0.01624
60	0.0812 <	0.2505	0.5837	0.3510	1.6167	1.5453	0.01825

General Footnote: [Adrenal weights for animals 12 and 66 were not used in statistical analysis || Pituitary weights from animals 49 and 71 were not used for statistical analysis]

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

750 mg/kg/day 2-EHP				
	Adrenals Wt Ratio (%)	Kidney Wt Ratio (%)	Liver Wt Ratio (%)	Pituitary Wt Ratio (%)
	Termination	Termination	Termination	Termination
49	0.013	0.69	4.71	0.007
50	0.012	0.73	4.60	0.002
51	0.013	0.62	3.89	0.002
52	0.012	0.66	4.42	0.003
53	0.010	0.65	4.29	0.003
54	0.011	0.70	4.60	0.003
55	0.009	0.65	4.40	0.003
56	0.011	0.74	4.93	0.004
57	0.014	0.76	5.02	0.004
58	0.013	0.85	4.50	0.003
59	0.014	0.76	4.90	0.003
60	0.009	0.68	4.62	0.003

General Footnote: [Adrenal weights for animals 12 and 66 were not used in statistical analysis || Pituitary weights from animals 49 and 71 were not used for statistical analysis]

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

750 mg/kg/day 2-EHP							
	Adrenal Gland Weight (g)	Epididymis Right Weight (g)	Epididymis Left Weight (g)	Kidneys Weight (g)	LABC Weight (g)	Liver Weight (g)	Pituitary Gland Weight (g)
	Termination	Termination	Termination	Termination	Termination	Termination	Termination
61	0.0363	0.1856	0.2181	1.9819	0.4906	12.5904	0.0074
62	0.0292 <	0.1796	0.1618	1.9236	0.4408	13.6770	0.0082
63	0.0422	0.2121	0.1973	2.2942	0.5172	16.1607	0.0096
64	0.0231 <	0.1776	0.1836	1.8866	0.3591	10.8494	0.0083
Mean	0.03339	0.19890	0.19873	2.02462	0.45549	13.17345	0.00924
SD	0.00572	0.02284	0.03046	0.15951	0.06946	1.41325	0.00370
N	16	16	16	16	16	16	16

General Footnote: [Adrenal weights for animals 12 and 66 were not used in statistical analysis || Pituitary weights from animals 49 and 71 were not used for statistical analysis]

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

750 mg/kg/day 2-EHP							
	DLP Gland Weight (g)	VP Gland Weight (g)	SV Weight (g)	SV - Blotted Weight (g)	Right Testis Weight (g)	Left Testis Weight (g)	Thyroid Weight (EDSP (g)
	Termination	Termination	Termination	Termination	Termination	Termination	Termination
61	0.0701 <	0.1872	0.5481	0.3300	1.3539	1.3427	0.01152
62	0.0486 <	0.2157	0.4545	0.2592 <	1.2175	1.2253	0.01727
63	0.0821 <	0.3130 >	0.4739	0.3435	1.4309	1.4330	0.02297 >
64	0.0610 <	0.2299	0.4180	0.2488 <	1.4546	1.4151	0.01263
Mean	0.08216	0.23046	0.47629	0.29256	1.43720	1.44739	0.016853
SD	0.02114	0.03518	0.07957	0.03995	0.10824	0.10908	0.002926
N	16	16	16	16	16	16	16

General Footnote: [Adrenal weights for animals 12 and 66 were not used in statistical analysis || Pituitary weights from animals 49 and 71 were not used for statistical analysis]

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

750 mg/kg/day 2-EHP				
	Adrenals Wt Ratio (%)	Kidney Wt Ratio (%)	Liver Wt Ratio (%)	Pituitary Wt Ratio (%)
	Termination	Termination	Termination	Termination
61	0.014	0.75	4.74	0.003
62	0.010	0.67	4.80	0.003
63	0.012	0.65	4.58	0.003
64	0.009	0.75	4.32	0.003
Mean	0.0116	0.707	4.583	0.0032
SD	0.0017	0.058	0.282	0.0012
N	16	16	16	16

General Footnote: [Adrenal weights for animals 12 and 66 were not used in statistical analysis || Pituitary weights from animals 49 and 71 were not used for statistical analysis]

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

1000 mg/kg/day 2-EHP							
	Adrenal Gland Weight (g)	Epididymis Right Weight (g)	Epididymis Left Weight (g)	Kidneys Weight (g)	LABC Weight (g)	Liver Weight (g)	Pituitary Gland Weight (g)
	Termination	Termination	Termination	Termination	Termination	Termination	Termination
65	0.0354	0.1884	0.1910	1.9237	0.3579	13.5281	0.0046 <
66	0.0154 <	0.2056	0.1830	1.9509	0.4486	12.1838	0.0063 <
67	0.0386	0.2105	0.2433	2.1922	0.4381	14.1937	0.0090
68	0.0325	0.1899	0.2296	2.0140	0.4244	11.6634	0.0084
69	0.0310	0.1531	0.1790	1.6660	0.3753	11.4771	0.0079
70	0.0255 <	0.3091	0.1770	1.6733	0.3707	9.7720	0.0173
71	0.0391	0.1635	0.1665	2.2000	0.4873	17.3779	0.0068 <
72	0.0367	0.1639	0.1658	2.4509	0.3557	16.1953	0.0092
73	0.0418	0.2545	0.2330	2.1926	0.4667	14.8045	0.0098
74	0.0258 <	0.1881	0.1846	2.0574	0.3838	11.9259	0.0065 <
75	0.0381	0.2363	0.2178	2.0087	0.4660	14.3820	0.0077
76	0.0293 <	0.2408	0.2262	1.8523	0.4135	10.9013	0.0070

General Footnote: [Adrenal weights for animals 12 and 66 were not used in statistical analysis || Pituitary weights from animals 49 and 71 were not used for statistical analysis]

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

1000 mg/kg/day 2-EHP							
	DLP Gland Weight (g)	VP Gland Weight (g)	SV Weight (g)	SV - Blotted Weight (g)	Right Testis Weight (g)	Left Testis Weight (g)	Thyroid Weight (EDSP (g)
	Termination	Termination	Termination	Termination	Termination	Termination	Termination
65	0.0649 <	0.2172	0.4593	0.2278 <	1.4390	1.4421	0.01945
66	0.0594 <	0.2610	0.5003	0.2795 <	1.4396	1.3942	0.01183
67	0.0670 <	0.2727	0.4848	0.3457	1.5708	1.5351	0.01758
68	0.0810 <	0.1925	0.3675	0.2186 <	1.4540	1.4303	0.01681
69	0.0998 <	0.2218	0.7133	0.3173	1.2849	1.3242	0.01530
70	0.0727 <	0.1719	0.5477	0.1771 <	1.3008	1.3049	0.01709
71	0.0681 <	0.1751	0.3969	0.2820	1.3819	1.3886	0.01699
72	0.0985 <	0.2316	0.6660	0.3979 >	1.5215	1.4969	0.01445
73	0.1084	0.2215	0.5275	0.2840	1.4572	1.4709	0.01827
74	0.0725 <	0.2335	0.5069	0.2680 <	1.3282	1.3359	0.01242
75	0.1343	0.1981	0.4427	0.2812	1.5824	1.5779	0.01720
76	0.1044	0.2505	0.5225	0.3069	1.3747	1.3656	0.01311

General Footnote: [Adrenal weights for animals 12 and 66 were not used in statistical analysis || Pituitary weights from animals 49 and 71 were not used for statistical analysis]



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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

1000 mg/kg/day 2-EHP				
	Adrenals Wt Ratio (%)	Kidney Wt Ratio (%)	Liver Wt Ratio (%)	Pituitary Wt Ratio (%)
	Termination	Termination	Termination	Termination
65	0.012	0.65	4.57	0.002
66	0.006	0.70	4.37	0.002
67	0.013	0.73	4.71	0.003
68	0.012	0.77	4.47	0.003
69	0.012	0.65	4.46	0.003
70	0.011	0.72	4.18	0.007
71	0.012	0.67	5.26	0.002
72	0.012	0.79	5.21	0.003
73	0.014	0.75	5.07	0.003
74	0.011	0.84	4.86	0.003
75	0.013	0.69	4.92	0.003
76	0.012	0.75	4.40	0.003

General Footnote: [Adrenal weights for animals 12 and 66 were not used in statistical analysis || Pituitary weights from animals 49 and 71 were not used for statistical analysis]

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

1000 mg/kg/day 2-EHP							
	Adrenal Gland Weight (g)	Epididymis Right Weight (g)	Epididymis Left Weight (g)	Kidneys Weight (g)	LABC Weight (g)	Liver Weight (g)	Pituitary Gland Weight (g)
	Termination	Termination	Termination	Termination	Termination	Termination	Termination
77	0.0429	0.1864	0.1986	1.9598	0.4429	12.2616	0.0089
78	0.0335	0.1724	0.2006	1.8835	0.3777	12.5718	0.0084
79	0.0317	0.2011	0.2096	2.1598	0.3415	13.2312	0.0083
80	0.0292 <	0.2042	0.1962	1.8992	0.4690	12.9956	0.0088
Mean	0.03291	0.20424	0.20011	2.00527	0.41369	13.09158	0.00843
SD	0.00703	0.03991	0.02425	0.20326	0.04761	1.96034	0.00271
N	16	16	16	16	16	16	16

General Footnote: [Adrenal weights for animals 12 and 66 were not used in statistical analysis || Pituitary weights from animals 49 and 71 were not used for statistical analysis]

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

1000 mg/kg/day 2-EHP							
	DLP Gland Weight (g)	VP Gland Weight (g)	SV Weight (g)	SV - Blotted Weight (g)	Right Testis Weight (g)	Left Testis Weight (g)	Thyroid Weight (EDSP (g)
	Termination	Termination	Termination	Termination	Termination	Termination	Termination
77	0.0609 <	0.2420	0.5347	0.3069	1.7376	1.4794	0.01512
78	0.1043	0.2168	0.3171	0.2135 <	1.3955	1.3576	0.01799
79	0.1012	0.1650	0.4968	0.2402 <	1.4717	1.4215	0.01739
80	0.0840 <	0.2880	0.3781	0.2086 <	1.5011	1.4067	0.01701
Mean	0.08634	0.22245	0.49138	0.27220	1.45256	1.42074	0.016126
SD	0.02150	0.03590	0.10274	0.05682	0.11572	0.07702	0.002202
N	16	16	16	16	16	16	16

General Footnote: [Adrenal weights for animals 12 and 66 were not used in statistical analysis || Pituitary weights from animals 49 and 71 were not used for statistical analysis]

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Sex: Male Day(s) Relative to Start Date

1000 mg/kg/day 2-EHP				
	Adrenals Wt Ratio (%)	Kidney Wt Ratio (%)	Liver Wt Ratio (%)	Pituitary Wt Ratio (%)
	Termination	Termination	Termination	Termination
77	0.016	0.74	4.66	0.003
78	0.013	0.73	4.89	0.003
79	0.012	0.80	4.90	0.003
80	0.011	0.70	4.77	0.003
Mean	0.0119	0.729	4.731	0.0031
SD	0.0022	0.054	0.311	0.0012
N	16	16	16	16

General Footnote: [Adrenal weights for animals 12 and 66 were not used in statistical analysis || Pituitary weights from animals 49 and 71 were not used for statistical analysis]

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
1	Termination	1	Male	03	Pituitary Gland Weight	Out of Range	<
1	Termination	1	Male	12	Adrenal Gland Weight	Out of Range	<
2	Termination	1	Male	01	SV - Blotted Weight	Out of Range	>
2	Termination	1	Male	02	DLP Gland Weight	Out of Range	<
2	Termination	1	Male	04	DLP Gland Weight	Out of Range	<
2	Termination	1	Male	04	SV - Blotted Weight	Out of Range	<
2	Termination	1	Male	04	Thyroid Weight (EDSP)	Out of Range	>>
			<i>Comment:</i> weight verified				
2	Termination	1	Male	05	DLP Gland Weight	Out of Range	<
2	Termination	1	Male	06	DLP Gland Weight	Out of Range	<
2	Termination	1	Male	06	VP Gland Weight	Out of Range	>
2	Termination	1	Male	06	SV - Blotted Weight	Out of Range	<
2	Termination	1	Male	07	DLP Gland Weight	Out of Range	<
2	Termination	1	Male	08	DLP Gland Weight	Out of Range	<
2	Termination	1	Male	10	DLP Gland Weight	Out of Range	<
2	Termination	1	Male	10	SV - Blotted Weight	Out of Range	>
2	Termination	1	Male	11	DLP Gland Weight	Out of Range	<
2	Termination	1	Male	11	VP Gland Weight	Out of Range	>
2	Termination	1	Male	12	DLP Gland Weight	Out of Range	<
2	Termination	1	Male	12	VP Gland Weight	Out of Range	>
4	Termination	1	Male	15	Adrenal Gland Weight	Out of Range	<
4	Termination	1	Male	16	LABC Weight	Out of Range	>
5	Termination	1	Male	13	DLP Gland Weight	Out of Range	<
5	Termination	1	Male	14	DLP Gland Weight	Out of Range	<
5	Termination	1	Male	15	DLP Gland Weight	Out of Range	<
5	Termination	1	Male	15	SV - Blotted Weight	Out of Range	<
5	Termination	1	Male	16	VP Gland Weight	Out of Range	>
5	Termination	1	Male	16	SV - Blotted Weight	Out of Range	>
7	Termination	2	Male	18	LABC Weight	Out of Range	>

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
7	Termination	2	Male	19	Pituitary Gland Weight	Out of Range	<
7	Termination	2	Male	22	Adrenal Gland Weight	Out of Range	<
7	Termination	2	Male	23	Adrenal Gland Weight	Out of Range	<
8	Termination	2	Male	18	VP Gland Weight	Out of Range	>
8	Termination	2	Male	19	VP Gland Weight	Out of Range	>
8	Termination	2	Male	20	DLP Gland Weight	Out of Range	<
8	Termination	2	Male	21	DLP Gland Weight	Out of Range	<
8	Termination	2	Male	22	DLP Gland Weight	Out of Range	<
8	Termination	2	Male	22	SV - Blotted Weight	Out of Range	<
8	Termination	2	Male	23	DLP Gland Weight	Out of Range	<
8	Termination	2	Male	23	SV - Blotted Weight	Out of Range	<
8	Termination	2	Male	25	DLP Gland Weight	Out of Range	<
8	Termination	2	Male	26	VP Gland Weight	Out of Range	>
8	Termination	2	Male	27	Thyroid Weight (EDSP	Out of Range	>
8	Termination	2	Male	28	DLP Gland Weight	Out of Range	<
8	Termination	2	Male	28	VP Gland Weight	Out of Range	>
10	Termination	2	Male	29	Adrenal Gland Weight	Out of Range	<
11	Termination	2	Male	29	DLP Gland Weight	Out of Range	<
11	Termination	2	Male	30	DLP Gland Weight	Out of Range	<
11	Termination	2	Male	31	DLP Gland Weight	Out of Range	<
11	Termination	2	Male	31	VP Gland Weight	Out of Range	>
11	Termination	2	Male	31	Thyroid Weight (EDSP	Out of Range	>>
			<i>Comment:</i> weight verified				
13	Termination	3	Male	37	Adrenal Gland Weight	Out of Range	<
13	Termination	3	Male	39	Adrenal Gland Weight	Out of Range	<
13	Termination	3	Male	41	Adrenal Gland Weight	Out of Range	<
14	Termination	3	Male	34	SV - Blotted Weight	Out of Range	<
14	Termination	3	Male	35	DLP Gland Weight	Out of Range	<
14	Termination	3	Male	35	SV - Blotted Weight	Out of Range	>
14	Termination	3	Male	37	DLP Gland Weight	Out of Range	<

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
14	Termination	3	Male	37	SV - Blotted Weight	Out of Range	<
14	Termination	3	Male	38	DLP Gland Weight	Out of Range	<
14	Termination	3	Male	38	SV - Blotted Weight	Out of Range	<
14	Termination	3	Male	39	DLP Gland Weight	Out of Range	<
14	Termination	3	Male	40	DLP Gland Weight	Out of Range	<
14	Termination	3	Male	41	DLP Gland Weight	Out of Range	<
14	Termination	3	Male	41	SV - Blotted Weight	Out of Range	<
14	Termination	3	Male	42	DLP Gland Weight	Out of Range	<
14	Termination	3	Male	42	SV - Blotted Weight	Out of Range	>
14	Termination	3	Male	43	SV - Blotted Weight	Out of Range	<
14	Termination	3	Male	44	DLP Gland Weight	Out of Range	<
14	Termination	3	Male	44	Thyroid Weight (EDSP	Out of Range	>
17	Termination	3	Male	45	DLP Gland Weight	Out of Range	<
17	Termination	3	Male	45	SV - Blotted Weight	Out of Range	<
17	Termination	3	Male	46	DLP Gland Weight	Out of Range	<
17	Termination	3	Male	47	DLP Gland Weight	Out of Range	<
17	Termination	3	Male	48	DLP Gland Weight	Out of Range	<
17	Termination	3	Male	48	SV - Blotted Weight	Out of Range	<
19	Termination	4	Male	49	Pituitary Gland Weight	Out of Range	>
19	Termination	4	Male	50	Pituitary Gland Weight	Out of Range	<
19	Termination	4	Male	51	Pituitary Gland Weight	Out of Range	<
19	Termination	4	Male	53	Adrenal Gland Weight	Out of Range	<
19	Termination	4	Male	55	Adrenal Gland Weight	Out of Range	<
19	Termination	4	Male	56	Adrenal Gland Weight	Out of Range	<
19	Termination	4	Male	58	Pituitary Gland Weight	Out of Range	<
19	Termination	4	Male	60	Adrenal Gland Weight	Out of Range	<
20	Termination	4	Male	49	DLP Gland Weight	Out of Range	<
20	Termination	4	Male	50	DLP Gland Weight	Out of Range	<
20	Termination	4	Male	50	VP Gland Weight	Out of Range	>
20	Termination	4	Male	52	DLP Gland Weight	Out of Range	<

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
20	Termination	4	Male	54	DLP Gland Weight	Out of Range	<
20	Termination	4	Male	54	SV - Blotted Weight	Out of Range	<
20	Termination	4	Male	55	DLP Gland Weight	Out of Range	<
20	Termination	4	Male	57	DLP Gland Weight	Out of Range	<
20	Termination	4	Male	57	SV - Blotted Weight	Out of Range	<
20	Termination	4	Male	58	SV - Blotted Weight	Out of Range	<
20	Termination	4	Male	59	DLP Gland Weight	Out of Range	<
20	Termination	4	Male	59	SV - Blotted Weight	Out of Range	<
20	Termination	4	Male	60	DLP Gland Weight	Out of Range	<
22	Termination	4	Male	62	Adrenal Gland Weight	Out of Range	<
22	Termination	4	Male	64	Adrenal Gland Weight	Out of Range	<
23	Termination	4	Male	61	DLP Gland Weight	Out of Range	<
23	Termination	4	Male	62	DLP Gland Weight	Out of Range	<
23	Termination	4	Male	62	SV - Blotted Weight	Out of Range	<
23	Termination	4	Male	63	DLP Gland Weight	Out of Range	<
23	Termination	4	Male	63	VP Gland Weight	Out of Range	>
23	Termination	4	Male	63	Thyroid Weight (EDSP	Out of Range	>
23	Termination	4	Male	64	DLP Gland Weight	Out of Range	<
23	Termination	4	Male	64	SV - Blotted Weight	Out of Range	<
25	Termination	5	Male	65	Pituitary Gland Weight	Out of Range	<
25	Termination	5	Male	66	Adrenal Gland Weight	Out of Range	<
25	Termination	5	Male	66	Pituitary Gland Weight	Out of Range	<
25	Termination	5	Male	70	Adrenal Gland Weight	Out of Range	<
25	Termination	5	Male	71	Pituitary Gland Weight	Out of Range	<
25	Termination	5	Male	74	Adrenal Gland Weight	Out of Range	<
25	Termination	5	Male	74	Pituitary Gland Weight	Out of Range	<
25	Termination	5	Male	76	Adrenal Gland Weight	Out of Range	<
26	Termination	5	Male	65	DLP Gland Weight	Out of Range	<
26	Termination	5	Male	65	SV - Blotted Weight	Out of Range	<
26	Termination	5	Male	66	DLP Gland Weight	Out of Range	<



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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

Comments and Markers

<u>Page</u>	<u>Day</u>	<u>Group</u>	<u>Sex</u>	<u>Subject</u>	<u>Measurement</u>	<u>Type</u>	<u>Marker</u>
26	Termination	5	Male	66	SV - Blotted Weight	Out of Range	<
26	Termination	5	Male	67	DLP Gland Weight	Out of Range	<
26	Termination	5	Male	68	DLP Gland Weight	Out of Range	<
26	Termination	5	Male	68	SV - Blotted Weight	Out of Range	<
26	Termination	5	Male	69	DLP Gland Weight	Out of Range	<
26	Termination	5	Male	70	DLP Gland Weight	Out of Range	<
26	Termination	5	Male	70	SV - Blotted Weight	Out of Range	<
26	Termination	5	Male	71	DLP Gland Weight	Out of Range	<
26	Termination	5	Male	72	DLP Gland Weight	Out of Range	<
26	Termination	5	Male	72	SV - Blotted Weight	Out of Range	>
26	Termination	5	Male	74	DLP Gland Weight	Out of Range	<
26	Termination	5	Male	74	SV - Blotted Weight	Out of Range	<
28	Termination	5	Male	80	Adrenal Gland Weight	Out of Range	<
29	Termination	5	Male	77	DLP Gland Weight	Out of Range	<
29	Termination	5	Male	78	SV - Blotted Weight	Out of Range	<
29	Termination	5	Male	79	SV - Blotted Weight	Out of Range	<
29	Termination	5	Male	80	DLP Gland Weight	Out of Range	<
29	Termination	5	Male	80	SV - Blotted Weight	Out of Range	<

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

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Key Page

**General Footnotes**

Adrenal weights for animals 12 and 66 were not used in statistical analysis  
Pituitary weights from animals 49 and 71 were not used for statistical analysis

**Measurement Descriptions**

<u>Headings Used</u>	<u>Description</u>
Adrenal Gland Weight	Adrenal Glands (Both) Weight
Epididymis Right Weight	Epididymis - Right Weight
Epididymis Left Weight	Epididymis - Left Weight
Kidneys Weight	Kidneys Weight
LABC Weight	LABC Muscle Weight
Liver Weight	Liver Weight
Pituitary Gland Weight	Pituitary Glands Weight
DLP Gland Weight	Prostate Gland, Dorsolateral Weight
VP Gland Weight	Prostate Gland, Ventral Weight
SV Weight	Seminal Vesicles Weight
SV - Blotted Weight	Seminal Vesicles - Blotted Weight
Right Testis Weight	Testis Weight- Right
Left Testis Weight	Testis Weight - Left
Thyroid Weight (EDSP)	Thyroid Weight (EDSP)
Adrenals Wt Ratio	Adrenals/Terminal Bodyweight Ratio
Kidney Wt Ratio	Kidney/Terminal Bodyweight Ratio
Liver Wt Ratio	Liver/Terminal Bodyweight Ratio
Pituitary Wt Ratio	Pituitary/Terminal Bodyweight Ratio

**Unit Descriptions**

<u>Headings Used</u>	<u>Description</u>
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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

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**Unit Descriptions (Continued)**

<u>Headings Used</u>	<u>Description</u>
%	%
g	g

**Time-Points/Ranges**

<u>Measurement</u>	<u>From</u>	<u>To</u>	<u>Report As</u>
Adrenal Gland Weight	-9,999	9,999	Termination
Epididymis Right Weight	-9,999	9,999	Termination
Epididymis Left Weight	-9,999	9,999	Termination
Kidneys Weight	-9,999	9,999	Termination
LABC Weight	-9,999	9,999	Termination
Liver Weight	-9,999	9,999	Termination
Pituitary Gland Weight	-9,999	9,999	Termination
DLP Gland Weight	-9,999	9,999	Termination
VP Gland Weight	-9,999	9,999	Termination
SV Weight	-9,999	9,999	Termination
SV - Blotted Weight	-9,999	9,999	Termination
Right Testis Weight	-9,999	9,999	Termination
Left Testis Weight	-9,999	9,999	Termination
Thyroid Weight (EDSP)	-9,999	9,999	Termination
Adrenals Wt Ratio	-9,999	9,999	Termination
Kidney Wt Ratio	-9,999	9,999	Termination
Liver Wt Ratio	-9,999	9,999	Termination
Pituitary Wt Ratio	-9,999	9,999	Termination

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

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Key Page

**Measurement/Statistics**

<u>Measurement</u>	<u>Descriptive</u>
Adrenal Gland Weight	Mean
	Standard Deviation
	Count (N)
Epididymis Right Weight	Mean
	Standard Deviation
	Count (N)
Epididymis Left Weight	Mean
	Standard Deviation
	Count (N)
Kidneys Weight	Mean
	Standard Deviation
	Count (N)
LABC Weight	Mean
	Standard Deviation
	Count (N)
Liver Weight	Mean
	Standard Deviation
	Count (N)
Pituitary Gland Weight	Mean
	Standard Deviation
	Count (N)
DLP Gland Weight	Mean
	Standard Deviation
	Count (N)
VP Gland Weight	Mean
	Standard Deviation
	Count (N)

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

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Key Page

**Measurement/Statistics (Continued)**

<u>Measurement</u>	<u>Descriptive</u>
SV Weight	Mean
	Standard Deviation
	Count (N)
SV - Blotted Weight	Mean
	Standard Deviation
	Count (N)
Right Testis Weight	Mean
	Standard Deviation
	Count (N)
Left Testis Weight	Mean
	Standard Deviation
	Count (N)
Thyroid Weight (EDSP	Mean
	Standard Deviation
	Count (N)
Adrenals Wt Ratio	Mean
	Standard Deviation
	Count (N)
Kidney Wt Ratio	Mean
	Standard Deviation
	Count (N)
Liver Wt Ratio	Mean
	Standard Deviation
	Count (N)
Pituitary Wt Ratio	Mean
	Standard Deviation
	Count (N)

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Generalised Results - Animals by Mixed Parameter / Time

10005.0102 - Pubertal Development and Thyroid Function in Intact  
Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

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Key Page

**Group Information**

<u>Short Name</u>	<u>Long Name</u>	<u>Report Headings 1-4</u>		
1	1	0	mg/kg/day	2-EHP
2	2	250	mg/kg/day	2-EHP
3	3	500	mg/kg/day	2-EHP
4	4	750	mg/kg/day	2-EHP
5	5	1000	mg/kg/day	2-EHP

**Comment Abbreviations**

RC = Result Comment

# APPENDIX V: Individual Animal Clinical Pathology Data

Group No.:	Animal No.:	Treatment/Dose Level	NA+ (MEQ/L)	K+ (MEQ/L)	CL- (MEQ/L)	Calcium (mg/dL)	Phosphorous (mg/dL)	AST (U/L)	ALT (U/L)	GGT (U/L)	ALP (U/L)	BUN (mg/dL)	Creatinine (mg/dL)	Total Bilirubin (mg/dL)	SDH (U/L)	Total Protein (g/dL)	Albumin (g/dL)
1	01	2-Ethylhexyl Paraben (0 mg/kg/day)	141	10.0	103	10.9	10.3	203	80	0	518	16	0.2	0.3	11.0	5.5	3.0
1	02		140	10.0	103	10.7	10.4	212	72	0	639	14	0.2	0.2	10.8	5.7	3.1
1	03		Sample not submitted due to processing unforeseen circumstance														
1	04		146	9.2	102	11.2	13.2	151	60	0	483	15	0.3	0.2	14.9	5.5	3.0
1	05		142	8.2	102	11.2	9.8	137	50	0	602	13	0.2	0.2	10.3	5.5	2.8
1	06		142	9.1	104	10.7	9.8	143	52	0	434	11	0.2	0.2	12.7	5.4	3.0
1	07		143	8.3	103	11.5	10.3	184	62	0	591	11	0.2	0.2	12.6	5.8	3.0
1	08		142	8.1	100	11.0	10.2	184	73	0	243	10	0.2	0.2	17.8	5.7	2.6
1	09		138	9.0	101	10.1	9.1	181	72	0	534	15	0.2	0.2	11.2	5.0	2.7
1	10		142	9.2	105	10.4	8.8	184	73	0	527	14	0.2	0.2	11.3	5.4	3.0
1	11		144	9.3	100	11.6	10.5	136	65	0	448	19	0.2	0.2	21.9	6.5	3.1
1	12		142	9.9	105	10.6	9.8	305	75	0	531	12	0.2	0.2	8.6	5.5	3.0
1	13		142	9.1	101	11.6	9.8	182	75	0	415	17	0.2	0.2	13.0	6.1	3.2
1	14		141	8.4	102	10.5	9.4	193	56	0	358	14	0.2	0.2	7.4	5.4	2.8
1	15		141	8.8	102	11.1	9.3	197	74	0	628	10	0.2	0.2	7.7	5.5	2.9
1	16		144	8.3	105	10.9	9.5	218	61	0	661	10	0.2	0.2	8.6	5.3	2.8
Mean			142	9.0	103	10.9	10.0	187	67	0	507	13	0.2	0.2	12.0	5.6	2.9
SD			2	0.6	2	0.4	1.0	42	9	0	114	3	0.0	0.0	3.9	0.4	0.2
CV			1	7.2	2	4.1	10.1	22	14	-	23	20	12.5	12.5	32.4	6.3	5.6



Group No.:	Animal No.:	Treatment/Dose Level	NA+ (MEQ/L)	K+ (MEQ/L)	CL- (MEQ/L)	Calcium (mg/dL)	Phosphorous (mg/dL)	AST (U/L)	ALT (U/L)	GGT (U/L)	ALP (U/L)	BUN (mg/dL)	Creatinine (mg/dL)	Total Bilirubin (mg/dL)	SDH (U/L)	Total Protein (g/dL)	Albumin (g/dL)	
2	17	2-Ethylhexyl Paraben (250 mg/kg/day)	142	10.0	102	11.1	9.6	229	82	0	558	15	0.2	0.2	18.8	6.2	3.2	
2	18		142	9.9	107	11.1	9.9	236	87	0	541	11	0.3	0.2	12.2	6.1	3.2	
2	19		141	8.5	103	11.0	10.0	141	66	0	429	13	0.2	0.2	11.4	5.4	2.9	
2	20		145	8.0	104	11.4	10.6	124	66	0	565	10	0.2	0.2	9.3	5.8	3.1	
2	21		146	8.6	102	11.1	10.0	156	57	0	386	12	0.3	0.2	10.3	5.5	2.9	
2	22		145	8.5	104	11.1	10.3	171	69	0	494	10	0.2	0.2	8.0	5.6	3.0	
2	23		145	8.3	106	11.0	9.2	196	78	0	311	9	0.2	0.2	8.8	5.2	2.5	
2	24		143	7.9	105	10.7	9.5	175	63	0	563	12	0.2	0.2	6.7	5.4	2.9	
2	25		141	9.6	102	10.6	8.1	194	66	0	419	13	0.2	0.2	9.9	5.7	3.0	
2	26		Sample not submitted due to processing unforeseen circumstance															
2	27		143	9.3	105	10.7	9.9	149	65	0	536	11	0.2	0.2	7.3	5.5	3.0	
2	28		142	9.1	102	11.0	9.4	121	54	0	439	11	0.2	0.2	10.1	5.5	2.9	
2	29		142	8.3	104	10.7	9.0	132	65	0	566	9	0.2	0.3	9.6	5.2	2.9	
2	30		143	8.5	103	10.7	11.2	219	82	0	636	9	0.2	0.2	9.4	5.3	2.9	
2	31		142	8.6	103	11.0	10.2	153	60	0	418	12	0.2	0.2	8.0	5.6	2.9	
2	32	141	8.2	105	10.7	9.3	224	66	0	737	9	0.2	0.2	6.3	5.6	3.1		
			Mean	143	8.8	104	10.9	9.7	175	68	0	507	11	0.2	0.2	9.7	5.6	3.0
			SD	2	0.7	2	0.2	0.7	39	10	0	108	2	0.0	0.0	3.0	0.3	0.2
			CV	1	7.6	2	2.1	7.5	23	14	-	21	16	16.5	12.5	30.8	5.2	5.7

Group No.:	Animal No.:	Treatment/Dose Level	NA+ (MEQ/L)	K+ (MEQ/L)	CL- (MEQ/L)	Calcium (mg/dL)	Phosphorous (mg/dL)	AST (U/L)	ALT (U/L)	GGT (U/L)	ALP (U/L)	BUN (mg/dL)	Creatinine (mg/dL)	Total Bilirubin (mg/dL)	SDH (U/L)	Total Protein (g/dL)	Albumin (g/dL)
3	33	2-Ethylhexyl Paraben (500 mg/kg/day)	142	10.0	104	10.6	9.6	229	70	0	488	11	0.3	0.2	8.3	5.4	3.0
3	34		143	9.2	106	10.7	9.9	201	87	0	468	11	0.2	0.2	7.8	5.6	3.0
3	35		Sample not submitted due to processing unforeseen circumstance														
3	36		142	8.0	105	10.7	9.5	172	66	0	631	7	0.2	0.3	6.8	5.3	3.0
3	37		145	9.4	107	10.9	8.7	211	95	0	340	9	0.3	0.2	8.2	5.6	2.6
3	38		143	8.4	107	10.4	10.2	164	75	0	462	5	0.2	0.2	13.6	5.6	3.0
3	39		145	7.5	105	10.9	9.8	170	95	0	546	9	0.2	0.2	11.0	5.2	2.8
3	40		145	8.5	105	10.9	10.8	216	85	0	394	8	0.2	0.2	11.3	5.4	2.9
3	41		143	9.5	104	10.5	9.2	148	91	0	680	13	0.2	0.2	9.4	5.1	2.9
3	42		142	9.1	106	10.4	9.0	154	83	0	745	13	0.2	0.2	9.5	5.5	3.0
3	43		141	9.4	107	10.6	9.7	157	73	0	504	12	0.2	0.2	6.4	5.1	2.8
3	44		140	7.6	104	10.3	8.7	123	69	0	351	8	0.2	0.2	7.9	5.6	3.0
3	45		147	9.5	106	11.3	9.2	267	97	0	519	9	0.2	0.3	15.6	5.6	3.1
3	46		143	8.3	104	11.1	10.4	101	67	0	707	11	0.2	0.2	10.8	6.0	3.1
3	47		142	8.3	103	10.6	9.2	190	78	0	465	7	0.2	0.2	12.5	5.5	3.0
3	48		142	6.9	105	10.1	7.7	122	59	0	579	5	0.2	0.2	7.6	5.3	2.8
Mean			143	8.6	105	10.7	9.4	175	79	0	525	9	0.2	0.2	9.8	5.5	2.9
SD			2	0.9	1	0.3	0.8	45	12	0	124	3	0.0	0.0	2.6	0.2	0.1
CV			1	10.3	1	3.0	8.1	26	15	-	24	28	16.5	16.5	27.1	4.3	4.6

Group No.:	Animal No.:	Treatment/Dose Level	NA+ (MEQ/L)	K+ (MEQ/L)	CL- (MEQ/L)	Calcium (mg/dL)	Phosphorous (mg/dL)	AST (U/L)	ALT (U/L)	GGT (U/L)	ALP (U/L)	BUN (mg/dL)	Creatinine (mg/dL)	Total Bilirubin (mg/dL)	SDH (U/L)	Total Protein (g/dL)	Albumin (g/dL)
4	49	2-Ethylhexyl Paraben (750 mg/kg/day)	139	9.6	105	10.2	9.4	250	94	0	494	8	0.2	0.2	10.8	5.2	2.9
4	50		141	9.5	102	10.2	9.2	226	80	0	399	9	0.2	0.2	19.0	5.4	3.0
4	51		141	9.3	105	10.8	9.3	179	79	0	526	9	0.2	0.2	9.9	5.6	3.0
4	52		143	9.6	105	10.6	10.2	173	83	0	554	10	0.2	0.2	8.7	5.5	3.0
4	53		142	9.0	104	11.1	9.8	214	79	0	501	8	0.3	0.2	11.8	6.2	3.2
4	54		143	9.3	104	11.0	10.8	177	73	0	343	8	0.2	0.2	17.3	6.2	3.1
4	55		141	8.0	106	10.2	9.8	296	96	0	401	5	0.2	0.2	19.6	5.4	2.8
4	56		142	7.9	107	10.1	9.7	193	79	0	448	5	0.2	0.2	13.1	5.2	2.8
4	57		142	8.4	101	10.5	8.3	173	76	0	654	8	0.2	0.2	14.9	5.3	2.9
4	58		144	8.1	105	10.2	9.1	153	87	0	581	8	0.2	0.2	8.3	5.1	2.8
4	59		142	8.1	105	10.0	8.7	181	79	0	462	7	0.2	0.2	9.0	5.2	2.9
4	60		140	7.8	105	10.3	9.4	152	90	0	511	7	0.2	0.2	7.1	5.5	2.9
4	61		143	8.6	100	10.7	9.0	193	61	0	406	10	0.2	0.2	8.4	5.6	2.9
4	62		145	9.2	106	10.8	9.1	152	74	0	319	10	0.3	0.2	9.5	5.7	3.0
4	63		143	7.8	103	10.9	9.7	130	75	0	404	10	0.2	0.2	8.1	5.5	3.0
4	64		146	7.6	108	10.8	9.7	177	70	0	586	3	0.2	0.2	6.5	5.5	2.9
Mean			142	8.6	104	10.5	9.5	189	80	0	474	8	0.2	0.2	11.4	5.5	2.9
SD			2	0.7	2	0.4	0.6	41	9	0	93	2	0.0	0.0	4.2	0.3	0.1
CV			1	8.5	2	3.4	6.3	22	11	-	20	26	16.1	0.0	37.0	5.8	3.7

Group No.:	Animal No.:	Treatment/Dose Level	NA+ (MEQ/L)	K+ (MEQ/L)	CL- (MEQ/L)	Calcium (mg/dL)	Phosphorous (mg/dL)	AST (U/L)	ALT (U/L)	GGT (U/L)	ALP (U/L)	BUN (mg/dL)	Creatinine (mg/dL)	Total Bilirubin (mg/dL)	SDH (U/L)	Total Protein (g/dL)	Albumin (g/dL)
5	65	2-Ethylhexyl Paraben (1000 mg/kg/day)	142	9.0	106	10.8	9.0	202	100	0	452	11	0.2	0.2	6.7	5.4	3.0
5	66		143	8.3	105	10.2	8.2	203	105	0	628	9	0.2	0.2	5.4	5.4	2.9
5	67		142	8.7	104	10.8	10.1	188	65	0	602	9	0.2	0.2	7.2	5.4	2.8
5	68		144	8.7	109	10.1	9.0	188	95	0	474	5	0.2	0.2	9.2	5.1	2.8
5	69		142	8.0	104	10.5	9.7	204	84	0	459	8	0.2	0.2	6.4	5.4	2.9
5	70		144	7.9	106	10.5	10.4	153	72	0	378	7	0.2	0.2	9.9	5.5	3.0
5	71		145	7.9	105	10.9	9.6	194	79	0	532	6	0.2	0.2	13.2	5.2	2.7
5	72		148	9.0	106	10.6	10.1	123	77	0	345	6	0.2	0.2	10.4	5.6	3.0
5	73		145	9.4	107	10.3	9.0	117	63	0	349	12	0.2	0.2	7.3	5.4	2.8
5	74		147	7.9	107	10.0	7.9	132	93	0	378	9	0.2	0.2	6.0	5.6	3.1
5	75		143	9.0	106	10.4	9.1	222	96	0	390	8	0.3	0.1	11.7	5.3	2.8
5	76		130	UDDH	99	11.5	12.3	363	90	UDDH	240	9	0.3	0.8	UDDH	7.1	3.6
5	77		145	7.7	108	10.0	8.9	134	103	0	438	8	0.2	0.2	8.6	4.9	2.7
5	78		143	8.6	105	10.6	9.6	172	87	0	616	7	0.2	0.2	7.2	5.7	3.0
5	79		143	7.4	105	10.5	9.3	130	74	0	392	5	0.2	0.2	6.0	5.4	3.0
5	80		145	7.7	106	10.5	9.3	186	99	0	411	5	0.2	0.2	7.9	5.4	2.9
Mean			144	8.3	106	10.4	9.3	170	86	0	456	8	0.2	0.2	8.2	5.4	2.9
SD			2	0.6	1	0.3	0.7	35	14	0	96	2	0.0	0.0	2.3	0.2	0.1
CV			1	7.3	1	2.7	7.3	21	16	-	21	28	12.5	13.4	27.6	3.7	4.2

Results from animal 76 not used for descriptive statistics or statistical analyses due to hemolysis.

Abbreviation: UDDH - unable to determine due to hemolysis

Group No.:	Animal No.:	Treatment/Dose Level	T <sub>4</sub> (µg/dL)	TSH (ng/mL)	T (ng/mL)
1	01	2-Ethylhexyl Paraben (0 mg/kg)	4.85	3.14	2.27
1	02		4.07	4.20	3.12
1	03		*	*	*
1	04		3.28	2.87	1.13
1	05		4.98	2.17	0.54
1	06		5.14	2.97	1.27
1	07		5.55	1.90	0.56
1	08		6.58	4.59	0.39
1	09		4.05	1.07	2.38
1	10		5.42	2.07	1.18
1	11		4.33	2.60	1.15
1	12		5.26	3.02	1.49
1	13		5.77	6.49	0.92
1	14		3.41	3.44	1.69
1	15		4.05	1.27	0.40
1	16		3.96	4.98	0.93
		Mean	4.71	3.12	1.29
		SD	0.93	1.46	0.79
		CV	19.8	46.7	61.0

\*Sample not processed due to unforeseen circumstance

Group No.:	Animal No.:	Treatment/Dose Level	T <sub>4</sub> (µg/dL)	TSH (ng/mL)	T (ng/mL)
2	17	2-Ethylhexyl Paraben (250 mg/kg)	6.49	3.40	1.68
2	18		6.92	1.78	1.37
2	19		4.75	4.45	0.91
2	20		4.06	3.31	1.02
2	21		4.06	0.92	1.24
2	22		7.20	2.04	0.97
2	23		5.18	1.52	0.24
2	24		5.68	1.18	0.16
2	25		5.51	2.84	0.64
2	26		*	*	*
2	27		4.12	4.93	1.30
2	28		4.00	2.71	1.22
2	29		4.69	3.85	0.15
2	30		4.01	1.03	0.12
2	31		4.71	1.89	0.26
2	32		5.98	1.65	0.71
		Mean	5.16	2.50	0.80
		SD	1.10	1.26	0.52
		CV	21.3	50.5	64.7

\*Sample not processed due to unforeseen circumstance

Group No.:	Animal No.:	Treatment/Dose Level	T <sub>4</sub> (µg/dL)	TSH (ng/mL)	T (ng/mL)
3	33	2-Ethylhexyl Paraben (500 mg/kg)	5.54	2.69	1.16
3	34		5.45	6.65	1.63
3	35		*	*	*
3	36		4.78	4.85	0.28
3	37		6.72	0.94	0.03
3	38		4.38	0.78	0.52
3	39		4.24	1.89	1.01
3	40		4.06	2.27	1.96
3	41		3.36	1.68	3.22
3	42		4.26	6.52	1.83
3	43		4.19	1.91	1.55
3	44		5.39	2.23	0.75
3	45		4.17	1.86	0.18
3	46		4.83	1.34	0.14
3	47		3.14	0.53	0.29
3	48		2.83	0.68	0.21
		Mean	4.49	2.45	0.98
		SD	1.01	1.98	0.91
		CV	22.6	80.6	92.3

\*Sample not processed due to unforeseen circumstance

Group No.:	Animal No.:	Treatment/Dose Level	T <sub>4</sub> (µg/dL)	TSH (ng/mL)	T (ng/mL)
4	49	2-Ethylhexyl Paraben (750 mg/kg)	4.27	3.90	2.23
4	50		3.27	2.83	1.94
4	51		3.58	2.61	0.41
4	52		4.42	2.14	0.63
4	53		5.27	2.36	0.68
4	54		5.08	0.81	0.36
4	55		4.18	0.86	0.60
4	56		3.84	1.34	1.93
4	57		3.70	1.76	0.86
4	58		3.52	3.72	0.36
4	59		3.11	1.15	0.31
4	60		4.31	1.68	0.51
4	61		5.15	6.30	1.44
4	62		4.78	1.68	0.31
4	63		7.12	1.05	0.65
4	64		3.74	1.47	0.14
		Mean	4.33	2.23	0.84
		SD	1.00	1.43	0.67
		CV	23.0	64.2	79.8

Group No.:	Animal No.:	Treatment/Dose Level	T <sub>4</sub> (µg/dL)	TSH (ng/mL)	T (ng/mL)
5	65	2-Ethylhexyl Paraben (1000 mg/kg)	4.10	1.09	0.79
5	66		3.81	3.08	0.69
5	67		3.46	2.07	0.28
5	68		3.32	1.13	0.41
5	69		3.63	1.34	0.29
5	70		3.03	0.68	0.39
5	71		4.29	0.80	1.26
5	72		4.90	1.56	0.70
5	73		5.76	2.60	0.76
5	74		4.79	3.34	1.18
5	75		4.75	1.35	0.40
5	76		2.88	2.49	0.10
5	77		3.77	1.02	0.28
5	78		5.04	0.80	0.23
5	79		4.56	0.83	1.07
5	80		4.30	1.14	0.60
		Mean	4.23	1.52	0.62
		SD	0.75	0.85	0.34
		CV	17.6	56.2	54.7

Results from animal 76 not used for descriptive statistics or statistical analyses due to hemolysis.

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**Quality Assurance Statement (QAS)**

**CONFIDENTIAL**

The study listed below has been inspected and the raw data and report(s) have been audited by the Quality Assurance (QA) Unit of Antech Diagnostics GLP in accordance with United States Environmental Protection Agency (EPA) Good Laboratory Practices (GLP) principles for non-clinical laboratory studies. The reported results accurately reflect the raw data of the study.

To: Integrated Laboratory Systems, Inc.

Protocol referenced: Pubertal Development and Thyroid Function  
in Intact Juvenile/Peripubertal Male Rats;  
2-Ethylhexyl Paraben (10005.0102)

Study Director: Jeffrey P. Davis (and Study Director's  
Management)

Principal Management (PI  
(and PI's Management): Charles Walker (David Brown)

From Quality Assurance Auditor: John Murphy

Timeperiod(s)	Material audited	Inspection date(s)	SD and SD's Management notified (Date sent)	PI and PI's Management notified (Date sent)	Auditor's Initials
0900-1300	Study Data	5/5/16	5/6/16	5/6/16	JEM

Printed Name: John Murphy

Signature: John Murphy Date: May 6, 2016

Title: QA Auditor

Initials: KP = Katie Powell JEM = John Murphy



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**Good Laboratory Practices Statement**

Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats;  
2-Ethylhexyl Paraben

Study Number: 10005.0102

Timeperiod	Collection Date	Date Samples were received (at Antech Diagnostics)	Date Samples were analyzed
0900-1300	3/21/16	3/28/16	3/28/16
0900-1300	3/22/16	3/28/16	3/28/16

Study Activities at Antech Diagnostics:

Start Date: 3/28/16

Completion Date of Analysis: 3/28/16

As Principal Investigator, I confirm that the clinical pathology portion of this study performed at Antech Diagnostics was in compliance with U.S. Environmental Protection Act (U.S. EPA) Good Laboratory Practice (GLP) Regulations (40 CFR Part 160).

Charles Walker  
Charles Walker, Laboratory Supervisor

06 MAY 16  
Date



200 Girard Street, Suite 200, Gaithersburg, MD 20877  
301-921-0168 Fax: 301-977-0433

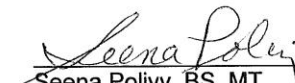
### PRINCIPAL INVESTIGATOR'S STATEMENT

**Study Title: Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben**

**ILS Study No.: 10005.0102**

**Study Director: Jeffrey P. Davis, B.S., LATG**

Sample analyses for this study were conducted in compliance with the requirements of the U.S. Environmental Protection Agency's (EPA) Good Laboratory Practice Standards for Nonclinical Studies, 40 CFR Part 160.

  
Seena Polivy, BS, MT  
Principal Investigator  
Ani-Lytics, Inc.

5/4/16  
Date



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### QUALITY ASSURANCE STATEMENT

Study No.: 10005.0102

Client ID: ILS

Client No.: 1824

Sample analysis for this study was audited according to the standard operating procedures of the Quality Assurance Unit of Ani Lytics, Inc., the study protocol and FDA (and/or OECD, MHLW, EPA) Good Laboratory Practice Standards regulations. The dates that the audits were performed and findings were reported are listed below.

Phases Inspected	Dates Inspected	Audit Report Number	Date findings reported to PI and test site management	Date findings reported to SD, Lead QAU and test facility management
Sample analysis	4/14/16	16-014	4/14/16	5/04/16
Data/Tables audit	4/26/16	16-023	4/26/16	5/04/16

Eva Zurek  
Eva Zurek, RQAP-GLP  
QAU Consultant  
Ani Lytics, Inc.

5/04/16  
Date

# APPENDIX VI: Histopathology Report and Peer Review Statement



## **PATHOLOGY REPORT**

### **Study Title**

**Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben**

### **ILS Project-Study Number**

**10005.0102**

### **Study Director**

**Jeffrey Davis, BS, LATG**

### **Study Pathologist**

**Rebecca R. Moore, DVM, DACVP**

### **Performing Laboratory**

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

### **Date of Submission**

**July 28, 2016**

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2-EHP = 2-Ethylhexyl Paraben

## OBJECTIVE

The objective of the pathology portion of this study was to identify the potential histologic effects of 2-ethylhexyl paraben on select endocrine and reproductive organs of intact juvenile/peripubertal male rats. The thyroid gland, left testis, left epididymis, left kidney, and mammary gland were evaluated microscopically following oral administration of 2-ethylhexyl paraben to male rats on postnatal days 23-53/54.

## STUDY DESIGN AND METHODS

The details of the experimental design are presented in Table 1.

**Table 1: Study Design**

Group	Animal			Test Substance / Control	Test Substance Dose Level (mg/kg/day)
	Number	Sex	Identification		
1	16	Male	01 - 16	Corn Oil (Vehicle)	0
2	16	Male	17 - 32	2-Ethylhexyl Paraben	250
3	16	Male	33 - 48	2-Ethylhexyl Paraben	500
4	16	Male	49 - 64	2-Ethylhexyl Paraben	750
5	16	Male	65 - 80	2-Ethylhexyl Paraben	1000

Eighty male Sprague Dawley CRL:CD(SD) IGS rats were administered one of four dose levels of 2-ethylhexyl paraben or corn oil (vehicle control) for 31 or 32 consecutive days via oral gavage beginning on postnatal day 23.

The left testis, left epididymis, thyroid gland, left kidney, and mammary gland were excised and fixed in 10% neutral buffered formalin for at least 24 hours. After fixation, tissues were washed in 70% histology grade alcohol, processed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). Mammary gland whole mounts were prepared as described in *Study Specific Procedure No. 1*. The tissues were then evaluated microscopically for potential 2-ethylhexyl paraben-related effects.

Two serial sections of each of the two lobes of the thyroid gland from each animal were subjectively assessed for follicular cell height and colloid area using a five point grading scale (1=shortest follicular cell height/least colloid area; 5=tallest follicular cell height/largest colloid area) (Capen and Martin, 1989; U.S. EPA, 2009).

Histologic evaluation of the left testis and left epididymis (including caput, corpus, and cauda) was guided by the U.S. EPA's Health Effects Test Guideline OPPTS 870.3800: Reproduction and Fertility Effects (U.S. EPA, 1998).

Mammary gland development was assessed by the microscopic evaluation of a single section of hematoxylin & eosin stained mammary gland as well as one mammary gland whole mount preparation. Mammary gland whole mount preparations were examined for lateral growth (width of



the gland), longitudinal growth, the number of primary ducts arising from the nipple, budding (branch density), lateral branching, and the number of terminal end buds (Elmore et al., 2016). Each whole mount preparation was assigned a developmental score ranging from 1 to 6. Whole mount sections from control rats were generally graded as 3. Lower grades (< 3) were assigned when glands displayed evidence of delayed development. Higher grades (> 3) were recorded when glands exhibited accelerated development.

For all non-quantitative endpoints, histologic diagnoses were graded using a scale of 1-4 where 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

## **RESULTS**

### **Testis**

There were no 2-ethylhexyl paraben-related alterations in the left testis. Histologic observations in the testis were considered incidental background findings unrelated to the administration of 2-ethylhexyl paraben. Summary data are presented in Appendix 1; individual animal data are in Appendices 2-3.

### **Epididymis**

No microscopic observations related to 2-ethylhexyl paraben administration were noted in the left epididymis. Histologic observations in the epididymis were considered incidental background findings unrelated to the administration of 2-ethylhexyl paraben. Summary data are presented in Appendix 4. Individual animal data are presented in Appendices 5-9.

### **Kidney**

There were no lesions that would indicate direct 2-ethylhexyl paraben-related renal toxicity. Summary data are presented in Appendix 10, and individual animal data are presented in Appendices 11-15.

### **Mammary Gland**

There were no microscopic findings in the mammary gland in this study. Summary data are presented in Appendix 16, and individual animal data are presented in Appendices 17-21. Mammary gland whole mount data are presented in Appendices 22-26.

### **Thyroid gland**

Administration of 2-ethylhexyl paraben was not associated with statistically significant differences in follicular cell height or colloid area in the thyroid glands of male rats compared to concurrent controls. Summary data are presented in Table 2, and individual animal data are presented in Appendices 27-31.

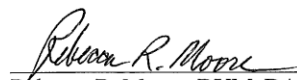
Table 2: Thyroid Gland: Summary of Histologic Findings

	Corn Oil (Vehicle)	2-Ethylhexyl Paraben			
		250 (mg/kg/day)	500 (mg/kg/day)	750 (mg/kg/day)	1000 (mg/kg/day)
No. Examined	16	16	16	16	16
<b>Follicular cell (height)*</b>					
1	7	6	12	10	9
2	6	9	4	5	6
3	3	1	0	1	1
4	0	0	0	0	0
5	0	0	0	0	0
<b>Follicular colloid (area)*</b>					
1	0	0	0	0	0
2	0	0	0	0	0
3	3	1	0	1	1
4	6	9	4	5	6
5	7	6	12	10	9
<b>Colloid, hyper eosinophilic</b>					
Not present	16	16	15	16	16
Minimal	0	0	1	0	0
Mild	0	0	0	0	0
<b>Hemorrhage</b>					
Not present	16	16	15	16	16
Moderate	0	0	1	0	0

\*Two serial sections of both thyroid lobes from each animal were subjectively assessed for follicular cell height and colloid area using a five-point grading scale (1=shortest follicular cell height/least colloid area; 5=tallest follicular cell height/largest colloid area). The two serial sections of thyroid were consistently graded the same

## SUMMARY

Oral (gavage) administration of 0, 250, 750, or 1000 mg/kg/day 2-ethylhexyl paraben for 31 or 32 days to juvenile Sprague Dawley male rats was not associated with histologic alterations in the left testis, left epididymis, left kidney, mammary gland, or thyroid gland.

  
Rebecca R. Moore, DVM, DACVP

  
Date

#### REFERENCES

Capen CC, Martin SL (1989). The effects of xenobiotics on the structure and function of thyroid follicular and C-cells. *Toxicol Pathol.* 17(2): 266-93.

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U.S. EPA (1998). Health Effects Test Guideline OPPTS 870.3800: Reproduction and Fertility Effects. Office of Prevention, Pesticides and Toxic Substances. U.S. EPA, Washington, DC.

U.S. EPA (2009). Endocrine disruptor screening program test guidelines. OPPTS 890.1500: Pubertal development and thyroid function in intact juvenile / peripubertal male rats. 740-C-09-012, Office of Prevention, Pesticides and Toxic Substances. U.S. EPA, Washington, DC.

**Appendix 1: Testis: Summary of Histologic Findings**

	Corn Oil (Vehicle)	2-Ethylhexyl Paraben			
		250 mg/kg/day	500 mg/kg/day	750 mg/kg/day	1000 mg/kg/day
No. Examined	16	16	15	16	16
<b>Seminiferous tubules:</b>					
<b>Giant cells</b>					
Not present	16	15	15	16	16
Minimal	0	1	0	0	0

**Appendix 2: Testis: Individual Male Rat Histology (Control, 250 and 500 mg/kg/day 2-Ethylhexyl Paraben)**

Corn Oil (Vehicle)		250 mg/kg/day 2-Ethylhexyl Paraben			500 mg/kg/day 2-Ethylhexyl Paraben	
Animal ID	Comment	Animal ID	Seminiferous tubules: Giant cells	Comment	Animal ID	Comment
1	Normal	17	—	Normal	33	Normal
2	Normal	18	—	Normal	34	Normal
3	Normal	19	—	Normal	35	Normal
4	Normal	20	—	Normal	36	Normal
5	Normal	21	—	Normal	37	Normal
6	Normal	22	—	Normal	38	Normal
7	Normal	23	—	Normal	39	Normal
8	Normal	24	—	Normal	40	Normal
9	Normal	25	—	Normal	41	Normal
10	Normal	26	—	Normal	42	Normal
11	Normal	27	—	Normal	43	Normal
12	Normal	28	—	Normal	44	Normal
13	Normal	29	—	Normal	45	Normal
14	Normal	30	—	Normal	46	Normal
15	Normal	31	1		47	Missing*
16	Normal	32	—	Normal	48	Normal

— = Not present

1 = Minimal

\* Left testis was not present in wet tissues for histological processing

**Appendix 3: Testis: Individual Male Rat Histology  
(750 and 1000 mg/kg/day 2-Ethylhexyl Paraben)**

750 mg/kg/day 2-Ethylhexyl Paraben		1000 mg/kg/day 2-Ethylhexyl Paraben	
Animal ID	Comment	Animal ID	Comment
49	Normal	65	Normal
50	Normal	66	Normal
51	Normal	67	Normal
52	Normal	68	Normal
53	Normal	69	Normal
54	Normal	70	Normal
55	Normal	71	Normal
56	Normal	72	Normal
57	Normal	73	Normal
58	Normal	74	Normal
59	Normal	75	Normal
60	Normal	76	Normal
61	Normal	77	Normal
62	Normal	78	Normal
63	Normal	79	Normal
64	Normal	80	Normal

**Appendix 4: Epididymis: Summary of Histologic Findings**

	Corn Oil (Vehicle)	2-Ethylhexyl Paraben			
		250 mg/kg/day	500 mg/kg/day	750 mg/kg/day	1000 mg/kg/day
No. Examined	16	16	16	16	16
<b>Inflammation</b>					
Not present	6	7	6	6	5
Minimal	10	9	10	10	11

**Appendix 5: Epididymis: Individual Male Rat Histology  
(Corn Oil, control)**

Animal ID	Inflammation
1	—
2	1
3	—
4	—
5	—
6	1
7	1
8	1
9	1
10	1
11	1
12	1
13	1
14	1
15	—
16	—

— = Not present

1 = Minimal

**Appendix 6: Epididymis: Individual Male Rat Histology  
(250 mg/kg/day 2-Ethylhexyl Paraben)**

Animal ID	Inflammation
17	—
18	1
19	—
20	—
21	1
22	—
23	—
24	1
25	1
26	1
27	—
28	1
29	1
30	1
31	1
32	—

— = Not present

1 = Minimal

**Appendix 7: Epididymis: Individual Male Rat Histology  
(500 mg/kg/day 2-Ethylhexyl Paraben)**

Animal ID	Inflammation
33	1
34	1
35	1
36	1
37	1
38	1
39	1
40	1
41	1
42	—
43	—
44	—
45	1
46	—
47	—
48	—

— = Not present

1 = Minimal

**Appendix 8: Epididymis: Individual Male Rat Histology  
(750 mg/kg/day 2-Ethylhexyl Paraben)**

Animal ID	Inflammation
49	—
50	1
51	1
52	1
53	1
54	1
55	1
56	—
57	—
58	1
59	—
60	1
61	—
62	1
63	1
64	—

— = Not present

1 = Minimal

**Appendix 9: Epididymis: Individual Male Rat Histology  
(1000 mg/kg/day 2-Ethylhexyl Paraben)**

Animal ID	Inflammation
65	—
66	—
67	1
68	1
69	1
70	1
71	—
72	—
73	1
74	1
75	1
76	1
77	1
78	—
79	1
80	1

— = Not present

1 = Minimal



**Appendix 10: Kidney: Summary of Histologic Findings**

	Corn Oil (Vehicle)	2-Ethylhexyl Paraben			
		250 mg/kg/day	500 mg/kg/day	750 mg/kg/day	1000 mg/kg/day
No. Examined	16	16	16	16	16
<b>Renal Tubule Carcinoma</b>					
Not present	16	15	16	16	16
Present	0	1	0	0	0
<b>Nephropathy</b>					
Not present	1	1	0	0	0
Minimal	13	14	15	15	15
Mild	2	1	1	1	1
<b>Hyaline droplets</b>					
Not present	3	1	0	0	2
Minimal	13	15	16	15	14
Mild	0	0	0	1	0
<b>Cyst</b>					
Not present	14	16	15	14	13
Present	2	0	1	2	3

**Appendix 11: Kidney: Individual Male Rat Histology (Corn Oil, control)**

Animal ID	Renal Tubule Carcinoma	Nephropathy	Hyaline Droplets	Cyst
1	—	1	1	—
2	—	1	1	—
3	—	1	1	—
4	—	1	1	—
5	—	1	—	—
6	—	1	1	—
7	—	2	1	P
8	—	2	1	—
9	—	1	1	—
10	—	1	1	P
11	—	1	1	—
12	—	1	1	—
13	—	1	1	—
14	—	1	—	—
15	—	—	—	—
16	—	1	1	—

— = Not present

1 = Minimal

2 = Mild

P = Present

**Appendix 12: Kidney: Individual Male Rat Histology (250 mg/kg/day 2-Ethylhexyl Paraben)**

Animal ID	Renal Tubule Carcinoma	Nephropathy	Hyaline Droplets	Cyst
17	—	1	1	—
18	—	1	1	—
19	—	1	1	—
20	—	1	1	—
21	P	1	1	—
22	—	1	1	—
23	—	1	—	—
24	—	1	1	—
25	—	1	1	—
26	—	1	1	—
27	—	1	1	—
28	—	2	1	—
29	—	1	1	—
30	—	1	1	—
31	—	—	1	—
32	—	1	1	—

— = Not present

1 = Minimal

2 = Mild

P = Present

**Appendix 13: Kidney: Individual Male Rat Histology (500 mg/kg/day 2-Ethylhexyl Paraben)**

Animal ID	Renal Tubule Carcinoma	Nephropathy	Hyaline Droplets	Cyst
33	—	1	1	—
34	—	1	1	—
35	—	1	1	—
36	—	1	1	—
37	—	1	1	—
38	—	1	1	—
39	—	1	1	—
40	—	1	1	—
41	—	2	1	—
42	—	1	1	—
43	—	1	1	P
44	—	1	1	—
45	—	1	1	—
46	—	1	1	—
47	—	1	1	—
48	—	1	1	—

— = Not present  
1 = Minimal  
2 = Mild  
P = Present

**Appendix 14: Kidney: Individual Male Rat Histology (750 mg/kg/day 2-Ethylhexyl Paraben)**

Animal ID	Renal Tubule Carcinoma	Nephropathy	Hyaline Droplets	Cyst
49	—	1	1	—
50	—	1	1	P
51	—	2	1	—
52	—	1	1	—
53	—	1	1	—
54	—	1	1	—
55	—	1	1	—
56	—	1	1	—
57	—	1	1	P
58	—	1	2	—
59	—	1	1	—
60	—	1	1	—
61	—	1	1	—
62	—	1	1	—
63	—	1	1	—
64	—	1	1	—

— = Not present  
1 = Minimal  
2 = Mild  
P = Present

**Appendix 15: Kidney: Individual Male Rat Histology (1000 mg/kg/day 2-Ethylhexyl Paraben)**

Animal ID	Renal Tubule Carcinoma	Nephropathy	Hyaline Droplets	Cyst
65	—	1	1	—
66	—	1	—	—
67	—	1	—	—
68	—	1	1	—
69	—	1	1	—
70	—	2	1	P
71	—	1	1	—
72	—	1	1	—
73	—	1	1	—
74	—	1	1	P
75	—	1	1	—
76	—	1	1	—
77	—	1	1	P
78	—	1	1	—
79	—	1	1	—
80	—	1	1	—

— = Not present  
1 = Minimal  
2 = Mild  
P = Present

**Appendix 16: Mammary Gland: Summary of Histologic Findings**

	Corn Oil (Vehicle)	2-Ethylhexyl Paraben			
		250 mg/kg/day	500 mg/kg/day	750 mg/kg/day	1000 mg/kg/day
No. Examined	16	16	16	16	16
Normal	16	16	16	16	16

**Appendix 17: Mammary Gland: Individual Male Rat Histology  
(Corn Oil, control)**

Animal ID	Comment
1	Normal
2	Normal
3	Normal
4	Normal
5	Normal
6	Normal
7	Normal
8	Normal
9	Normal
10	Normal
11	Normal
12	Normal
13	Normal
14	Normal
15	Normal
16	Normal

**Appendix 18: Mammary Gland: Individual Male Rat Histology  
(250 mg/kg/day 2-Ethylhexyl Paraben)**

Animal ID	Comment
17	Normal
18	Normal
19	Normal
20	Normal
21	Normal
22	Normal
23	Normal
24	Normal
25	Normal
26	Normal
27	Normal
28	Normal
29	Normal
30	Normal
31	Normal
32	Normal

**Appendix 19: Mammary Gland: Individual Male Rat Histology  
(500 mg/kg/day 2-Ethylhexyl Paraben)**

Animal ID	Comment
33	Normal
34	Normal
35	Normal
36	Normal
37	Normal
38	Normal
39	Normal
40	Normal
41	Normal
42	Normal
43	Normal
44	Normal
45	Normal
46	Normal
47	Normal
48	Normal

**Appendix 20: Mammary Gland: Individual Male Rat Histology  
(750 mg/kg/day 2-Ethylhexyl Paraben)**

Animal ID	Comment
49	Normal
50	Normal
51	Normal
52	Normal
53	Normal
54	Normal
55	Normal
56	Normal
57	Normal
58	Normal
59	Normal
60	Normal
61	Normal
62	Normal
63	Normal
64	Normal

**Appendix 21: Mammary Gland: Individual Male Rat Histology  
(1000 mg/kg/day 2-Ethylhexyl Paraben)**

Animal ID	Comment
65	Normal
66	Normal
67	Normal
68	Normal
69	Normal
70	Normal
71	Normal
72	Normal
73	Normal
74	Normal
75	Normal
76	Normal
77	Normal
78	Normal
79	Normal
80	Normal

**Appendix 22: Mammary Gland Whole Mounts  
Individual Male Rat Histology (Corn Oil, Control)**

Animal ID	Score
1	3
2	3
3	3
4	3
5	3
6	3
7	3
8	3
9	3
10	3
11	3
12	3
13	3
14	3
15	3
16	3

3 = normal developmental stage

**Appendix 23: Mammary Gland Whole Mounts  
Individual Male Rat Histology (250 mg/kg/day 2-Ethylhexyl Paraben)**

Animal ID	Score
17	3
18	3
19	3
20	3
21	3
22	3
23	3
24	3
25	3
26	3
27	3
28	3
29	3
30	3
31	3
32	3

3 = normal developmental stage



**Appendix 24: Mammary Gland Whole Mounts  
Individual Male Rat Histology (500 mg/kg/day 2-Ethylhexyl Paraben)**

Animal ID	Score
33	3
34	3
35	3
36	3
37	3
38	3
39	3
40	3
41	3
42	3
43	3
44	3
45	3
46	3
47	3
48	3

3 = normal developmental stage

**Appendix 25: Mammary Gland Whole Mounts  
Individual Male Rat Histology (750 mg/kg/day 2-Ethylhexyl Paraben)**

Animal ID	Score
49	3
50	3
51	3
52	3
53	3
54	3
55	3
56	3
57	3
58	3
59	3
60	3
61	3
62	3
63	3
64	3

3 = normal developmental stage

**Appendix 26: Mammary Gland Whole Mounts**  
**Individual Male Rat Histology (1000 mg/kg/day 2-Ethylhexyl Paraben)**

Animal ID	Score
65	3
66	3
67	3
68	3
69	3
70	3
71	3
72	3
73	3
74	3
75	3
76	3
77	3
78	3
79	3
80	3

3 = normal developmental stage

**Appendix 27: Thyroid Gland: Individual Male Rat Histology (Corn Oil, control)**

Animal ID	Follicular cell (height) <sup>1</sup>	Follicular colloid (area) <sup>1</sup>	Hypereosinophilic Colloid <sup>2</sup>
1	2	4	—
2	3	3	—
3	2	4	—
4	1	5	—
5	3	3	—
6	1	5	—
7	2	4	—
8	2	4	—
9	3	3	—
10	1	5	—
11	2	4	—
12	1	5	—
13	2	4	—
14	1	5	—
15	1	5	—
16	1	5	—

<sup>1</sup>Two serial sections of thyroid gland evaluated, both were graded the same

Follicular cell height and colloid area assessed as 1=shortest follicular cell height/least colloid area to 5=tallest follicular cell height/largest colloid area.

<sup>2</sup> — =not present

**Appendix 28: Thyroid Gland: Individual Male Rat Histology (250 mg/kg/day 2-Ethylhexyl Paraben)**

Animal ID	Follicular cell (height) <sup>1</sup>	Follicular colloid (area) <sup>1</sup>	Hypereosinophilic Colloid <sup>2</sup>
17	1	5	—
18	2	4	—
19	2	4	—
20	1	5	—
21	1	5	—
22	2	4	—
23	2	4	—
24	2	4	—
25	3	3	—
26	2	4	—
27	1	5	—
28	2	4	—
29	2	4	—
30	1	5	—
31	1	5	—
32	2	4	—

<sup>1</sup>Two serial sections of thyroid gland evaluated, both were graded the same

Follicular cell height and colloid area assessed as 1=shortest follicular cell height/least colloid area to 5=tallest follicular cell height/largest colloid area.

<sup>2</sup> — =not present

**Appendix 29: Thyroid Gland: Individual Male Rat Histology (500 mg/kg/day 2-Ethylhexyl Paraben)**

Animal ID	Follicular cell (height) <sup>1</sup>	Follicular colloid (area) <sup>1</sup>	Hypereosinophilic Colloid <sup>2</sup>	Hemorrhage <sup>2</sup>
33	1	5	—	—
34	2	4	—	—
35	1	5	—	—
36	1	5	1	—
37	2	4	—	—
38	1	5	—	—
39	1	5	—	—
40	2	4	—	—
41	1	5	—	—
42	1	5	—	—
43	2	4	—	—
44	1	5	—	—
45	1	5	—	—
46	1	5	—	3
47	1	5	—	—
48	1	5	—	—

<sup>1</sup>Two serial sections of thyroid gland evaluated, both were graded the same  
Follicular cell height and colloid area assessed as 1=shortest follicular cell height/least colloid area; 5=tallest follicular cell height/largest colloid area.

<sup>2</sup>—=not present, 1=minimal, 3=moderate

**Appendix 30: Thyroid Gland: Individual Male Rat Histology (750 mg/kg/day 2-Ethylhexyl Paraben)**

Animal ID	Follicular cell (height) <sup>1</sup>	Follicular colloid (area) <sup>1</sup>	Hypereosinophilic Colloid <sup>2</sup>
49	1	5	—
50	1	5	—
51	1	5	—
52	1	5	—
53	1	5	—
54	1	5	—
55	1	5	—
56	1	5	—
57	3	3	—
58	2	4	—
59	1	5	—
60	1	5	—
61	2	4	—
62	2	4	—
63	2	4	—
64	2	4	—

<sup>1</sup>Two serial sections of thyroid gland evaluated, both were graded the same  
Follicular cell height and colloid area assessed as 1=shortest follicular cell height/least colloid area; 5=tallest follicular cell height/largest colloid area.

<sup>2</sup>—=not present

**Appendix 31: Thyroid Gland: Individual Male Rat Histology (1000 mg/kg/day 2-Ethylhexyl Paraben)**

Animal ID	Follicular cell (height) <sup>1</sup>	Follicular colloid (area) <sup>1</sup>	Hypereosinophilic Colloid <sup>2</sup>
65	1	5	—
66	3	3	—
67	1	5	—
68	1	5	—
69	2	4	—
70	1	5	—
71	1	5	—
72	1	5	—
73	2	4	—
74	2	4	—
75	2	4	—
76	2	4	—
77	2	4	—
78	1	5	—
79	1	5	—
80	1	5	—

<sup>1</sup>Two serial sections of thyroid gland evaluated, both were graded the same  
Follicular cell height and colloid area assessed as 1=shortest follicular cell height/least colloid area; 5=tallest follicular cell height/largest colloid area.

<sup>2</sup> — =not present



Experimental Pathology Laboratories, Inc.

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RTI INTERNATIONAL  
RTI STUDY NUMBER 0213211.013.005.006  
ILS STUDY NUMBER 10005.0102  
EPL PROJECT NUMBER 229-192

PEER REVIEW OF HISTOLOGIC SECTIONS AND WHOLE MOUNT  
PREPARATIONS OF MAMMARY GLANDS FROM MALE RATS  
EXPOSED TO 2-ETHYLHEXYL PARABEN

PEER REVIEW REPORT

Submitted by:

Experimental Pathology Laboratories, Inc.

Street Address:	Mailing Address:
45600 Terminal Drive	P.O. Box 169
Sterling, VA 20166	Sterling, VA 20167-0169

(703) 471-7060

Submitted to:

RTI International  
Research Triangle Park, NC 27709

February 8, 2017

**FINAL REPORT**



Experimental Pathology Laboratories, Inc.

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PEER REVIEW SUMMARY





Experimental Pathology Laboratories, Inc.

RTI INTERNATIONAL  
RTI STUDY NUMBER 0213211.013.005.006  
ILS STUDY NUMBER 10005.0102  
EPL PROJECT NUMBER 229-192

PEER REVIEW OF HISTOLOGIC SECTIONS AND WHOLE MOUNT  
PREPARATIONS OF MAMMARY GLANDS FROM MALE RATS EXPOSED TO  
2-ETHYLHEXYL PARABEN

PEER REVIEW SUMMARY

**INTRODUCTION**

The objective of this project was to review all histologic sections and whole mount preparations of mammary glands from male rats exposed to 2-ethylhexyl paraben (2-EHPB) via oral gavage for 31 or 32 days.

The experimental design is presented in Table 1.

Table 1. Experimental Design		
Group	2-Ethylhexyl Paraben Concentration (mg/kg/day)	Number of Male Rats
1 (control)	0	16
2	250	16
3	500	16
4	750	16
5	1000	16
Total		80

**METHODS**

A single H&E-stained histologic section and a single carmine-stained whole mount were received per rat. The pathologist evaluated all original slides using brightfield microscopy, and addressed each original diagnosis with one of three responses: 1) Agree; 2) Disagree; or 3) Disagree and provide alternate finding(s). Added findings were graded according to the following scale: Grade 1 = minimal, Grade 2 = mild, Grade 3 =



Experimental Pathology Laboratories, Inc.

RTI International Study Number 0213211.013.005.006  
ILS Study # 10005.0102

moderate, Grade 4 = marked, Grade 5 = severe. References consulted during the course of this review included Lucas et al., 2007 and Hovey et al., 2002. Because the quality of mammary gland whole mounts varied considerably, and some were of limited diagnostic value, the pathologist assigned a quality grade of good, fair, poor, or very poor for each whole mount specimen.

#### **RESULTS AND DISCUSSION**


Differences between original and review diagnoses are presented in Appendix A.

There were no original findings for the H&E histologic sections, and the reviewing pathologist added a single diagnosis of Alveolar Hypoplasia, Grade 1, in a 1000 mg/kg/day dose group male (Animal No. 73).

There were no original findings for the whole mount preparations, and the reviewing pathologist agreed with those assessments.

Although the quality of the histologic sections was generally excellent, the quality of the mammary gland whole mounts in this study varied from good to poor. Fifty-five percent of whole mounts were judged to be of good to fair quality, whereas the quality of the remaining 45% was poor. Major problems involved folding or bunching of the specimens, and failure to adequately clear the adipose tissue, which resulted in obscured, indistinct, and/or artifactually thickened ductal profiles. The amount of diagnostic information that could be ascertained from specimens graded as poor was very limited.

Based on the nearly complete absence of findings, there were no mammary gland effects attributed to BBP exposure, and the overall outcome of the study remained unchanged following this review.

  
JEFFREY C. WOLF, DVM, Diplomate, ACVP  
Senior Anatomic Pathologist

2-8-17  
Date

JCW/cb



Experimental Pathology Laboratories, Inc.

RTI International Study Number 0213211.013.005.006

ILS Study # 10005.0102

#### REFERENCES

Hovey RC, Trott JF, Vonderhaar BK (2002) Establishing a framework for the functional mammary gland: from endocrinology to morphology. J Mammary Gland Biol Neoplasia, 7(1):17-38.

Lucas JN, Rudmann DG, Credille KM, Irizarry AR, Peter A, Snyder PW (2007) The rat mammary gland: morphologic changes as an indicator of systemic hormonal perturbations induced by xenobiotics. Toxicol Pathol, 35(2):199-207.

APPENDIX A  
PEER REVIEW DIAGNOSES



Experimental Pathology Laboratories, Inc.

RTI International Study Number 0213211.013.005.006  
ILS Study # 10005.0102

**PEER REVIEW DIAGNOSIS  
(H&E Slides)**

Test Article: Corn Oil, Control

Mammary Gland: Individual Male Rat Histology

Animal ID	Original Pathologist Findings	Reviewing Pathologist Agree / Disagree	Reviewing Pathologist Findings
01	Within Normal Limits	Agree	No Findings
02	Within Normal Limits	Agree	No Findings
03	Within Normal Limits	Agree	No Findings
04	Within Normal Limits	Agree	No Findings
05	Within Normal Limits	Agree	No Findings
06	Within Normal Limits	Agree	No Findings
07	Within Normal Limits	Agree	No Findings
08	Within Normal Limits	Agree	No Findings
09	Within Normal Limits	Agree	No Findings
10	Within Normal Limits	Agree	No Findings
11	Within Normal Limits	Agree	No Findings
12	Within Normal Limits	Agree	No Findings
13	Within Normal Limits	Agree	No Findings
14	Within Normal Limits	Agree	No Findings
15	Within Normal Limits	Agree	No Findings
16	Within Normal Limits	Agree	No Findings

A-1



Experimental Pathology Laboratories, Inc.

RTI International Study Number 0213211.013.005.006  
ILS Study # 10005.0102

**PEER REVIEW DIAGNOSIS  
(H&E Slides)**

Test Article: 250 mg/kg/day; 2-Ethylhexyl Paraben

Mammary Gland: Individual Male Rat Histology

Animal ID	Original Pathologist Findings	Reviewing Pathologist Agree / Disagree	Reviewing Pathologist Findings
17	Within Normal Limits	Agree	No Findings
18	Within Normal Limits	Agree	No Findings
19	Within Normal Limits	Agree	No Findings
20	Within Normal Limits	Agree	No Findings
21	Within Normal Limits	Agree	No Findings
22	Within Normal Limits	Agree	No Findings
23	Within Normal Limits	Agree	No Findings
24	Within Normal Limits	Agree	No Findings
25	Within Normal Limits	Agree	No Findings
26	Within Normal Limits	Agree	No Findings
27	Within Normal Limits	Agree	No Findings
28	Within Normal Limits	Agree	No Findings
29	Within Normal Limits	Agree	No Findings
30	Within Normal Limits	Agree	No Findings
31	Within Normal Limits	Agree	No Findings
32	Within Normal Limits	Agree	No Findings

A-2



Experimental Pathology Laboratories, Inc.

RTI International Study Number 0213211.013.005.006  
ILS Study # 10005.0102

**PEER REVIEW DIAGNOSIS  
(H&E Slides)**

Test Article: 500 mg/kg/day; 2-Ethylhexyl Paraben

Mammary Gland: Individual Male Rat Histology

Animal ID	Original Pathologist Findings	Reviewing Pathologist Agree / Disagree	Reviewing Pathologist Findings
33	Within Normal Limits	Agree	No Findings
34	Within Normal Limits	Agree	No Findings
35	Within Normal Limits	Agree	No Findings
36	Within Normal Limits	Agree	No Findings
37	Within Normal Limits	Agree	No Findings
38	Within Normal Limits	Agree	No Findings
39	Within Normal Limits	Agree	No Findings
40	Within Normal Limits	Agree	No Findings
41	Within Normal Limits	Agree	No Findings
42	Within Normal Limits	Agree	No Findings
43	Within Normal Limits	Agree	No Findings
44	Within Normal Limits	Agree	No Findings
45	Within Normal Limits	Agree	No Findings
46	Within Normal Limits	Agree	No Findings
47	Within Normal Limits	Agree	No Findings
48	Within Normal Limits	Agree	No Findings

A-3



Experimental Pathology Laboratories, Inc.

RTI International Study Number 0213211.013.005.006  
ILS Study # 10005.0102

**PEER REVIEW DIAGNOSIS  
(H&E Slides)**

Test Article: 750 mg/kg/day; 2-Ethylhexyl Paraben

Mammary Gland: Individual Male Rat Histology

Animal ID	Original Pathologist Findings	Reviewing Pathologist Agree / Disagree	Reviewing Pathologist Findings
49	Within Normal Limits	Agree	No Findings
50	Within Normal Limits	Agree	No Findings
51	Within Normal Limits	Agree	No Findings
52	Within Normal Limits	Agree	No Findings
53	Within Normal Limits	Agree	No Findings
54	Within Normal Limits	Agree	No Findings
55	Within Normal Limits	Agree	No Findings
56	Within Normal Limits	Agree	No Findings
57	Within Normal Limits	Agree	No Findings
58	Within Normal Limits	Agree	No Findings
59	Within Normal Limits	Agree	No Findings
60	Within Normal Limits	Agree	No Findings
61	Within Normal Limits	Agree	No Findings
62	Within Normal Limits	Agree	No Findings
63	Within Normal Limits	Agree	No Findings
64	Within Normal Limits	Agree	No Findings





Experimental Pathology Laboratories, Inc.

RTI International Study Number 0213211.013.005.006  
ILS Study # 10005.0102

**PEER REVIEW DIAGNOSIS  
(H&E Slides)**

Test Article: 1000 mg/kg/day; 2-Ethylhexyl Paraben

Mammary Gland: Individual Male Rat Histology

Animal ID	Original Pathologist Findings	Reviewing Pathologist Agree / Disagree	Reviewing Pathologist Findings
65	Within Normal Limits	Agree	No Findings
66	Within Normal Limits	Agree	No Findings
67	Within Normal Limits	Agree	No Findings
68	Within Normal Limits	Agree	No Findings
69	Within Normal Limits	Agree	No Findings
70	Within Normal Limits	Agree	No Findings
71	Within Normal Limits	Agree	No Findings
72	Within Normal Limits	Agree	No Findings
73	Within Normal Limits	Disagree	Alveolar Hypoplasia, Grade 1
74	Within Normal Limits	Agree	No Findings
75	Within Normal Limits	Agree	No Findings
76	Within Normal Limits	Agree	No Findings
77	Within Normal Limits	Agree	No Findings
78	Within Normal Limits	Agree	No Findings
79	Within Normal Limits	Agree	No Findings
80	Within Normal Limits	Agree	No Findings

A-5



Experimental Pathology Laboratories, Inc.

RTI International Study Number 0213211.013.005.006  
ILS Study # 10005.0102

**PEER REVIEW DIAGNOSIS  
(Whole Mounts)**

Test Article: Corn Oil, Control

Mammary Gland: Individual Male Rat Histology

Animal ID	Original Pathologist Findings	Reviewing Pathologist Agree/Disagree	Reviewing Pathologist Findings	Slide Quality
01	Normal Developmental Stage	Agree	No Findings	Fair
02	Normal Developmental Stage	Agree	No Findings	Poor
03	Normal Developmental Stage	Agree	No Findings	Poor
04	Normal Developmental Stage	Agree	No Findings	Fair
05	Normal Developmental Stage	Agree	No Findings	Fair
06	Normal Developmental Stage	Agree	No Findings	Poor
07	Normal Developmental Stage	Agree	No Findings	Fair
08	Normal Developmental Stage	Agree	No Findings	Poor
09	Normal Developmental Stage	Agree	No Findings	Fair
10	Normal Developmental Stage	Agree	No Findings	Poor
11	Normal Developmental Stage	Agree	No Findings	Fair
12	Normal Developmental Stage	Agree	No Findings	Poor
13	Normal Developmental Stage	Agree	No Findings	Fair
14	Normal Developmental Stage	Agree	No Findings	Fair
15	Normal Developmental Stage	Agree	No Findings	Fair
16	Normal Developmental Stage	Agree	No Findings	Fair

A-6



Experimental Pathology Laboratories, Inc.

RTI International Study Number 0213211.013.005.006  
ILS Study # 10005.0102

**PEER REVIEW DIAGNOSIS  
(Whole Mounts)**

Test Article: 250 mg/kg/day; 2-Ethylhexyl Paraben

Mammary Gland: Individual Male Rat Histology

Animal ID	Original Pathologist Findings	Reviewing Pathologist Agree/Disagree	Reviewing Pathologist Findings	Slide Quality
17	Normal Developmental Stage	Agree	No Findings	Poor
18	Normal Developmental Stage	Agree	No Findings	Fair
19	Normal Developmental Stage	Agree	No Findings	Fair
20	Normal Developmental Stage	Agree	No Findings	Fair
21	Normal Developmental Stage	Agree	No Findings	Fair
22	Normal Developmental Stage	Agree	No Findings	Poor
23	Normal Developmental Stage	Agree	No Findings	Poor
24	Normal Developmental Stage	Agree	No Findings	Poor
25	Normal Developmental Stage	Agree	No Findings	Poor
26	Normal Developmental Stage	Agree	No Findings	Fair
27	Normal Developmental Stage	Agree	No Findings	Poor
28	Normal Developmental Stage	Agree	No Findings	Poor
29	Normal Developmental Stage	Agree	No Findings	Poor
30	Normal Developmental Stage	Agree	No Findings	Fair
31	Normal Developmental Stage	Agree	No Findings	Poor
32	Normal Developmental Stage	Agree	No Findings	Good

A-7



Experimental Pathology Laboratories, Inc.

RTI International Study Number 0213211.013.005.006  
ILS Study # 10005.0102

**PEER REVIEW DIAGNOSIS  
(Whole Mounts)**

Test Article: 500 mg/kg/day; 2-Ethylhexyl Paraben

Mammary Gland: Individual Male Rat Histology

Animal ID	Original Pathologist Findings	Reviewing Pathologist Agree/Disagree	Reviewing Pathologist Findings	Slide Quality
33	Normal Developmental Stage	Agree	No Findings	Poor
34	Normal Developmental Stage	Agree	No Findings	Fair
35	Normal Developmental Stage	Agree	No Findings	Fair
36	Normal Developmental Stage	Agree	No Findings	Fair
37	Normal Developmental Stage	Agree	No Findings	Fair
38	Normal Developmental Stage	Agree	No Findings	Fair
39	Normal Developmental Stage	Agree	No Findings	Poor
40	Normal Developmental Stage	Agree	No Findings	Fair
41	Normal Developmental Stage	Agree	No Findings	Poor
42	Normal Developmental Stage	Agree	No Findings	Fair
43	Normal Developmental Stage	Agree	No Findings	Fair
44	Normal Developmental Stage	Agree	No Findings	Poor
45	Normal Developmental Stage	Agree	No Findings	Poor
46	Normal Developmental Stage	Agree	No Findings	Poor
47	Normal Developmental Stage	Agree	No Findings	Poor
48	Normal Developmental Stage	Agree	No Findings	Fair



Experimental Pathology Laboratories, Inc.

RTI International Study Number 0213211.013.005.006  
ILS Study # 10005.0102

**PEER REVIEW DIAGNOSIS  
(Whole Mounts)**

Test Article: 750 mg/kg/day; 2-Ethylhexyl Paraben

Mammary Gland: Individual Male Rat Histology

Animal ID	Original Pathologist Findings	Reviewing Pathologist Agree/Disagree	Reviewing Pathologist Findings	Slide Quality
49	Normal Developmental Stage	Agree	No Findings	Fair
50	Normal Developmental Stage	Agree	No Findings	Fair
51	Normal Developmental Stage	Agree	No Findings	Fair
52	Normal Developmental Stage	Agree	No Findings	Fair
53	Normal Developmental Stage	Agree	No Findings	Poor
54	Normal Developmental Stage	Agree	No Findings	Fair
55	Normal Developmental Stage	Agree	No Findings	Poor
56	Normal Developmental Stage	Agree	No Findings	Fair
57	Normal Developmental Stage	Agree	No Findings	Fair
58	Normal Developmental Stage	Agree	No Findings	Poor
59	Normal Developmental Stage	Agree	No Findings	Fair
60	Normal Developmental Stage	Agree	No Findings	Poor
61	Normal Developmental Stage	Agree	No Findings	Poor
62	Normal Developmental Stage	Agree	No Findings	Poor
63	Normal Developmental Stage	Agree	No Findings	Fair
64	Normal Developmental Stage	Agree	No Findings	Fair



Experimental Pathology Laboratories, Inc.

RTI International Study Number 0213211.013.005.006  
ILS Study # 10005.0102

**PEER REVIEW DIAGNOSIS  
(Whole Mounts)**

Test Article: 1000 mg/kg/day; 2-Ethylhexyl Paraben

Mammary Gland: Individual Male Rat Histology

Animal ID	Original Pathologist Findings	Reviewing Pathologist Agree/Disagree	Reviewing Pathologist Findings	Slide Quality
65	Normal Developmental Stage	Agree	No Findings	Fair
66	Normal Developmental Stage	Agree	No Findings	Fair
67	Normal Developmental Stage	Agree	No Findings	Fair
68	Normal Developmental Stage	Agree	No Findings	Fair
69	Normal Developmental Stage	Agree	No Findings	Poor
70	Normal Developmental Stage	Agree	No Findings	Poor
71	Normal Developmental Stage	Agree	No Findings	Fair
72	Normal Developmental Stage	Agree	No Findings	Poor
73	Normal Developmental Stage	Agree	No Findings	Fair
74	Normal Developmental Stage	Agree	No Findings	Poor
75	Normal Developmental Stage	Agree	No Findings	Poor
76	Normal Developmental Stage	Agree	No Findings	Poor
77	Normal Developmental Stage	Agree	No Findings	Poor
78	Normal Developmental Stage	Agree	No Findings	Poor
79	Normal Developmental Stage	Agree	No Findings	Fair
80	Normal Developmental Stage	Agree	No Findings	Fair

A-10

# APPENDIX VII: Certificate 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	Lot: 7CZZO	
CAS: 6153-25-3		
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: +1-800-423-8816 / +1-503-283-1681  
Fax: +1-888-520-1075 / +1-503-283-1987  
E-mail: Sales-US@TCIchemicals.com



## 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 9/22/16  
Sherry Black Date  
Task Leader

Approved by:

Hernan Navarro 9/22/16  
Hernan Navarro Date  
Principal Investigator



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



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).

A handwritten signature in black ink, appearing to read "Phillip S. Anderson", written over a horizontal line.

Phillip S. Anderson

Quality Assurance Specialist

A handwritten date "09/22/16" in black ink, written over a horizontal line.

Date

*turning knowledge into practice*

2-Ethylhexyl Paraben

RTI Study Numbers: 0213211.013.002.001 and 0213211.013.002.006

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-Ethylhexyl Paraben

RTI Study Numbers: 0213211.013.002.001 and 0213211.013.002.006

## 1.0 INTRODUCTION

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.

**Name:** 2-Ethylhexyl paraben

**Vendor:** TCI America

**Lot #:** 7CZZO

**CAS #:** 5153-25-3

**RTI Log No:** 091515-A-01

An aliquot from Log #091515-A-01 was analyzed by  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR for confirmation of identity and by HPLC for purity.

## 2.0 SPECTROSCOPY

### 2.1 Nuclear Magnetic Resonance (Proton)

#### 2.1.1 NMR Instrument Parameters

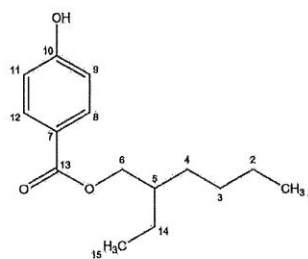
System	Bruker Avance DPX-300 NMR
Software	Topspin 1.3
Sweep Frequency	300 MHz
Pulse Width	10 $\mu\text{sec}$
Solvent	$\text{CDCl}_3$
Reference	Solvent

2-Ethylhexyl Paraben

RTI Study Numbers: 0213211.013.002.001 and 0213211.013.002.006

### 2.1.2 NMR Results

Structure and Assignments:



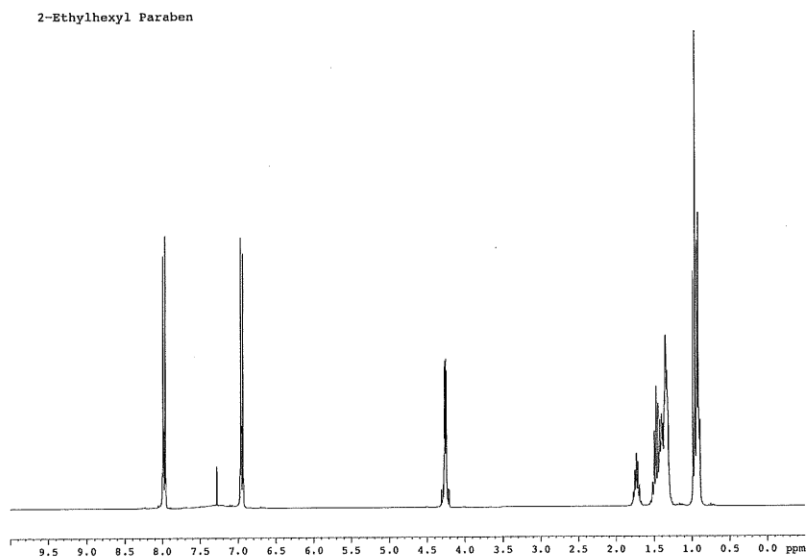
Assignment	Chemical Shift (ppm)	Integral Ratio
1, 15	0.9 - 1	6H
2, 3, 4, 14	1.3-1.5	8H
5	1.7-1.8	1H
6	4.2-4.3	2H
9, 11	7.0	2H
8, 12	8.0	2H
solvent	7.3	

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-Ethylhexyl Paraben

RTI Study Numbers: 0213211.013.002.001 and 0213211.013.002.006

**Figure 1.  $^1\text{H}$  NMR of 2-Ethylhexyl Paraben (Lot # 7CZZO) in  $\text{CDCl}_3$**



## 2.2 Nuclear Magnetic Resonance (Carbon)

### 2.2.1 NMR Instrument Parameters

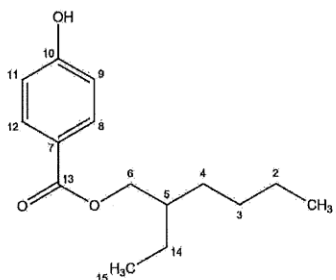
System	Bruker Avance DPX-300 NMR
Software	Topspin 1.3
Sweep Frequency	75 MHz
Sweep Width	23810 Hz
Pulse Width	5.8 $\mu\text{sec}$
Solvent	$\text{CDCl}_3$
Reference	Solvent

2-Ethylhexyl Paraben

RTI Study Numbers: 0213211.013.002.001 and 0213211.013.002.006

## 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

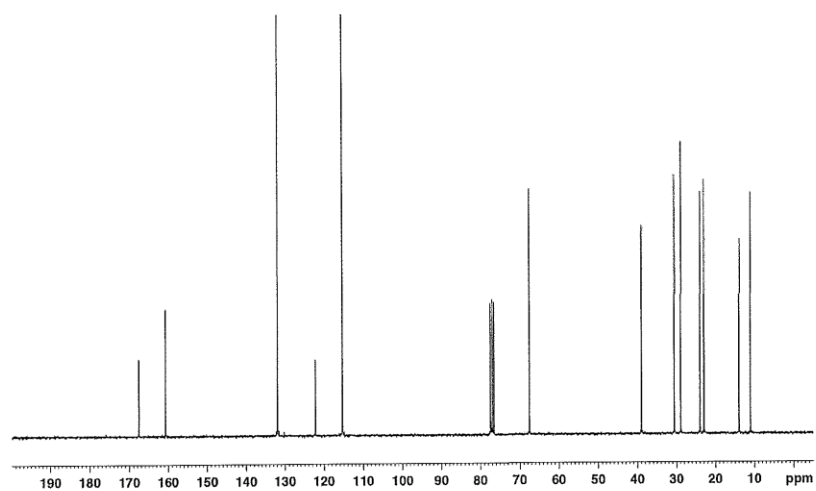
The observed spectrum (Figure 2) shows chemical shifts consistent with the structure of 2-ethylhexyl paraben and with the predicted spectrum generated using ACD/C + H NMR Predictors and DB (V.10.02).

2-Ethylhexyl Paraben

RTI Study Numbers: 0213211.013.002.001 and 0213211.013.002.006

**Figure 2.  $^{13}\text{C}$  NMR of 2-Ethylhexyl Paraben (Lot # 7CZZO) in  $\text{CDCl}_3$**

2-Ethylhexyl Paraben





2-Ethylhexyl Paraben

RTI Study Numbers: 0213211.013.002.001 and 0213211.013.002.006

### 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

<b>Instrument</b>	Waters Alliance 2695
<b>Column</b>	Phenomenex Prodigy ODS3, 5 µm; 250 x 4.6 mm w/ Phenomenex Prodigy ODS3, 5 µm precolumn
<b>Column Temperature</b>	Room Temperature
<b>Mobile Phase</b>	A: Water 0.1% Formic Acid B: Acetonitrile 0.1% Formic Acid
<b>Gradient Conditions</b>	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
<b>Flow Rate</b>	1 mL/min
<b>Injection Volume</b>	10 µL
<b>Detector</b>	Waters W2487, UV monitored at 280 nm
<b>Data System</b>	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*

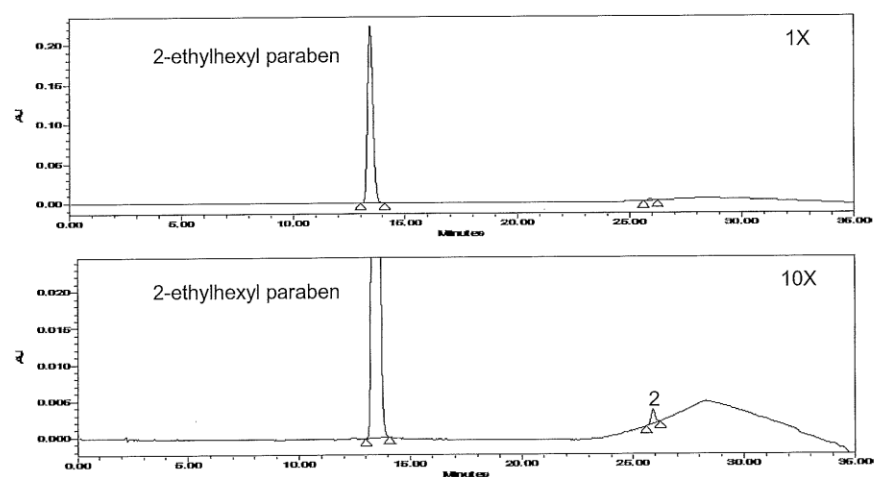
\*Average of two determinations.

2-Ethylhexyl Paraben

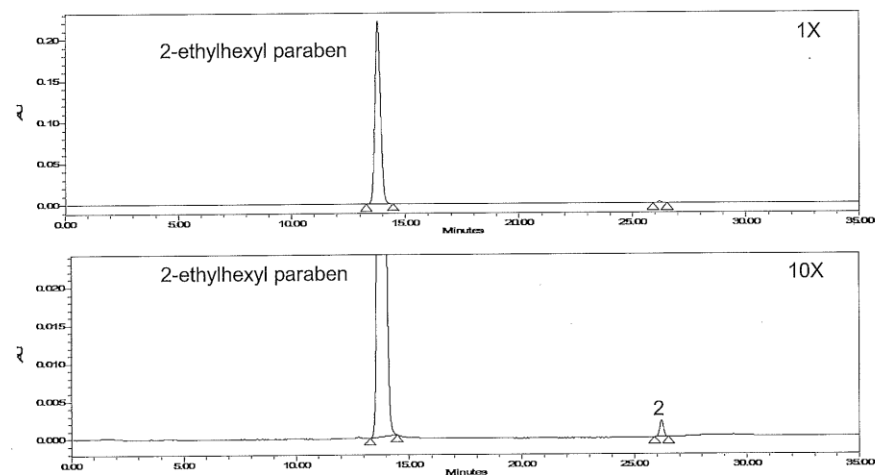
RTI Study Numbers: 0213211.013.002.001 and 0213211.013.002.006

Figure 3. HPLC Chromatograms of 2-Ethylhexyl Paraben

Analysis date: 11/02/15



Analysis date: 03/15/16



# APPENDIX VIII: Dose Formulation Stability and Sample Analysis Reports

**Study Title**

Storage Stability of 2-Ethylhexyl Paraben in Corn Oil

**Data Requirement**

OCSP 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

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ILS Project/Study No. 10005/0104

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**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: \_\_\_\_\_

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ILS Project/Study No. 10005/0104


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### 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  
Study Director

29 Apr 16  
\_\_\_\_\_  
Date

INTEGRATED LABORATORY SYSTEMS, INC.

\_\_\_\_\_  
Study Sponsor

\_\_\_\_\_  
Date

\_\_\_\_\_  
Submitter

\_\_\_\_\_  
Date

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

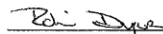
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### QUALITY ASSURANCE STATEMENT

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.

<u>Inspection Date</u>	<u>Inspection Types</u>	<u>Reported to Study Director/Management</u>
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

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#### 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



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## **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

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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.

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## 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<sup>®</sup>-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.

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### 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:

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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 <sup>a</sup>	0.3991– 0.4010 <sup>a</sup>	2.00

<sup>a</sup> 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 <sup>a</sup>	2.00	50.0	0.100	20.0	0.100	10.0	500,000

<sup>a</sup> 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

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average recovery of  $105 \pm 3.21\%$  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.



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#### **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.

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- 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 <sup>a</sup>
C12-15-78		1.00	1.08	108			
C12-15-79		1.00	1.06	106			
C12-15-89		200	189	94.4			
C12-15-90		200	145	72.5			
C12-15-91	10	200	178	89.0	1.01	0.0622	-5.05
C12-15-80		1.00	0.946	94.6			
C12-15-81		1.00	1.02	102			
C12-15-82		1.00	1.07	107			
C12-15-92		200	216	108			
C12-15-93	20	200	217	108	0.914	0.0115	-14.3
C12-15-94		200	224	112			
C12-15-83		1.00	0.903	90.3			
C12-15-84		1.00	0.914	91.4			
C12-15-85		1.00	0.926	92.6			
C12-15-95	20	200	193	96.3	193 <sup>b</sup>	0.707 <sup>b</sup>	13.2
C12-15-96		200	192	96.1			
C12-15-97		101 <sup>b</sup>	98.3	97.3			

<sup>a</sup> NA = Not Applicable.

<sup>b</sup> 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.

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**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	10	1.00	1.06	106
C12-15-141	QC #2		1.00	1.07	107
C12-15-142	QC #3		200	217	109
C12-15-164	QC #1	20	1.00	0.933	93.3
C12-15-165	QC #2		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.

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**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

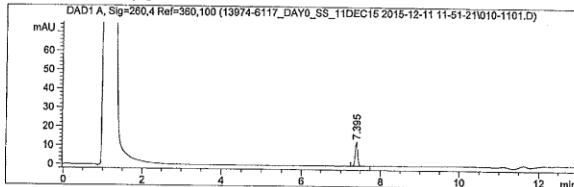
Last changed : 12/9/2015 1:50:47 PM by Xianai Wu

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\13974-6117\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

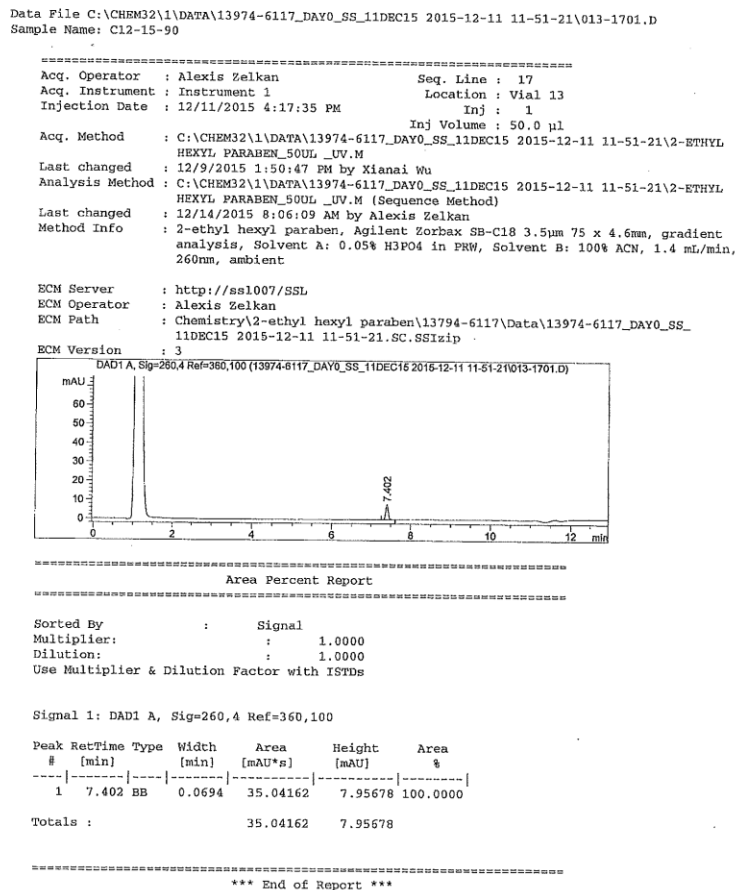
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Note: 2-ethylhexyl paraben is ordinarily detected at a retention time of approximately 7.4 minutes.

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**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.**



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

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







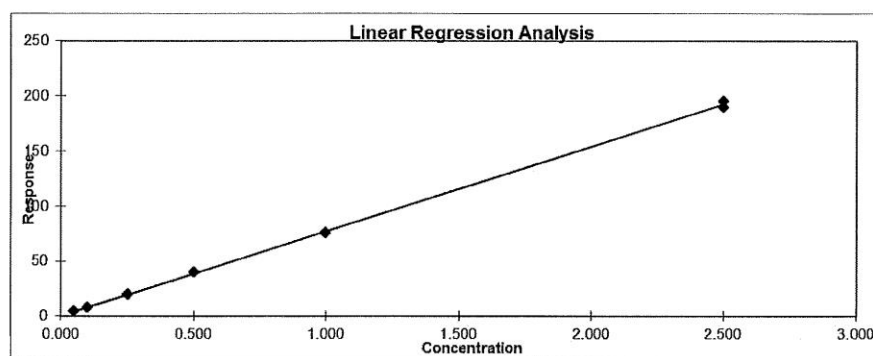




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**Figure 7.** A typical regression analysis for the calibration standards used to quantitate 2-ethylhexyl paraben during the 20-day storage stability experiment.



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**

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TEST PROTOCOL

Title: Storage Stability of 2-Ethylhexyl Paraben in Corn Oil

Data Requirement(s): OCSPP 860.1380, 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](mailto: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  
  
Study Director Signature  
02 December 2015  
Date  
02 Dec 2015  
Study Initiation Date

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Smithers Viscient LLC  
[www.smithersviscient.com](http://www.smithersviscient.com)  
790 Main Street | Wareham, MA 02571 | p 508.295.2550  
2900 Quakerbush Road | Snow Camp, NC 27349 | p 336.376.0141

#### Storage Stability 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 designates.

#### 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
	Day 11 ± 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

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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

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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.

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## **APPENDIX 2 - CERTIFICATE OF ANALYSIS**

Smithers Viscient Study No. 13974.6117

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### Certificate of Analysis

Sep 25, 2015 (JST)

TOKYO CHEMICAL INDUSTRY CO., LTD.  
4-10-1 Nishinarasawa-cho, Chuo-ku, Tokyo 100-0023 Japan

Chemical Name: 2-Ethylhexyl 4-Hydroxybenzoate			
Product Number: F0505		Lot: TCZ70	
CAS: 9159-25-3			
Tests		Results	Specifications
Purity (PLC)		99.3 area%	min. 99.0 area%
Purity (Neutralization titration)		99.0 %	min. 99.0 %
Specific gravity (20/20)		1.0382	1.0360 to 1.0360
Refractive index n <sub>D</sub> 20		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: +1-800-422-6616 / +1-603-285-1891  
Fax: +1-603-500-1075 / +1-603-285-1897  
E-mail: Sales-US@TCIchemicals.com

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### **APPENDIX 3 - ANALYTICAL SUMMARY**

Smithers Viscient Study No. 13974.6117

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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 $\mu$ m, 75 x 4.6 mm																		
Mobile Phase (A):	0.05% phosphoric acid in purified reagent water																		
Mobile Phase (B):	100% acetonitrile																		
Gradient:	<table><thead><tr><th>Time (min.)</th><th>Solvent A (%)</th><th>Solvent B (%)</th></tr></thead><tbody><tr><td>0.00</td><td>60.0</td><td>40.0</td></tr><tr><td>1.00</td><td>60.0</td><td>40.0</td></tr><tr><td>10.00</td><td>0.00</td><td>100.0</td></tr><tr><td>12.00</td><td>0.00</td><td>100.0</td></tr><tr><td>13.00</td><td>60.0</td><td>40.0</td></tr></tbody></table>	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
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 $\mu$ L																		
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

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ILS Projects/Study Numbers 10005.0103 and 10005.0102

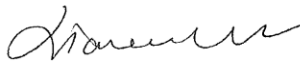
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### GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

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



Date



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


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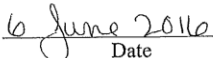
### QUALITY ASSURANCE STATEMENT

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

  
Date

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

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#### **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

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## 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

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**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:

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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



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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

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## 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

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## 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%  $\pm$  3.21% from corn oil (Wu, 2016). Defined limits for acceptance of

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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

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## 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 $\mu$ m, 75 x 4.6 mm		
Mobile Phase (A):	0.05% phosphoric acid in purified reagent water		
Mobile Phase (B):	100% acetonitrile		
Gradient:	<u>Time (min.)</u>	<u>Solvent A (%)</u>	<u>Solvent B (%)</u>
	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 $\mu$ L		
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  $96.5 \pm 1.13\%$  for 50.0 mg/mL,  $95.2 \pm 1.26\%$  for 100 mg/mL,  $92.4 \pm 2.33\%$  for 150 mg/mL, and  $90.4 \pm 6.20\%$  for 200 mg/mL with all controls below the limit of quantitation (LOQ).

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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  $99.5 \pm 3.76\%$  for 50.0 mg/mL,  $99.2 \pm 4.88\%$  for 100 mg/mL,  $99.4 \pm 2.27\%$  for 150 mg/mL, and  $98.5 \pm 2.90\%$  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

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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  $90.5 \pm 4.82\%$  for 50.0 mg/mL,  $87.9 \pm 2.20\%$  for 100 mg/mL,  $92.7 \pm 2.85\%$  for 150 mg/mL, and  $91.7 \pm 5.36\%$  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.



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- 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.

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**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 <sup>a</sup>	Homogeneity - Control	0.00	< 0.122 <sup>b</sup>	NA <sup>c</sup>
15-206-14T-2 <sup>a</sup>	Homogeneity - Control	0.00	< 0.122	NA
15-206-14B-1 <sup>a</sup>	Homogeneity - Control	0.00	< 0.122	NA
15-206-14B-2 <sup>a</sup>	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 <sup>d</sup>
C01-16-95	QC #2	100	101	101 <sup>e</sup>
C01-16-96	QC #3	200	203	102 <sup>e</sup>

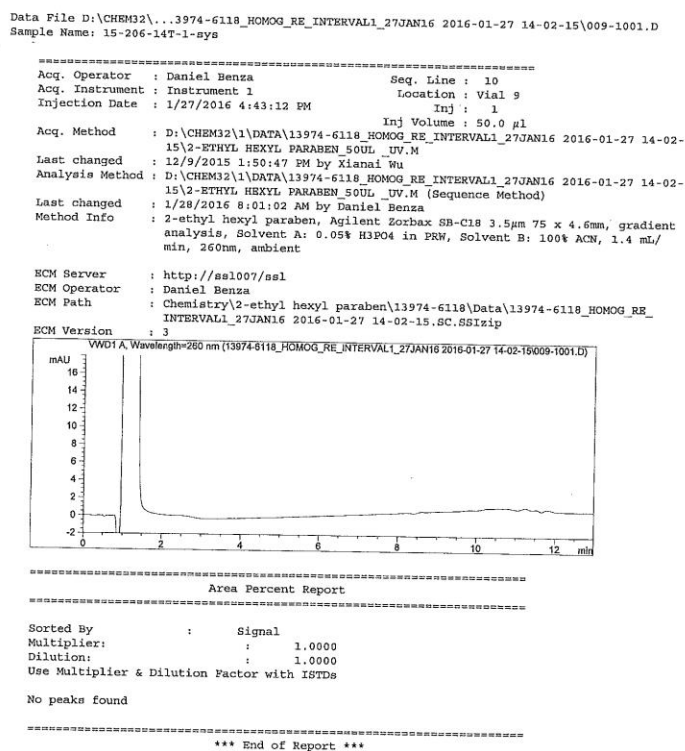
<sup>a</sup> Two control samples (ILS code 15-206-14) were received. SMV assigned unique sample identifiers.  
<sup>b</sup> Concentrations expressed as less than values were below the limit of quantitation (LOQ). The LOQ for each analysis is dependent upon the regression, the area of the low standards and the dilution factor of the controls.  
<sup>c</sup> NA = Not Applicable.  
<sup>d</sup> This quality control sample was prepared on 26 January 2016 and reinjected to demonstrate storage stability of the homogeneity samples.  
<sup>e</sup> 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.



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**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.**



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

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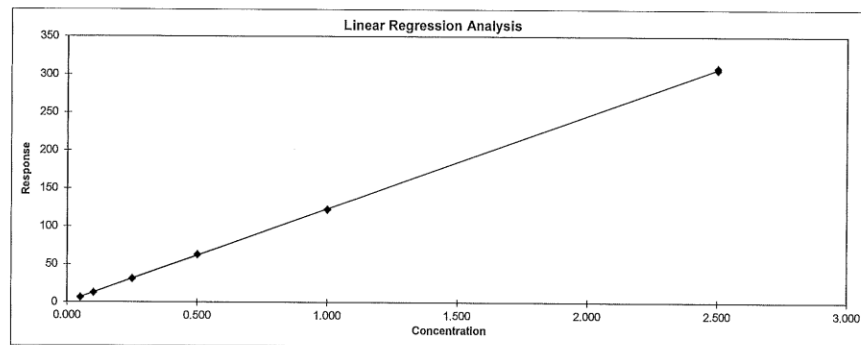




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**Figure 6. ILS 10005.0103: A typical regression analysis for the calibration standards used to quantitate 2-ethylhexyl paraben during analysis of dose formulation samples.**



$$R^2 = 0.99994$$

$$y = 122.833x + 0.5304$$

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



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**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 <sup>a</sup>	NA <sup>b</sup>
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

<sup>a</sup> 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.

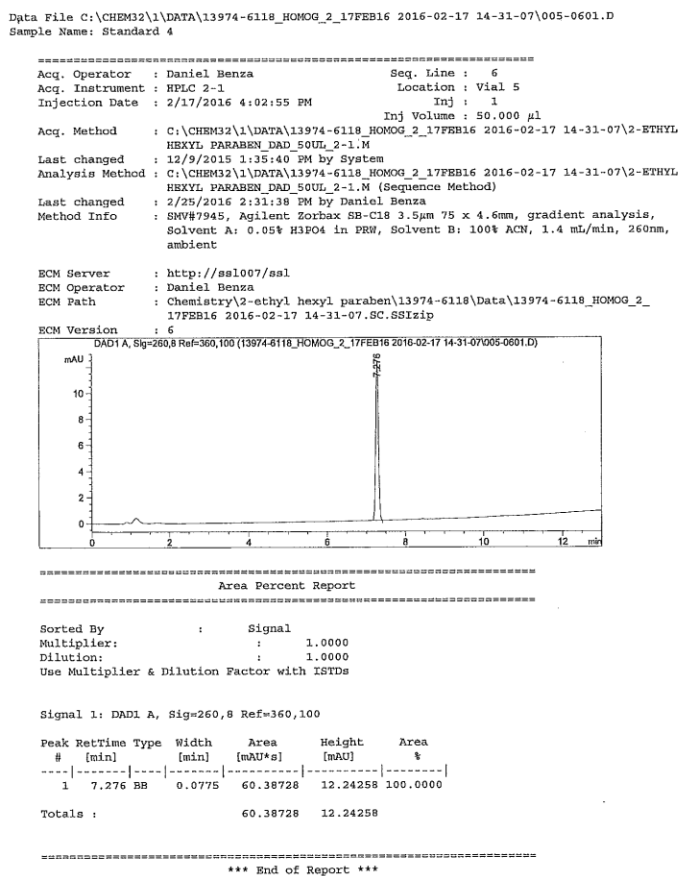
<sup>b</sup> NA = Not Applicable.

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

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

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**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.**



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

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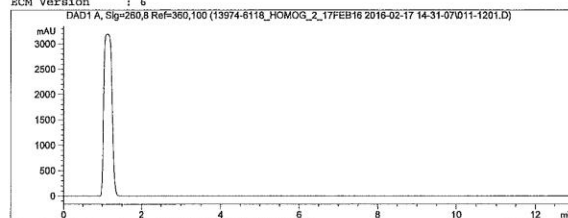
Smithers Viscient Study Number 13974.6118  
ILS Projects/Study Numbers 10005.0103 and 10005.0102

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**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     : SMVH7945, 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

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

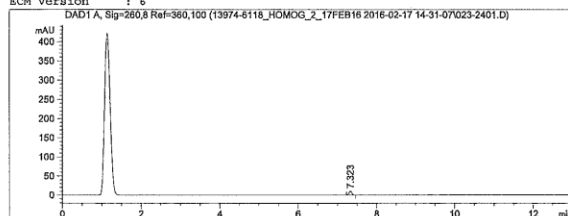
Data File C:\CHEM32\1\DATA\13974-6118\_HOMOG\_2\_17FEB16 2016-02-17 14-31-07\023-2401.D  
Sample Name: 15-172-76B-1

=====

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 2:11:38 PM by Daniel Benza  
Method Info : SMV#7945, Agilent Xorbax SB-C18 3.5µm 75 x 4.6mm, gradient analysis,  
Solvent A: 0.05% H3PO4 in PRN, 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.SSIaip  
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

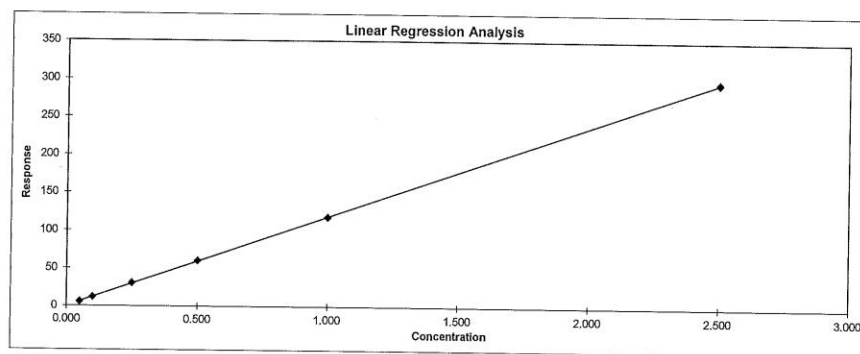




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**Figure 12.** ILS 10005.0102 (17 February 2016): A typical regression analysis for the calibration standards used to quantitate 2-ethylhexyl paraben during analysis of dose formulation samples.



$$R^2 = 0.99997$$

$$y = 118.230x + 0.5499$$

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

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**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 <sup>a</sup>	NA <sup>b</sup>
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 <sup>c</sup>	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

<sup>a</sup> 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.

<sup>b</sup> NA = Not Applicable.

<sup>c</sup> 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



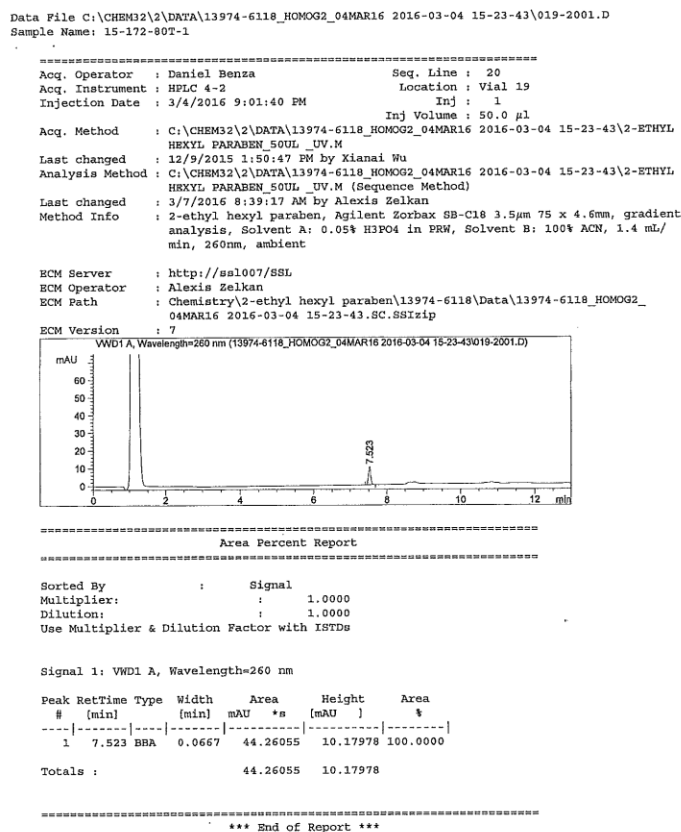




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 ILS Projects/Study Numbers 10005.0103 and 10005.0102

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



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

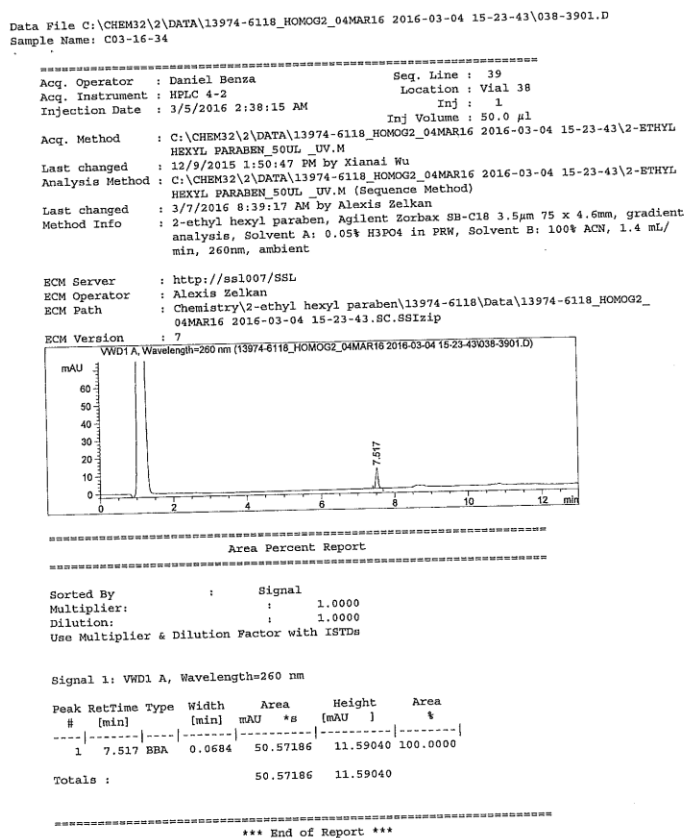
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 ILS Projects/Study Numbers 10005.0103 and 10005.0102

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**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.**



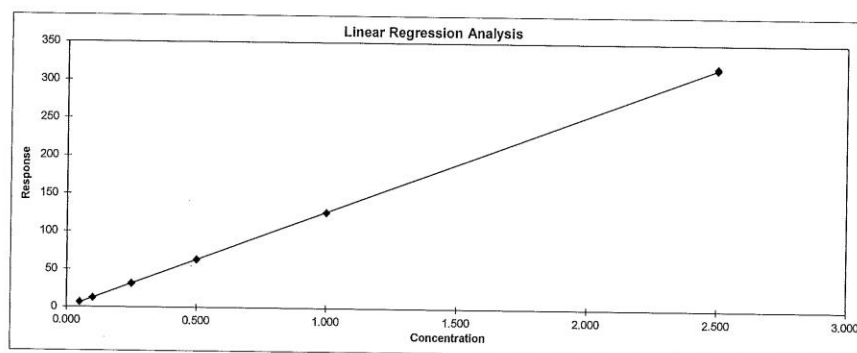
HPLC 4-2 3/7/2016 8:41:42 AM Alexis Zelkan

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 ILS Projects/Study Numbers 10005.0103 and 10005.0102

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**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

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ILS Projects/Study Numbers 10005.0103 and 10005.0102

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## **APPENDIX 1 – CERTIFICATE OF ANALYSIS**

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Certificate of Analysis

Rev 28, 2015 (JST)

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

Chemical Name: 2-Ethylhexyl 4-Hydroxybenzoate		
Product Number: H0509		
CAS: 9152-29-3		
Lot: FC220		
Tests	Results	Specifications
Purity (HPLC)	99.3 area%	min. 99.0 area%
Purity (Refractometer)	99.5 %	min. 99.0 %
Specific gravity (20/20)	1.0393	1.0380 to 1.0390
Refractive index (20/20)	1.5210	1.5190 to 1.5220

TCI Lot numbers are 1-5 characters in length.  
Characters listed after the first 5 characters are control numbers for internal purpose only.

Customer service:  
TCI AMERICA  
Tel: +1-800-222-6910 / +1-513-251-1881  
Fax: +1-800-525-1075 / +1-513-251-1997  
E-mail: Sales-US@TCI-america.com



# APPENDIX IX: Study Protocol, Amendments, Study Specific Procedure, and Protocol Deviations



**Study Title**

**Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben**

**Guideline Number**

**OPPTS 890.1500**

**ILS Project-Study Number**

**10005.0102**

**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**

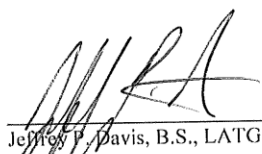
ILS Project No. - Study No.: 10005.0102; Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

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**Study Protocol Approval**

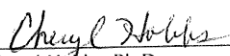
  
Sherry Black, B.S.  
Sponsor Representative

1-4-2016  
Date

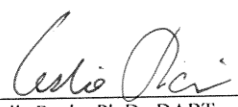
  
Jeffrey P. Davis, B.S., LATG  
Study Director  
Integrated Laboratory Systems, Inc.

06 January 2016  
Date

Reviewed by:

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

05 Jan 2016  
Date

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

05 Jan 2015 2016  
Date (2) lk 05 Jan 2016

ILS Project No. – Study No.: 10005.0102; Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

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## 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 this assay is to identify the potential of 2-ethylhexyl paraben to interact with the endocrine system, by identifying effects on pubertal development and thyroid function in the intact juvenile/peripubertal male rat (U.S. EPA, 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 890.1500: Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats (U.S. EPA, 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).

Animals will not be transported to a holding room the night before euthanasia as stated in Section j of the male pubertal test guideline, but will be maintained and dosed in the study room and taken to the necropsy room at least two hours prior to euthanasia. Reverse osmosis treated water will be supplied to the animals in lieu of deionized water recommended in Section d of the male pubertal test guideline. A 3 mL syringe may be used in place of a 1 mL (disposable) tuberculin syringe if the dose volume is greater than 1 mL for dose administration as stated in

ILS Project No. – Study No.: 10005.0102; Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

section h of the male pubertal test guideline. In addition, an appropriately sized gavage needle may be used as dictated by the size of the animal.

A QA inspection of critical phases will be conducted 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 will provide a QA inspection statement to the Study Director to be included in the final report.

QA functions related to the clinical pathology will be the responsibility of the QA Unit at AniLytics, Inc. All findings will be reported to the Study Director, and the QA Unit at AniLytics, Inc. 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](mailto: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](mailto:jdavis@ils-inc.com)

ILS Project No. – Study No.: 10005.0102; Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

---

#### 1.6 Proposed Study Dates

Animal Arrival:	07 January 2016
Experimental Start Date:	13 February 2016
Experimental In-Life Termination Date:	15 March 2016
Experimental Termination Date:	15 May 2016

#### TEST 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 <sub>15</sub> H <sub>22</sub> O <sub>3</sub>
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 at dose concentrations and frequency to be added by protocol amendment. Prepared dose formulations will be dispensed into vials to be used daily during the study.
Storage:	
Test Substance:	Room temperature and protected from light
Dose Formulation:	Will be determined upon completion of storage stability testing and details added by protocol amendment.
Stability:	
Dose Formulation:	Storage stability testing will be completed prior to dose administration and stability information added by protocol amendment.

ILS Project No. – Study No.: 10005.0102; Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

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<b>2.2 Vehicle:</b>	<b>Corn oil</b>
CAS No.	8001-30-7
Source:	Animal Health International (Greeley, CO)
Lot/Batch No.:	16303-100175
ILS Repository No.:	15-206
Formula:	C <sub>27</sub> H <sub>50</sub> O <sub>6</sub>
Description:	Yellow Oil
Storage:	Room temperature and protected from light

### 2.3 Archival Samples

Approximately a 1 mg sample of the test substance 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. The test substance will be maintained by ILS for five years following finalization of the study report.

### 2.4 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

Fifty timed pregnant nulliparous dams (F<sub>0</sub>) will be received and allowed to naturally deliver their litters (F<sub>1</sub>). Eighty F<sub>1</sub> males at postnatal day (PND) 21 will be allocated to one of five dose groups. On PND 21, female offspring will be transferred to the study entitled "Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Female Rats (OPPTS 890.1450); Benzyl Butyl Phthalate" (ILS Project-Study Number 10005.0302).



ILS Project No. – Study No.: 10005.0102; Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats; 2-Ethylhexyl Paraben

---

### Male Pubertal Study Design

F<sub>1</sub> males will be administered one of four dose levels, based upon daily body weights, of 2-ethylhexyl paraben or the vehicle control (corn oil) for 31 or 32 consecutive days via oral gavage. Preputial separation (PPS) observations will be performed daily beginning on PND 30 until complete PPS is observed in all animals. Approximately two hours following the final dose administration, the animals will be humanely euthanized; trunk blood will be collected and the adrenals, kidneys, liver, thyroid, ventral prostate, dorsolateral prostate, seminal vesicle, levator ani plus bulbocavernous muscle complex (LABC), epididymides, testes, and pituitary will be excised and weights recorded. Serum will be prepared to measure thyroxine (T<sub>4</sub>), thyroid stimulating hormone (TSH), testosterone (T), and clinical chemistry endpoints (total protein, albumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transferase, total bilirubin, sorbitol dehydrogenase, sodium, potassium, chloride, calcium, phosphorous, creatinine, and blood urea nitrogen). The left testis, left epididymis, left kidney, and thyroid will be histopathologically evaluated.

#### **3.1 Test System**

Species:	Rat, <i>Rattus norvegicus</i>
Strain:	Sprague Dawley Crl:CD®(SD) IGS
Source:	Charles River Laboratories International, Inc. (Raleigh, NC)
Number/Sex F <sub>0</sub> :	50 timed pregnant nulliparous dams at gestation day (GD) 8 to generate the F <sub>1</sub> animals
	Note: GD 0 is the day of sperm positive identification.
Culling/Standardized Litters:	Between PND 3 and 5, litters with the same date of birth will be standardized to include either 8 or 10 pups with an equal number of each sex. Dams that give birth to less than 8 pups will be excluded from the study.
Number/Sex F <sub>1</sub> :	80 Males
Acclimation:	From PND 0 to PND 22
Age at Initial Dose Administration:	PND 23
	Note: PND 0 is the day the pup was first seen during a morning room check.

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Weight at Initial Dose Administration: 25-100 grams

Identification: F<sub>0</sub> animals will be identified by cage cards located on the animals' cages. F<sub>1</sub> animals will be identified by markings on the tail for randomization purposes and ear punched when assigned to treatment groups.

Justification: Test system and treatment are in accordance with the Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats test guideline (U.S. EPA, 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).

Housing F<sub>0</sub>: 1 per cage (with litter)

Housing F<sub>1</sub>: 2 per cage at allocation

Cage Type: Polycarbonate with micro-isolator lids

Cage Size: 23 cm wide by 44 cm long (1012 cm<sup>2</sup> area) and 21 cm high

Bedding: Absorbent heat-treated hardwood bedding (Northeastern Products Corp., Warrensburg, NY)

Cage Changes F<sub>0</sub>: Once per week without litter, twice per week with litter

Cage Changes F<sub>1</sub>: 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* provided 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 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

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	diet will be used during the acclimatization and treatment phase 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) <i>ad libitum</i>
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.
Water Bottle Changes:	Once per week
Animal Room Conditions:	
Temperature:	20-25°C
Humidity	30-70%
Lighting:	14/10 hour light/dark cycle (lights on: lights off; 0500-1900 hours)
Enrichment:	None

### 3.3 Allocation

The animals will be assigned to a dose group with care taken to avoid littermates in the same dose group, using a procedure that stratifies animals across groups by body weight such that mean body weight of each group is not statistically different from any other group using analysis of variance (ANOVA) (Statistical Analysis System version 9.2, SAS Institute, Cary, NC). Only clinically healthy animals will be used for allocation.

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### 3.4 Group Designation

**Table 1. Group Designation, Animal Identification, and Dose Levels**

Group Number	Animal Identification	Test Substance	Dose Level (mg/kg/day)
1	01-16	2-Ethylhexyl Paraben	0
2	17-32	2-Ethylhexyl Paraben	*
3	33-48	2-Ethylhexyl Paraben	*
4	49-64	2-Ethylhexyl Paraben	*
5	65-80	2-Ethylhexyl Paraben	*

\*Dose levels will be added by protocol amendment.

### 3.5 Dose Administration

Males (PND 23 - 53 or 54) will be administered dose formulations by oral gavage at a dosing volume of 5 mL/kg body weight. Dose formulations will be administered daily between 0700 and 0900 using an 18 gauge appropriate length stainless steel gavage needle and a 1 or 3 mL syringe. Dose volume will be based on individual animal daily body weight and recorded. Dose formulations will be placed on a stir plate at least 30 minutes prior to dosing and continuously stirred. The dosing sequence will be stratified across dose groups; one animal from each group and then repeated until all animals are administered the test substance.

#### 3.5.1 Justification of Route of Administration

Selection of the route of administration is in accordance with the Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats test guideline (U.S. EPA, 2009).

#### 3.5.2 Justification of Dose Levels

Justification of dose levels will be added by protocol amendment.

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### 3.5.3 Disposal of Dose Formulations

Dose formulations will be disposed of as hazardous material.

### 3.6 In-Life Animal Observations

Mortality/Moribundity:	Twice daily on weekdays, once daily on weekends/holidays
Clinical Observations:	<p>F<sub>0</sub> animals will be observed within two days of arrival. F<sub>1</sub> animals will be observed for allocation of animals to study groups, daily prior to dose administration, and prior to euthanasia.</p> <p>If adverse clinical signs are seen additional observations may be recorded.</p>
Cage-Side Observations:	Observations will be performed 1 hour ( $\pm$ 30 minutes) following dosing each day.
Body Weights:	Body weights will be collected on F <sub>0</sub> dams within two days of arrival. Body weights will be collected on F <sub>1</sub> animals weekly following birth (litter weights), for allocation of animals to study groups, daily prior to dose administration, and prior to euthanasia.
Preputial Separation:	Beginning on PND 30, males will be examined for PPS status. Progression not initiated, the appearance of partial separation, a persistent thread of tissue between the glans and prepuce, or complete PPS will be recorded each day.

### 3.7 Termination

Moribunds/Unscheduled:	<p>Tissue collection will not be performed on accidental deaths, moribunds, or animals found dead during the acclimation period.</p> <p>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<sub>2</sub>) inhalation and death confirmed by cervical dislocation.</p>
Scheduled:	F <sub>1</sub> animals culled between PND 3 and 5 will be humanely euthanized by CO <sub>2</sub> asphyxiation confirmed by decapitation. F <sub>0</sub> animals and F <sub>1</sub> animals not assigned to a dose group will be humanely euthanized by CO <sub>2</sub> asphyxiation confirmed by cervical dislocation following allocation on PND 21.

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	<p>Animals will be moved to the necropsy holding room at least two hours before euthanasia. F<sub>1</sub> males will be humanely euthanized on PND 53 or 54. Animals will be euthanized at least two hours following that day's dose administration and between the hours of 0900 and 1300. Animals will be humanely euthanized by decapitation, in the same order as they were dosed.</p>
Blood Collection:	<p>Prior to tissue collection, trunk blood will be collected. Blood will be collected in a serum separator tube and stored on wet ice until centrifugation. Blood will be centrifuged at 3000 g for 30 minutes at 4°C. If lipemia is observed, serum will be re-centrifuged at 10,000 g for 10 minutes at 4°C. Serum will be aliquoted into siliconized microcentrifuge tubes and stored at or below -70°C.</p>
Tissue Collection:	<p>Gross observations of the tissues that are being excised for tissue weights will be recorded.</p>
Tissue Weights:	<p>From the F<sub>1</sub> males, the following tissues will be excised, trimmed of excess adhering tissue and fat, and weights recorded to the nearest 0.1 mg, except thyroid which will be weighed to the nearest 0.01 mg.</p> <ol style="list-style-type: none"><li>1. Adrenals (paired)</li><li>2. Kidneys (paired)</li><li>3. Liver</li><li>4. Thyroid, post fixation (paired)*</li><li>5. Ventral prostate</li><li>6. Dorsolateral prostate</li><li>7. Seminal vesicle with coagulating gland (with and without fluid)</li><li>8. Levator ani plus bulbocavernous muscle complex</li><li>9. Epididymides (left and right separately)</li><li>10. Testes (left and right separately)</li><li>11. Pituitary</li></ol> <p>*The thyroid with attached trachea will be fixed in 10% neutral buffered formalin (NBF) for at least 24 hours. The thyroid will then be dissected from the trachea, blotted, weighed to the nearest 0.01 mg, and transferred to 70% histology grade alcohol. The fixed thyroid dissection will be performed by one individual to reduce variability.</p>
Clinical Pathology:	<p>T, T<sub>4</sub>, and TSH will be measured in duplicate in serum collected from male rats using radioimmunoassays (RIA). Multiple quality control samples will be dispersed among the test samples.</p>

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Serum samples will be evaluated for clinical chemistry endpoints (total protein, albumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transferase, total bilirubin, sorbitol dehydrogenase, sodium, potassium, chloride, calcium, phosphorous, creatinine, and blood urea nitrogen).

For all measurements, serum aliquot(s) will be transported on dry ice to:

Principal Investigator- Seena Polivy  
AniLytics Inc.  
20 Girard Street  
Suite 200  
Gaithersburg, MD 20877

**Histopathology:**

The left kidney and thyroid will be fixed in 10% NBF for at least 24 hours, transferred to 70% histology grade alcohol, histologically processed, and embedded in paraffin; 5 µm sections will be cut and stained with hematoxylin and eosin (H&E). Sections of the thyroid will include two serial sections obtained approximately halfway into the tissue.

The left testis and left epididymis will be placed in Bouin's fixative for 18 to 24 hours and then washed in 70% histology grade alcohol. After fixation, tissues will be transferred to 70% histology grade alcohol, histologically processed, embedded, sectioned, and stained with H&E.

Histologically processed tissues will be microscopically evaluated by the study pathologist. A minimum of two sections of each of the two lobes of the thyroid will be examined and evaluated for follicular cell height and colloid area using a five-point grading scale; other lesions or abnormalities will be noted.

**3.8 Statistical Analysis**

Descriptive statistics (mean, standard deviation, coefficient of variance, and sample size) will be calculated in Provantis version 9.3.1 (Instem, Philadelphia, PA). Each data set listed below will be analyzed using Statistical Analysis System version 9.2 (SAS Institute, Cary, NC). First, studentized residual plots will be used to detect possible outliers in the data and homogeneity of variance will be analyzed using Levene's test. If the data are heterogeneous, then transformation of the data will be performed (logarithm, square root, or multiplicative inverse). Transformed data will be analyzed for homogeneity of variance.

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Homogenous data sets listed below will be analyzed using a one-way analysis of variance followed by pairwise comparisons performed using Dunnett's t tests. If a data set cannot be transformed to become homogenous, the heterogeneous data sets will be analyzed using appropriate non-parametric tests.

Initial body weights  
Final body weight (last day all body weights collected)  
Final body weight gain (last day all body weights collected)  
Age and body weight at PPS

Data sets listed below will be analyzed using a two-way analysis of variance with treatment and necropsy day (if > 1 day) as main effects. Pair-wise comparisons will be performed using Dunnett's t tests.

Tissue weights  
Relative tissue weights (liver, kidneys, pituitary, and adrenals only)  
Hormone levels  
Clinical chemistry levels

The data sets listed below will be analyzed using analysis of covariance with PND 21 body weight as the covariable. Pair-wise comparisons will be performed using Dunnett's t tests.

Initial body weights  
Final body weight (last day all body weights collected)  
Final body weight gain (last day all body weights collected)  
Age and body weight at PPS  
Tissue weights

For the instances in which PPS has not occurred prior to necropsy, the last day of examination plus one will be used as the age at PPS. For the instances in which PPS has not occurred prior to necropsy, body weights on the last day that all body weights were collected will be used for determining body weight at PPS. In instances that at least one animal in any group exhibits incomplete separation, including partial threads for > 3 days, the day partial separation was first recorded will be statistically analyzed.

Dose-dependent changes will be evaluated using a linear regression model for both adjusted and unadjusted values if data are not significant.

Statistical analyses of histopathology results will be included as appropriate.

### 3.9 Performance Criteria

Data generated from the vehicle control rats will be compared to the performance criteria set forth by the EPA (U.S. EPA, 2009).



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**Table 2. Performance Criteria for Male Vehicle Control Sprague Dawley Rats**

Endpoint	Mean	2 SD	Acceptable Range	CV	1.5 CV	Top of Acceptable Range <sup>a</sup>
Ventral Prostate (g)						
	0.246	0.086	0.160 to 0.332	16.67	5.65	22.32
LABC (g)						
	0.651	.204	0.447 to 0.855	15.77	11.33	27.100
Epididymis (g)						
	0.446	0.082	0.364 to 0.528	10.94	5.45	16.39
Seminal Vesicles (g)						
	0.507	0.212	0.295 to 0.719	20.61	0.45	21.06
Testis (g)						
	-	-	-	9.27	8.35	17.62
T <sub>4</sub> (µg/dL)						
	5.716	1.660	4.056 to 7.376	18.27	9.20	27.46
Thyroid Weight (mg)						
	20	6	14 to 26	15.39	8.24	23.63
TSH (ng/mL)						
	14.162	9.950	4.212 to 24.112	34.04	24.26	58.29
Age at PPS (postnatal day, where day of birth=PND 0)						
	43.147	3.366	39.781 to 46.513	3.64	2.03	5.67
Body weight at PPS (g)						
	222.223	33.946	188.277 to 256.169	7.54	0.03	7.57
Testosterone (ng/mL)						
	2.110	1.850	0.260 to 3.960	58.82	30.88	89.70
Final Body Weight (g)						
	295.647	36.412	259.235 to 332.059	6.62	0.85	7.47
Adrenals (mg)						
	46.478	14.636	31.842 to 61.114	15.42	7.34	22.77
Kidneys (g)						
	2.646	0.404	2.242 to 3.050	9.56	5.20	14.76
Liver (g)						
	12.670	2.680	9.990 to 15.350	10.24	4.69	14.93
Pituitary (mg)						
	10.354	2.544	7.810 to 12.898	12.14	3.83	15.98
Weaning Body Weight (g)						
	52.642	7.170	45.472 to 59.812	8.04	2.21	10.25

Source: U.S. EPA 2009

<sup>a</sup> Bottom of acceptable range for coefficient of variation is zero.

Abbreviations: SD- standard deviation, CV- coefficient of variation, LABC- levator ani plus bulbocavernous muscle complex, T<sub>4</sub>- thyroxine, TSH- thyroid stimulating hormone.

## 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 as well as a presentation of all data collected in the study, including an executive summary. 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.

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

#### RECORD RETENTION

Upon acceptance of the final report, remaining serum samples and the sample of the diet used will be discarded. 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.

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.

U.S. EPA (Environmental Protection Agency). (2009). Endocrine Disruptor Screening Program Test Guidelines. OPPTS 890.1500: Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats. EPA 740-C-09-012. 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., D.V.M.
Necropsy Manager:	John Pope, B.S.
Histology Manager:	John Pope, B.S.
Study Pathologist:	Rebecca Moore, D.V.M., DACVP
Attending Veterinarian:	Alyssa McIntyre, D.V.M., DACLAM
Health and Safety Officer:	Michael Streicker, B.S., LATG

ILS-A-065  
Last Revised: 06/08/12

**Integrated Laboratory Systems, Inc.  
Protocol Amendment**

ILS Project No.-Study No.: 10005.0102

Sponsor Study No.: NA

Protocol Amendment No.: 1

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Section Amended: 2.1

Amendment Made: 2-Ethylhexyl paraben will be prepared in corn oil twice during the study and stored between 1 and 10°C.

Reason for Amendment: At the time the study protocol was approved, storage stability testing of 2-ethylhexyl paraben was not complete. 2-Ethylhexyl paraben prepared in corn oil at concentrations of 1 and 200 mg/mL stored between 1 and 10°C were found to be stable for at least 20 days.

ILS-A-065  
Last Revised: 06/08/12

Section Amended: 3.4

Amendment Made: Added dose levels.

Group Number	Animal Identification	Test Substance	Dose Level (mg/kg/day)
1	01-16	2-Ethylhexyl Paraben	0
2	17-32	2-Ethylhexyl Paraben	250
3	33-48	2-Ethylhexyl Paraben	500
4	49-64	2-Ethylhexyl Paraben	750
5	65-80	2-Ethylhexyl Paraben	1000

Reason for Amendment: At the time the study protocol was approved, dose levels had not been selected and were to be added by amendment following review of the results of the range finding study.

ILS-A-065  
Last Revised: 06/08/12

Section Amended: 3.5.2

Amendment Made: Justification of the selected dose levels.

In a recent range finding study conducted at ILS, all immature male rats administered 2-ethylhexyl paraben for 14 days survived to study termination. No clinical signs of toxicity were observed prior to or following dose administration for animals administered 0, 200, 400, 600, or 800 mg/kg/day 2-ethylhexyl paraben. Lethargy was noted in two animals administered 1000 mg/kg/day 2-ethylhexyl paraben post-dose on Study Day 1; however, there were no further signs of toxicity during the dose administration period.

There was not a significant change in final body weight or body weight gain following 14 days of 2-ethylhexyl paraben dose administration. Final body weights averaged 93.0%, 94.0%, 97.4%, 89.7% (93.5% if an animal with a marked overnight decrease in body weight overnight is excluded), and 91.4% of controls in animals administered 200, 400, 600, 800, or 1000 mg/kg/day 2-ethylhexyl paraben, respectively. Mean liver, kidney, and ventral prostate weights (absolute and relative) in all dose groups were not significantly different from the control group.

Based on the results from the dose range finding assay, 1000 mg/kg/day has been selected to be the top dose evaluated in this study.

Reason for Amendment: At the time the study protocol was approved, dose levels had not been selected and were to be added by amendment following review of the results of the range finding study.

ILS-A-065  
Last Revised: 06/08/12

Section Amended: 3.7

Amendment Made: Left and right 4th and 5th mammary glands will be collected at termination. Left intact mammary gland will be collected as described in Study Specific Procedure 1. Right mammary gland will be collected, fixed in 10% neutral buffered formalin, histologically processed, stained with hematoxylin and eosin, and microscopically evaluated.

Reason for Amendment: The Sponsor requested mammary gland collection and microscopic evaluation.

Section Amended: 3.7

Amendment Made: Samples for clinical pathology will be sent to:

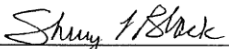
Principal Investigator: Charles Walker

Antech GLP  
600 Airport Blvd  
Suite 500  
Morrisville, NC 27560

Quality Assurance functions related to the analyses will be the responsibility of the QA Unit at Antech GLP.


Reason for Amendment: AniLytics Inc. no longer offers clinical chemistry testing.

ILS-A-065  
Last Revised: 06/08/12

  
Sherry Black, B.S.  
Sponsor Representative

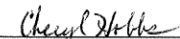
2/22/2016  
Date

Approval via  
e-mail. JD  
22 Feb 2016

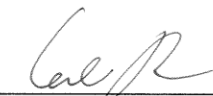
  
Jeffrey P. Davis, B.S., LATG  
Study Director  
Integrated Laboratory Systems, Inc.

22 Feb 2016  
Date

Reviewed by:

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

22 Feb 2016  
Date

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

24 Feb 2016  
Date

**Jeffrey Davis**

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**From:** Black, Sherry L. <sherryb@rti.org>  
**Sent:** 08 February 2016 16:17  
**To:** Jeffrey Davis  
**Cc:** Les Recio  
**Subject:** RE: Protocol amendments

Hi Jeff –

These amendments all look fine. Can you provide them as pdfs? I will sign and return the approval.

Thanks  
Sherry

---

**From:** Jeffrey Davis [mailto:jdavis@ils-inc.com]  
**Sent:** Thursday, February 04, 2016 3:59 PM  
**To:** Black, Sherry L. <sherryb@rti.org>  
**Cc:** Les Recio <lrecio@ils-inc.com>  
**Subject:** Protocol amendments

Hi Sherry,

Attached are study protocol amendments for the male pubertal evaluating 2-EHP, the female pubertal evaluating BBP, and the dose range finding study for triclosan. Dose levels of 2-EHP are added as stipulated in the study protocol, storage stability testing is now complete and the frequency of preparations and storage of prepared doses are added, and mammary gland collection and evaluation is added. I also am amending the clin path lab in both pubertal protocols. AniLytics no longer provides clinical chemistry testing as of 01Jan. It definitely caught us off guard. We have used Antech previously to perform clinical chemistry and hormone analysis.

I also provided an amendment on the triclosan RF assay to clarify the dosing period and to request a broadening of the dosing window. We are keeping the DBP and octylphenol RF dosing windows from 0700 to 0900 to match the pubertal assay TGs, but would like an additional hour on this one to ensure we can administer all doses within protocol stated times.

Regards,  
Jeff

Jeffrey P. Davis, MBA, LATG  
Investigative Toxicology Program Manager  
ILS, Inc.  
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[www.ils-inc.com](http://www.ils-inc.com)



ILS-A-065  
Last Revised: 06/08/12

**Integrated Laboratory Systems, Inc.  
Protocol Amendment**

ILS Project No.-Study No.: 10005.0102

Sponsor Study No.: NA

Protocol Amendment No.: 2

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Section Amended: 3.7

Amendment Made: Samples for clinical chemistry tests will be sent to:

Principal Investigator: Charles Walker

Antech GLP  
600 Airport Blvd  
Suite 500  
Morrisville, NC 27560

Quality Assurance functions related to the analyses will be the responsibility of the QA Unit at Antech GLP.

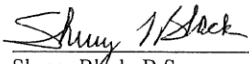
Samples for serum hormone analysis will be sent to:

Principal Investigator- Seena Polivy  
AniLytics Inc.  
20 Girard Street  
Suite 200  
Gaithersburg, MD 20877

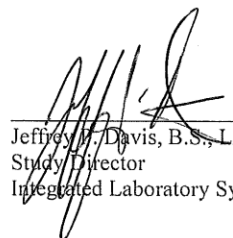
Quality Assurance functions related to the analyses will be the responsibility of the QA Unit at AniLytics Inc.

Reason for Amendment: While AniLytics Inc. no longer offers clinical chemistry testing; they continue to offer serum hormone analyses. ILS maintains a robust historical control range of serum hormones evaluated using radioimmunoassay kits for reference, the procedure utilized by AniLytics Inc.

ILS-A-065  
Last Revised: 06/08/12

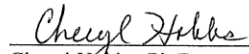
  
Sherry Black, B.S.  
Sponsor Representative

3/15/16  
Date

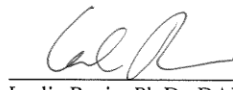
  
Jeffrey J. Davis, B.S., LATG  
Study Director  
Integrated Laboratory Systems, Inc.

23 March 2016  
Date

Reviewed by:

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

15 March 2016  
Date

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

21 March 2016  
Date

ILS-A-065  
Last Revised: 06/08/12

**Integrated Laboratory Systems, Inc.  
Protocol Amendment**

ILS Project No.-Study No.: 10005.0102

Sponsor Study No.: NA

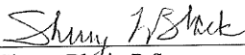
Protocol Amendment No.: 3


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Section Amended: 3.7

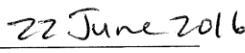
Amendment Made: Left 4th and 5th mammary glands (whole mount) will be evaluated by the study pathologist.

Reason for Amendment: Amendment 1 and the Study Specific Procedure detail the collection and processing of whole mammary glands, but do not explicitly state the glands will be evaluated.

  
Sherry Black, B.S.  
Sponsor Representative

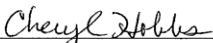
  
Date

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


  
Date

ILS-A-065  
Last Revised: 06/08/12

Reviewed by:

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

20 June 2016  
Date

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

20 June 2016  
Date

## Study Specific Procedure No. 1

Project No.-Study No.: 10005.0102

Title: Necropsy Procedure for Mammary Gland Whole Mount

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### I. Purpose:

To establish a procedure for the collection, mounting, and staining of whole mammary glands.

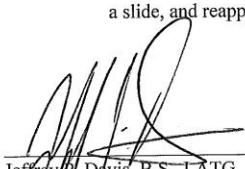
### I. Collection Procedure

- a. Euthanize animals as designated in the study protocol.
- b. Lay the animal on its back on a dissecting board.
- c. Wet the animal's abdomen and rear legs with 70% ethanol (necessary to reduce hair in the gland preparation).
- d. Make a skin incision from the pubis to the rib cage, being careful not to cut through the internal abdominal wall.
- e. Make an incision from the origin of the first incision (at the pubis) along the medial aspect of each rear limb, forming an inverted Y.
- f. Remove the skin from the abdomen with the mammary glands attached.
- g. Pin the skin to the dissection board (or other flat surface such as a cooler top). This exposes the 4<sup>th</sup> and 5<sup>th</sup> mammary glands (and 6<sup>th</sup> in the rat). In the rat, the 5<sup>th</sup> and 6<sup>th</sup> glands may need to be separated by cutting through the gland at the leg.
- h. The left gland will be collected for whole mount. The right gland will be preserved in 10%NBF for paraffin embedding and histopathologic evaluation.
- i. Gently separate the fat pad containing the 4<sup>th</sup> and 5<sup>th</sup> glands (leave the 6<sup>th</sup> gland in rats) from the skin at the point where the skin is pinned and lift it away from the skin, cutting the attachments to the skin as you go (with fine scissors or sharp blade).
- j. When the entire fat pad (to the back of the animal) with the 4<sup>th</sup> and 5<sup>th</sup> mammary glands has been separated from the skin, make a straight cut parallel to the animal's body and detach the fat pad/mammary glands.
- k. Spread the mammary tissue on a dry slide, skin side down, with the forceps (completely spread out), one fat pad/mammary gland set (4<sup>th</sup> and 5<sup>th</sup> glands as a single unit) per slide. The thickest part of the gland should be adjacent to the slide label and the lymph node should be near the center of the slide. Spread the gland to the original size in the body, trying to lay it flat, without bubbles under it.

### II. Fixation and Staining Procedure

- a. Once the mammary tissue has been spread onto a slide, press the tissue with your gloved fingers to remove bubbles that may be under the tissue, place a 2x3 inch rectangle of Parafilm on the gland and cover with another glass slide. Weight the cover glass.
- b. Compress the mammary tissue in a refrigerator for 30 minutes to several hours, depending on its thickness (rat PND 45 for ~1-2 hours).

- c. Remove top glass slide, and peel the Parafilm back from one end, being careful not to loosen the gland from the slide. Place the slides in a glass staining tray. Fix the tissue in Carnoy's Fixative for 4 to 48 hours, depending on thickness, at room temperature. Most mammary tissue from PND 32-45 rats and smaller can be fixed for 18-24 hours (overnight). If white areas are present in the mammary tissue after fixing (more opaque than rest of gland), change the fixative and allow those glands to fix for an additional 24 hours.
- d. Soak in 70% ethanol for 15-30 minutes.
- e. Change gradually to water. (Pour out 1/3, add water, let sit 5 minutes, repeat 3 times).
- f. Equilibrate in water for 5 minutes.
- g. Stain in Carmine alum stain for 12-24 hours, depending on thickness (longer does not hurt it, but be consistent for all tissues of same age). The stain is reusable.
- h. Soak in water for 30 seconds.
- i. Soak in 70% ethanol for 15-30 minutes.
- j. Soak in 95% ethanol for 15-30 minutes.
- k. Soak in 100% ethanol for 20-30 minutes.
- l. Clear in xylene for 4 to 72 hours, depending on the thickness of gland. The gland should be translucent after clearing. If any opaque (whitish) areas remain, place those slides in larger containers with xylene until translucent (clear – this could take a week for thick glands).
- m. Pipet enough mounting media onto the tissue to cover the specimen and place a cover slip on top, being careful to avoid air bubbles. If bubbles form, lift the cover slip, pop the bubbles with the edge of a slide, and reapply the cover slip (additional mounting media may be necessary).

  
Jeffrey F. Davis, B.S., LATG  
Study Director  
Investigative Toxicology Division  
Integrated Laboratory Systems, Inc.

16 March 2016  
Date

**Integrated Laboratory Systems, Inc.  
Protocol Deviation**

ILS Project No.-Study No.: 10005.0102

Sponsor Study No.: NA

Protocol Deviation No.: 2

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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.

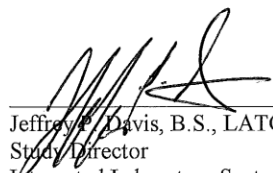
Maximum Temp °C	24.8		Maximum Humidity %	85.6
Minimum Temp °C	19.1		Minimum Humidity %	24.9
Mean Temp °C	21.7		Mean Humidity %	46.1
No. of Points Out of Range	5		No. of Points Out of Range	136
Time out of Range (min)	75		Time out of Range (min)	2040
% Out of Range	0.1%		% Out of Range	2.1%
Total Days Out	1		Total Days Out	9

Reason for Deviation: On 29 January through 01 February 2016 and again on 25 February 2016, a chiller malfunction caused the humidity to fluctuate and be out of range.

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.

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

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

28 July 2016  
Date



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

**Integrated Laboratory Systems, Inc.  
Protocol Deviation**

ILS Project No.-Study No.: 10005.0102

Sponsor Study No.: NA

Protocol Deviation No.: 1

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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 two weeks 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 H. Davis, B.S., LATG  
Study Director  
Integrated Laboratory Systems, Inc.

28 July 2016  
\_\_\_\_\_  
Date

**Integrated Laboratory Systems, Inc.**  
**Protocol Deviation**

ILS Project No.-Study No.: 10005.0102

Sponsor Study No.: NA

Protocol Deviation No.: 3

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Protocol Section Deviated from: 3.7

Nature of Deviation: Rats were humanely euthanized between 0900 and 1300 with the exception of two rats that were euthanized between 1300 and 1321 on the first day of necropsy.

Animals 56: 750 mg/kg/day

Animals 72: 1000 mg/kg/day

Reason for Deviation: The number of animals coupled with the addition of mammary gland collection resulted in a longer necropsy time per animal than anticipated.

Corrective Action: For the second day of necropsy, an additional certified prosector assisted with the necropsy.

Impact on Study: None. Clinical pathology results were evaluated with and without the two animals euthanized after 1300 hours. No differences were observed between inclusion and exclusion of the two rats, with one exception. There was not a statistically significant increase in calcium concentration when the two rats were excluded following administration of 500 mg/kg/day compared to the control group. Previous analysis including data from the two rats euthanized after 1300 hours did result in a significant pair-wise comparison (p-value: 0.0001).

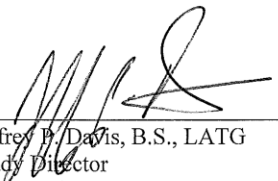
ILS-A-066  
Last Revised: 06/08/12

Protocol Section Deviated from:	3.1
Nature of Deviation:	<p>Litters were culled to four males and four females, with the exception of four litters.</p> <p>Dam 25: Three males and five females Dam 30: Five males and three females Dam 34: Five males and three females Dam 40: Five males and three females</p> <p>The following offspring were allocated to a dose group.</p> <p>Dam 30, pup 2: Animal #06, 0 mg/kg/day Dam 30, pup 1: Animal #22, 250 mg/kg/day Dam 30, pup 3: Animal #55, 750 mg/kg/day</p>
Reason for Deviation:	To ensure enough litters were available for allocation of offspring to dose groups while avoiding littermates in the same group, four litters with unequal number of males and females following culling were maintained.
Corrective Action:	None.
Impact on Study:	None.
Protocol Section Deviated from:	3.7
Nature of Deviation:	The left testis and left epididymides were not fixed in Bouin's fixative, but rather fixed in 10% neutral buffered formalin.
Reason for Deviation:	Oversight by the necropsy coordinator.
Corrective Action:	None.

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

Impact on Study:

While fixation in 10% neutral buffered formalin is less optimal than fixation in Bouin's fixative, microscopic evaluation was not impacted.

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

28 July 2016  
Date

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

**Integrated Laboratory Systems, Inc.**  
**Protocol Deviation**

ILS Project No.-Study No.: 10005.0102

Sponsor Study No.: NA

Protocol Deviation No.: 4

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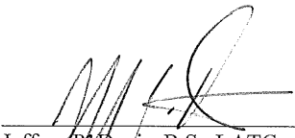
Protocol Section Deviated from: 3.7

Nature of Deviation: Lipemic samples were re-centrifuged; however, it was not properly documented. Serum conditions were recorded and when cloudy conditions were present samples were re-centrifuged per protocol, but not was explicitly recorded.

Reason for Deviation: Oversight by technical staff.

Corrective Action: Staff were reminded to document all procedures.

Impact on Study: None. Serum sample conditions were recorded and document the samples were in fact cloudy (lipemic).

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

01 August 2016  
Date

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

**Integrated Laboratory Systems, Inc.  
Protocol Deviation**

ILS Project No.-Study No.: 10005.0102

Sponsor Study No.: NA

Protocol Deviation No.: 5

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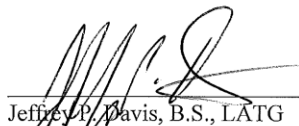
Protocol Section Deviated from: 3.7

Nature of Deviation: Tissues collected for histopathology were transferred to 80% histology grade alcohol during processing rather than 70% histology grade alcohol.

Reason for Deviation: Oversight by the Necropsy/Histology Coordinator.

Corrective Action: None.

Impact on Study: None. The minimal difference in percent of histology grade alcohol did not impact histological processing or the microscopic evaluation of the tissues.

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

22 August 2016  
\_\_\_\_\_  
Date