FINAL REPORT

Study Title

The Hershberger Bioassay for Padimate-O and Homosalate

ILS Project-Study Number

N135-249

Guideline Reference Number

OPPTS 890.1400

Author

[redacted]

Performing Laboratory

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Sponsor

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P.O. Box 12233
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Date of Completion

07 November 2012

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Sponsor:	National Institute of Environmental Health Sciences
Sponsor Representative:	[redacted]
Title:	Contract Officer Technical Representative
Signature:	[redacted]
Date:	11/7/12

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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 §160 with the following exceptions:

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

Dose formulation analyses were performed at Research Triangle Institute, International (Research Triangle Park, NC) with [redacted] as the Study Director at the request of the sponsor.

Study Director: [redacted]

Signature: [redacted] Date: 11-7-12

Typed Name of Laboratory: Integrated Laboratory Systems, Inc.

Typed Name of Study Monitor/Sponsor/Submitter: [redacted]

Signature: [redacted] Date: 11/7/12

Typed Name of Company: National Institute of Environmental Health Sciences

This final report has been reviewed by:

[redacted] 11-7-12

Date:

Study Toxicologist
Investigative Toxicology Division
Integrated Laboratory Systems, Inc.

QUALITY ASSURANCE INSPECTION STATEMENT

Laboratory Project ID - Study No.: N135-249

Study Title: The Hershberger Bioassay for Padimate-O and Homosalate

This study was inspected by one or more persons of the Quality Assurance Unit of ILS, Inc., Research Triangle Park, NC, US, and written status reports were submitted on the following dates:

Inspection/Audit:	Date(s) Performed:	Dates Reported to Study Director / Management:
Study Protocol:	22 March 2012	22 March 2012/22 March 2012
Dose Formulation:	19 March 2012	19 March 2012/19 March 2012
Data Audit:	27/28 June, 01-03 July 2012	03 July 2012/12 July 2012
Draft Report:	27/28 June, 01-03 July 2012	03 July 2012/12 July 2012
Final Report:	02 November 2012	02 November 2012/02 November 2012

[redacted]
Quality Assurance Auditor

07 Nov 2012
Date

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SUMMARY

The purpose of this Hershberger Bioassay was to screen Padimate-O and Homosalate for their ability to elicit biological activities consistent with androgen agonists, antagonists, or 5α-reductase inhibitors using the adult castrated rat model. This assay is one of a suite of assays selected for Tier 1 Screening as part of U.S. EPA's Endocrine Disruptor Screening Program (EDSP).

One hundred four castrated male Sprague-Dawley (SD) rats were allocated to 1 of 13 designated dose groups. To evaluate the test substance for agonist properties, animals were administered 1 of 2 dose levels (320 or 1000 mg/kg/day) of Padimate-O or Homosalate, the vehicle control (corn oil), or the agonist reference substance testosterone propionate (TP, 0.4 mg/kg/day). To evaluate Padimate-O or Homosalate for antagonist properties animals were co-administered 1 of 3 dose levels (100, 320, or 1000 mg/kg/day) of Padimate-O, or Homosalate with TP. Flutamide (FT, 3 mg/kg/day, antagonist positive control) with TP (0.4 mg/kg/day) was utilized as the antagonist positive control.

Animals were dosed for 10 consecutive days via oral gavage (Padimate-O, Homosalate, or FT) and subcutaneous injection (TP). Approximately 24-hours following the final dose administration, the animals were humanely euthanized; the glans penis, ventral prostate, levator ani plus bulbocavernous muscle complex (LABC), Cowper's gland, and seminal vesicle with coagulating gland with fluid were excised and weighed. Changes in androgen-dependent tissue weights were evaluated to determine the ability of Padimate-O or Homosalate to act as androgen agonists/antagonists or inhibitors of 5α-reductase.

In the agonist assay, final body weight and body weight gain were not significantly different in animals administered 320 mg/kg/day Padimate-O compared to vehicle control animals (corn oil). Body weight gain was significantly decreased in animals administered 1000 mg/kg/day Padimate-O (93.1%) compared to vehicle controls. Final body weight was not significantly different in animals administered 1000 mg/kg/day Padimate-O compared to vehicle controls. The glans penis, ventral prostate, LABC, Cowper's gland, and seminal vesicle with coagulating gland with fluid were not significantly different in animals administered 320 or 1000 mg/kg/day Padimate-O compared to vehicle control animals (corn oil).

In the antagonist assay, final body weights and body weight gain of animals co-administered 100 or 320 mg/kg/day Padimate-O and TP were not statistically different compared to vehicle control animals (corn oil and TP). Final body weights and body weight gain of animals administered 1000 mg/kg/day of Padimate-O and TP were significantly decreased (89.6% of controls) compared to vehicle control animals (corn oil and TP). In animals co-administered 1000 mg/kg/day Padimate-O and TP, the glans penis, LABC, and seminal vesicle weights were significantly lower compared to vehicle control animals (corn oil and TP). The glans penis, LABC, and seminal vesicle weights at dose levels of 100 or 320 mg/kg/day were not different compared to vehicle controls

(corn oil and TP). The ventral prostate and Cowper's gland weights were not different compared to vehicle control weights at any dose level of Padimate-O.

In the agonist assay, final body weight and body weight gain were not significantly different in animals administered 320 mg/kg/day Homosalate compared to vehicle control animals (corn oil). Compared to vehicle control animals, body weight gain (90.9%), but not final body weight, was significantly decreased in animals administered 1000 mg/kg/day Homosalate. The glans penis, ventral prostate, LABC, Cowper's gland, and seminal vesicle with coagulating gland with fluid weights were not significantly different in animals administered 320 or 1000 mg/kg/day compared to vehicle control animals (corn oil).

In the antagonist assay, final body weights and body weight gain of animals administered 100 or 320 mg/kg/day Homosalate and TP were not statistically different compared to vehicle control animals (corn oil and TP). Final body weights and body weight gain of animals administered 1000 mg/kg/day Homosalate and TP were significantly decreased (89.4% of controls) compared to control animals (corn oil and TP). Homosalate co-administered with TP at 1000 mg/kg/day significantly decreased the LABC weights, but not any other androgen-dependent tissue weights.

Based on these findings using the castrated rat model, oral administration of Homosalate, up to the limit dose of 1000 mg/kg/day, did not show any androgen agonist/antagonist activity, or 5α-reductase inhibition.

Administration of Padimate-O, up to the limit dose of 1000 mg/kg/day, was positive in the antagonist Hershberger Bioassay with a significant decrease in three androgen-dependent tissue weights.

INTRODUCTION

1.1 Study Title

The Hershberger Bioassay for Padimate-O and Homosalate

1.2 Laboratory Project Identification

ILS Project No.-Study No.: N135-249

1.3 Background

The Endocrine Disruptor Screening Program (EDSP) reflects a two-tiered approach to implement the statutory testing requirements of FFDCA section 408(p) (21 U.S.C. 346a). The U.S. EPA will use the data collected under the EDSP, along with other information to determine if a pesticide, chemical, or other substances may pose a risk to human health or the environment due to disruption of the endocrine system.

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 the potential of each test substance activity will be made on a weight-of-evidence basis taking into account data from the Tier 1 assays and other available scientifically-relevant information. The fact that a substance may interact with a hormone system, however, does not mean that when the substance is used it will cause adverse effects in humans or ecological systems. The Hershberger Bioassay (OPPTS 890.1400) is used as an *in vivo* screening assay for androgen agonists, androgen antagonists, and 5α-reductase inhibitors and is one of four *in vivo* mammalian assays in the EDSP Tier 1 battery of assays.

1.4 Purpose of the Study

The purpose of the Hershberger Bioassay assay was to screen Padimate-O and Homosalate for their ability to elicit biological activities consistent with androgen agonists, antagonists, or 5α-reductase inhibition using the castrated rat model (OPPTS 890.1400).

1.5 Sponsor

National Institute of Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, NC 27709

NIEHS Investigator
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Telephone No.: [redacted]
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Study Monitor
[redacted].
Contract Officer Technical Representative
Telephone No.: [redacted]
E-mail: [redacted]

1.6 Testing Facility **Integrated Laboratory Systems, Inc. (ILS)**

Shipping Address: 601 Keystone Park Drive, Suite 100
Durham, NC 27713 USA

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

Study Director
[redacted]
Telephone No.: [redacted]
Facsimile No.: [redacted]
E-mail: [redacted]

1.7 Study Dates

Study Initiation Date: 13 April 2012
Animal Arrival Date: 12 April 2012
Experimental Start Date: 21 April 2012
Experimental End Date: 02 May 2012

TEST SUBSTANCE

2.1 Test Substance: **2-Ethylhexyl-P-Dimethyl-Aminobenzoate (Padimate-O)**

CAS No. 21245-02-3

Source: Sigma-Aldrich Company

Lot/Batch No.: MKBF0590V

Expiration: 21 February 2014

ILS Repository No.: 12-26

Formula: $C_{17}H_{27}NO_2$

Description:	Colorless liquid
Purity:	98.1%
Dose Formulation:	Test substance formulations were prepared at ILS one time. Padimate-O formulations were prepared using corn oil as the vehicle at concentrations of 20, 64, or 200 mg/ml and dispensed into 15 mL amber vials that were used for daily dosing throughout the study.
Storage:	
Test Substance:	Ambient temperature
Dose Formulation:	Ambient temperature protected from light
Stability:	
Dose Formulation:	Padimate-O in corn oil stored at ambient temperature was shown to be stable for 43 days (Blake, 2011).

2.2 Test Substance: 3,3,5-Trimethylclohexyl Salicylate (Homosalate)

CAS No.	118-56-9
Source:	Spectrum Laboratory Products Inc.
Lot/Batch No.:	YT0976
Expiration:	11 November 2012
ILS Repository No.:	12-24
Formula:	$C_{16}H_{22}O_3$
Description:	Colorless to light yellow liquid
Purity:	99.3%
Dose Formulation:	Test substance formulations were prepared at ILS one time. Homosalate formulations were prepared using corn oil as the vehicle at concentrations of 20, 64, or 200 mg/ml and dispensed into 15 mL

amber vials that were used for daily dosing throughout the study.

Storage:

Test Substance: Ambient temperature

Dose Formulation: Ambient temperature protected from light

Stability:

Dose Formulation: Homosalate in corn oil stored at ambient temperature was shown to be stable for 42 days (Blake, 2012a).

2.3 Reference Substance: Testosterone Propionate (TP)

CAS No. 57-85-2

Source: Sigma-Aldrich Company

Lot/Batch No.: 051M1803V

Expiration: 06 March 2014

ILS Repository Number: 09-26

Formula: $C_{22}H_{32}O_3$

Description: White to off-white powder

Purity: 100%

Dose Formulation: TP was prepared at ILS in corn oil once at a dose level of 0.8 mg/mL and dispensed into vials used daily during the study.

Storage:

Reference Substance: Ambient temperature, protected from light

Dose Formulation: Between 1-10°C

Stability:

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

2.4	**Reference Substance:**	**Flutamide (FT)**
	CAS No.	13311-84-7
	Source:	Sigma-Aldrich Company
	Lot/Batch No.:	021M1406V
	Expiration:	27 June 2012
	ILS Repository No.:	11-77
	Formula:	$C_{11}H_{11}F_3N_2O_3$
	Description:	Yellow powder
	Purity:	>99%
	Dose Formulation:	FT was prepared at ILS in corn oil once at a dose level of 0.6 mg/mL and dispensed into vials used daily during the study.
	Storage:	
	Reference Substance:	Ambient temperature, protected from light
	Dose Formulation:	Between 1-10°C
	Stability:	
	Dose Formulation:	FT prepared in corn oil stored between 1-10°C was shown to be stable for 42 days (Graves, 2001).
2.5	**Vehicle**	**Corn Oil**
	CAS No.:	8001-30-7
	Source:	MP Biomedicals, LLC
	Lot/Batch No.:	7862K
	ILS Repository No.:	11-121
	Formula:	$C_{27}H_{50}O_6$

Description: Yellow oil

Storage: Room temperature

2.6 Archival Samples

Approximately a 1 g sample of the neat test substance and 1 mg of the reference substances are stored between 0 and -30°C. One mL of the vehicle and each dose formulation are stored between 0 and -30°C until acceptance of the final report; after acceptance of the report by the Sponsor archival dose formulations will be discarded. The archival test substance and reference substance samples will be maintained by ILS for 5 years following submission of the final report to the Sponsor.

2.7 Dose Formulation Analysis

Dose formulations were prepared at ILS then sent and analyzed at Research Triangle Institute (RTI) International in accordance with GLP regulations as promulgated by the U.S. EPA (40 CFR Part 160).

Research Triangle Institute, International
[redacted]
Materials Handling Facility
East Institute Drive
Research Triangle Park, NC 27709

Three samples (top, middle, and bottom) of the test substance were analyzed for concentration and homogeneity. Concentration results were acceptable if the mean concentration was within 10% of the target concentration. Homogeneity results were acceptable if the coefficient of variation was ≤5%.

EXPERIMENTAL DESIGN

3.1 Test System

Species: Rat, *Rattus norvegicus*

Strain: Sprague-Dawley Crl:CD®(SD) IGS

Source: Charles River Laboratories International, Inc. (Raleigh, NC)

Number/Sex: 104/castrated males; surgical manipulation performed by Charles River Laboratories International, Inc., rats were postnatal day (PND) 44 at surgery.

	Note: PND 0 is the day of birth
Date of birth:	22 February 2012
Age at arrival:	PND 50
Acclimation:	Animals were acclimated in the study room for 8 days.
Age at dose administration:	PND 59/60
Weight at dose administration:	268.3 - 337.3 grams
Identification:	Each animal was uniquely identified by ear punch prior to dose administration. Until the animals were ear punched, they were identified by the temporary numbers located on the animal's cage.
Justification:	Animal model used was in accordance with OPPTS 890.1400: Hershberger Bioassay (U.S. EPA, 2009).

3.2 Animal Husbandry

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

Housing (pre-allocation):	1 per cage
Housing (post allocation):	2 per cage
Cage Changes:	At least twice per week
Cage Type:	Polycarbonate with micro-isolator top
Cage Size:	23 cm wide by 44 cm long (1012 cm^2 area) and 21 cm high
Bedding:	Absorbent heat-treated hardwood bedding (Northeastern Products Corp., Warrensburg, NY)
Diet:	Teklad Global 16% Protein Rodent Diet (Teklad Diets, Madison, WI) *ad libitum*

Prior to shipment rats were given Autoclaved Purina 5L79 Rat and Mouse diet *ad libitum* at Charles River Laboratories International, Inc. A copy of the diet composition is included in the raw data.

Analysis: The manufacturer's analytical results is included in the raw data and reviewed prior to animal arrival. The total genistein equivalent of genistein plus daidzein (as described by Owens et al., 2003) was determined to be 3.8 µg/g of feed.

Water: Reverse osmosis treated tap water (City of Durham, NC) *ad libitum*

Supplied: Glass water bottles with stainless steel sipper tubes

Analysis: The results of the current annual comprehensive chemical analyses of water from National Testing Laboratories, Inc. (Cleveland, OH) were reviewed prior to initiation of the study and are included in the raw data.

Water Bottle Changes: At least once per week

Animal Room Conditions:

Temperature: 21.6-25.1°C (See Protocol Deviation 1)

Humidity 28-60% (See Protocol Deviation 1)

Lighting: 12/12 hour light/dark cycle

Cleaning: The room was sanitized within 1 day of animal receipt.

Enrichment: None

STUDY DESIGN

4.1 Allocation

The animals were assigned to a dose group using a procedure that allocated 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].

4.2 Group Designation

Table 1. Androgen Agonist - Group Number, Animal Identification, Dose Group and Level

Group Number	Animal Identification	Test Substance / Control	Test Substance Dose Level (mg/kg/day)
1	001-008	Vehicle Control (Corn Oil)	0
2	009-016	Padimate-O	320
3	017-024	Padimate-O	1000
4	025-032	Homosalate	320
5	033-040	Homosalate	1000

Table 2. Androgen Antagonist - Group Number, Animal Identification, Dose Group and Level

Group Number	Animal Identification	Test/Reference Substance / Control	Test/Reference Dose Level Substance (mg/kg/day)
6[§]	041-048	Vehicle Control (Corn Oil) + TP	0 + 0.4
7	049-056	Padimate-O + TP	100 + 0.4
8	057-064	Padimate-O + TP	320 + 0.4
9	065-072	Padimate-O + TP	1000 + 0.4
10	073-080	Homosalate + TP	100 + 0.4
11	081-088	Homosalate + TP	320 + 0.4
12	089-096	Homosalate + TP	1000 + 0.4
13	097-104	FT + TP	3 + 0.4

[§] Group served as the positive control for the agonist assay and control in the antagonist assay

4.3 Dose Administration

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

The dose formulations were administered on a staggered start for 10 consecutive days (PND 59/60 through PND 68/69). The first 4 animals from each group were dosed beginning on PND 59 and the second 4 from each group on PND 60. Dosing occurred 24-hours (± 2 hours) from the previous dose. Dose volume was determined on individual animal daily body weight. The dosing sequence was stratified across dose groups; 1 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 is in accordance with OPPTS 890.1400: Hershberger Bioassay (U.S. EPA, 2009).

4.3.2 Justification of Dose Levels

OPPTS 890.1400 specifies to select doses that ensure animal survival and that are without significant toxicity or distress to the animals after ten consecutive days of chemical administration, and the highest dose should not cause a reduction in the final body weight of the animals greater than 10% of control weight.

Selection of the highest dose level for each test substance was based upon the available LD_{50} and/or acute toxicity information in order to avoid death, severe suffering, or distress in the animals and second, takes into consideration available information on the doses used in other studies. In general, the highest dose should not cause a reduction in the final body weight of the animals greater than 10% of control body weight. The highest dose should ensure animal survival and that is without significant toxicity or distress to the animals after 10 consecutive days of administration up to a maximal dose of 1000 mg/kg/day.

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.
Clinical Observations:	Observed within 2 days of arrival, again for allocation of animals to study groups, daily prior to dose administration, and prior to euthanasia.
Body Weights:	Collected within 2 days of arrival, again for allocation of animals to study groups, daily prior to dose administration, and prior to euthanasia.

4.5 Termination

Scheduled:	Twenty-four hours (± 2 hours)* after the final dose administration, animals were humanely euthanized by carbon dioxide (CO_2) asphyxiation with death confirmed by cervical dislocation in the same order as they were dosed.

*2 animals were necropsied <22-hours post-dose administration (See protocol deviation 1)

Tissue Collection: Gross observations of the tissues that were excised for tissue weights were recorded.

Tissue Weights: The following tissues were excised, trimmed of excess adhering tissue and fat, and weighed to the nearest 0.0001 g.

1. Ventral Prostate
2. Seminal vesicle with coagulating gland with fluid
3. Levator ani plus bulbocavernous muscle complex (LABC)
4. Cowper's gland (weighed as a pair)
5. Glans penis

4.6 Statistical Analysis

Descriptive statistics (mean, standard deviation, and coefficient of variance) were calculated using MS Excel. Final body weight, body weight gain, and tissue weights were analyzed using SAS version 9.2 (Cary, NC). Studentized residual plots were used to detect possible outliers and Levene's test was used to assess homogeneity of variance.

Final body weight, body weight gain, and androgen-dependent tissue weights were analyzed by one-way ANOVA followed by pair-wise comparisons using a Dunnett's one tailed t test (tissues weights) or Dunnett's two tailed t test (final body weight and body weight gain). Positive controls were analyzed by the t-test procedure. Statistically-significant effects were reported when $p<0.05$.

4.7 Record Retention

All original data [including the original signed study protocol and all amendments (if any), test substance information, animal receipt records, animal caretaker records, observations, body weight records, clinical observations, etc.] and the original final report will be transferred to the National Toxicology Program Archives following finalization of the study report to the address below:
NTP Archives
[redacted]
615 Davis Drive, Suite 300
Durham, NC 27713

RESULTS

5.1 Dose Formulation Analysis

Actual concentration and homogeneity results of each dose formulation used in the study were within the acceptance criteria (Appendix II).

Table 3. Analytical Results for Dose Formulations
Preparation Date: 22 March 2012

Test Substance	Nominal Dose Formulation Concentration (mg/mL)	Actual Dose Formulation Concentration* (mg/mL) [Percent from Nominal]	Percent CV* (Homogeneity)	Nominal Dose Level (mg/kg/day)	Actual Dose Level (mg/kg/day)
Padimate-O	20	19.5 [2.7]	3.0	100	97.5
Padimate-O	64	62.9 [1.7]	3.1	320	314.5
Padimate-O	200	191 [4.5]	3.1	1000	955
Homosalate	20	19.2 [4.2]	1.4	100	96.0
Homosalate	64	61.4 [4.1]	1.8	320	307.0
Homosalate	200	194 [3.2]	3.5	1000	970

*Source: Blake (2012b,c)
Abbreviation: CV – coefficient of variation

5.2 In Life Animal Observations

Mortality/Moribundity

Androgen Agonist (Groups 1-6[§])

All animals survived to scheduled euthanasia with none showing signs of moribundity.

Androgen Antagonist (Groups 6[§]-13)

All animals survived to the scheduled euthanasia with none showing signs of moribundity.

[§]Group 6 served as the positive control for the agonist assay and control in the antagonist assay

Clinical Observations

Clinical observations were recorded for all animals' 24-hours post-dose administration. A scab was noted on one animal in Group 4 for 7 days, 2 animals in Group 5 were noted as thin on 1 study day, and 1 animal in group 12 was noted as thin for 7 study days. No other adverse observations were noted throughout the study. Individual animal data are listed in Appendix IV.

Androgen Agonist (Groups 1-6[§])

No clinical signs of toxicity were observed in any animals administered vehicle control, 320, or 1000 mg/kg/day Padimate-O or Homosalate (Groups 1-5), or TP alone (Group 6) 24-hours post-dose. One animal administered 320 mg/kg/day Homosalate was noted with a scab proximal to the left and right eye for 7 days, and two animals administered 1000 mg/kg/day Homosalate were observed as thin for one day.

Androgen Antagonist (Groups 6[§]-13)

No clinical signs of toxicity were observed in any animals administered vehicle control and TP (Group 6), or 100, 320, or 1000 mg/kg/day Padimate-O or Homosalate and TP (Groups 7-12), or FT and TP (Group 13) 24-hours post-dose. One animal administered 1000 mg/kg/day Homosalate was observed as thin for eight days.

Body Weights

Group mean initial and final body weights and body weight changes for animals euthanized following 10 consecutive days of Padimate-O or Homosalate administration are presented in Table 4 (agonist assay) and Table 5 (antagonist assay). Individual animal data are listed in Appendix V.

Androgen Agonist (Groups 1-6[§], Table 4)

No significant change in mean final body weight was observed in animals administered 320 or 1000 mg/kg/day Padimate-O or Homosalate (Groups 2-5) compared to vehicle control group (Group 1). No significant change in body weight gain was observed in animals administered 320 mg/kg/day Padimate-O or Homosalate (Groups 2, 4) compared to the vehicle control group (Group 1), however, both 1000 mg/kg Padimate-O (Group 3) and Homosalate (Group 5)

body weight gain were significantly decreased compared to the vehicle control group (Group 1). Positive control animals administered vehicle control and TP (Group 6) showed a statistically significant increase in body weight gain as compared to vehicle control group (Group 1).

[§]Group 6 served as the positive control for the agonist assay and control in the antagonist assay

Androgen Antagonist (Groups 6[§]-13, Table 5)

No significant change in body weight or body weight gain was observed in animals co-administered 100 or 320 mg/kg/day Padimate-O or Homosalate and TP, or positive control animals administered FT and TP (Groups 7-8, 10-11) compared to vehicle control and TP (Group 6). Animals co-administered 1000 mg/kg/day Padimate-O or Homosalate and TP (Groups 9 and 12) had a significantly lower final body weight and body weight gain than control animals (Group 6).

[§]Group 6 served as the positive control for the agonist assay and control in the antagonist assay

Table 4. Androgen Agonist; Body Weight Changes

Dose Group	Test/Reference Substance / Control	Test Substance Dose Level (mg/kg/day)	n	Initial Body Weight Mean (g) ± SD	Final Body Weight Mean (g) ± SD	Body Weight Gain Mean (g) ± SD	Final Body Weight (% of Control)
1	Vehicle Control (Corn Oil)	0	8	302.7 ± 21.7	340.6 ± 37.3	38.0 ± 17.1	-
2	Padimate-O	320	8	302.2 ± 22.3	335.8 ± 26.0	33.6 ± 7.6	98.6
3	Padimate-O	1000	8	304.1 ± 20.5	317.0 ± 24.4	**12.9 ± 11.4***	93.1
4	Homosalate	320	8	304.4 ± 21.1	330.7 ± 26.8	26.2 ± 10.6	97.1
5	Homosalate	1000	8	302.9 ± 16.1	309.6 ± 20.0	**6.7 ± 11.0***	90.9
6[§]	Vehicle Control + TP (Positive Control)	0 + 0.4	8	304.1 ± 17.4	365.5 ± 19.9	**61.4 ± 9.8**[†]	-

Abbreviation: SD - standard deviation, TP- Testosterone Propionate
*Statistically significant ($p<0.05$) compared to the vehicle control mean (Dunnett's test)
[†]Statistically significant ($p<0.05$) compared to the vehicle control mean (t-test)
[§]Group served as the positive control for the agonist assay and control in the antagonist assay

Table 5. Androgen Antagonist; Body Weight Changes

Dose Group	Test/Reference Substance / Control	Test Substance Dose Level (mg/kg/day)	n	Initial Body Weight Mean (g) ± SD	Final Body Weight Mean (g) ± SD	Body Weight Gain Mean (g) ± SD	Final Body Weight (% of Control)
6[§]	Vehicle Control + TP (Control)	0 + 0.4	8	304.1 ± 17.4	365.5 ± 19.9	61.4 ± 9.8	-
7	Padimate-O + TP	100 + 0.4	8	303.9 ± 18.2	364.3 ± 23.9	60.4 ± 10.4	99.7
8	Padimate-O + TP	320 + 0.4	8	301.4 ± 20.0	351.5 ± 23.5	50.1 ± 10.9	96.2
9	Padimate-O + TP	1000 + 0.4	8	304.3 ± 17.9	**327.4 ± 35.9***	**23.1 ± 23.3***	89.6
10	Homosalate + TP	100 + 0.4	8	304.5 ± 18.5	374.3 ± 26.9	69.8 ± 15.0	102.4
11	Homosalate + TP	320 + 0.4	8	302.9 ± 18.0	371.3 ± 26.6	68.4 ± 12.1	101.6
12	Homosalate + TP	1000 + 0.4	8	300.0 ± 16.7	**326.7 ± 22.1***	**26.7 ± 7.3***	89.4
13	FT + TP (Positive Control)	3 + 0.4	8	302.7 ± 18.1	361.6 ± 27.5	58.9 ± 11.1	-

Abbreviation: SD - standard deviation, TP- Testosterone Propionate, FT- Flutamide
*Statistically significant ($p<0.05$) compared to the vehicle control mean (Dunnett's test)
[§]Group served as the positive control for the agonist assay and control in the antagonist assay

5.3 Necropsy

Gross Observations

One animal in Group 8 was observed as having a discolored yellow right lobe of the ventral prostate, a small right seminal vesicle and the bladder mucosa was thickened and contained multiple calcali. One animal in Group 10 was observed as having a small right Cowper's gland and one animal in Group 13 was observed as having a small left Cowper's gland. All remaining animals were observed as normal.

Tissue Weights

Group mean weights of glans penis, Cowper's gland, LABC, ventral prostate and seminal vesicle for animals euthanized following 10 consecutive days of Padimate-O or Homosalate administration are presented in Table 6 (agonist assay) and Table 7 (antagonist assay). Individual animal tissue weight data are listed in Appendix VI.

Androgen Agonist (Groups 1-6[§], Table 6)

Administration of 320 or 1000 mg/kg/day Padimate-O or Homosalate (Groups 2-5) did not affect glans penis, Cowper's gland, LABC, ventral prostate, or seminal vesicle weights in the agonist assay. All five androgen-dependent tissues weights were significantly increased in the positive control group (vehicle and TP; Group 6) as compared to vehicle control group (Group 1).

Androgen Antagonist (Groups 6[§]-13, Table 7)

Co-administration of 100 or 320 Padimate-O and TP (Groups 7-8) did not affect glans penis, Cowper's gland, LABC, ventral prostate, or seminal vesicle weights compared to vehicle control and TP (Group 6). Co-administration of 1000 mg/kg/day Padimate-O and TP significantly decreased LABC, glans penis, and ventral prostate weights, but not seminal vesicle or Cowper's gland as compared to tissue weights in vehicle control animals.

Co-administration of 100 or 320 Homosalate and TP (Groups 10-11) did not affect glans penis, Cowper's gland, LABC, ventral prostate, or seminal vesicle weights compared to vehicle control and TP (Group 6). Co-administration of 1000 mg/kg/day Homosalate and TP significantly decreased LABC tissue weights, however no significant differences were observed in the glans penis, seminal vesicle, ventral prostate, or Cowper's gland as compared to tissue weights in vehicle control animals.

All five androgen-dependent tissues weights were significantly decreased in the positive control group, (FT and TP; Group 13) compared to the vehicle control and TP control group (Group 6).

[§]Group 6 served as the positive control for the agonist assay and control in the antagonist assay

Table 6. Androgen Agonist; Androgen Dependent Tissue Weights

Dose Group	Test/Reference Substance / Control	Test Substance Dose Level (mg/kg/day)	n	Glans Penis Weight (mg) Mean ± SD (CV)	Cowper's Gland Weight (mg) Mean ± SD (CV)	LABC Weight (mg) Mean ± SD (CV)	Ventral Prostate Weight (mg) Mean ± SD (CV)	Seminal Vesicle Weight (mg) Mean ± SD (CV)
1	Vehicle Control	0	8	65.7 ± 4.4 (6.6)	8.1 ± 2.8 (34.7)	142.3 ± 31.0 (21.8)	21.1 ± 3.1 (14.8)	70.6 ± 10.9 (15.5)
2	Padimate-O	320	8	62.7 ±4.1¥ (6.6)	9.1 ± 0.8 (9.0)	161.4 ± 16.8 (10.4)	21.7 ± 2.9 (13.4)	64.6 ± 12.6 (19.6)
3	Padimate-O	1000	8	60.8 ± 10.1¥ (16.6)	7.1 ± 2.5 (34.9)	117.7 ± 15.2 (12.9)	22.3 ± 5.9 (26.3)	59.5 ± 9.6 (16.1)
4	Homosalate	320	8	62.6 ± 3.4 (5.5)	7.4 ± 1.6 (21.6)	148.0 ± 23.9 (16.2)	20.0 ± 3.9 (19.4)	64.1 ± 7.3# (11.4)
5	Homosalate	1000	8	62.1 ± 4.7 (7.5)	8.4 ± 2.1 (24.4)	140.6 ± 23.9 (17.0)	21.1 ± 3.9 (18.4)	67.1 ± 18.8# (28.0)
6§	Vehicle Control + TP (Positive Control)	0 + 0.4	8	**102.6 ± 5.5† (5.3)**	**45.5 ± 7.3† (16.1)**	**405.9 ± 57.1† (14.1)**	**207.1 ± 18.9† (9.1)**	**770.8 ± 68.2† (8.9)**

Abbreviations: SD - standard deviation; TP- Testosterone Propionate; LABC-levator ani plus bulbocavernous muscle complex; CV- Coefficient of Variation

*Statistically significant ($p<0.05$) compared to the vehicle control mean (Dunnett's test)

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

§Group served as the positive control for the agonist assay and control in the antagonist assay

Data was log transformed; ¥ Dunn's test

Table 7. Androgen Antagonist; Androgen Dependent Tissue Weights

Dose Group	Test/Reference Substance / Control	Test Substance Dose Level (mg/kg/day)	n	Glans Penis Weight (mg) Mean ± SD (CV)	Cowper's Gland Weight (mg) Mean ± SD (CV)	LABC Weight (mg) Mean ± SD (CV)	Ventral Prostate Weight (mg) Mean ± SD (CV)	Seminal Vesicle Weight (mg) Mean ± SD (CV)
6[§]	Vehicle Control + TP (Control)	0 + 0.4	8	102.6 ± 5.5 (5.3)	45.5 ± 7.3 (16.1)	405.9 ± 57.1 (14.1)	207.1 ± 18.9 (9.1)	770.8 ± 68.2 (8.9)
7	Padimate-O + TP	100 + 0.4	8	102.9 ± 7.3 (7.1)	50.1 ± 4.6 (9.1)	406.9 ± 48.9 (12.0)	181.0 ± 48.9 (27.0)	872.7 ± 151.0 (17.3)
8	Padimate-O + TP	320 + 0.4	8	102.7 ± 3.7 (3.6)	54.1 ± 10.3 (19.0)	404.4 ± 54.4 (13.5)	205.9 ± 44.4 (21.6)	881.1 ± 151.2 (17.2)
9	Padimate-O + TP	1000 + 0.4	8	**94.7 ± 6.7* (7.0)**	40.0 ± 6.4 (16.0)	**277.6 ± 41.1* (14.8)**	**131.9 ± 22.0* (16.7)**	693.3 ± 113.9 (16.4)
10	Homosalate + TP	100 + 0.4	8	108.4 ± 6.1 (5.6)	44.8 ± 7.8 (17.5)	411.4 ± 46.8 (11.4)	224.2 ± 33.1 (14.8)	812.2 ± 79.6 (9.8)
11	Homosalate + TP	320 + 0.4	8	104.4 ± 3.2 (3.1)	47.6 ± 11.3 (23.8)	382.6 ± 51.9 (13.6)	213.6 ± 44.6 (20.9)	839.5 ± 72.8 (8.7)
12	Homosalate + TP	1000 + 0.4	8	102.8 ± 5.0 (4.9)	46.9 ± 7.1 (15.1)	**309.1 ± 54.0* (17.5)**	182.9 ± 31.6 (17.3)	734.4 ± 131.0 (17.8)
13	FT + TP (Positive Control)	3 + 0.4	8	**78.6 ± 5.7† (7.2)**	**18.0 ± 7.8† (43.3)**	**202.4 ± 47.4† (23.4)**	**55.4 ± 14.9† (26.8)**	**165.9 ± 58.9† (35.5)**

Abbreviations: SD - standard deviation; TP- Testosterone Propionate; FT- Flutamide; LABC-levator ani plus bulbocavernous muscle complex; CV- Coefficient of Variation

*Statistically significant ($p<0.05$) compared to the vehicle control mean (Dunnett's test)

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

[§]Group served as the positive control for the agonist assay and control in the antagonist assay

Performance Criteria

Agonist

All tissue CVs met performance criteria for the agonist assay (Table 6 and 8).

Antagonist

All tissue CVs met performance criteria for the antagonist assay (Table 7 and 8).

Table 8. Maximum allowable Coefficient of Variations

Tissue	Androgen Agonist	Androgen Antagonist
Glans Penis	22%	17%
Cowper's Glands	55%	35%
LABC	30%	20%
Ventral Prostate	45%	40%
Seminal Vesicle	40%	40%

Source: U.S. EPA (2009)

CONCLUSION

Castrated SD male rats were orally administered Padimate-O or Homosalate alone or co-administered subcutaneously with TP, for 10 consecutive days. Approximately 24-hours following the final dose administration, the animals were humanely euthanized; the glans penis, ventral prostate, LABC, Cowper's gland, and seminal vesicle with coagulating gland with fluid were excised and weighed.

In the agonist assay, final body weight and body weight gain were not significantly different in animals administered 320 mg/kg/day Padimate-O compared to vehicle control animals (corn oil). Body weight gain was significantly decreased in animals administered 1000 mg/kg/day Padimate-O (93.1%) compared to vehicle controls. Final body weight was not significantly different in animals administered 1000 mg/kg/day Padimate-O compared to vehicle controls. The glans penis, ventral prostate, LABC, Cowper's gland, and seminal vesicle with coagulating gland with fluid were not significantly different in animals administered 320 or 1000 mg/kg/day Padimate-O compared to vehicle control animals (corn oil).

In the antagonist assay, final body weights and body weight gain of animals co-administered 100 or 320 mg/kg/day Padimate-O and TP were not statistically different compared to vehicle control animals (corn oil and TP). Final body weights and body weight gain of animals administered 1000 mg/kg/day of Padimate-O and TP were significantly decreased (89.6% of controls) compared to vehicle control animals (corn oil and TP). In animals co-administered 1000 mg/kg/day Padimate-O and TP, the glans penis, LABC, and seminal vesicle weights were significantly lower compared to vehicle control animals (corn oil and TP). The glans penis, LABC, and seminal vesicle weights at dose levels of 100 or 320 mg/kg/day were not different compared to vehicle controls (corn oil and TP). The ventral prostate and Cowper's gland weights were not different compared to vehicle control weights at any dose level of Padimate-O.

In the agonist assay, final body weight and body weight gain were not significantly different in animals administered 320 mg/kg/day Homosalate compared to vehicle control animals (corn oil). Compared to vehicle control animals, body weight gain (90.9%), but not final body weight, was significantly decreased in animals administered 1000 mg/kg/day Homosalate. The glans penis, ventral prostate, LABC, Cowper's gland, and seminal vesicle with coagulating gland with fluid weights were not significantly different in animals administered 320 or 1000 mg/kg/day compared to vehicle control animals (corn oil).

In the antagonist assay, final body weights and body weight gain of animals administered 100 or 320 mg/kg/day Homosalate and TP were not statistically different compared to vehicle control animals (corn oil and TP). Final body weights and body weight gain of animals administered 1000 mg/kg/day Homosalate and TP were significantly decreased (89.4% of controls) compared to control animals (corn oil and TP). Homosalate co-administered with TP at 1000 mg/kg/day significantly decreased the LABC weights, but not any other androgen-dependent tissue weights.

Based on these findings using the castrated rat model, oral administration of Homosalate, up to the limit dose of 1000 mg/kg/day, did not show any androgen agonist/antagonist activity, or 5α-reductase inhibition.

Administration of Padimate-O, up to the limit dose of 1000 mg/kg/day, was positive in the antagonist Hershberger Bioassay with a significant decrease in three androgen-dependent tissue weights.

REFERENCES

Blake, J. (2011). Padimate-O in Corn Oil Dose Formulation Development. RTI Project Number-ChemTask Number: 0212839.100.003.034-Chem11137. Unpublished study report prepared by Research Triangle Institute, International.

Blake, J. (2012a). Homosalate in Corn Oil Dose Formulation Development. RTI Project Number-ChemTask Number: 0212839.200.003.063-Chem11139. Unpublished study report prepared by Research Triangle Institute, International.

Blake, J. (2012b). Homosalate in Corn Oil Formulation Analysis. RTI Project Number-ChemTask Number: 0212839.200.003.077-Chem11720. Unpublished study report prepared by Research Triangle Institute, International.

Blake, J. (2012c). Padimate-O in Corn Oil Formulation Analysis. RTI Project Number-ChemTask Number: 0212839.200.003.076-Chem11719. Unpublished study report prepared by Research Triangle Institute, International.

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

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

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

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

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

KEY PERSONNEL

Study Director:
Study Toxicologist:
Toxicology Study Manager:
Animal Facility Operations Manager:
Necropsy Manager:
Facility Veterinarian:
Health and Safety Manager:
Dose Formulations:

Appendix I:

Certificate of Analysis

RTI INTERNATIONAL
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Analytical Chemistry Services for the NTP
NIH Contract No. HHSN273201100003C
RTI Project 0212839.200.003.081
ChemTask No. CHEM11787
CAS No. 21245-02-3

This pdf is an exact duplicate of the original approved report.

[redacted]
Program Information Coordinator

2-ETHYLHEXYL-P-DIMETHYL-AMINOBENZOATE (PADIMATE O)

CHEMICAL REANALYSIS

September 5, 2012

Prepared by:

[redacted] 09-05-12
Date
Task Leader

Approved by:

[redacted] 09/05/12
Reshan Fernando, Ph.D. Date
Principal Investigator

Submitted to:

[redacted]
National Institute of Environmental Health Sciences
P.O. Box 12233
111 T. W. Alexander Drive
Research Triangle Park, NC 27709-2233

2-ETHYLHEXYL-P-DIMETHYL-AMINOBENZOATE (PADIMATE O)

CAS No.: 21245-02-3

RTI Chemical ID Code: L98

ChemTask No.: CHEM11787

RTI Log Nos. (Amt. Received):
Bulk Analytical: 082010-B-14 (~50 g)
Reference: 082010-B-05 (~5 g)

Program Supported: TOX

Analysis Dates: May 21-22, 24, 2012

Interim Results Date: May 30, 2012

Study Lab: (Investigator): ILS ([redacted]

Lot No. (Vendor): MKBF0590V (Aldrich)

Vendor Purity: 98.3% (Aldrich COA)

Receipt Date: Aug 20, 2010 (Bulk)

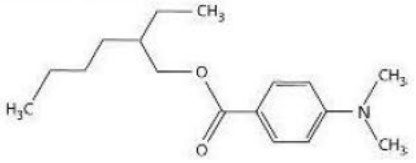
Bulk Receipt Condition: Good, room temperature

Submitter: [redacted] RTI)

Shipping Container: NA (in-house transfer)

Storage Conditions:
Bulk: Room temperature
Reference: Freezer (~-20 °C)

STRUCTURE



MOL. WT.
277.40

MOL. FORMULA
$C_{19}H_{29}NO_2$

EXECUTIVE SUMMARY

In support of the Toxicity Testing Program, an aliquot of padimate O was submitted for bulk chemical reanalysis. Chemical purity of the bulk sample was determined relative to a reference standard of the same lot/batch number which had been stored at RTI under freezer conditions. Analytical results obtained by a GC/FID chromatographic method indicated that the sample had a percent relative purity of 98.1% when compared to the frozen reference standard. The FTIR spectrum of the bulk sample matched the spectrum of the frozen reference and was consistent with an identity of padimate O.

Certificate of Analysis

SIGMA-ALDRICH

Product Name	2-Ethylhexyl 4-(dimethylamino)benzoate, 98%
Product Number	437395
Product Brand	ALDRICH
CAS Number	21245-02-3
Molecular Formula	$(CH_3)_2NC_6H_4CO_2CH_2CH(C_2H_5)(CH_2)_3CH_3$
Molecular Weight	277.40

TEST	SPECIFICATION	LOT MKBF0590V RESULTS
Appearance (Color)	Colorless to Yellow	Colorless
Appearance (Form)	Liquid	Liquid
Infrared spectrum	Conforms to Structure	Conforms
Purity (GC)	≥97.5 %	98.3 %
Specification Date:		JUN 2010
Date of QC Release:		AUG 2010
Print Date:		AUG 02 2010

[redacted]
[redacted] Supervisor
Quality Control
Milwaukee, Wisconsin USA

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Analytical Chemistry Services for the NTP
NIH Contract No. HHSN273201100003C
RTI Project 0212839.200.003.082
ChemTask No. CHEM11788
CAS No. 118-56-9

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[redacted]
Program Information Coordinator

HOMOSALATE

CHEMICAL REANALYSIS

September 5, 2012

[redacted]
09-05-12
Date
Task Leader

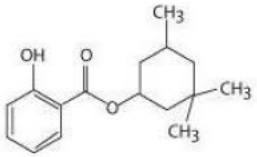
Approved by:
[redacted]
09/05/12
Reshan Fernando, Ph.D. Date
Principal Investigator

Submitted to:
[redacted]
National Institute of Environmental Health Sciences
P.O. Box 12233
111 T. W. Alexander Drive
Research Triangle Park, NC 27709-2233

HOMOSALATE

CAS No.: 118-56-9	Study Lab: (Investigator): ILS [redacted]
RTI Chemical ID Code: N67	Lot No. (Vendor): YT0976 (Spectrum)
ChemTask No.: CHEM11788	Vendor Purity: 99.88% (Spectrum COA)
RTI Log Nos. (Amt. Received): Analytical: 091410-A-14 (~50 g) Reference: 091410-A-05 (~5 g)	Receipt Date: Sep 14, 2010 (Bulk) Receipt Condition: No damage noted
Program Supported: TOX	Submitter: [redacted] RTI)
Analysis Date: May 11, 21-23, 2012	Shipping Container: NA (in-house transfer)
Interim Results Date: May 29, 2012	Storage Conditions: Bulk: Room temperature Reference: Freezer (~-20 °C)

STRUCTURE



MOL. WT.
262.34

MOL. FORMULA
$C_{16}H_{22}O_3$

EXECUTIVE SUMMARY

In support of the Toxicity Testing Program, an aliquot of homosalate was submitted for bulk chemical reanalysis. Chemical purity of the bulk sample was determined relative to a reference standard of the same lot/batch number which had been stored at RTI under freezer conditions. Analytical results obtained by a GC/FID chromatographic method indicated that the sample had a percent relative purity of 99.3% when compared to the frozen reference standard. The FTIR spectrum of the bulk sample matched the spectrum of the frozen reference and was consistent with an identity of homosalate.

CERTIFICATE OF ANALYSIS

Printed: 9/8/2010 — Page 1 of 1

Customer No : 23385 — Customer : RESEARCH TRIANGLE INSTITUTE — Customer PO : CT151563

Order Number : 2472349 — Delivery # : 11205793

Catalog : H1121 — Homosalate, USP — Lot : YT0976

Chemical Formula : $C_{16}H_{22}O_3$ — Formula Weight : 262.36

CAS# : 118-56-9

Test	Limit Min.	Limit Max.	Results
ASSAY	90.0 - 110%		99.88%
SPECIFIC GRAVITY	1.049 - 1.056		1.0505
REFRACTIVE INDEX @ 20°C	1.516 - 1.519		1.5183
IDENTIFICATION	TO PASS TEST		PASSES TEST
EXPIRATION DATE			08-APR-2012
RESIDUAL SOLVENTS	TO PASS TEST		
CLASS 2 (SOLVENT) / METHANOL			< 3000 ppm
CLASS 3 (solvent) / ISOPROPYL ACETATE			< 5000 ppm
MANUFACTURE DATE			24-APR-2009
APPEARANCE			CLEAR, COLORLESS LIQUID

spectrum CHEMICAL MFG CORP

AN ISO 9001:2000 REGISTERED COMPANY

Corporate Headquarters: 14422 S. San Pedro St. Gardena, CA 90248 (310) 516-8000 • Fax (310) 516-9143. • East Coast Plant: 769 Jersey Ave. New Brunswick, NJ 08901 (732) 214-1300

[redacted] CofA Coordinator

Gardena, California Plant

N135-249
[redacted] 3-2012

From: [redacted]
To: [redacted]
Subject: C of A for lot 7862K
Date: Tuesday, April 05, 2011 3:18:17 PM
Attachments: ATT00002.jpe

----- Forwarded by [redacted] 04/05/2011 03:12 PM -----

MP Biomedicals, LLC	29525 Fountain Parkway Solon, Ohio 44139	Telephone: 440/337-1200 Toll Free: 800/854-0530 Fax: 440/337-1180 web: www.mpbio.com

Certificate of Analysis

Product Description: Corn Oil
Catalog Number: 901414
Lot: 7862K

Formula: N/A **CAS #:** 8001-30-7 **Physical Description:** Yellow Oil	**Formula Weight:** N/A **Storage:** Room Temperature

Test	Specification	Result
Identity	Passes	Passes

Color (Lovibond): 1.6

Free Fatty Acid: 0.045%
Peroxide: 0.5 meq/kg
Iodine: 126.85
Cold Test: 5.5 Clear & Brilliant
Additives: None

[redacted]

08/17/2010

[redacted]

MP Biomedicals, LLC.
Technical Director

This is an electronically generated document
mailto:biotech@mpbio.com
http://www.mpbio.com
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N135-249
[redacted] 7/5/12

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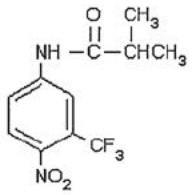
Website: www.sigmaaldrich.com
Email USA: techserv@sial.com
Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name:
Flutamide

Product Number:	**F9397**
Lot Number:	**021M1406V**
Brand:	SIGMA
CAS Number:	13311-84-7
MDL Number:	MFCD00072009
Formula:	C11H11F3N2O3
Formula Weight:	276.21 g/mol
Quality Release Date:	01 MAR 2011



Test	Specification	Result
Appearance (Color)	Yellow	Light Yellow
Appearance (Form)	Powder	Powder
Solubility (Color)	Yellow to Yellow-Green	Yellow - Green
Solubility (Turbidity) 50 mg/mL, EtOH	Clear to Hazy	Clear
Carbon	46.8 - 49.8%	48.0%
Nitrogen	9.8 - 10.4%	10.1%
Purity (TLC)	≥ 99%	100%

[redacted]
[redacted] Manager
Analytical Services
St. Louis, Missouri US

Sigma-Aldrich warrants, that at the time of the quality release or subsequent retest date this product conformed to the information ntained in this publication. The current Specification sheet may be available at Sigma-Aldrich.com. For further inquiries, please contact .echnical Service. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Version Number: 1

Page 1 of 1

N135-249 [redacted] 3-26-12

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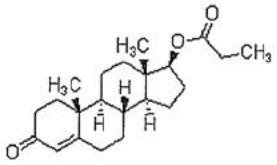
Website: www.sigmaaldrich.com
Email USA: techserv@sial.com
Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name:
Testosterone propionate - solid

Product Number:	**T1875**
Lot Number:	**051M1803V**
Brand:	SIGMA
CAS Number:	57-85-2
MDL Number:	MFCD00003653
Formula:	C22H32O3
Formula Weight:	344.49 g/mol
Quality Release Date:	28 JUN 2011



Test	Specification	Result
Appearance (Color)	White to Off-White	White
Appearance (Form)	Powder	Powder
Solubility (Color)	Colorless to Faint Yellow	Very Faint Yellow
Solubility (Turbidity) 50 mg/mL, CHCl3	Clear	Clear
Infrared spectrum	Conforms to Structure	Conforms
Specific Rotation (+), C = 2 in dioxane at 25 deg C	82 - 87 °	85 °
Purity (HPLC)	≥ 98 %	102 %

[redacted]

[redacted] Manager
Analytical Services
St. Louis, Missouri US

Sigma-Aldrich warrants, that at the time of the quality release or subsequent retest date this product conformed to the information contained in this publication. The current Specification sheet may be available at Sigma-Aldrich.com. For further inquiries, please contact Technical Service. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Version Number: 1

Page 1 of 1

Appendix II:

Dose Formulation Analysis

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Analytical Chemistry Services for the NTP
NIH Contract No. HHSN273201100003C
RTI Project 0212839.200.003.076
ChemTask No. CHEM11719
CAS No. 21245-02-3

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[redacted]
Program Information Coordinator

PADIMATE O

IN CORN OIL

FORMULATION ANALYSIS

Mix Date: March 22, 2012

July 17, 2012

Prepared by:
[redacted] 07-17-12
Task Leader Date

Approved by:
[redacted] 07-17-12
Reshan Fernando, Ph.D. Date
Principal Investigator

Submitted to:

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PADIMATE O

CAS No.: 21245-02-3

ChemTask No.: CHEM11719

RTI Chemical ID Code: L98

Program Supported: TOX

Analysis Dates: Mar 26-29, 2012

Interim Results Date: Mar 29, 2012

Mix Date: Mar 22, 2012

Lot No. (Vendor): MKBF0590V (Aldrich)

Vendor Purity: 98.3% (Aldrich COA)

Vehicle: Corn oil

Vehicle Lot No.: (Vendor): unknown

Samples Received: 9 x 30 mL and 1 x 100 mL

Dose Formulation Concentrations (RTI Log Nos.):
200 mg/mL (032312-C-01 to -03);
64 mg/mL (032312-C-04 to -06);
20 mg/mL (032312-C-07 to -09);
0 mg/mL (032312-C-10)

Sample Receipt Date: Mar 23, 2012

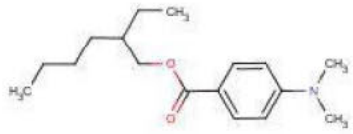
Submitter: ILS

Study Lab (Investigator): ILS ([redacted])

Sample Containers: Amber glass bottles

Receipt Condition: No damage noted

Storage Condition: Room temperature

STRUCTURE	MOL. WT.	MOL. FORMULA
	277.40	$C_{19}H_{27}NO_2$

EXECUTIVE SUMMARY

In support of the Toxicity Testing Program, a formulation analysis was performed to determine the padimate O concentration and confirm homogeneity of dose formulations prepared in corn oil, submitted by the study lab. In addition a single control sample was received for analysis.

Analyses conducted using a GC/FID method yielded results ranging from 95.5% to 98.3% of the nominal concentrations; analytical precision was demonstrated at each dose concentration with relative standard deviation values ≤ 3.1%. The accuracy and homogeneity of these test mixes were confirmed. No test chemical was detected in the blank sample (detection limit was 0.39 mg/mL).

RTI INTERNATIONAL

Quality Assurance Statement

Chemical Name: **Padimate O**

Task Type: **Formulation Analysis**

RTI Task Number: **0212839.200.003.076**

Chem Task Number: **CHEM11719**

This study/task was audited by the Regulatory and Quality Assurance (RQA) – Quality Assurance Unit and the results of the inspections and audits were reported to the task leader/study director and management as identified below. To the best of our knowledge, the reported results accurately describe the study methods and procedures used, and the reported results accurately reflect the raw data.

Inspections and Audits	Inspection and Audit Date(s)	Date Inspection/Audit Report Sent to Task Leader/ Management
Process Inspection - Formulation Analysis	03/27/2012	03/28/2012
Data and Report Audit	06/12/2012	06/12/2012

Prepared by:

[redacted]
Quality Assurance Specialist

7/17/2012
Date

Reviewed by:

[redacted]
Quality Assurance Specialist

7-17-2012
Date

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Figures

PADIMATE O

1.0 INTRODUCTION

The purpose of this work was to determine the padimate O concentration in corn oil formulation samples submitted by the study lab. To accomplish this objective, a formulation analysis was performed.

2.0 SAMPLE IDENTIFICATION

The following samples were received at RTI analytical laboratory on March 23, 2012, and analyzed for padimate O.

RTI Log Nos.	Target Conc. (mg/mL)	Sample ID	Expiration Date
032312-C-01	200	12-26-1T	May 4, 2012
032312-C-02	200	12-26-1M	May 4, 2012
032312-C-03	200	12-26-1B	May 4, 2012
032312-C-04	64	12-26-2T	May 4, 2012
032312-C-05	64	12-26-2M	May 4, 2012
032312-C-06	64	12-26-2B	May 4, 2012
032312-C-07	20	12-26-3T	May 4, 2012
032312-C-08	20	12-26-3M	May 4, 2012
032312-C-09	20	12-26-3B	May 4, 2012
032312-C-10	0	N135-11-121-32212	May 4, 2012

3.0 SAMPLE ANALYSIS

The methodology used for determining the dose formulations is described in the RTI International report "2-Ethylhexyl-p-dimethylaminobenzoate (Padimate O) in Corn Oil, Dose Formulation Development", (CHEM11137), October 25, 2011. A summary of the method is attached as an appendix to this report.

4.0 SAMPLE RESULTS

The concentrations of padimate O found in the dose formulations and homogeneity results are provided below. Found concentrations are reported in units of mg/mL, and percent recovery (versus the nominal concentration) was calculated using these values.

RTI Log No	Nominal Conc. (mg/mL)	Found Conc.[a] (mg/mL)	Mean Found Conc. (n=3) (mg/mL)	Mean Found/ Nominal	Mean Found Conc. (n=9) (mg/mL)	Mean Found/ Nominal
032312-C-01-1[b]	200	184	186	93.0%		
032312-C-01-2	(top)	195	(4.1% RSD)			
032312-C-01-3		180				
032312-C-02-1	200	190	192	95.9%	191	95.5%
032312-C-02-2	(middle)	190	(1.5% RSD)		(3.1% RSD)	
032312-C-02-3		195				
032312-C-03-1	200	198	195	97.5%		
032312-C-03-2	(bottom)	193	(1.4% RSD)			
032312-C-03-3		194				
032312-C-04-1[b]	64	61.6	61.5	96.0%		
032312-C-04-2	(top)	59.5	(3.0% RSD)			
032312-C-04-3		63.2				
032312-C-05-1	64	61.9	62.3	97.3%	62.9	98.3%
032312-C-05-2	(middle)	62.5	(0.6% RSD)		(3.1% RSD)	
032312-C-05-3		62.5				
032312-C-06-1	64	64.1	65.0	102%		
032312-C-06-2	(bottom)	65.0	(1.3% RSD)			
032312-C-06-3		65.9				
032312-C-07-1[b]	20	19.3	18.9	94.5%		
032312-C-07-2	(top)	18.6	(2.0% RSD)			
032312-C-07-3		18.8				
032312-C-08-1	20	20.4	19.7	98.5%	19.5	97.3%
032312-C-08-2	(middle)	19.3	(3.2% RSD)		(3.0% RSD)	
032312-C-08-3		19.3				
032312-C-09-1	20	20.0	19.8	99.0%		
032312-C-09-2	(bottom)	20.0	(1.4% RSD)			
032312-C-09-3		19.5				
032312-C-10-1[b]	0	ND[c]	NA	NA	NA	NA
032312-C-10-2						
032312-C-10-3						

[a]Quantitation was based on the weighted ($1/x^2$) linear regression equation: y = 0.5739x - 0.1113, r = 0.9998.

[b]Sample numerical suffixes (1, 2, 3) indicate RTI analytical aliquots.

[c]ND = Not detected; Limit of detection (LOD) = 0.39 mg/mL; Limit of quantitation (LOQ) = 1.3 mg/mL.

Note: Value have been rounded to the appropriate number of significant figures after their performing all calculations in order to minimize round-off error. Some summary parameters

presented in the table may not be accurately reproduced using the rounded values presented elsewhere in the table.

Based on these results, it appears that the mixes are homogeneous and acceptable for use as their average percent found concentrations were within 95.5% and 98.3% of their nominal concentrations and acceptable analytical precision was demonstrated with percent relative standard deviations less than or equal to 3.1%. The two quality control (QC) standards prepared at equivalent concentrations of VA3 (12.0 mg/mL) and VB1 (180 mg/mL) had relative errors of -10% and -7.2% respectively, demonstrating acceptable analytical control.

Representative chromatograms are shown in Figure 1. The vehicle standards plot is illustrated in Figure 2 for the weighted ($1/x^2$) linear regression equations $y = 0.5739x - 0.1113$, $r = 0.9998$.

5.0 ACKNOWLEDGMENT

Personnel contributing to the performance of this task included: [redacted]

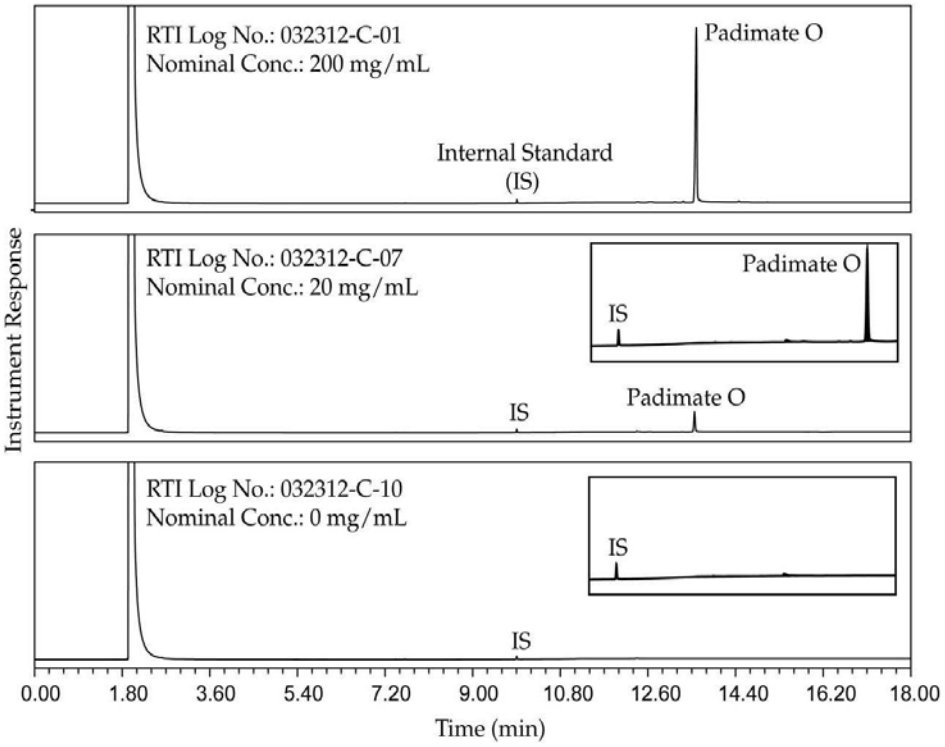

Figure 1. Representative Gas Chromatograms of Padimate O in Corn Oil

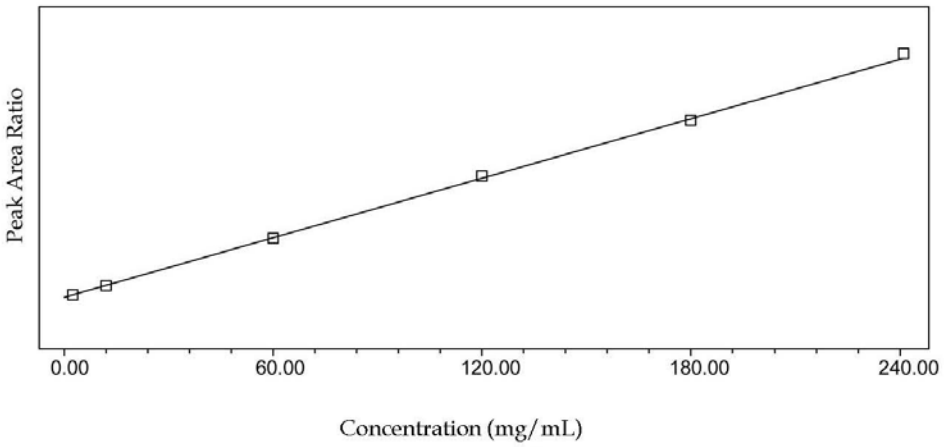

Figure 2. Plot of Vehicle Standards Data – Padimate O in Corn Oil

APPENDIX

Method Summary

Determination of Padimate O in Corn Oil

This appendix summarizes the method used to prepare formulation samples of padimate O in corn oil for analysis, and describes the gas chromatography method.

Preparation of the Internal Standard

An internal standard (IS) stock solution was prepared by transferring 258.11 mg of octanophenone into a 100-mL volumetric flask and diluting to volume with methylene chloride. The IS stock (2.58 mg/mL) was mixed by inversion and transferred to an amber bottle for use and refrigerator storage.

A working IS solution was prepared by transferring 1.0 mL of the IS stock solution to a 100-mL volumetric flask, diluting to volume with methylene chloride, and then mixing by inversion. The working IS solution (0.0258 mg/mL) was transferred to an amber bottle for refrigerator storage.

Preparation of Vehicle Stock Standards

Two vehicle stock standards (VA and VB) were prepared by weighing out aliquots of padimate O and dissolving them in 25 mL of the corn oil vehicle.

VA was prepared with ~7.5 g of padimate O and had a final concentration of 301 mg/mL. VB was prepared from ~ 6.0 g of padimate O and had a final concentration of 240 mg/mL.

Preparation of Vehicle and QC Standards

The standards were prepared by diluting the spiking solutions in corn oil vehicle as described in the table below. The vehicle standards were mixed by inversion. Two additional vehicle standards were prepared as quality control (QC) standards at the VB1 and VA3 concentrations.

Vehicle Standards

Vehicle Std ID	Spiking Solution	Spike Volume (mL)	Final Volume (mL)	Nominal Vehicle Std Conc. (mg/mL)	Actual Vehicle Std Conc.[a] (mg/mL)
VA1	VA	4.0	5	240	241
VB1	VB	3.75	5	180	180
VA2	VA	2.0	5	120	120
VB2	VB	2.5	10	60	60
VA3	VA	1.0	25	12.0	12.0
VB3	VB	0.5	50	2.40	2.40

[a]Example Calculation, VA1: 301 mg/mL x 4.0 mL/5.0 mL = 241 mg/mL.

For each vehicle standard, blank (unspiked corn oil was used for the vehicle blank) and QC standard, 1.0 mL was transferred to a 50-mL volumetric flask and diluted to volume with methylene chloride and mixed by inversion. One milliliter of this primary dilution was transferred to a scintillation vial and 1 mL of the WIS was added, and the sample mixed by inversion. An aliquot was transferred to an autosampler vial for analysis.

Preparation of Formulations Sample for Analysis

Three 1-mL aliquots of each dose formulation sample were transferred to three separate 50-mL volumetric flasks and diluted to volume with methylene chloride, and mixed by inversion. One milliliter of each primary dilution was transferred to a scintillation vial and 1 mL of the WIS was added, and the sample mixed by inversion. An aliquot was transferred to an autosampler vial for analysis.

GC Analysis

Instrument	Agilent 6890N
Column	Phenomenex ZB-5MS (30 m x 0.25 mmID, 0.50 µm film) with 5 m pre-guard
Data System	Empower 2; Build 2154
Inlet Temperature	250 °C
Column Program	70 °C for 1 min., ramp to 270 °C at 20 °C/min., hold for 7 min.
Column Flow	Helium ~1.5 mL/min
Injection Mode	Split ~20:1
Injection Volume	1 µL
Detector: Gas flows	FID at 290 °C: H_2 at 30 mL/min, air at 300 mL/min, N_2 make-up at 30 mL/min

For each dose formulation, the peak area ratio (normalized if required) of each aliquot was calculated (sample area ÷ IS peak area). The found concentration of the analyte was calculated using the peak area ratios and the linear regression equation (weighted $1/x^2$). A mean found concentration was determined for each sample, and for all nine samples at each concentration.

Acceptance criteria for each formulation were a final found concentrations within +/- 10% of the nominal concentration, and a precision (expressed as relative standard deviation for the triplicate preparations) of ≤ 10%.

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NTP Analytical Chemistry Services

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This pdf is an exact duplicate of the original approved report.

[redacted]
Program Information Coordinator

Analytical Chemistry Services for the NTP
NIH Contract No. HHSN273201100003C
RTI Project 0212839.200.003.077
ChemTask No. CHEM11720
CAS No. 118-56-9

HOMOSALATE

IN CORN OIL

FORMULATION ANALYSIS

Mix Dates: March 22, 2012

July 13, 2012

Prepared by:

[redacted] 07-13-12
Date
Task Leader

Approved by:

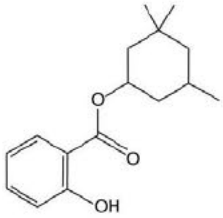
[redacted] 07/13/12
Reshan Fernando, Ph.D. Date
Principal Investigator

Submitted to:

[redacted]
National Institute of Environmental Health Sciences
P.O. Box 12233
111 T. W. Alexander Drive
Research Triangle Park, NC 27709-2233

HOMOSALATE

CAS No.: 118-56-9	Samples Received: 9 x 30 mL and 1 x 100 mL
ChemTask No.: CHEM11720	Dose Formulation Concentrations (RTI Log Nos.): 032312-A-01 to -03: 200 mg/mL; 032312-A-04 to -06: 64 mg/mL: 032312-A-07 to -09: 20 mg/mL; , 032312-A-10: 0 mg/mL
RTI Chemical ID Code: N67	
Program Supported: TOX	
Analysis Dates: Mar 26-28, 2012	Sample Receipt Date: Mar 23, 2012
Interim Results Date: Mar 29, 2012	Submitter: ILS
Mix Dates: Mar 22, 2012	Study Lab (Investigator): ILS ([redacted]
Lot No. (Vendor):YT0976 (Spectrum)	Sample Containers: Amber glass bottles
Vendor Purity: 99.88% (Spectrum COA)	Receipt Condition: No damage noted
Vehicle: Corn oil	Storage Condition: Room temperature
Vehicle Lot No.: (Vendor): unknown	

STRUCTURE	MOL. WT.	MOL. FORMULA
	262.34	$C_{16}H_{22}O_3$

EXECUTIVE SUMMARY

In support of the Toxicity Testing Program, a formulation analysis was performed to determine the homosalate content and homogeneity (top, middle and bottom sampling locations) of dose formulations and one vehicle blank prepared in corn oil, submitted by the study lab. Each formulation sample was prepared and analyzed in triplicate.

Analyses conducted using a GC/FID method yielded results ranging from 95.8% to 96.8% of the nominal concentrations; analytical precision was demonstrated at each dose concentration with relative standard deviation values ≤ 3.5%. The accuracy of these test mixes were confirmed. No test chemical was detected in the blank sample (estimated detection limit was 0.09 mg/mL).

In addition these results confirm the homogeneity of each dose formulation over the three sampling locations (top, middle and bottom).

RTI INTERNATIONAL

Quality Assurance Statement

Chemical Name: **Homosolate**

Task Type: **Formulation Analysis**

RTI Task Number: **0212839.200.003.077**

Chem Task Number: **CHEM11720**

This study/task was audited by the Regulatory and Quality Assurance (RQA) – Quality Assurance Unit and the results of the inspections and audits were reported to the task leader/study director and management as identified below. To the best of our knowledge, the reported results accurately describe the study methods and procedures used, and the reported results accurately reflect the raw data.

Inspections and Audits	Inspection and Audit Date(s)	Date Inspection/Audit Report Sent to Task Leader/ Management
Process Inspection - Formulation Analysis	03/27/2012	03/29/2012
Data and Report Audit	06/13/2012	06/13/2012

Prepared by:

[redacted]
Quality Assurance Specialist

7/13/2012
Date

Reviewed by:

[redacted]
Quality Assurance Specialist

7/13/2012
Date

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Figures

HOMOSALATE

1.0 INTRODUCTION

The purpose of this work was to determine the homosalate content and assess homogeneity of corn oil formulation submitted by the study lab. To accomplish this objective, a formulation analysis was performed.

2.0 SAMPLE IDENTIFICATION

The following samples were received at RTI analytical laboratory on March 23, 2012, and analyzed for homosalate.

RTI Log Nos.	Target Conc. (mg/mL)	Sample ID	Expiration Date
032312-A-01	200	12-24-1T	May 4, 2012
032312-A-02	200	12-24-1M	May 4, 2012
032312-A-03	200	12-24-1B	May 4, 2012
032312-A-04	64	12-24-2T	May 4, 2012
032312-A-05	64	12-24-2M	May 4, 2012
032312-A-06	64	12-24-2B	May 4, 2012
032312-A-07	20	12-24-3T	May 4, 2012
032312-A-08	20	12-24-3M	May 4, 2012
032312-A-09	20	12-24-3B	May 4, 2012
032312-A-10	0	N135-11-121-32212	May 4, 2012

3.0 SAMPLE ANALYSIS

The methodology used for determining the dose formulations is described in the RTI International report "Homosalate in Corn Oil, Dose Formulation Development", (CHEM11139), January 10, 2012. A summary of the method is attached as an appendix to this report.

4.0 SAMPLE RESULTS

The concentrations of homosalate found in the dose formulations are tabulated below. Found concentrations are calculated using the summed area of the two homosalate peaks, reported in units of mg/mL; percent recovery (versus the nominal concentration) was calculated using these values.

RTI Log No.	Nominal Conc. (mg/mL) (Sampling Location)	Found Conc.[a] (mg/mL)	Mean Found Conc. (n=3) (mg/mL)	Mean Found/ Nominal	Mean Found Conc. (n=9) (mg/mL)	Mean Found/ Nominal
032312-A-01-1[b] 032312-A-01-2 032312-A-01-3	200 (top)	190 192 211	197 (5.9% RSD)	98.7%		
032312-A-02-1 032312-A-02-2 032312-A-02-3	200 (middle)	194 190 190	191 (1.2% RSD)	95.6%	194 (3.5% RSD)	96.8%
032312-A-03-1 032312-A-03-2 032312-A-03-3	200 (bottom)	193 190 193	192 (0.9% RSD)	96.0%		
032312-A-04-1[b] 032312-A-04-2 032312-A-04-3	64 (top)	60.8 63.1 62.9	62.3 (2.0% RSD)	97.3%		
032312-A-05-1 032312-A-05-2 032312-A-05-3	64 (middle)	62.0 60.2 60.7	60.9 (1.6% RSD)	95.2%	61.4 (1.8%)	95.9%
032312-A-06-1 032312-A-06-2 032312-A-06-3	64 (bottom)	60.2 61.2 61.3	60.9 (1.0% RSD)	95.2%		
032312-A-07-1[b] 032312-A-07-2 032312-A-07-3	20 (top)	19.6 19.1 19.2	19.3 (1.2% RSD)	96.6%		
032312-A-08-1 032312-A-08-2 032312-A-08-3	20 (middle)	19.1 19.0 19.3	19.1 (0.7% RSD)	95.7%	19.2 (1.4%)	95.8%
032312-A-09-1 032312-A-09-2 032312-A-09-3	20 (bottom)	19.0 18.7 19.5	19.1 (2.3% RSD)	95.3%		
032312-A-10-1[b] 032312-A-10-2 032312-A-10-3	0	ND[c]	NA	NA	NA	NA

[a] Quantitation was based on the weighted ($1/x^2$) linear regression equation: $y = 0.6364x - 0.1406$, $r = 0.9999$.

[b] Sample suffixes (1, 2, 3) indicates RTI analytical aliquots.

[c] ND = Not detected; Limit of detection (LOD) = 0.09 mg/mL; Limit of quantitation (LOQ) = 0.3 mg/mL.

Based on these results, it appears that the mixes are acceptable for use as their average percent found concentrations were within 95.8% and 96.8% of their nominal concentrations and acceptable analytical precision was also demonstrated with percent relative standard deviations less than or equal to 3.5%. In addition these results confirm the homogeneity of the three dose formulations. The two quality control (QC) standards prepared at equivalent concentrations of VA3 (12.0 mg/mL) and VB1 (180 mg/mL) had percent relative errors of -3.3 and -4.7 respectively, demonstrating acceptable analytical control.

Representative chromatograms are shown in Figure 1. The vehicle standards plot is illustrated in Figure 2 for the weighted ($1/x^2$) linear regression equations $y = 0.6364x - 0.1406$, $r=0.9999$.

5.0 ACKNOWLEDGMENT

Personnel contributing to the performance of this task included: [redacted]

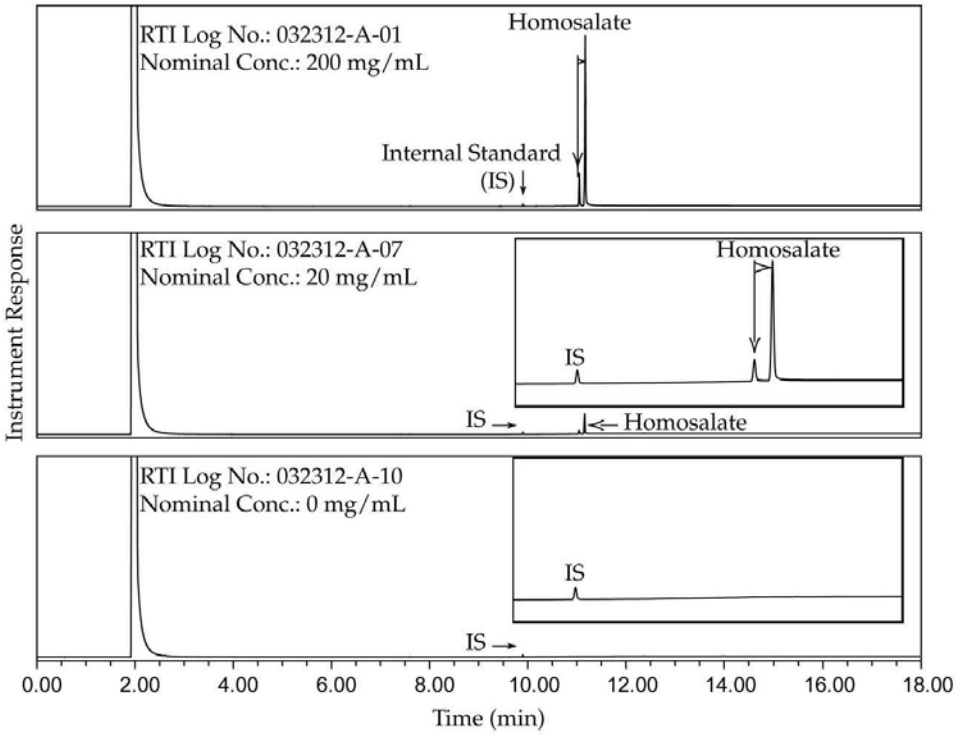

Figure 1. Representative Gas Chromatograms of Homosalate in Corn Oil

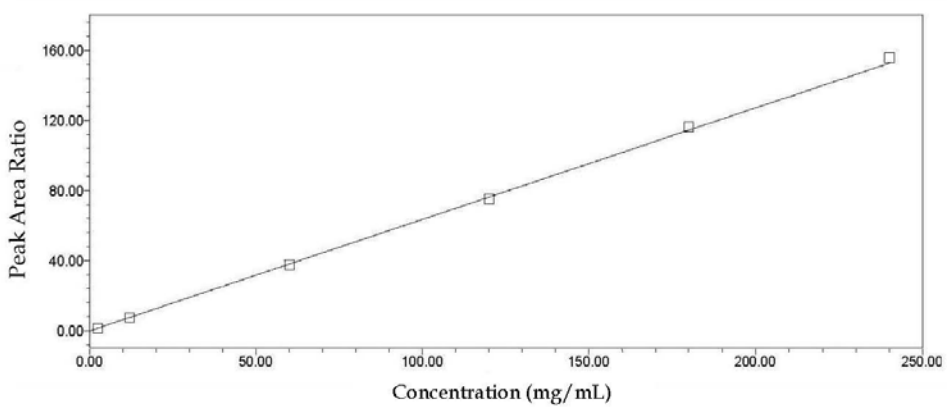

Figure 2. Plot of Vehicle Standards Data – Homosalate in Corn Oil

APPENDIX

Method Summary

Determination of Homosalate in Corn Oil

This appendix summarizes the method used to prepare formulation samples of homosalate in corn oil for analysis, and describes the gas chromatography method.

Preparation of the Internal Standard

An internal standard (IS) stock solution was prepared by transferring 258.11 mg of octanophenone into a 100-mL volumetric flask and diluting to volume with methylene chloride. The IS stock (2.58 mg/mL) was mixed by inversion.

A working IS solution was prepared by transferring 1.0 mL of the IS stock solution to a 100-mL volumetric flask, diluting to volume with methylene chloride, and then mixing by inversion. The working IS solution (0.0258 mg/mL) was transferred to an amber bottle for use.

Preparation of Vehicle Stock Standards

Two vehicle stock standards (VA and VB) were prepared by weighing out aliquots of homosalate and dissolving them in 25 mL of the corn oil vehicle.

VA was prepared with 7.50973 g of homosalate and had a final concentration of 300 mg/mL. VB was prepared from 6.00431 g of homosalate and had a final concentration of 240 mg/mL.

Preparation of Vehicle and QC Standards

The standards were prepared by diluting the spiking solutions in corn oil vehicle as described in the table below. The vehicle standards were mixed by inversion. Two additional vehicle standards were prepared as quality control (QC) standards at the VB1 and VA3 concentrations.

Vehicle Standards

Vehicle Std ID	Spiking Solution	Spike Volume (mL)	Final Volume (mL)	Nominal Vehicle Std Conc. (mg/mL)	Actual Vehicle Std Conc.[a] (mg/mL)
VA1	VA	4.0	5	240	240
VB1	VB	3.75	5	180	180
VA2	VA	2.0	5	120	120
VB2	VB	2.5	10	60	60
VA3	VA	1.0	25	12.0	12.0
VB3	VB	0.5	50	2.40	2.40

[a]Example Calculation, VA1: 300 mg/mL x 4.0 mL/5.0 mL = 240 mg/mL.

For each vehicle standard, blank (unspiked corn oil was for the vehicle blank) and QC standard, 1.0 mL was transferred to a 50-mL volumetric flask and diluted to volume with

methylene chloride and mixed by inversion. One milliliter of this primary dilution was transferred to a scintillation vial and 1 mL of the WIS was added, and the sample mixed by inversion. An aliquot was transferred to an autosampler vial for analysis.

Preparation of Formulations Sample for Analysis

Three 1-mL aliquots of each dose formulation sample were transferred to a 50-mL volumetric flask and diluted to volume with methylene chloride, and mixed by inversion. One milliliter of each primary dilution was transferred to a scintillation vial and 1 mL of the WIS was added, and the sample mixed by inversion. An aliquot was transferred to an autosampler vial for analysis.

GC Analysis

Instrument	Agilent 6890N
Column	Phenomenex ZB-5MS (30 m x 0.25 mmID, 0.50 µm film) with 5 m pre-guard
Data System	Empower 2; Build 2154
Inlet Temperature	250 °C
Column Program	70 °C for 1 min., ramp to 270 °C at 20 °C/min., hold for 7 min.
Column Flow	Helium ~1.5 mL/min
Injection Mode	Split ~20:1
Injection Volume	1 µL
Detector: Temp	FID 290 °C

For each dose formulation, the peak area of the two homosalate peaks was summed, then a peak area ratio (normalized if required) was calculated (sample area ÷ IS peak area). The found concentration of the analyte was calculated using the peak area ratios and the linear regression equations (weighted $1/x^2$). A mean found concentration was determined for each sampling location (n=3), and for overall homogeneity confirmation of each formulation (n=9).

Acceptance criteria for each formulation were a final found concentrations within +/- 10% of the nominal concentration, and a precision (expressed as percent relative standard deviation for the triplicate preparations) of ≤ 10%.

Appendix III:

Dose Times, Volumes and Dose Administration

Dose Administration

					Day 1 (21 & 22 April 2012)			Day 2 (22 & 23 April 2012)			Day 3 (23 & 24 April 2012)			Day 4 (24 & 25 April 2012)			Day 5 (25 & 26 April 2012)			Day 6 (26 & 27 April 2012)		
Group No.:	Animal No.:	Test Substance Dose Level	Actual Dose Concentration (mg/mL)	Age of Animal (PND)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)
1	01	Corn Oil Control	0	59	10:46	1.4	0.0	10:45	1.4	0.0	10:30	1.4	0.0	10:33	1.5	0.0	10:42	1.5	0.0	10:30	1.5	0.0
1	02			59	11:03	1.4	0.0	11:01	1.5	0.0	10:45	1.4	0.0	10:43	1.5	0.0	10:51	1.5	0.0	10:45	1.5	0.0
1	03			59	11:18	1.5	0.0	11:14	1.5	0.0	11:00	1.5	0.0	10:53	1.5	0.0	10:59	1.5	0.0	10:59	1.5	0.0
1	04			59	11:32	1.7	0.0	11:29	1.7	0.0	11:15	1.8	0.0	11:03	1.8	0.0	11:07	1.8	0.0	11:13	1.9	0.0
1	05			60	11:44	1.6	0.0	11:29	1.6	0.0	11:16	1.6	0.0	11:15	1.6	0.0	11:32	1.7	0.0	11:12	1.7	0.0
1	06			60	11:59	1.4	0.0	11:43	1.4	0.0	11:26	1.4	0.0	11:22	1.4	0.0	11:48	1.4	0.0	11:20	1.4	0.0
1	07			60	12:11	1.6	0.0	11:58	1.6	0.0	11:40	1.6	0.0	11:31	1.7	0.0	12:05	1.7	0.0	11:27	1.7	0.0
1	08			60	12:22	1.5	0.0	12:12	1.5	0.0	11:52	1.6	0.0	11:39	1.6	0.0	12:28	1.6	0.0	11:35	1.7	0.0

					Day 7 (27 & 28 April 2012)			Day 8 (28 & 29 April 2012)			Day 9 (29 & 30 April 2012)			Day 10 (30 April & 01 May 2012)			Day 11 (01 & 02 May 2012)	
Group No.:	Animal No.:	Test Substance Dose Level	Actual Dose Concentration (mg/mL)	Age of Animal (PND)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Death	Time From Last Administration
1	01	Corn Oil Control	0	59	10:40	1.5	0.0	10:30	1.5	0.0	10:30	1.5	0.0	10:40	1.5	0.0	8:33	21:53
1	02			59	10:49	1.5	0.0	10:44	1.5	0.0	10:42	1.5	0.0	10:47	1.5	0.0	9:35	22:48
1	03			59	10:57	1.6	0.0	10:58	1.6	0.0	10:56	1.6	0.0	10:55	1.6	0.0	10:28	23:33
1	04			59	11:05	1.9	0.0	11:13	1.9	0.0	11:09	2.0	0.0	11:02	2.0	0.0	11:23	24:21
1	05			60	11:27	1.7	0.0	11:24	1.8	0.0	11:09	1.8	0.0	10:31	1.8	0.0	8:51	22:20
1	06			60	11:41	1.5	0.0	11:38	1.5	0.0	11:17	1.5	0.0	10:44	1.5	0.0	9:45	23:01
1	07			60	11:55	1.8	0.0	11:53	1.8	0.0	11:26	1.8	0.0	11:00	1.9	0.0	10:52	23:52
1	08			60	12:11	1.7	0.0	12:08	1.7	0.0	11:33	1.7	0.0	11:15	1.7	0.0	11:42	24:27

					Day 1 (21 & 22 April 2012)			Day 2 (22 & 23 April 2012)			Day 3 (23 & 24 April 2012)			Day 4 (24 & 25 April 2012)			Day 5 (25 & 26 April 2012)			Day 6 (26 & 27 April 2012)		
Group No.:	Animal No.:	Test Substance Dose Level	Actual Dose Concentration (mg/mL)	Age of Animal (PND)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)
2	09	Padimate-O (320 mg/kg)	62.9	59	10:47	1.5	323.4	10:46	1.5	322.8	10:31	1.5	319.3	10:34	1.5	308.8	10:43	1.5	309.2	10:32	1.5	307.2
2	10			59	11:03	1.5	315.4	11:02	1.5	309.6	10:46	1.5	305.1	10:44	1.6	315.6	10:51	1.6	312.1	10:46	1.6	318.9
2	11			59	11:19	1.3	304.5	11:15	1.5	309.2	11:01	1.4	319.5	10:53	1.4	319.2	10:59	1.4	313.0	11:00	1.4	313.0
2	12			59	11:33	1.4	314.4	11:30	1.4	314.1	11:16	1.4	311.9	11:04	1.4	305.0	11:08	1.5	324.3	11:14	1.5	321.2
2	13			60	11:45	1.7	317.0	11:30	1.7	315.5	11:17	1.7	311.8	11:15	1.7	306.7	11:33	1.8	322.2	11:12	1.8	321.8
2	14			60	12:00	1.5	309.8	11:45	1.5	307.5	11:27	1.6	324.0	11:23	1.6	315.3	11:49	1.6	316.3	11:20	1.6	311.5
2	15			60	12:11	1.6	317.5	11:59	1.6	318.8	11:40	1.6	311.7	11:31	1.6	306.8	12:06	1.6	306.5	11:28	1.6	313.9
2	16			60	12:22	1.6	315.1	12:12	1.6	317.1	11:53	1.6	305.5	11:39	1.7	320.1	12:29	1.7	317.4	11:35	1.7	314.7

					Day 7 (27 & 28 April 2012)			Day 8 (28 & 29 April 2012)			Day 9 (29 & 30 April 2012)			Day 10 (30 April & 01 May 2012)			Day 11 (01 & 02 May 2012)	
Group No.:	Animal No.:	Test Substance Dose Level	Actual Dose Concentration (mg/mL)	Age of Animal (PND)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Death	Time From Last Administration
2	09	Padimate-O (320 mg/kg)	62.9	59	10:41	1.5	304.5	10:31	1.6	322.2	10:30	1.6	319.7	10:41	1.6	317.4	8:37	21:56
2	10			59	10:49	1.6	307.9	10:45	1.7	322.9	10:43	1.7	314.2	10:48	1.7	311.0	9:39	22:51
2	11			59	10:57	1.4	310.7	10:59	1.4	304.4	10:57	1.5	318.4	10:56	1.5	320.8	10:32	23:36
2	12			59	11:05	1.5	319.3	11:14	1.5	315.4	11:10	1.5	310.9	11:02	1.5	308.1	11:26	24:24
2	13			60	11:28	1.8	315.6	11:26	1.8	314.7	11:10	1.8	311.5	10:32	1.8	307.8	8:56	22:24
2	14			60	11:42	1.7	324.0	11:39	1.7	319.3	11:17	1.7	317.3	10:45	1.7	317.8	9:49	23:04
2	15			60	11:56	1.7	321.0	11:54	1.7	314.9	11:26	1.7	316.1	11:01	1.7	311.6	10:56	23:55
2	16			60	12:12	1.7	308.5	12:09	1.7	306.3	11:34	1.7	305.7	11:15	1.8	319.6	11:46	24:31

Dose Administration

					Day 1 (21 & 22 April 2012)			Day 2 (22 & 23 April 2012)			Day 3 (23 & 24 April 2012)			Day 4 (24 & 25 April 2012)			Day 5 (25 & 26 April 2012)			Day 6 (26 & 27 April 2012)		
Group No.:	Animal No.:	Test Substance Dose Level	Actual Dose Concentration (mg/mL)	Age of Animal (PND)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)
3	17	Padimate-O (1000 mg/kg)	191	59	10:48	1.5	981.8	10:46	1.5	976.5	10:32	1.5	945.2	10:34	1.5	936.6	10:44	1.5	926.9	10:33	1.5	936.9
3	18			59	11:04	1.3	925.5	11:03	1.4	987.8	10:47	1.3	938.8	10:44	1.4	990.0	10:52	1.4	973.1	10:47	1.4	975.2
3	19			59	11:20	1.5	938.7	11:16	1.5	957.2	11:02	1.5	941.5	10:54	1.5	942.7	10:59	1.6	980.1	11:01	1.6	977.6
3	20			59	11:34	1.7	966.7	11:31	1.7	968.7	11:16	1.7	969.5	11:04	1.8	964.4	11:08	1.7	943.3	11:15	1.7	934.1
3	21			60	11:46	1.6	961.3	11:31	1.6	964.3	11:17	1.6	963.4	11:16	1.6	934.3	11:34	1.6	937.1	11:13	1.7	978.0
3	22			60	12:00	1.6	965.9	11:46	1.5	924.8	11:28	1.5	947.4	11:24	1.5	970.9	11:50	1.5	983.5	11:21	1.6	967.1
3	23			60	12:12	1.5	936.6	12:00	1.5	946.8	11:41	1.5	939.3	11:32	1.6	976.4	12:07	1.6	982.3	11:28	1.6	969.9
3	24			60	12:23	1.5	982.5	12:13	1.4	944.9	11:53	1.4	925.9	11:39	1.5	960.4	12:30	1.5	966.9	11:35	1.4	925.6

					Day 7 (27 & 28 April 2012)			Day 8 (28 & 29 April 2012)			Day 9 (29 & 30 April 2012)			Day 10 (30 April & 01 May 2012)			Day 11 (01 & 02 May 2012)	
Group No.:	Animal No.:	Test Substance Dose Level	Actual Dose Concentration (mg/mL)	Age of Animal (PND)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Death	Time From Last Administration
3	17	Padimate-O (1000 mg/kg)	191	59	10:41	1.6	976.7	10:32	1.6	958.0	10:31	1.6	961.3	10:41	1.6	974.5	8:43	22:02
3	18			59	10:50	1.4	966.0	10:46	1.4	961.2	10:45	1.4	962.2	10:48	1.4	966.7	9:43	22:55
3	19			59	10:58	1.6	979.5	11:00	1.6	977.9	10:58	1.6	959.2	10:56	1.5	929.3	10:35	23:39
3	20			59	11:06	1.8	959.3	11:15	1.8	972.6	11:11	1.8	965.7	11:03	1.8	977.8	11:31	24:28
3	21			60	11:29	1.6	947.0	11:27	1.6	964.6	11:10	1.6	965.9	10:33	1.6	961.0	9:00	22:27
3	22			60	11:43	1.6	974.2	11:40	1.5	925.4	11:17	1.5	929.9	10:47	1.5	929.9	9:59	23:12
3	23			60	11:58	1.6	977.9	11:55	1.6	966.8	11:27	1.5	927.2	11:02	1.6	981.1	10:59	23:57
3	24			60	12:13	1.5	953.1	12:10	1.5	938.1	11:34	1.5	934.4	11:16	1.6	978.5	11:50	24:34

					Day 1 (21 & 22 April 2012)			Day 2 (22 & 23 April 2012)			Day 3 (23 & 24 April 2012)			Day 4 (24 & 25 April 2012)			Day 5 (25 & 26 April 2012)			Day 6 (26 & 27 April 2012)		
Group No.:	Animal No.:	Test Substance Dose Level	Actual Dose Concentration (mg/mL)	Age of Animal (PND)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)
4	25	Homosalate (320 mg/kg)	61.4	59	10:49	1.5	298.0	10:47	1.5	303.26	10:33	1.5	302.26	10:35	1.5	297.77	10:44	1.6	314.47	10:34	1.6	314.4
4	26			59	11:05	1.6	311.9	11:03	1.6	314.87	10:48	1.6	312.67	10:45	1.6	304.81	10:53	1.6	301.91	10:48	1.6	299.0
4	27			59	11:21	1.4	304.1	11:17	1.4	305.58	11:03	1.4	302.25	10:55	1.4	297.75	11:00	1.4	300.24	11:02	1.5	317.1
4	28			59	11:35	1.4	299.3	11:32	1.4	301.51	11:17	1.4	300.03	11:05	1.5	317.37	11:09	1.5	313.59	11:16	1.5	316.6
4	29			60	11:46	1.5	298.3	11:32	1.5	303.86	11:18	1.5	300.29	11:16	1.5	298.93	11:34	1.5	300.29	11:13	1.6	307.7
4	30			60	12:01	1.7	309.5	11:47	1.7	310.47	11:29	1.7	302.90	11:24	1.7	302.11	11:51	1.8	315.59	11:21	1.8	309.6
4	31			60	12:13	1.4	312.4	12:01	1.4	315.22	11:42	1.4	305.36	11:32	1.4	302.78	12:07	1.4	301.19	11:29	1.5	316.0
4	32			60	12:24	1.6	306.8	12:14	1.6	309.61	11:54	1.6	303.77	11:40	1.6	303.02	12:31	1.6	303.02	11:36	1.7	315.8

					Day 7 (27 & 28 April 2012)			Day 8 (28 & 29 April 2012)			Day 9 (29 & 30 April 2012)			Day 10 (30 April & 01 May 2012)			Day 11 (01 & 02 May 2012)	
Group No.:	Animal No.:	Test Substance Dose Level	Actual Dose Concentration (mg/mL)	Age of Animal (PND)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Death	Time From Last Administration
4	25	Homosalate (320 mg/kg)	61.4	59	10:42	1.6	308.2	10:33	1.6	304.9	10:32	1.6	304.1	10:42	1.6	301.7	8:47	22:05
4	26			59	10:50	1.7	307.1	10:47	1.7	312.6	10:46	1.7	306.3	10:49	1.7	302.0	9:46	22:57
4	27			59	10:58	1.5	315.4	11:01	1.5	312.1	10:59	1.5	304.6	10:57	1.5	306.5	10:38	23:41
4	28			59	11:06	1.5	307.9	11:16	1.5	306.1	11:12	1.5	305.1	11:03	1.5	298.4	11:36	24:33
4	29			60	11:30	1.6	311.5	11:28	1.6	311.3	11:11	1.6	313.4	10:34	1.6	313.8	9:06	22:32
4	30			60	11:44	1.8	306.7	11:41	1.8	302.7	11:18	1.8	306.2	10:48	1.9	314.6	10:02	23:14
4	31			60	11:59	1.5	315.2	11:56	1.5	310.3	11:27	1.5	310.5	11:03	1.5	302.6	11:02	23:59
4	32			60	12:14	1.7	313.7	12:11	1.7	314.1	11:35	1.7	311.1	11:18	1.7	312.3	11:53	24:35

Dose Administration

					Day 1 (21 & 22 April 2012)			Day 2 (22 & 23 April 2012)			Day 3 (23 & 24 April 2012)			Day 4 (24 & 25 April 2012)			Day 5 (25 & 26 April 2012)			Day 6 (26 & 27 April 2012)		
Group No.:	Animal No.:	Test Substance Dose Level	Actual Dose Concentration (mg/mL)	Age of Animal (PND)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)
5	33	Homosalate (1000 mg/kg)	194	59	10:50	1.6	952.7	10:48	1.6	970.61	10:34	1.6	960.10	10:35	1.6	962.18	10:45	1.6	966.68	10:35	1.6	992.0
5	34			59	11:06	1.5	996.6	11:04	1.4	946.34	10:49	1.4	950.98	10:46	1.4	974.87	10:53	1.4	990.88	10:49	1.3	963.3
5	35			59	11:21	1.4	989.8	11:18	1.4	1000.74	11:04	1.3	939.64	10:55	1.3	956.03	11:01	1.3	965.54	11:03	1.3	974.1
5	36			59	11:36	1.5	978.8	11:33	1.5	966.46	11:19	1.5	956.61	11:06	1.5	955.04	11:09	1.5	939.32	11:17	1.5	939.6
5	37			60	11:47	1.5	949.7	11:33	1.5	960.08	11:18	1.5	949.74	11:16	1.5	943.58	11:35	1.5	966.78	11:14	1.5	955.4
5	38			60	12:02	1.5	940.2	11:48	1.5	958.18	11:29	1.5	946.34	11:24	1.5	949.12	11:52	1.5	944.19	11:21	1.5	945.1
5	39			60	12:14	1.6	973.3	12:01	1.6	970.30	11:42	1.6	989.80	11:32	1.5	954.41	12:08	1.5	979.80	11:29	1.4	968.3
5	40			60	12:25	1.5	972.6	12:15	1.5	988.12	11:54	1.4	951.98	11:40	1.4	977.33	12:33	1.4	978.74	11:36	1.5	993.2

					Day 7 (27 & 28 April 2012)			Day 8 (28 & 29 April 2012)			Day 9 (29 & 30 April 2012)			Day 10 (30 April & 01 May 2012)			Day 11 (01 & 02 May 2012)	
Group No.:	Animal No.:	Test Substance Dose Level	Actual Dose Concentration (mg/mL)	Age of Animal (PND)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Death	Time From Last Administration
5	33	Homosalate (1000 mg/kg)	194	59	10:43	1.6	962.78	10:34	1.6	942.6	10:33	1.7	975.2	10:42	1.7	978.6	8:51	22:09
5	34			59	10:51	1.4	1002.58	10:48	1.4	970.7	10:47	1.4	952.6	10:49	1.4	951.6	9:49	23:00
5	35			59	10:59	1.3	943.86	11:02	1.3	944.2	11:00	1.4	989.8	10:57	1.4	989.4	10:42	23:45
5	36			59	11:06	1.6	995.83	11:17	1.6	986.6	11:13	1.6	969.1	11:03	1.6	971.5	11:40	24:37
5	37			60	11:32	1.6	995.51	11:29	1.6	992.0	11:11	1.6	999.4	10:35	1.6	999.7	9:08	22:33
5	38			60	11:45	1.6	971.82	11:42	1.6	982.0	11:18	1.6	980.7	10:49	1.6	968.8	10:05	23:16
5	39			60	12:00	1.4	983.70	11:57	1.4	1001.8	11:28	1.5	986.4	11:04	1.5	966.1	11:06	24:02
5	40			60	12:15	1.5	961.67	12:13	1.5	1003.1	11:35	1.5	947.3	11:19	1.6	1000.6	11:57	24:38

Dose Administration

						Day 1 (21 & 22 April 2012)			Day 2 (22 & 23 April 2012)			Day 3 (23 & 24 April 2012)			Day 4 (24 & 25 April 2012)			Day 5 (25 & 26 April 2012)			Day 6 (26 & 27 April 2012)		
Group No.:	Animal No.:	Test Substance Dose Level	Dose Route	Actual Dose Concentration (mg/mL)	Age of Animal (PND)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)
6	41	Corn Oil Control (0 mg/kg) + TP (0.4 mg/kg)	Gavage	0	59	10:51	1.6	0.00	10:50	1.6	0.00	10:36	1.6	0.00	10:36	1.7	0.00	10:46	1.7	0.00	10:36	1.7	0.00
			Subcutaneous				0.16			0.16			0.16			0.17			0.17			0.17	
6	42		Gavage	0	59	11:07	1.4	0.00	11:06	1.5	0.00	10:50	1.5	0.00	10:47	1.5	0.00	10:53	1.6	0.00	10:50	1.6	0.00
			Subcutaneous				0.14			0.15			0.15			0.15			0.16			0.16	
6	43		Gavage	0	59	11:22	1.5	0.00	11:20	1.5	0.00	11:05	1.5	0.00	10:56	1.6	0.00	11:01	1.6	0.00	11:04	1.7	0.00
			Subcutaneous				0.15			0.15			0.15			0.16			0.16			0.17	
6	44		Gavage	0	59	11:37	1.5	0.00	11:34	1.5	0.00	11:19	1.6	0.00	11:07	1.6	0.00	11:10	1.6	0.00	11:18	1.6	0.00
			Subcutaneous				0.15			0.15			0.16			0.16			0.16			0.16	
6	45		Gavage	0	60	11:50	1.6	0.00	11:34	1.6	0.00	11:19	1.6	0.00	11:17	1.7	0.00	11:36	1.7	0.00	11:15	1.8	0.00
			Subcutaneous				0.16			0.16			0.16			0.17			0.17			0.18	
6	46		Gavage	0	60	12:03	1.4	0.00	11:49	1.4	0.00	11:32	1.4	0.00	11:25	1.5	0.00	11:53	1.5	0.00	11:22	1.5	0.00
			Subcutaneous				0.14			0.14			0.14			0.15			0.15			0.15	
6	47		Gavage	0	60	12:14	1.7	0.00	12:03	1.7	0.00	11:43	1.7	0.00	11:33	1.7	0.00	12:09	1.8	0.00	11:30	1.8	0.00
			Subcutaneous				0.17			0.17			0.17			0.17			0.18			0.18	
6	48		Gavage	0	60	12:26	1.6	0.00	12:16	1.5	0.00	11:56	1.6	0.00	11:42	1.6	0.00	12:36	1.7	0.00	11:37	1.7	0.00
			Subcutaneous				0.16			0.15			0.16			0.16			0.17			0.17	

					Day 7 (27 & 28 April 2012)			Day 8 (28 & 29 April 2012)			Day 9 (29 & 30 April 2012)			Day 10 (30 April & 01 May 2012)			Day 11 (01 & 02 May 2012)	
Group No.:	Animal No.:	Test Substance Dose Level	Dose Route	Actual Dose Concentration (mg/mL)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Death	Time From Last Administration
6	41	Corn Oil Control (0 mg/kg) + TP (0.4 mg/kg)	Gavage	0	10:43	1.8	0.00	10:35	1.7	0.00	10:34	1.8	0.00	10:42	1.8	0.00	8:56	22:14
			Subcutaneous			0.18			0.17			0.18			0.18			
6	42		Gavage	0	10:52	1.6	0.00	10:49	1.6	0.00	10:48	1.7	0.00	10:50	1.7	0.00	9:52	23:02
			Subcutaneous			0.16			0.16			0.17			0.17			
6	43		Gavage	0	11:00	1.7	0.00	11:04	1.8	0.00	11:01	1.8	0.00	10:58	1.8	0.00	10:46	23:48
			Subcutaneous			0.17			0.18			0.18			0.18			
6	44		Gavage	0	11:07	1.7	0.00	11:18	1.7	0.00	11:14	1.8	0.00	11:05	1.8	0.00	11:44	24:39
			Subcutaneous			0.17			0.17			0.18			0.18			
6	45		Gavage	0	11:33	1.8	0.00	11:30	1.8	0.00	11:12	1.9	0.00	10:36	1.9	0.00	9:12	22:36
			Subcutaneous			0.18			0.18			0.19			0.19			
6	46		Gavage	0	11:46	1.6	0.00	11:44	1.6	0.00	11:19	1.6	0.00	10:50	1.7	0.00	10:10	23:20
			Subcutaneous			0.16			0.16			0.16			0.17			
6	47		Gavage	0	12:01	1.8	0.00	11:58	1.9	0.00	11:29	1.9	0.00	11:05	1.9	0.00	11:11	24:06
			Subcutaneous			0.18			0.19			0.19			0.19			
6	48		Gavage	0	12:16	1.7	0.00	12:13	1.8	0.00	11:36	1.8	0.00	11:20	1.8	0.00	12:02	24:42
			Subcutaneous			0.17			0.18			0.18			0.18			

Dose Administration

						Day 1 (21 & 22 April 2012)			Day 2 (22 & 23 April 2012)			Day 3 (23 & 24 April 2012)			Day 4 (24 & 25 April 2012)			Day 5 (25 & 26 April 2012)			Day 6 (26 & 27 April 2012)		
Group No.:	Animal No.:	Test Substance Dose Level	Dose Route	Actual Dose Concentration (mg/mL)	Age of Animal (PND)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)
7	49	Padimate-O (100 mg/kg) + TP (0.4 mg/kg)	Gavage	19.5	59	10:52	1.5	99.52	10:51	1.5	98.85	10:38	1.5	97.08	10:37	1.5	94.72	10:46	1.6	99.43	10:37	1.6	97.84
			Subcutaneous				0.15			0.15			0.15			0.15			0.16			0.16	
7	50		Gavage	19.5	59	11:09	1.6	97.38	11:07	1.6	95.33	10:51	1.7	99.94	10:47	1.7	97.99	10:54	1.7	95.51	10:51	1.8	99.89
			Subcutaneous				0.16			0.16			0.17			0.17			0.17			0.18	
7	51		Gavage	19.5	59	11:23	1.4	94.27	11:20	1.5	99.22	11:07	1.5	95.53	10:57	1.6	99.43	11:02	1.6	96.98	11:05	1.7	99.22
			Subcutaneous				0.14			0.15			0.15			0.16			0.16			0.17	
7	52		Gavage	19.5	59	11:39	1.6	100.13	11:34	1.6	98.45	11:20	1.6	97.23	11:08	1.6	95.30	11:10	1.7	99.19	11:20	1.7	96.48
			Subcutaneous				0.16			0.16			0.16			0.16			0.17			0.17	
7	53		Gavage	19.5	60	11:51	1.6	97.59	11:36	1.6	100.10	11:20	1.6	96.65	11:18	1.6	95.12	11:37	1.7	98.51	11:15	1.7	99.31
			Subcutaneous				0.16			0.16			0.16			0.16			0.17			0.17	
7	54		Gavage	19.5	60	12:04	1.4	98.88	11:50	1.4	97.74	11:33	1.4	95.32	11:26	1.4	94.66	11:55	1.5	98.42	11:23	1.4	95.19
			Subcutaneous				0.14			0.14			0.14			0.14			0.15			0.14	
7	55		Gavage	19.5	60	12:15	1.5	99.86	12:04	1.5	98.09	11:44	1.5	97.89	11:34	1.5	95.28	12:11	1.6	98.61	11:30	1.6	96.47
			Subcutaneous				0.15			0.15			0.15			0.15			0.16			0.16	
7	56		Gavage	19.5	60	12:27	1.6	95.41	12:17	1.6	95.38	11:57	1.7	98.02	11:42	1.7	95.92	12:37	1.8	100.14	11:38	1.8	96.09
			Subcutaneous				0.16			0.16			0.17			0.17			0.18			0.18	

					Day 7 (27 & 28 April 2012)			Day 8 (28 & 29 April 2012)			Day 9 (29 & 30 April 2012)			Day 10 (30 April & 01 May 2012)			Day 11 (01 & 02 May 2012)	
Group No.:	Animal No.:	Test Substance Dose Level	Dose Route	Actual Dose Concentration (mg/mL)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Death	Time From Last Administration
7	49	Padimate-O (100 mg/kg) + TP (0.4 mg/kg)	Gavage	19.5	10:44	1.6	95.53	10:36	1.6	95.01	10:35	1.7	98.78	10:42	1.7	97.04	9:04	22:22
			Subcutaneous			0.16			0.16			0.17			0.17			
7	50		Gavage	19.5	10:52	1.8	97.53	10:50	1.9	98.96	10:49	1.9	98.33	10:50	1.9	98.02	9:58	23:08
			Subcutaneous			0.18			0.19			0.19			0.19			
7	51		Gavage	19.5	11:00	1.7	97.87	11:05	1.7	95.45	11:02	1.8	98.96	10:58	1.8	98.71	10:51	23:53
			Subcutaneous			0.17			0.17			0.18			0.18			
7	52		Gavage	19.5	11:08	1.8	100.20	11:19	1.8	97.91	11:15	1.9	100.00	11:05	1.9	99.84	11:48	24:43
			Subcutaneous			0.18			0.18			0.19			0.19			
7	53		Gavage	19.5	11:34	1.6	95.76	11:31	1.8	97.91	11:13	1.8	97.42	10:37	1.8	96.11	9:17	22:40
			Subcutaneous			0.16			0.18			0.18			0.18			
7	54		Gavage	19.5	11:47	1.4	97.53	11:45	1.5	94.97	11:20	1.6	98.52	10:52	1.6	99.49	10:15	23:23
			Subcutaneous			0.14			0.15			0.16			0.16			
7	55		Gavage	19.5	12:02	1.6	95.18	12:00	1.7	99.79	11:29	1.7	98.37	11:06	1.7	96.09	11:16	24:10
			Subcutaneous			0.16			0.17			0.17			0.17			
7	56		Gavage	19.5	12:17	1.9	98.12	12:14	1.9	96.76	11:37	1.9	95.71	11:21	2.0	98.71	12:06	24:45
			Subcutaneous			0.19			0.19			0.19			0.20			

Dose Administration

						Day 1 (21 & 22 April 2012)			Day 2 (22 & 23 April 2012)			Day 3 (23 & 24 April 2012)			Day 4 (24 & 25 April 2012)			Day 5 (25 & 26 April 2012)			Day 6 (26 & 27 April 2012)		
Group No.:	Animal No.:	Test Substance Dose Level	Dose Route	Actual Dose Concentration (mg/mL)	Age of Animal (PND)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)
8	57	Padimate-O (320 mg/kg) + TP (0.4 mg/kg)	Gavage	62.9	59	10:55	1.6	316.48	10:53	1.6	315.68	10:39	1.6	313.23	10:38	1.6	306.08	10:47	1.7	323.93	10:38	1.7	319.19
			Subcutaneous				0.16			0.16			0.16			0.16			0.17			0.17	
8	58		Gavage	62.9	59	11:10	1.5	308.94	11:08	1.5	309.24	10:52	1.5	305.83	10:48	1.6	322.67	10:55	1.6	316.78	10:52	1.6	316.48
			Subcutaneous				0.15			0.15			0.15			0.16			0.16			0.16	
8	59		Gavage	62.9	59	11:24	1.5	322.45	11:21	1.5	319.07	11:08	1.5	312.00	10:57	1.5	306.43	11:02	1.6	317.78	11:06	1.6	313.13
			Subcutaneous				0.15			0.15			0.15			0.15			0.16			0.16	
8	60		Gavage	62.9	59	11:40	1.4	318.94	11:35	1.4	314.72	11:21	1.4	306.19	11:09	1.5	324.00	11:11	1.5	318.32	11:23	1.5	314.19
			Subcutaneous				0.14			0.14			0.14			0.15			0.15			0.15	
8	61		Gavage	62.9	60	11:52	1.6	307.77	11:37	1.6	309.00	11:21	1.7	316.27	11:19	1.7	309.22	11:38	1.7	306.92	11:16	1.8	317.23
			Subcutaneous				0.16			0.16			0.17			0.17			0.17			0.18	
8	62		Gavage	62.9	60	12:05	1.6	311.19	11:51	1.6	313.62	11:34	1.6	306.46	11:27	1.7	314.78	11:56	1.7	307.62	11:23	1.8	322.66
			Subcutaneous				0.16			0.16			0.16			0.17			0.17			0.18	
8	63		Gavage	62.9	60	12:16	1.4	315.85	12:05	1.4	312.60	11:45	1.5	324.56	11:35	1.5	317.14	12:14	1.5	307.63	11:31	1.5	306.73
			Subcutaneous				0.14			0.14			0.15			0.15			0.15			0.15	
8	64		Gavage	62.9	60	12:28	1.4	303.76	12:18	1.4	306.30	11:57	1.5	315.34	11:43	1.5	311.69	12:39	1.6	319.90	11:38	1.6	315.39
			Subcutaneous				0.14			0.14			0.15			0.15			0.16			0.16	

					Day 7 (27 & 28 April 2012)			Day 8 (28 & 29 April 2012)			Day 9 (29 & 30 April 2012)			Day 10 (30 April & 01 May 2012)			Day 11 (01 & 02 May 2012)	
Group No.:	Animal No.:	Test Substance Dose Level	Dose Route	Actual Dose Concentration (mg/mL)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Death	Time From Last Administration
8	57	Padimate-O (320 mg/kg) + TP (0.4 mg/kg)	Gavage	62.9	10:45	1.7	311.84	10:37	1.7	310.12	10:35	1.7	309.58	10:44	1.8	322.84	9:09	22:25
			Subcutaneous			0.17			0.17			0.17			0.18			
8	58		Gavage	62.9	10:53	1.6	310.04	10:52	1.6	310.14	10:50	1.6	305.25	10:51	1.7	323.44	10:04	23:13
			Subcutaneous			0.16			0.16			0.16			0.17			
8	59		Gavage	62.9	11:01	1.6	306.55	11:06	1.7	320.15	11:03	1.7	315.06	10:58	1.7	306.92	10:55	23:57
			Subcutaneous			0.16			0.17			0.17			0.17			
8	60		Gavage	62.9	11:08	1.5	310.77	11:20	1.6	322.25	11:16	1.6	320.00	11:06	1.6	316.88	11:55	24:49
			Subcutaneous			0.15			0.16			0.16			0.16			
8	61		Gavage	62.9	11:35	1.8	312.50	11:32	1.8	307.00	11:13	1.9	319.29	10:38	1.9	313.59	9:21	22:43
			Subcutaneous			0.18			0.18			0.19			0.19			
8	62		Gavage	62.9	11:48	1.8	312.07	11:46	1.8	313.80	11:22	1.9	318.52	10:53	1.9	319.37	10:18	23:25
			Subcutaneous			0.18			0.18			0.19			0.19			
8	63		Gavage	62.9	12:04	1.6	317.98	12:01	1.6	313.52	11:30	1.6	309.09	11:08	1.6	307.96	11:19	24:11
			Subcutaneous			0.16			0.16			0.16			0.16			
8	64		Gavage	62.9	12:19	1.6	310.23	12:15	1.7	322.08	11:37	1.7	318.24	11:22	1.7	318.91	12:10	24:48
			Subcutaneous			0.16			0.17			0.17			0.17			

Dose Administration

						Day 1 (21 & 22 April 2012)			Day 2 (22 & 23 April 2012)			Day 3 (23 & 24 April 2012)			Day 4 (24 & 25 April 2012)			Day 5 (25 & 26 April 2012)			Day 6 (26 & 27 April 2012)		
Group No.:	Animal No.:	Test Substance Dose Level	Dose Route	Actual Dose Concentration (mg/mL)	Age of Animal (PND)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)
9	65	Padimate-O (1000 mg/kg) + TP (0.4 mg/kg)	Gavage	191	59	10:57	1.4	938.25	10:54	1.4	938.25	10:40	1.4	926.54	10:39	1.5	985.21	10:48	1.4	924.30	10:39	1.4	941.88
			Subcutaneous				0.14			0.14			0.14			0.15			0.14			0.14	
9	66		Gavage	191	59	11:12	1.4	979.49	11:09	1.4	977.70	10:54	1.3	922.02	10:49	1.4	950.92	10:56	1.4	971.30	10:53	1.3	937.69
			Subcutaneous				0.14			0.14			0.13			0.14			0.14			0.13	
9	67		Gavage	191	59	11:26	1.6	944.67	11:22	1.6	943.79	11:09	1.6	928.03	10:58	1.7	970.12	11:03	1.7	976.84	11:07	1.7	981.56
			Subcutaneous				0.16			0.16			0.16			0.17			0.17			0.17	
9	68		Gavage	191	59	11:41	1.5	924.79	11:36	1.6	983.90	11:23	1.6	967.70	11:10	1.6	963.43	11:12	1.6	930.86	11:25	1.7	973.03
			Subcutaneous				0.15			0.16			0.16			0.16			0.16			0.17	
9	69		Gavage	191	60	11:53	1.5	937.19	11:38	1.5	955.00	11:22	1.5	934.44	11:19	1.5	941.51	11:40	1.6	967.70	11:16	1.6	972.32
			Subcutaneous				0.15			0.15			0.15			0.15			0.16			0.16	
9	70		Gavage	191	60	12:06	1.6	942.34	11:52	1.7	974.78	11:35	1.6	929.44	11:27	1.7	975.37	11:57	1.7	962.93	11:24	1.7	942.53
			Subcutaneous				0.16			0.17			0.16			0.17			0.17			0.17	
9	71		Gavage	191	60	12:17	1.5	953.73	12:06	1.5	948.36	11:46	1.5	950.56	11:36	1.5	946.17	12:16	1.5	972.51	11:32	1.5	977.48
			Subcutaneous				0.15			0.15			0.15			0.15			0.15			0.15	
9	72		Gavage	191	60	12:29	1.6	976.98	12:19	1.6	971.08	11:59	1.6	968.01	11:44	1.5	935.05	12:42	1.5	956.28	11:39	1.5	934.75
			Subcutaneous				0.16			0.16			0.16			0.15			0.15			0.15	

					Day 7 (27 & 28 April 2012)			Day 8 (28 & 29 April 2012)			Day 9 (29 & 30 April 2012)			Day 10 (30 April & 01 May 2012)			Day 11 (01 & 02 May 2012)	
Group No.:	Animal No.:	Test Substance Dose Level	Dose Route	Actual Dose Concentration (mg/mL)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Death	Time From Last Administration
9	65	Padimate-O (1000 mg/kg) + TP (0.4 mg/kg)	Gavage	191	10:45	1.5	943.37	10:38	1.6	984.22	10:37	1.5	964.65	10:44	1.5	986.91	9:13	22:29
			Subcutaneous			0.15			0.16			0.15			0.15			
9	66		Gavage	191	10:54	1.4	957.74	10:53	1.4	958.08	10:51	1.4	971.30	10:52	1.3	920.65	10:07	23:15
			Subcutaneous			0.14			0.14			0.14			0.13			
9	67		Gavage	191	11:02	1.7	943.07	11:08	1.7	928.51	11:04	1.7	933.31	10:59	1.7	929.57	11:01	24:02
			Subcutaneous			0.17			0.17			0.17			0.17			
9	68		Gavage	191	11:09	1.7	966.66	11:21	1.7	950.81	11:17	1.7	953.60	11:06	1.7	981.26	12:01	24:55
			Subcutaneous			0.17			0.17			0.17			0.17			
9	69		Gavage	191	11:36	1.6	957.69	11:33	1.6	938.57	11:14	1.6	953.21	10:39	1.6	953.21	9:25	22:46
			Subcutaneous			0.16			0.16			0.16			0.16			
9	70		Gavage	191	11:50	1.7	934.12	11:47	1.7	951.36	11:23	1.7	957.25	10:54	1.7	933.05	10:26	23:32
			Subcutaneous			0.17			0.17			0.17			0.17			
9	71		Gavage	191	12:06	1.5	967.91	12:02	1.5	941.81	11:30	1.5	957.87	11:09	1.5	954.68	11:23	24:14
			Subcutaneous			0.15			0.15			0.15			0.15			
9	72		Gavage	191	12:20	1.5	941.20	12:16	1.5	959.80	11:38	1.4	924.94	11:23	1.4	949.91	12:14	24:51
			Subcutaneous			0.15			0.15			0.14			0.14			

Dose Administration

						Day 1 (21 & 22 April 2012)			Day 2 (22 & 23 April 2012)			Day 3 (23 & 24 April 2012)			Day 4 (24 & 25 April 2012)			Day 5 (25 & 26 April 2012)			Day 6 (26 & 27 April 2012)		
Group No.:	Animal No.:	Test Substance Dose Level	Dose Route	Actual Dose Concentration (mg/mL)	Age of Animal (PND)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)
10	73	Homosalate (100 mg/kg) + TP (0.4 mg/kg)	Gavage	19.2	59	10:58	1.4	98.79	10:55	1.4	96.55	10:41	1.4	94.75	10:40	1.5	98.19	10:48	1.5	97.86	10:40	1.5	96.00
			Subcutaneous				0.14			0.14			0.14			0.15			0.15			0.15	
10	74		Gavage	19.2	59	11:13	1.5	98.50	11:10	1.5	97.04	10:55	1.5	95.18	10:49	1.6	98.71	10:56	1.6	96.27	10:54	1.6	95.46
			Subcutaneous				0.15			0.15			0.15			0.16			0.16			0.16	
10	75		Gavage	19.2	59	11:27	1.6	94.52	11:23	1.6	93.12	11:10	1.7	95.27	10:59	1.7	93.39	11:04	1.8	96.46	11:08	1.8	95.44
			Subcutaneous				0.16			0.16			0.17			0.17			0.18			0.18	
10	76		Gavage	19.2	59	11:43	1.6	95.26	11:38	1.7	98.73	11:24	1.7	97.84	11:11	1.7	95.02	11:12	1.8	96.64	11:26	1.8	95.29
			Subcutaneous				0.16			0.17			0.17			0.17			0.18			0.18	
10	77		Gavage	19.2	60	11:55	1.6	98.24	11:39	1.6	97.12	11:23	1.6	95.17	11:20	1.6	93.20	11:41	1.7	96.91	11:17	1.7	94.69
			Subcutaneous				0.16			0.16			0.16			0.16			0.17			0.17	
10	78		Gavage	19.2	60	12:07	1.5	96.32	11:53	1.5	94.89	11:36	1.6	97.83	11:28	1.6	96.33	11:58	1.6	94.99	11:25	1.7	98.73
			Subcutaneous				0.15			0.15			0.16			0.16			0.16			0.17	
10	79		Gavage	19.2	60	12:18	1.5	98.36	12:07	1.5	95.78	11:47	1.6	98.08	11:36	1.6	95.31	12:19	1.7	97.14	11:32	1.7	95.24
			Subcutaneous				0.15			0.15			0.16			0.16			0.17			0.17	
10	80		Gavage	19.2	60	12:30	1.6	96.18	12:20	1.6	96.09	12:00	1.7	96.43	11:45	1.7	95.44	12:44	1.7	94.50	11:39	1.8	97.63
			Subcutaneous				0.16			0.16			0.17			0.17			0.17			0.18	

					Day 7 (27 & 28 April 2012)			Day 8 (28 & 29 April 2012)			Day 9 (29 & 30 April 2012)			Day 10 (30 April & 01 May 2012)			Day 11 (01 & 02 May 2012)	
Group No.:	Animal No.:	Test Substance Dose Level	Dose Route	Actual Dose Concentration (mg/mL)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Death	Time From Last Administration
10	73	Homosalate (100 mg/kg) + TP (0.4 mg/kg)	Gavage	19.2	10:46	1.5	95.71	10:40	1.6	98.87	10:38	1.6	97.03	10:45	1.6	95.76	9:18	22:33
			Subcutaneous			0.15			0.16			0.16			0.16			
10	74		Gavage	19.2	10:55	1.7	98.79	10:54	1.7	97.32	10:52	1.7	94.94	10:53	1.7	94.44	10:12	23:19
			Subcutaneous			0.17			0.17			0.17			0.17			
10	75		Gavage	19.2	11:03	1.8	93.76	11:09	1.9	96.00	11:05	1.9	94.58	11:00	2.0	97.07	11:05	24:05
			Subcutaneous			0.18			0.19			0.19			0.20			
10	76		Gavage	19.2	11:09	1.8	93.73	11:22	1.9	96.05	11:18	1.9	94.07	11:07	2.0	97.34	12:05	24:58
			Subcutaneous			0.18			0.19			0.19			0.20			
10	77		Gavage	19.2	11:37	1.8	97.52	11:34	1.8	95.87	11:15	1.8	94.19	10:40	1.9	98.57	9:29	22:49
			Subcutaneous			0.18			0.18			0.18			0.19			
10	78		Gavage	19.2	11:51	1.7	97.64	11:48	1.7	95.38	11:23	1.7	94.86	10:55	1.8	97.00	10:30	23:35
			Subcutaneous			0.17			0.17			0.17			0.18			
10	79		Gavage	19.2	12:07	1.8	96.89	12:04	1.8	94.37	11:31	1.9	98.20	11:10	1.9	95.32	11:27	24:17
			Subcutaneous			0.18			0.18			0.19			0.19			
10	80		Gavage	19.2	12:21	1.8	95.52	12:18	1.9	97.64	11:39	1.9	97.12	11:24	1.9	96.23	12:18	24:54
			Subcutaneous			0.18			0.19			0.19			0.19			

Dose Administration

						Day 1 (21 & 22 April 2012)			Day 2 (22 & 23 April 2012)			Day 3 (23 & 24 April 2012)			Day 4 (24 & 25 April 2012)			Day 5 (25 & 26 April 2012)			Day 6 (26 & 27 April 2012)		
Group No.:	Animal No.:	Test Substance Dose Level	Dose Route	Actual Dose Concentration (mg/mL)	Age of Animal (PND)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)
11	81	Homosalate (320 mg/kg) + TP (0.4 mg/kg)	Gavage	61.4	59	10:59	1.6	314.87	10:57	1.6	315.58	10:42	1.6	306.62	10:40	1.6	298.97	10:49	1.7	311.95	10:41	1.7	307.27
			Subcutaneous				0.16			0.16			0.16			0.16			0.17			0.17	
11	82		Gavage	61.4	59	11:14	1.4	299.83	11:11	1.4	302.04	10 56	1.5	314.44	10:50	1.5	304.66	10:57	1.5	301.28	10:56	1.6	313.67
			Subcutaneous				0.14			0.14			0.15			0.15			0.15			0.16	
11	83		Gavage	61.4	59	11:29	1.4	315.57	11:25	1.4	303.21	11:12	1.4	298.68	11:00	1.5	314.01	11:04	1.5	302.86	11:09	1.5	298.25
			Subcutaneous				0.14			0.14			0.14			0.15			0.15			0.15	
11	84		Gavage	61.4	59	11:44	1.6	307.96	11:40	1.6	304.24	11:25	1.7	316.02	11:12	1.7	305.74	11:12	1.7	300.12	11:27	1.8	303.46
			Subcutaneous				0.16			0.16			0.17			0.17			0.17			0.18	
11	85		Gavage	61.4	60	11:56	1.5	308.34	11:40	1.5	307.20	11:23	1.5	303.06	11:20	1.5	303.26	11:42	1.6	315.78	11:18	1.6	302.93
			Subcutaneous				0.15			0.15			0.15			0.15			0.16			0.16	
11	86		Gavage	61.4	60	12:08	1.6	316.70	11:54	1.6	316.39	11:37	1.6	303.77	11:28	1.6	302.00	12:00	1.7	312.70	11:26	1.7	303.43
			Subcutaneous				0.16			0.16			0.16			0.16			0.17			0.17	
11	87		Gavage	61.4	60	12:19	1.6	299.88	12:08	1.7	309.92	11:48	1.7	305.92	11:36	1.8	311.15	12:21	1.8	308.28	11:33	1.8	302.38
			Subcutaneous				0.16			0.17			0.17			0.18			0.18			0.18	
11	88		Gavage	61.4	60	12:31	1.5	310.62	12:21	1.5	306.39	12:01	1.5	299.41	11:45	1.6	311.77	12:46	1.6	301.16	11:40	1.6	299.60
			Subcutaneous				0.15			0.15			0.15			0.16			0.16			0.16	

					Day 7 (27 & 28 April 2012)			Day 8 (28 & 29 April 2012)			Day 9 (29 & 30 April 2012)			Day 10 (30 April & 01 May 2012)			Day 11 (01 & 02 May 2012)	
Group No.:	Animal No.:	Test Substance Dose Level	Dose Route	Actual Dose Concentration (mg/mL)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Death	Time From Last Administration
11	81	Homosalate (320 mg/kg) + TP (0.4 mg/kg)	Gavage	61.4	10:47	1.8	315.23	10:41	1.8	310.80	10:39	1.8	301.23	10:46	1.8	300.00	9:22	22:36
			Subcutaneous			0.18			0.18			0.18			0.18			
11	82		Gavage	61.4	10:55	1.6	304.34	10:55	1.6	298.97	10:53	1.7	307.72	10:53	1.7	305.03	10:16	23:23
			Subcutaneous			0.16			0.16			0.17			0.17			
11	83		Gavage	61.4	11:03	1.6	312.07	11:10	1.6	303.49	11:06	1.6	299.97	11:00	1.7	313.64	11:10	24:10
			Subcutaneous			0.16			0.16			0.16			0.17			
11	84		Gavage	61.4	11:10	1.9	311.76	11:23	1.9	301.68	11:19	2.0	310.96	11:08	2.0	306.23	12:09	25:01
			Subcutaneous			0.19			0.19			0.20			0.20			
11	85		Gavage	61.4	11:38	1.7	315.82	11:35	1.7	305.12	11:15	1.7	301.07	10:41	1.7	304.23	9:33	22:52
			Subcutaneous			0.17			0.17			0.17			0.17			
11	86		Gavage	61.4	11:52	1.7	299.34	11:49	1.8	309.06	11:24	1.8	302.21	10:56	1.8	300.08	10:34	23:38
			Subcutaneous			0.17			0.18			0.18			0.18			
11	87		Gavage	61.4	12:08	1.9	310.60	12:05	1.9	300.59	11:32	2.0	308.31	11:11	2.0	310.73	11:31	24:20
			Subcutaneous			0.19			0.19			0.20			0.20			
11	88		Gavage	61.4	12:22	1.7	307.09	12:19	1.8	315.05	11:39	1.8	311.24	11:25	1.8	310.10	12:22	24:57
			Subcutaneous			0.17			0.18			0.18			0.18			

Dose Administration

						Day 1 (21 & 22 April 2012)			Day 2 (22 & 23 April 2012)			Day 3 (23 & 24 April 2012)			Day 4 (24 & 25 April 2012)			Day 5 (25 & 26 April 2012)			Day 6 (26 & 27 April 2012)		
Group No.:	Animal No.:	Test Substance Dose Level	Dose Route	Actual Dose Concentration (mg/mL)	Age of Animal (PND)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)
12	89	Homosalate (1000 mg/kg) + TP (0.4 mg/kg)	Gavage	194	59	11:00	1.5	956.61	10:58	1.5	951.60	10:43	1.5	947.57	10:41	1.5	958.81	10:50	1.5	943.89	10:43	1.6	996.47
			Subcutaneous				0.15			0.15			0.15			0.15			0.15			0.16	
12	90		Gavage	194	59	11:16	1.6	958.32	11:12	1.6	972.74	10:58	1.6	975.18	10:52	1.6	953.32	10:57	1.7	985.95	10:57	1.6	959.21
			Subcutaneous				0.16			0.16			0.16			0.16			0.17			0.16	
12	91		Gavage	194	59	11:30	1.5	949.12	11:26	1.5	958.18	11:13	1.5	942.66	11:02	1.5	939.32	11:06	1.6	973.04	11:10	1.6	971.82
			Subcutaneous				0.15			0.15			0.15			0.15			0.16			0.16	
12	92		Gavage	194	59	11:45	1.4	994.87	11:41	1.3	947.41	11:26	1.3	976.38	11:13	1.2	934.56	11:13	1.3	1000.40	11:29	1.3	968.88
			Subcutaneous				0.14			0.13			0.13			0.12			0.13			0.13	
12	93		Gavage	194	60	11:57	1.5	973.57	11:41	1.5	988.12	11:24	1.5	991.14	11:21	1.4	946.34	11:45	1.4	959.04	11:18	1.4	968.62
			Subcutaneous				0.15			0.15			0.15			0.14			0.14			0.14	
12	94		Gavage	194	60	12:09	1.4	970.00	11:56	1.4	987.28	11:38	1.4	1001.47	11:29	1.4	979.44	12:03	1.4	969.31	11:26	1.5	995.21
			Subcutaneous				0.14			0.14			0.14			0.14			0.14			0.15	
12	95		Gavage	194	60	12:20	1.5	970.97	12:09	1.5	986.78	11:49	1.5	975.20	11:38	1.5	998.28	12:25	1.5	994.87	11:33	1.5	968.71
			Subcutaneous				0.15			0.15			0.15			0.15			0.15			0.15	
12	96		Gavage	194	60	12:32	1.6	989.80	12:22	1.5	945.11	12:02	1.6	984.15	11:47	1.5	946.65	12:47	1.5	954.41	11:41	1.5	945.42
			Subcutaneous				0.16			0.15			0.16			0.15			0.15			0.15	

					Day 7 (27 & 28 April 2012)			Day 8 (28 & 29 April 2012)			Day 9 (29 & 30 April 2012)			Day 10 (30 April & 01 May 2012)			Day 11 (01 & 02 May 2012)	
Group No.:	Animal No.:	Test Substance Dose Level	Dose Route	Actual Dose Concentration (mg/mL)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Actual Dose Received (mg/kg)	Time of Death	Time From Last Administration
12	89	Homosalate (1000 mg/kg) + TP (0.4 mg/kg)	Gavage	194	10:47	1.6	962.18	10:42	1.6	978.56	10:40	1.6	948.66	10:46	1.7	995.47	9:26	22:40
			Subcutaneous			0.16			0.16			0.16			0.17			
12	90		Gavage	194	10:56	1.7	997.88	10:56	1.7	995.17	10:54	1.7	950.71	10:54	1.8	974.33	10:20	23:26
			Subcutaneous			0.17			0.17			0.17			0.18			
12	91		Gavage	194	11:04	1.6	960.69	11:11	1.6	945.48	11:07	1.7	985.36	11:01	1.7	964.05	11:14	24:13
			Subcutaneous			0.16			0.16			0.17			0.17			
12	92		Gavage	194	11:11	1.4	994.14	11:24	1.4	971.74	11:20	1.4	964.15	11:08	1.5	979.14	12:16	25:08
			Subcutaneous			0.14			0.14			0.14			0.15			
12	93		Gavage	194	11:39	1.4	937.85	11:36	1.5	955.35	11:16	1.5	942.97	10:42	1.6	987.91	9:38	22:56
			Subcutaneous			0.14			0.15			0.15			0.16			
12	94		Gavage	194	11:53	1.5	1000.00	11:51	1.5	994.53	11:25	1.5	985.77	10:57	1.5	986.11	10:37	23:40
			Subcutaneous			0.15			0.15			0.15			0.15			
12	95		Gavage	194	12:09	1.6	993.28	12:06	1.6	976.10	11:32	1.6	945.76	11:13	1.6	946.63	11:34	24:21
			Subcutaneous			0.16			0.16			0.16			0.16			
12	96		Gavage	194	12:23	1.6	996.79	12:20	1.6	975.49	11:40	1.6	964.87	11:27	1.6	954.78	12:26	24:59
			Subcutaneous			0.16			0.16			0.16			0.16			

Dose Administration

					Day 1 (21 & 22 April 2012)		Day 2 (22 & 23 April 2012)		Day 3 (23 & 24 April 2012)		Day 4 (24 & 25 April 2012)		Day 5 (25 & 26 April 2012)		Day 6 (26 & 27 April 2012)	
Group No.:	Animal No.:	Test Substance Dose Level	Dose Route	Age of Animal (PND)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Time of Administration (Hour:Minute)	Volume Administered (mL)
13	97	Flutamide (3.0 mg/kg) + TP (0.4 mg/kg)	Gavage	59	11:01	1.4	11:00	1.5	10:44	1.5	10:42	1.6	10:50	1.6	10:44	1.6
			Subcutaneous			0.14		0.15		0.15		0.16		0.16		0.16
13	98		Gavage	59	11:17	1.4	11:13	1.4	10:59	1.4	10:52	1.4	10:58	1.5	10:58	1.5
			Subcutaneous			0.14		0.14		0.14		0.14		0.15		0.15
13	99		Gavage	59	11:31	1.6	11:28	1.7	11:14	1.7	11:02	1.7	11:07	1.7	11:12	1.8
			Subcutaneous			0.16		0.17		0.17		0.17		0.17		0.18
13	100		Gavage	59	11:47	1.6	11:42	1.6	11:27	1.7	11:14	1.7	11:13	1.8	11:30	1.8
			Subcutaneous			0.16		0.16		0.17		0.17		0.18		0.18
13	101		Gavage	60	11:58	1.6	11:42	1.6	11:25	1.6	11:21	1.6	11:47	1.7	11:19	1.7
			Subcutaneous			0.16		0.16		0.16		0.16		0.17		0.17
13	102		Gavage	60	12:10	1.5	11:57	1.5	11:39	1.6	11:30	1.6	12:04	1.6	11:27	1.7
			Subcutaneous			0.15		0.15		0.16		0.16		0.16		0.17
13	103		Gavage	60	12:21	1.6	12:11	1.6	11:51	1.6	11:38	1.6	12:27	1.7	11:34	1.7
			Subcutaneous			0.16		0.16		0.16		0.16		0.17		0.17
13	104		Gavage	60	12:33	1.4	12:23	1.4	12:03	1.5	11:47	1.5	12:49	1.5	11:41	1.5
			Subcutaneous			0.14		0.14		0.15		0.15		0.15		0.15

					Day 7 (27 & 28 April 2012)		Day 8 (28 & 29 April 2012)		Day 9 (29 & 30 April 2012)		Day 10 (30 April & 01 May 2012)		Day 11 (01 & 02 May 2012)	
Group No.:	Animal No.:	Test Substance Dose Level	Dose Route	Age of Animal (PND)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Time of Administration (Hour:Minute)	Volume Administered (mL)	Time of Death	Time From Last Administration
13	97	Flutamide (3.0 mg/kg) + TP (0.4 mg/kg)	Gavage	59	10:48	1.7	10:43	1.7	10:41	1.7	10:47	1.8	9:31	22:44
			Subcutaneous			0.17		0.17		0.17		0.18		
13	98		Gavage	59	10:56	1.5	10:57	1.5	10:55	1.6	10:54	1.6	10:24	23:30
			Subcutaneous			0.15		0.15		0.16		0.16		
13	99		Gavage	59	11:04	1.8	11:12	1.8	11:08	1.9	11:01	1.9	11:19	24:18
			Subcutaneous			0.18		0.18		0.19		0.19		
13	100		Gavage	59	11:11	1.8	11:25	1.9	11:21	1.9	11:09	1.9	12:20	25:11
			Subcutaneous			0.18		0.19		0.19		0.19		
13	101		Gavage	60	11:40	1.7	11:37	1.8	11:16	1.8	10:43	1.8	9:41	22:58
			Subcutaneous			0.17		0.18		0.18		0.18		
13	102		Gavage	60	11:54	1.7	11:52	1.8	11:26	1.8	10:58	1.8	10:40	23:42
			Subcutaneous			0.17		0.18		0.18		0.18		
13	103		Gavage	60	12:10	1.7	12:07	1.8	11:33	1.8	11:13	1.8	11:38	24:25
			Subcutaneous			0.17		0.18		0.18		0.18		
13	104		Gavage	60	12:24	1.5	12:21	1.6	11:40	1.6	11:28	1.6	12:29	25:01
			Subcutaneous			0.15		0.16		0.16		0.16		

Appendix IV:

Clinical Observation Data

Clinical Observations

				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Terminal
Group No.:	**Animal No.:**	**Sex**	**Test Substance Dose Level**	**Clinical Observation**	**Clinical Observation**	**Clinical Observation**	**Clinical Observation**	**Clinical Observation**	**Clinical Observation**	**Clinical Observation**	**Clinical Observation**	**Clinical Observation**	**Clinical Observation**	**Clinical Observation**
1	01	M	Corn Oil Control	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
1	02	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
1	03	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
1	04	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
1	05	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
1	06	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
1	07	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
1	08	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

Group No.:	**Animal No.:**	**Sex**	**Test Substance Dose Level**	**Clinical Observation**	**Clinical Observation**	**Clinical Observation**	**Clinical Observation**	**Clinical Observation**	**Clinical Observation**	**Clinical Observation**	**Clinical Observation**	**Clinical Observation**	**Clinical Observation**	**Clinical Observation**
2	09	M	Padimate-O (320 mg/kg)	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
2	10	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
2	11	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
2	12	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
2	13	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
2	14	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
2	15	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
2	16	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

Group No.:	**Animal No.:**	**Sex**	**Test Substance Dose Level**	**Clinical Observation**	**Clinical Observation**	**Clinical Observation**	**Clinical Observation**	**Clinical Observation**	**Clinical Observation**	**Clinical Observation**	**Clinical Observation**	**Clinical Observation**	**Clinical Observation**	**Clinical Observation**
3	17	M	Padimate-O (1000 mg/kg)	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
3	18	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
3	19	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
3	20	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
3	21	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
3	22	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
3	23	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
3	24	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

Clinical Observations

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation
4	25	M	Homosalate (320 mg/kg)	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Nornal	Normal
4	26	M		Normal	Normal	Normal	Normal	Scab - Left Eye	Scab-left eye	Scab-left/right eye	Scab-left/right eye	Scab-left/right eye	Scab-left/right eye	Scab-left/right eye
4	27	M		Normal	Normal	Normal	Normal	Diarrhea	Normal	Normal	Normal	Normal	Nornal	Normal
4	28	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Nornal	Normal
4	29	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Nornal	Normal
4	30	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Nornal	Normal
4	31	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Nornal	Normal
4	32	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Nornal	Normal

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation
5	33	M	Homosalate (1000 mg/kg)	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
5	34	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
5	35	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
5	36	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
5	37	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
5	38	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Thin(mild)	Normal	Normal
5	39	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Thin(mild)	Normal	Normal	Normal
5	40	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation
6	41	M	Corn Oil Control (0 mg/kg) + TP (0.4 mg/kg)	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
6	42	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
6	43	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
6	44	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
6	45	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
6	46	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
6	47	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
6	48	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

Clinical Observations

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation
7	49	M	Padimate-O (100 mg/kg) + TP (0.4 mg/kg)	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
7	50	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
7	51	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
7	52	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
7	53	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
7	54	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
7	55	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
7	56	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation
8	57	M	Padimate-O (320 mg/kg) + TP (0.4 mg/kg)	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
8	58	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
8	59	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
8	60	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
8	61	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
8	62	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
8	63	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
8	64	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation
9	65	M	Padimate-O (1000 mg/kg) + TP (0.4 mg/kg)	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
9	66	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
9	67	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
9	68	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
9	69	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
9	70	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
9	71	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
9	72	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

Clinical Observations

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation
10	73	M	Homosalate (100 mg/kg) + TP (0.4 mg/kg)	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
10	74	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
10	75	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
10	76	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
10	77	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
10	78	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
10	79	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
10	80	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation
11	81	M	Homosalate (320 mg/kg) + TP (0.4 mg/kg)	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
11	82	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
11	83	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
11	84	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
11	85	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
11	86	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
11	87	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
11	88	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

Clinical Observations

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation
12	89	M	Homosalate (1000 mg/kg) + TP (0.4 mg/kg)	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Nomal	Normal
12	90	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Nomal	Normal
12	91	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Nomal	Normal
12	92	M		Normal	Normal	Thin	Thin-mild	Thin	Thin-Moderate	Thin-Moderate	Thin-Moderate	Thin-Moderate	Normal	Normal
12	93	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Nomal	Normal
12	94	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Nomal	Normal
12	95	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Nomal	Normal
12	96	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Nomal	Normal

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation	Clinical Observation
13	97	M	Flutamide (3.0 mg/kg) + TP (0.4 mg/kg)	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
13	98	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
13	99	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
13	100	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
13	101	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
13	102	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
13	103	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
13	104	M		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

Appendix V:

Body Weight Data

Body Weights

				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Terminal	
Group No.:	**Animal No.:**	**Sex**	**Test Substance Dose Level**	**Body Weight (g)**	**Body Weight (g)**	**Body Weight (g)**	**Body Weight (g)**	**Body Weight (g)**	**Body Weight (g)**	**Body Weight (g)**	**Body Weight (g)**	**Body Weight (g)**	**Body Weight (g)**	**Body Weight (g)**	**Body Weight Gain (g)**
1	01	M	Corn Oil Control	279.2	283.7	289.3	292.4	291.4	291.9	292.9	297.1	303.5	302.7	305.9	26.7
1	02	M		286.8	290.9	289.3	298.4	300.2	303.3	304.1	305.2	309.7	302.6	313.1	26.3
1	03	M		300.3	299.8	302.9	301.4	303.9	304.4	310.0	313.7	318.2	314.9	317.1	16.8
1	04	M		336.8	346.0	353.6	363.5	369.9	376.5	376.1	387.2	396.4	402.0	402.4	65.6
1	05	M		313.9	322.9	327.8	325.2	331.1	337.8	344.0	351.7	350.7	350.2	361.9	48.0
1	06	M		274.1	275.0	277.8	280.5	281.6	289.3	290.4	296.3	295.4	297.9	301.2	27.1
1	07	M		320.7	320.8	327.3	337.8	342.7	347.1	356.8	365.9	368.1	370.9	377.3	56.6
1	08	M		309.5	309.4	318.5	319.1	322.3	330.5	333.8	338.9	343.4	344.8	346.2	36.7
			Mean	**302.7**	**306.1**	**310.8**	**314.8**	**317.9**	**322.6**	**326.0**	**332.0**	**335.7**	**335.8**	**340.6**	**38.0**
			SD	**21.7**	**23.4**	**25.4**	**27.1**	**29.4**	**30.6**	**31.5**	**34.2**	**35.2**	**37.7**	**37.3**	**17.1**
			Count	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**

Group No.:	**Animal No.:**	**Sex**	**Test Substance Dose Level**	**Body Weight (g)**	**Body Weight (g)**	**Body Weight (g)**	**Body Weight (g)**	**Body Weight (g)**	**Body Weight (g)**	**Body Weight (g)**	**Body Weight (g)**	**Body Weight (g)**	**Body Weight (g)**	**Body Weight (g)**	**Body Weight Gain (g)**
2	09	M	Padimate-O (320 mg/kg)	291.7	292.3	295.5	305.5	305.1	307.1	309.9	312.4	314.8	317.1	322.6	30.9
2	10	M		299.1	304.7	309.2	318.9	322.5	315.6	326.9	331.2	340.3	343.8	343.2	44.1
2	11	M		268.5	305.1	275.6	275.9	281.3	281.3	283.4	289.3	296.3	294.1	292.9	24.4
2	12	M		280.1	280.4	282.3	288.7	290.9	293.7	295.5	299.1	303.5	306.2	307.7	27.6
2	13	M		337.3	338.9	342.9	348.6	351.4	351.8	358.7	359.8	363.5	367.8	365.6	28.3
2	14	M		304.6	306.8	310.6	319.2	318.2	323.1	330.0	334.9	337.0	336.5	341.6	37.0
2	15	M		317.0	315.7	322.9	328.0	328.3	320.6	333.1	339.6	338.3	343.2	348.5	31.5
2	16	M		319.4	317.4	329.4	334.0	336.9	339.8	346.6	349.1	349.8	354.3	364.1	44.7
			Mean	**302.2**	**307.7**	**308.6**	**314.9**	**316.8**	**316.6**	**323.0**	**326.9**	**330.4**	**332.9**	**335.8**	**33.6**
			SD	**22.3**	**17.4**	**23.2**	**23.9**	**23.4**	**23.0**	**25.3**	**24.5**	**23.3**	**25.0**	**26.0**	**7.6**
			Count	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**
			% of Control	**99.9**	**100.5**	**99.3**	**100.0**	**99.7**	**98.1**	**99.1**	**98.5**	**98.4**	**99.1**	**98.6**	

Group No.:	**Animal No.:**	**Sex**	**Test Substance Dose Level**	**Body Weight (g)**	**Body Weight (g)**	**Body Weight (g)**	**Body Weight (g)**	**Body Weight (g)**	**Body Weight (g)**	**Body Weight (g)**	**Body Weight (g)**	**Body Weight (g)**	**Body Weight (g)**	**Body Weight (g)**	**Body Weight Gain (g)**
3	17	M	Padimate-O (1000 mg/kg)	291.8	293.4	303.1	305.9	309.1	305.8	312.9	319.0	317.9	313.6	314.0	22.2
3	18	M		268.3	270.7	264.5	270.1	274.8	274.2	276.8	278.2	277.9	276.6	276.2	7.9
3	19	M		305.2	299.3	304.3	303.9	311.8	312.6	312.0	312.5	318.6	308.3	322.2	17.0
3	20	M		335.9	335.2	334.9	356.5	344.2	347.6	358.4	353.5	356.0	351.6	365.3	29.4
3	21	M		317.9	316.9	317.2	327.1	326.1	332.0	322.7	316.8	316.4	318.0	322.8	4.9
3	22	M		316.4	309.8	302.4	295.1	291.3	316.0	313.7	309.6	308.1	308.1	310.0	-6.4
3	23	M		305.9	302.6	305.0	313.0	311.1	315.1	312.5	316.1	309.0	311.5	313.9	8.0
3	24	M		291.6	283.0	288.8	298.3	296.3	288.9	300.6	305.4	306.6	312.3	311.7	20.1
			Mean	**304.1**	**301.4**	**302.5**	**308.7**	**308.1**	**311.5**	**313.7**	**313.9**	**313.8**	**312.5**	**317.0**	**12.9**
			SD	**20.5**	**20.0**	**20.4**	**25.2**	**21.3**	**23.0**	**22.7**	**20.6**	**21.5**	**20.3**	**24.4**	**11.4**
			Count	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**
			% of Control	**100.5**	**98.5**	**97.3**	**98.1**	**96.9**	**96.6**	**96.2**	**94.5**	**93.5**	**93.1**	**93.1**	

Body Weights

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight Gain (g)
4	25	M	Homosalate (320 mg/kg)	309.1	303.7	304.7	309.3	312.4	312.5	318.8	322.2	323.1	325.6	334.1	25.0
4	26	M		315.0	312.0	314.2	322.3	325.4	328.6	333.9	333.9	340.8	345.6	354.6	39.6
4	27	M		282.7	281.3	284.4	288.7	286.3	290.4	292.0	295.1	302.4	300.5	300.5	17.8
4	28	M		287.2	285.1	286.5	290.2	293.7	290.9	299.1	300.9	301.9	308.6	309.4	22.2
4	29	M		308.7	303.1	306.7	308.1	306.7	319.3	315.4	315.6	313.5	313.1	318.5	9.8
4	30	M		337.3	336.2	344.6	345.5	350.2	357.0	360.3	365.1	360.9	370.8	377.9	40.6
4	31	M		275.2	272.7	281.5	283.9	285.4	291.5	292.2	296.8	296.6	304.4	307.0	31.8
4	32	M		320.2	317.3	323.4	324.2	324.2	330.5	332.7	332.3	335.5	334.2	343.3	23.1
			Mean	**304.4**	**301.4**	**305.8**	**309.0**	**310.5**	**315.1**	**318.1**	**320.2**	**321.8**	**325.4**	**330.7**	**26.2**
			SD	**21.1**	**21.0**	**21.7**	**21.2**	**22.4**	**23.8**	**23.8**	**23.7**	**22.5**	**24.0**	**26.8**	**10.6**
			Count	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**
			% of Control	**100.6**	**98.5**	**98.4**	**98.2**	**97.7**	**97.7**	**97.6**	**96.5**	**95.9**	**96.9**	**97.1**	

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight Gain (g)
5	33	M	Homosalate (1000 mg/kg)	325.8	319.8	323.3	322.6	321.1	312.9	322.4	329.3	338.2	337.0	336.4	10.6
5	34	M		292.0	287.0	285.6	278.6	274.1	261.8	270.9	279.8	285.1	285.4	290.1	-1.9
5	35	M		274.4	271.4	268.4	263.8	261.2	258.9	267.2	267.1	274.4	274.5	272.5	-1.9
5	36	M		297.3	301.1	304.2	304.7	309.8	309.7	311.7	314.6	320.3	319.5	324.0	26.7
5	37	M		306.4	303.1	306.4	308.4	301.0	304.6	311.8	312.9	310.6	310.5	309.5	3.1
5	38	M		309.5	303.7	307.5	306.6	308.2	307.9	319.4	316.1	316.5	320.4	319.2	9.7
5	39	M		318.9	319.9	313.6	304.9	297.0	280.5	276.1	271.1	295.0	301.2	311.4	-7.5
5	40	M		299.2	294.5	285.3	277.9	277.5	293.0	302.6	290.1	307.2	310.2	313.6	14.4
			Mean	**302.9**	**300.1**	**299.3**	**295.9**	**293.7**	**291.2**	**297.8**	**297.6**	**305.9**	**307.3**	**309.6**	**6.7**
			SD	**16.1**	**16.2**	**18.0**	**20.0**	**20.7**	**21.7**	**22.7**	**23.5**	**20.5**	**20.0**	**20.0**	**11.0**
			Count	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**
			% of Control	**100.1**	**98.0**	**96.3**	**94.0**	**92.4**	**90.3**	**91.3**	**89.6**	**91.1**	**91.5**	**90.9**	

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight Gain (g)
6	41	M	Corn Oil Control (0 mg/kg) + TP (0.4 mg/kg)	316.4	317.3	322.8	331.8	336.2	340.9	350.3	348.9	353.8	361.3	367.3	50.9
6	42	M		285.8	294.5	301.5	305.1	312.2	313.1	317.8	325.0	334.0	334.8	337.8	52.0
6	43	M		292.2	297.3	302.2	311.9	325.2	331.5	345.5	351.9	356.8	363.3	369.0	76.8
6	44	M		303.8	307.9	312.7	317.3	321.0	329.0	338.3	347.7	352.5	357.6	360.5	56.7
6	45	M		312.2	317.7	325.8	336.1	338.9	350.4	354.4	368.6	376.4	380.7	387.8	75.6
6	46	M		279.3	278.4	283.1	296.0	302.0	309.7	314.2	320.7	325.8	331.6	337.8	58.5
6	47	M		331.7	332.8	340.9	346.5	354.2	358.0	367.7	374.3	374.7	380.4	391.1	59.4
6	48	M		311.4	307.8	319.8	325.0	331.0	338.1	349.2	357.3	361.7	361.5	372.4	61.0
			Mean	**304.1**	**306.7**	**313.6**	**321.2**	**327.6**	**333.8**	**342.2**	**349.3**	**354.5**	**358.9**	**365.5**	**61.4**
			SD	**17.4**	**16.7**	**17.8**	**16.8**	**16.3**	**16.8**	**18.2**	**18.8**	**17.7**	**18.1**	**19.9**	**9.8**
			Count	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**

Body Weights

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight Gain (g)
7	49	M	Padimate-O (100 mg/kg) + TP (0.4 mg/kg)	293.9	295.9	301.3	308.8	313.8	318.9	326.6	328.4	335.6	341.6	345.5	51.6
7	50	M		320.4	327.3	331.7	338.3	347.1	351.4	359.9	374.4	376.8	378.0	382.4	62.0
7	51	M		289.6	294.8	306.2	313.8	321.7	334.1	338.7	347.3	354.7	355.6	365.3	75.7
7	52	M		311.6	316.9	320.9	327.4	334.2	343.6	350.3	358.5	370.5	371.1	373.7	62.1
7	53	M		319.7	311.7	322.8	328.0	336.5	333.8	325.8	358.5	360.3	365.2	371.1	51.4
7	54	M		276.1	279.3	286.4	288.4	297.2	286.8	279.9	308.0	316.7	313.6	324.1	48.0
7	55	M		292.9	298.2	298.8	307.0	316.4	323.4	327.8	332.2	337.0	345.0	350.6	57.7
7	56	M		327.0	327.1	338.2	345.6	350.5	365.3	377.6	382.9	387.1	395.1	401.3	74.3
			Mean	**303.9**	**306.4**	**313.3**	**319.7**	**327.2**	**332.2**	**335.8**	**348.8**	**354.8**	**358.2**	**364.3**	**60.4**
			SD	**18.2**	**17.1**	**17.9**	**18.7**	**18.1**	**23.7**	**29.1**	**25.0**	**23.7**	**25.1**	**23.9**	**10.4**
			Count	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**
			% of Control	**99.9**	**99.9**	**99.9**	**99.5**	**99.9**	**99.5**	**98.1**	**99.8**	**100.1**	**99.8**	**99.7**	

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight Gain (g)
8	57	M	Padimate-O (320 mg/kg) + TP (0.4 mg/kg)	318.0	318.8	321.3	328.8	330.1	335.0	342.9	344.8	345.4	350.7	359.0	41.0
8	58	M		305.4	305.1	308.5	311.9	317.7	318.0	324.6	324.5	329.7	330.6	334.3	28.9
8	59	M		292.6	295.7	302.4	307.9	316.7	321.4	328.3	334.0	339.4	348.4	345.7	53.1
8	60	M		276.1	279.8	287.6	291.2	296.4	300.3	303.6	312.3	314.5	317.6	323.6	47.5
8	61	M		327.0	325.7	338.1	345.8	348.4	356.9	362.3	368.8	374.3	381.1	383.8	56.8
8	62	M		323.4	320.9	328.4	339.7	347.6	350.9	362.8	360.8	375.2	374.2	387.1	63.7
8	63	M		278.8	281.7	290.7	297.5	306.7	307.6	316.5	321.0	325.6	326.8	332.8	54.0
8	64	M		289.9	287.5	299.2	302.7	314.6	319.1	324.4	332.0	336.0	335.3	345.8	55.9
			Mean	**301.4**	**301.9**	**309.5**	**315.7**	**322.3**	**326.2**	**333.2**	**337.3**	**342.5**	**345.6**	**351.5**	**50.1**
			SD	**20.0**	**18.4**	**18.1**	**20.1**	**18.5**	**20.0**	**21.2**	**19.6**	**22.0**	**22.6**	**23.5**	**10.9**
			Count	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**
			% of Control	**99.1**	**98.4**	**98.7**	**98.3**	**98.4**	**97.7**	**97.4**	**96.6**	**96.6**	**96.3**	**96.2**	

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight Gain (g)
9	65	M	Padimate-O (1000 mg/kg) + TP (0.4 mg/kg)	285.0	285.0	288.6	290.8	289.3	283.9	303.7	310.5	297.0	290.3	292.4	7.4
9	66	M		273.0	273.5	269.3	281.2	275.3	264.8	279.2	279.1	275.3	269.7	280.9	7.9
9	67	M		323.5	323.8	329.3	334.7	332.4	330.8	344.3	349.7	347.9	349.3	357.8	34.3
9	68	M		309.8	310.6	315.8	317.2	328.3	333.7	335.9	341.5	340.5	330.9	319.1	9.3
9	69	M		305.7	300.0	306.6	304.3	315.8	314.3	319.1	325.6	320.6	320.6	323.6	17.9
9	70	M		324.3	333.1	328.8	332.9	337.2	344.5	347.6	341.3	339.2	348.0	351.3	27.0
9	71	M		300.4	302.1	301.4	302.8	294.6	293.1	296.0	304.2	299.1	300.1	306.5	6.1
9	72	M		312.8	314.7	315.7	306.4	299.6	306.5	304.4	298.5	289.1	281.5	387.5	74.7
			Mean	**304.3**	**305.4**	**306.9**	**308.8**	**309.1**	**309.0**	**316.3**	**318.8**	**313.6**	**311.3**	**327.4**	**23.1**
			SD	**17.9**	**19.6**	**20.4**	**18.8**	**22.6**	**27.3**	**24.6**	**24.8**	**27.1**	**30.3**	**35.9**	**23.3**
			Count	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**
			% of Control	**100.1**	**99.6**	**97.9**	**96.1**	**94.3**	**92.5**	**92.4**	**91.3**	**88.5**	**86.7**	**89.6**	

Body Weights

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight Gain (g)
10	73	M	Homosalate (100 mg/kg) + TP (0.4 mg/kg)	272.1	278.4	283.7	293.3	294.3	300.0	300.9	310.7	316.6	320.8	325.7	53.6
10	74	M		292.4	296.8	302.6	311.2	319.1	321.8	330.4	335.4	343.8	345.6	352.7	60.3
10	75	M		325.0	329.9	342.6	349.5	358.3	362.1	368.6	380.0	385.7	395.6	398.1	73.1
10	76	M		322.5	330.6	333.6	343.5	357.6	362.7	368.7	379.8	387.8	394.5	402.2	79.7
10	77	M		312.7	316.3	322.8	329.6	336.8	344.7	354.4	360.5	366.9	370.1	377.8	65.1
10	78	M		299.0	303.5	314.0	318.9	323.4	330.6	334.3	342.2	344.1	356.3	356.1	57.1
10	79	M		292.8	300.7	313.2	322.3	336.0	342.7	356.7	366.2	371.5	382.7	393.1	100.3
10	80	M		319.4	319.7	338.5	342.0	345.4	354.0	361.8	373.6	375.6	379.1	388.3	68.9
			Mean	**304.5**	**309.5**	**318.9**	**326.3**	**333.9**	**339.8**	**347.0**	**356.1**	**361.5**	**368.1**	**374.3**	**69.8**
			SD	**18.5**	**17.9**	**19.8**	**18.8**	**21.4**	**21.5**	**23.5**	**24.6**	**24.6**	**25.8**	**26.9**	**15.0**
			Count	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**
			% of Control	**100.1**	**100.9**	**101.7**	**101.6**	**101.9**	**101.8**	**101.4**	**101.9**	**102.0**	**102.6**	**102.4**	

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight Gain (g)
11	81	M	Homosalate (320 mg/kg) + TP (0.4 mg/kg)	312.0	311.3	320.4	328.6	334.6	339.7	350.6	355.6	366.9	368.4	375.6	63.6
11	82	M		286.7	284.6	292.9	302.3	305.7	313.2	322.8	328.6	339.2	342.2	344.1	57.4
11	83	M		272.4	283.5	287.8	293.3	304.1	308.8	314.8	323.7	327.5	332.8	340.8	68.4
11	84	M		319.0	322.9	330.3	341.4	347.8	364.2	374.2	386.7	394.9	401.0	407.4	88.4
11	85	M		298.7	299.8	303.9	303.7	311.1	324.3	330.5	342.1	346.7	343.1	349.4	50.7
11	86	M		310.2	310.5	323.4	325.3	333.8	344.0	348.7	357.6	365.7	368.3	376.9	66.7
11	87	M		327.6	336.8	341.2	355.2	358.5	365.5	375.6	388.1	398.3	395.2	408.9	81.3
11	88	M		296.5	300.6	307.6	315.1	326.2	327.9	339.9	350.8	355.1	356.4	366.9	70.4
			Mean	**302.9**	**306.3**	**313.4**	**320.6**	**327.7**	**336.0**	**344.6**	**354.2**	**361.8**	**363.4**	**371.3**	**68.4**
			SD	**18.0**	**18.2**	**18.5**	**21.1**	**19.8**	**21.4**	**22.3**	**23.8**	**25.2**	**24.8**	**26.6**	**12.1**
			Count	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**
			% of Control	**99.6**	**99.8**	**99.9**	**99.8**	**100.0**	**100.6**	**100.7**	**101.4**	**102.1**	**101.3**	**101.6**	

Body Weights

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight Gain (g)
12	89	M	Homosalate (1000 mg/kg) + TP (0.4 mg/kg)	304.2	305.8	307.1	303.5	308.3	311.5	322.6	317.2	327.2	331.3	336.3	32.1
12	90	M		323.9	319.1	318.3	325.6	334.5	323.6	330.5	331.4	346.9	358.4	356.3	32.4
12	91	M		306.6	303.7	308.7	309.8	319.0	319.4	323.1	328.3	334.7	342.1	342.7	36.1
12	92	M		273.0	266.2	258.3	249.1	252.1	260.3	273.2	279.5	281.7	297.2	290.0	17.0
12	93	M		298.9	294.5	293.6	287.0	283.2	280.4	289.6	304.6	308.6	314.2	315.1	16.2
12	94	M		280.0	275.1	271.2	277.3	280.2	292.4	291.0	292.6	295.2	295.1	303.7	23.7
12	95	M		299.7	294.9	298.4	291.5	292.5	300.4	312.5	318.0	328.2	327.9	329.2	29.5
12	96	M		313.6	307.9	315.4	307.4	304.9	307.8	311.4	318.2	321.7	325.1	339.9	26.3
			Mean	**300.0**	**295.9**	**296.4**	**293.9**	**296.8**	**299.5**	**306.7**	**311.2**	**318.0**	**323.9**	**326.7**	**26.7**
			SD	**16.7**	**17.6**	**21.4**	**23.5**	**25.6**	**21.2**	**20.0**	**17.8**	**21.5**	**21.5**	**22.1**	**7.3**
			Count	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**
			% of Control	**98.6**	**96.5**	**94.5**	**91.5**	**90.6**	**89.7**	**89.6**	**89.1**	**89.7**	**90.3**	**89.4**	

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight (g)	Body Weight Gain (g)
13	97	M	Flutamide (3.0 mg/kg) + TP (0.4 mg/kg)	289.9	295.7	306.5	310.8	321.5	329.6	333.0	340.7	346.2	354.5	354.4	64.5
13	98	M		277.6	281.4	284.6	285.9	292.4	297.6	302.3	305.1	312.9	317.9	322.6	45.0
13	99	M		324.1	332.6	335.3	347.8	347.8	351.8	360.5	369.2	375.5	381.7	393.3	69.2
13	100	M		324.0	328.1	334.7	343.7	355.0	354.9	362.6	373.3	382.1	384.0	394.8	70.8
13	101	M		312.3	315.7	316.0	326.8	333.0	336.0	342.5	353.9	359.4	360.5	367.2	54.9
13	102	M		301.9	304.3	311.9	319.5	323.9	335.0	346.3	355.0	364.6	367.3	368.6	66.7
13	103	M		310.0	314.6	322.8	329.9	333.0	338.1	349.5	355.7	360.0	368.5	368.7	58.7
13	104	M		281.9	282.7	290.5	292.6	296.5	300.9	306.6	310.4	315.9	314.4	323.0	41.1
			Mean	**302.7**	**306.9**	**312.8**	**319.6**	**325.4**	**330.5**	**337.9**	**345.4**	**352.1**	**356.1**	**361.6**	**58.9**
			SD	**18.1**	**19.3**	**18.6**	**22.3**	**22.2**	**21.1**	**22.7**	**25.3**	**25.6**	**26.5**	**27.5**	**11.1**
			Count	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**
			% of Control	**99.5**	**100.1**	**99.7**	**99.5**	**99.3**	**99.0**	**98.8**	**98.9**	**99.3**	**99.2**	**98.9**	

Appendix VI:

Tissue Weight Data

Tissue Weights

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Glans Penis Weight (g)	Ventral Prostate Weight (g)	Seminal Vesicle Weight (g)	Cowper's Gland Weight (g)	LABC Weight (g)
1	01	M	Corn Oil Control	0.0652	0.0199	0.0832	0.0039	0.1661
1	02	M		0.0671	0.0230	0.0843	0.0105	0.1692
1	03	M		0.0694	0.0235	0.0668	0.0128	0.1299
1	04	M		0.0617	0.0222	0.0636	0.0087	0.1854
1	05	M		0.0688	0.0206	0.0620	0.0081	0.1347
1	06	M		0.0575	0.0161	0.0582	0.0054	0.0867
1	07	M		0.0651	0.0179	0.0637	0.0067	0.1254
1	08	M		0.0706	0.0257	0.0829	0.0088	0.1413
			Mean	**0.0657**	**0.0211**	**0.0706**	**0.0081**	**0.1423**
			SD	**0.0044**	**0.0031**	**0.0109**	**0.0028**	**0.0310**
			CV	**6.6**	**14.8**	**15.5**	**34.7**	**21.8**

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Glans Penis Weight (g)	Ventral Prostate Weight (g)	Seminal Vesicle Weight (g)	Cowper's Gland Weight (g)	LABC Weight (g)
2	09	M	Padimate-O (320 mg/kg)	0.0598	0.0184	0.0604	0.0077	0.1490
2	10	M		0.0713	0.0223	0.0614	0.0086	0.1676
2	11	M		0.0644	0.0240	0.0737	0.0092	0.1594
2	12	M		0.0649	0.0220	0.0743	0.0103	0.1404
2	13	M		0.0620	0.0231	0.0678	0.0097	0.1825
2	14	M		0.0588	0.0180	0.0778	0.0088	0.1883
2	15	M		0.0595	0.0263	0.0639	0.0097	0.1507
2	16	M		0.0606	0.0193	0.0376	0.0086	0.1534
			Mean	**0.0627**	**0.0217**	**0.0646**	**0.0091**	**0.1614**
			SD	**0.0041**	**0.0029**	**0.0126**	**0.0008**	**0.0168**
			CV	**6.6**	**13.4**	**19.6**	**9.0**	**10.4**

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Glans Penis Weight (g)	Ventral Prostate Weight (g)	Seminal Vesicle Weight (g)	Cowper's Gland Weight (g)	LABC Weight (g)
3	17	M	Padimate-O (1000 mg/kg)	0.0599	0.0189	0.0449	0.0063	0.1142
3	18	M		0.0727	0.0187	0.0728	0.0068	0.1191
3	19	M		0.0701	0.0211	0.0664	0.0054	0.1070
3	20	M		0.0614	0.0275	0.0663	0.0077	0.1040
3	21	M		0.0478	0.0118	0.0523	0.0070	0.1174
3	22	M		0.0481	0.0282	0.0601	0.0087	0.1167
3	23	M		0.0548	0.0233	0.0498	0.0032	0.1101
3	24	M		0.0716	0.0287	0.0634	0.0117	0.1530
			Mean	**0.0608**	**0.0223**	**0.0595**	**0.0071**	**0.1177**
			SD	**0.0101**	**0.0059**	**0.0096**	**0.0025**	**0.0152**
			CV	**16.6**	**26.3**	**16.1**	**34.9**	**12.9**

Tissue Weights

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Glans Penis Weight (g)	Ventral Prostate Weight (g)	Seminal Vesicle Weight (g)	Cowper's Gland Weight (g)	LABC Weight (g)
4	25	M	Homosalate (320 mg/kg)	0.0655	0.0226	0.0640	0.0050	0.1666
4	26	M		0.0642	0.0277	0.0645	0.0063	0.1297
4	27	M		0.0633	0.0188	0.0781	0.0081	0.1360
4	28	M		0.0595	0.0182	0.0692	0.0105	0.1361
4	29	M		0.0576	0.0176	0.0618	0.0075	0.1533
4	30	M		0.0679	0.0220	0.0612	0.0067	0.1947
4	31	M		0.0599	0.0164	0.0528	0.0080	0.1192
4	32	M		0.0627	0.0166	0.0608	0.0072	0.1486
			Mean	**0.0626**	**0.0200**	**0.0641**	**0.0074**	**0.1480**
			SD	**0.0034**	**0.0039**	**0.0073**	**0.0016**	**0.0239**
			CV	**5.5**	**19.4**	**11.4**	**21.6**	**16.2**

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Glans Penis Weight (g)	Ventral Prostate Weight (g)	Seminal Vesicle Weight (g)	Cowper's Gland Weight (g)	LABC Weight (g)
5	33	M	Homosalate (1000 mg/kg)	0.0580	0.0182	0.0734	0.0052	0.1697
5	34	M		0.0671	0.0266	0.0880	0.0100	0.1750
5	35	M		0.0652	0.0178	0.0355	0.0067	0.1221
5	36	M		0.0572	0.0199	0.0492	0.0068	0.1121
5	37	M		0.0605	0.0225	0.0873	0.0085	0.1441
5	38	M		0.0566	0.0155	0.0592	0.0089	0.1253
5	39	M		0.0638	0.0230	0.0631	0.0100	0.1545
5	40	M		0.0686	0.0253	0.0809	0.0113	0.1216
			Mean	**0.0621**	**0.0211**	**0.0671**	**0.0084**	**0.1406**
			SD	**0.0047**	**0.0039**	**0.0188**	**0.0021**	**0.0239**
			CV	**7.5**	**18.4**	**28.0**	**24.4**	**17.0**

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Glans Penis Weight (g)	Ventral Prostate Weight (g)	Seminal Vesicle Weight (g)	Cowper's Gland Weight (g)	LABC Weight (g)
6	41	M	Corn Oil Control (0 mg/kg) + TP (0.4 mg/kg)	0.0954	0.2070	0.6977	0.0375	0.4681
6	42	M		0.1023	0.2028	0.7064	0.0532	0.3754
6	43	M		0.0972	0.2094	0.8713	0.0426	0.4681
6	44	M		0.1060	0.2311	0.7367	0.0383	0.3253
6	45	M		0.1117	0.2273	0.7613	0.0398	0.4574
6	46	M		0.1052	0.1922	0.7213	0.0535	0.3356
6	47	M		0.0979	0.1724	0.8560	0.0552	0.4025
6	48	M		0.1051	0.2145	0.8159	0.0442	0.4150
			Mean	**0.1026**	**0.2071**	**0.7708**	**0.0455**	**0.4059**
			SD	**0.0055**	**0.0189**	**0.0682**	**0.0073**	**0.0571**
			CV	**5.3**	**9.1**	**8.9**	**16.1**	**14.1**

Tissue Weights

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Glans Penis Weight (g)	Ventral Prostate Weight (g)	Seminal Vesicle Weight (g)	Cowper's Gland Weight (g)	LABC Weight (g)
7	49	M	Padimate-O (100 mg/kg) + TP (0.4 mg/kg)	0.1006	0.1310	1.1371	0.0524	0.4645
7	50	M		0.1153	0.2035	0.9065	0.0514	0.4620
7	51	M		0.1088	0.2327	0.9096	0.0434	0.3423
7	52	M		0.1063	0.1888	0.9502	0.0559	0.3373
7	53	M		0.1048	0.1887	0.7809	0.0552	0.4350
7	54	M		0.0951	0.0956	0.6338	0.0501	0.4152
7	55	M		0.0937	0.1679	0.9101	0.0476	0.3856
7	56	M		0.0987	0.2398	0.7531	0.0448	0.4135
			Mean	**0.1029**	**0.1810**	**0.8727**	**0.0501**	**0.4069**
			SD	**0.0073**	**0.0489**	**0.1510**	**0.0046**	**0.0489**
			CV	**7.1**	**27.0**	**17.3**	**9.1**	**12.0**

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Glans Penis Weight (g)	Ventral Prostate Weight (g)	Seminal Vesicle Weight (g)	Cowper's Gland Weight (g)	LABC Weight (g)
8	57	M	Padimate-O (320 mg/kg) + TP (0.4 mg/kg)	0.1006	0.2090	0.7540	0.0647	0.4389
8	58	M		0.1085	0.2427	1.1344	0.0644	0.4582
8	59	M		0.1058	0.1695	0.7537	0.0516	0.4672
8	60	M		0.1027	0.2734	0.9555	0.0464	0.2992
8	61	M		0.1049	0.1964	0.9146	0.0473	0.4115
8	62	M		0.0972	0.1265	0.7607	0.0572	0.3930
8	63	M		0.1027	0.2173	1.0329	0.0370	0.4004
8	64	M		0.0990	0.2122	0.7426	0.0643	0.3665
			Mean	**0.1027**	**0.2059**	**0.8811**	**0.0541**	**0.4044**
			SD	**0.0037**	**0.0444**	**0.1512**	**0.0103**	**0.0544**
			CV	**3.6**	**21.6**	**17.2**	**19.0**	**13.5**

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Glans Penis Weight (g)	Ventral Prostate Weight (g)	Seminal Vesicle Weight (g)	Cowper's Gland Weight (g)	LABC Weight (g)
9	65	M	Padimate-O (1000 mg/kg) + TP (0.4 mg/kg)	0.0969	0.1334	0.7952	0.0408	0.3135
9	66	M		0.1003	0.1236	0.7767	0.0413	0.2992
9	67	M		0.0953	0.1342	0.7422	0.0454	0.3298
9	68	M		0.1064	0.1495	0.6631	0.0518	0.2511
9	69	M		0.0852	0.1391	0.6906	0.0357	0.2726
9	70	M		0.0900	0.1534	0.6073	0.0323	0.2343
9	71	M		0.0935	0.1390	0.8030	0.0340	0.3056
9	72	M		0.0898	0.0826	0.4680	0.0390	0.2146
			Mean	**0.0947**	**0.1319**	**0.6933**	**0.0400**	**0.2776**
			SD	**0.0067**	**0.0220**	**0.1139**	**0.0064**	**0.0411**
			CV	**7.0**	**16.7**	**16.4**	**16.0**	**14.8**

Tissue Weights

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Glans Penis Weight (g)	Ventral Prostate Weight (g)	Seminal Vesicle Weight (g)	Cowper's Gland Weight (g)	LABC Weight (g)
10	73	M	Homosalate (100 mg/kg) + TP (0.4 mg/kg)	0.1003	0.2230	0.9409	0.0464	0.3979
10	74	M		0.1155	0.2224	0.7749	0.0443	0.3999
10	75	M		0.1083	0.2508	0.8473	0.0482	0.4482
10	76	M		0.1050	0.2917	0.8440	0.0284	0.3322
10	77	M		0.1098	0.1922	0.8311	0.0430	0.4596
10	78	M		0.1174	0.2149	0.8405	0.0567	0.3711
10	79	M		0.1096	0.1944	0.6784	0.0449	0.4124
10	80	M		0.1016	0.2041	0.7406	0.0464	0.4698
			Mean	**0.1084**	**0.2242**	**0.8122**	**0.0448**	**0.4114**
			SD	**0.0061**	**0.0331**	**0.0796**	**0.0078**	**0.0468**
			CV	**5.6**	**14.8**	**9.8**	**17.5**	**11.4**

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Glans Penis Weight (g)	Ventral Prostate Weight (g)	Seminal Vesicle Weight (g)	Cowper's Gland Weight (g)	LABC Weight (g)
11	81	M	Homosalate (320 mg/kg) + TP (0.4 mg/kg)	0.1002	0.1842	0.8172	0.0404	0.4424
11	82	M		0.1048	0.1977	0.7867	0.0491	0.3256
11	83	M		0.1022	0.1787	0.9260	0.0474	0.4118
11	84	M		0.1070	0.2730	0.9759	0.0628	0.4505
11	85	M		0.1038	0.1809	0.8143	0.0426	0.3871
11	86	M		0.1048	0.2657	0.7870	0.0655	0.3912
11	87	M		0.1017	0.1678	0.7739	0.0333	0.3182
11	88	M		0.1104	0.2604	0.8349	0.0395	0.3342
			Mean	**0.1044**	**0.2136**	**0.8395**	**0.0476**	**0.3826**
			SD	**0.0032**	**0.0446**	**0.0728**	**0.0113**	**0.0519**
			CV	**3.1**	**20.9**	**8.7**	**23.8**	**13.6**

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Glans Penis Weight (g)	Ventral Prostate Weight (g)	Seminal Vesicle Weight (g)	Cowper's Gland Weight (g)	LABC Weight (g)
12	89	M	Homosalate (1000 mg/kg) + TP (0.4 mg/kg)	0.1004	0.1440	0.6354	0.0369	0.2350
12	90	M		0.1038	0.1530	0.9685	0.0528	0.3821
12	91	M		0.0978	0.1824	0.6688	0.0426	0.2988
12	92	M		0.0956	0.1861	0.6492	0.0513	0.2477
12	93	M		0.1119	0.2471	0.8100	0.0406	0.3559
12	94	M		0.1049	0.1715	0.6412	0.0462	0.3267
12	95	M		0.1053	0.1791	0.8712	0.0587	0.3531
12	96	M		0.1028	0.1999	0.6305	0.0460	0.2733
			Mean	**0.1028**	**0.1829**	**0.7344**	**0.0469**	**0.3091**
			SD	**0.0050**	**0.0316**	**0.1310**	**0.0071**	**0.0540**
			CV	**4.9**	**17.3**	**17.8**	**15.1**	**17.5**

Group No.:	Animal No.:	Sex	Test Substance Dose Level	Glans Penis Weight (g)	Ventral Prostate Weight (g)	Seminal Vesicle Weight (g)	Cowper's Gland Weight (g)	LABC Weight (g)
13	97	M	Flutamide (3.0 mg/kg) + TP (0.4 mg/kg)	0.0773	0.0602	0.1749	0.0251	0.2222
13	98	M		0.0796	0.0476	0.1178	0.0179	0.2329
13	99	M		0.0666	0.0564	0.0873	0.0133	0.1644
13	100	M		0.0786	0.0572	0.2121	0.0220	0.2448
13	101	M		0.0782	0.0330	0.0952	0.0040	0.1134
13	102	M		0.0840	0.0642	0.2482	0.0294	0.2272
13	103	M		0.0855	0.0820	0.1969	0.0161	0.2430
13	104	M		0.0787	0.0424	0.1949	0.0159	0.1712
			Mean	**0.0786**	**0.0554**	**0.1659**	**0.0180**	**0.2024**
			SD	**0.0057**	**0.0149**	**0.0589**	**0.0078**	**0.0474**
			CV	**7.2**	**26.8**	**35.5**	**43.3**	**23.4**

Appendix VII:

Study Protocol

Study Title
The Hershberger Bioassay for Padimate-O and Homosalate

ILS Project-Study Numbers
N135-249

Performing Laboratory
Integrated Laboratory Systems, Inc.
601 Keystone Park Drive, Suite 100
Durham, NC 27713

Sponsor
National Toxicology Program
National Institute of Environmental Health Sciences
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Research Triangle Park, NC 27709

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Study Protocol Approval

[redacted]

Chief, Toxicology Branch
National Toxicology Program, NIEHS

4/10/12
Date

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Contract Office Technical Representative
National Toxicology Program, NIEHS

4/12/12
Date

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Study Director
Investigative Toxicology Division
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Investigative Toxicology Division
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4/12/12
Date

TABLE OF CONTENTS

INTRODUCTION

1.1 Background

The Endocrine Disruptor Screening Program (EDSP) reflects a two-tiered approach to implement the statutory testing requirements of FFDCA section 408(p) (21 U.S.C. 346a). EPA will use the data collected under the EDSP, along with other information to determine if a pesticide chemical, or other substances, may pose a risk to human health or the environment due to disruption of the endocrine system.

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 the potential of each test substance activity will be made on a weight-of-evidence basis taking into account data from the Tier 1 assays and other scientifically relevant information available. The fact that a substance may interact with a hormone system, however, does not mean that when the substance is used it will cause adverse effects in humans or ecological systems. The Hershberger Bioassay (OPPTS 890.1400) is used as an *in vivo* screening assay for androgen agonists, androgen antagonists, and 5α-reductase inhibitors and is one of four in vivo mammalian assays in the EDSP Tier 1 battery of assays.

1.2 Purpose

The purpose of this assay is to screen two test substances selected by the National Toxicology Program for androgen agonist/antagonist activity and 5α-reductase inhibition properties using a castrated rat model (OPPTS 890.1400).

1.3 Regulatory Compliance

This study will be conducted in accordance with Good Laboratory Practice regulations as promulgated by the United States Environmental Protection Agency's (U.S. EPA) Good Laboratory Practice (GLP) Regulations (40 CFR Part 160), the Endocrine Disruptor Screening Program Test Guideline OPPTS 890.1400: Hershberger Bioassay (U.S. EPA), OECD Guideline 441 Hershberger Bioassay in Rats: A Short-term Screening Assay for (Anti) Androgenic Properties (adopted 7 September 2009) and ILS SOP's. 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.

Flutamide and testosterone propionate will not be analyzed as stated in 40 CFR 160.113(a)(1) of the U.S. EPA GLP requirements, a positive response in the test system following administration will be evident following statistical analysis of the tissue weights.

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.

1.4 Sponsor

National Institutes of Environmental Health Science
P.O. Box 12233
Research Triangle Park, NC 27709

[redacted]
Contract Office Technical Representative
NTP, NIEHS

National Toxicology Program Investigator

[redacted]
Telephone No.:
Facsimile No.:
E-mail:

1.5 Testing Facility

Integrated Laboratory Systems, Inc. (ILS)

Shipping Address: 601 Keystone Park Drive, Suite 100
Durham, NC 27713

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

Study Director

[redacted]
Telephone No.:
Facsimile No.:
E-mail:

1.6 Study Dates

Animal Arrival Dates:	12 April 2012
Experimental Start Date:	21 April 2012
Experimental Termination Date:	02 May 2012

TEST SUBSTANCES, REFERENCE SUBSTANCES, VEHICLE

2.1 Test Substance: 2-Ethylhexyl-P-Dimethyl-Aminobenzoate (Padimate-O)

CAS No.	21245-02-3
Source:	Sigma-Aldrich Company
Lot/Batch No.:	MKBF0590V
ILS Repository No.:	12-26

Formula:	$C_{17}H_{27}NO_2$
Description:	Colorless liquid
Purity:	98.3%
Dose Formulation:	Padimate-0 will be prepared at ILS in corn oil once at dose levels of 20, 64, and 200 mg/mL and dispensed into vials to be used daily during the study. Dose formulations will be stirred for at least 30 minutes prior to dose administration, and continuously during dose administration.
Storage	
Test Substance:	Ambient temperature
Dose Formulation:	Ambient temperature protected from light
Stability	
Dose Formulation:	Padimate-O in corn oil stored at ambient temperature was shown to be stable for 43 days (Blake, 2011).

2.2 Test Substance: 3,3,5-Trimethylclohexyl Salicylate (Homosalate)

CAS No.	118-56-9
Source:	Spectrum Laboratory Products Inc
Lot/Batch No.:	YT0976
ILS Repository No.:	12-24
Formula:	$C_{16}H_{22}O_3$
Description:	Colorless to light yellow liquid
Purity:	99.88%
Dose Formulation:	Homosalate will be prepared at ILS in corn oil once at dose levels of 20, 64, and 200 mg/mL and dispensed into vials to be used daily during the study. Dose formulations will be stirred for at least 30 minutes prior to dose administration, and continuously during dose administration.

Storage:

Test Substance:	Ambient temperature
Dose Formulation:	Ambient temperature protected from light

Stability:

Dose Formulation:	Homosalate in corn oil stored at ambient temperature was shown to be stable for 42 days (Blake, 2012).

2.3 Reference Substance: Testosterone Propionate (Androgen agonist)

CAS No.	57-85-2
Source:	Sigma-Aldrich Company
Lot/Batch No.:	051M1803V
ILS Repository No.:	12-29
Formula:	$C_{22}H_{32}O_3$
Description:	White to off-white powder
Purity:	100%
Dose Formulation:	Testosterone propionate will be prepared at ILS in corn oil once at a dose level of 0.08 mg/mL and dispensed into vials to be used daily during the study. Dose formulations will be stirred for at least 30 minutes prior to dose administration, and continuously during dose administration.

Storage:

Reference Substance:	Room temperature, protected from light
Dose Formulation:	Between 1-10°C (Smith, 2011)

2.4 Reference Substance: Flutamide (Androgen antagonist)

CAS No.	13311-84-7
Source:	Sigma-Aldrich Company
Lot/Batch No.:	021M1406V

ILS Repository No.:	11-77
Formula:	$C_{11}H_{11}F_3N_2O_3$
Description:	Yellow powder
Purity:	>99%
Dose Formulation:	Flutamide will be prepared at ILS in corn oil once at a dose level of 0.6 mg/mL and dispensed into vials to be used daily during the study. Dose formulations will be stirred for at least 30 minutes prior to dose administration, and continuously during dose administration.
Storage:	
Reference Substance:	Room temperature, protected from light
Dose Formulation:	Between 1-10°C
Stability:	
Dose Formulation:	Flutamide in corn oil stored between 1-10°C was demonstrated to be stable for 42 days (Graves, 2001).

2.5 Vehicle: Corn Oil

CAS No.:	8001-30-7
Source:	MP Biomedicals, LLC
Lot/Batch No.:	7862K
ILS Repository No.:	11-121
Formula:	$C_{27}H_{50}O_6$
Description:	Yellow oil
Storage:	
Vehicle:	Room temperature

2.6 Archive Samples

A ~1 g sample of the neat test substances, a ~1 mg sample reference substances (Flutamide Lot 021M1406V and testosterone propionate Lot 051M1803V), and 1 mL of the vehicle and dose formulations will be stored at room temperature until acceptance of the final report; after acceptance of the report by the sponsor, dose formulation samples will be discarded.

2.7 Dose Formulation Analysis

Dose formulations will be prepared at ILS and analyzed at Research Triangle Institute (RTI) International 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 10% of the target concentration. Homogeneity results will be acceptable if the coefficient of variation is ≤5%. Samples will be shipped overnight to the following addresses for analysis prior to administration:

Research Triangle Institute, International
Attn: [redacted]
Materials Handling Facility
East Institute Drive
Research Triangle Park, NC 27709

EXPERIMENTAL DESIGN

One hundred and four castrated male Sprague-Dawley rats will be allocated to one of thirteen designated dose groups. To evaluate the test substance for agonist properties, animals will be administered one of two dose levels, or the vehicle control. To evaluate for antagonist properties animals will be administered one of three dose levels of the test substance and co-administered testosterone propionate (0.4 mg/kg, agonist). A vehicle control group will be administered corn oil and testosterone propionate (0.4 mg/kg) and serve as the positive control for the agonist group and the negative control for the antagonist group. Flutamide will be administered orally to animals that are co-administered with 0.4 mg/kg testosterone propionate and serve as a positive antagonist control.

Animals will be dosed for 10 consecutive days via oral gavage (test substances and flutamide) and subcutaneous injection (testosterone propionate) based upon daily body weights. Approximately 24-hours following the final dose administration, the animals will be humanely euthanized; the glans penis, ventral prostate, levator ani plus bulbocavernous muscle, Cowper's glands, and seminal vesicles with coagulating gland and fluid will be excised and weights recorded. Changes in androgen dependent tissue weights will be evaluated to determine the ability of the test substances to act as an androgen agonist/antagonist or 5α-reductase inhibitor.

3.1 Test System

Species:	Rats, *Rattus norvegicus*
Strain:	Sprague-Dawley Crl:CD®(SD) IGS
Source:	Charles River Laboratories International, Inc. (Raleigh, NC)
Number/Sex:	104/Castrated males. Surgical manipulation performed by Charles River Laboratories International, Inc.
Acclimation:	Animals will be allowed to recover from the surgical manipulation for at least five days at Charles River Laboratories International, Inc. The animals will then be acclimated to ILS for at least seven days in the room where the study will occur.
Age at administration:	Postnatal Day (PND) 59/60 Note: PND 0 is the day of birth
Weight at administration:	250-350 grams
Identification:	Animals will be identified by the temporary numbers located on the animal's cage until allocation. Each animal will be uniquely identified by ear punch prior to dose administration.
Justification:	Animal model used is in accordance with OPPTS 890.1400: Hershberger Bioassay (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 (pre-allocation):	1 per cage
Housing (post-allocation):	2 per cage
Cage Type:	Polycarbonate with micro-isolator top
Cage Size:	23 cm wide by 44 cm long (1012 cm^2 area) and 21 cm high
Bedding:	Absorbent heat-treated hardwood bedding (Northeastern Bedding Corp., Warrensburg, NY)
Cage Changes:	At least once per week while single housed and twice per week while multi-housed.
Diet:	Teklad Global 16% Protein Rodent Diet (Teklad Diets, Madison WI) *ad libitum* Autoclaved Purina 5L79 Rat and Mouse diet *ad libitum* given at Charles River Laboratories International, Inc. prior to shipment. 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 does not exceed 350 µg/g (as described by Owens et al., 2003).
Archival:	A sample of the diet (~200 g) will be retained and stored between 0 and -30°C until acceptance of the final report.
Water:	Reverse osmosis treated tap water (City of Durham, NC) *ad libitum*
Supplied:	Glass water bottles with stainless steel sipper tube
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:	At least once per week

Animal Room Conditions:

Temperature:	19-25°C
Humidity	30-70%
Lighting:	12/12 hour light/dark cycle

Enrichment: None

3.3 Allocation

The animals will be assigned to a dose group 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.

3.4 Group Designation

Table 1. Androgen Agonist

Group Number	Animal Identification	Test Substance/Controls	Test Substance Dose Level (mg/kg/day)
1	001-008	Corn Oil Control	0
2	009-016	Padimate-O	320
3	017-024	Padimate-O	1000
4	025-032	Homosalate	320
5	033-040	Homosalate	1000

Table 2. Androgen Antagonist

Group Number	Animal Identification	Test Substance/Controls	Test Substance Dose Level (mg/kg/day)
6*	041-048	Corn Oil Control + Testosterone Propionate	0 + 0.4
7	049-056	Padimate-O+ Testosterone Propionate	100 + 0.4
8	057-064	Padimate-O+ Testosterone Propionate	320 + 0.4
9	065-072	Padimate-O+ Testosterone Propionate	1000 + 0.4
10	073-080	Homosalate + Testosterone Propionate	100 + 0.4
11	081-088	Homosalate + Testosterone Propionate	320 + 0.4
12	089-096	Homosalate+ Testosterone Propionate	1000 + 0.4
13	097-104	Flutamide + Testosterone Propionate	3.0 + 0.4

*Group will serve as the positive control for the androgen agonist assay

3.5 Dose Administration

The test substances, flutamide dose formulations, and the vehicle control dose formulations will be administered by oral gavage at a dosing volume of 5 mL/kg body weight. Testosterone propionate dose formulations will be administered by subcutaneous injection into the dorsoscapular region at a dosing volume of 0.5 mL/kg body weight. In co-administered animals, oral gavage will precede subcutaneous injections.

The dose formulations will be administered on a staggered start for 10 consecutive days (PND 59/60 through PND 68/69). The first four animals from each group will be dosed beginning on PND 59, and the second four animals from each group will begin on PND 60. Dosing will occur 24 hours (± 2 hours) from the previous dose. Dose volume will be determined on individual animal daily body weight. The dosing sequence will be

stratified across dosing groups; one animal from each group and then repeated until all animals are dosed.

3.5.1 Justification of Route of Administration

Selection of the route of administration is in accordance with OPPTS 890.1400: Hershberger Bioassay (U.S. EPA, 2009).

3.5.2 Justification of Dose Levels

Selection of the highest dose level for each test substance was based upon the available LD50 and/or acute toxicity information in order to avoid death, severe suffering, or distress in the animals and second, takes into consideration available information on the doses used in other studies. In general, the highest dose should not cause a reduction in the final body weight of the animals greater than 10% of control body weight. The highest dose should ensure animal survival and that is without significant toxicity or distress to the animals after 10 consecutive days of administration up to a maximal dose of 1000 mg/kg/day.

3.5.3 Disposal of Dose Formulations

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

3.6 In-Life Animal Observations

Mortality/Moribundity:	Twice daily on weekdays, once daily on weekends/holidays
Clinical Observations:	Observed within 2 days of arrival, again for allocation of animals to study groups, daily prior to dose administration, and prior to euthanasia. If adverse clinical signs are seen additional observations may be recorded.
Preputial Separation (PPS):	Animals will be evaluated for preputial separation upon arrival. Separation not initiated, partial separation, a persistent thread of tissue between the glans penis and prepuce, or complete PPS will be recorded.
Body Weights:	Collected within 2 days of arrival, again for allocation of animals to study groups, daily prior to dose administration, and prior to euthanasia.

3.7 Termination

Moribunds/Unscheduled:	Tissue collection will not be performed on accidental deaths, moribund, or animals found dead during the acclimation period.

	Beginning on the first day of dose administration, any animals found moribund or dead will be necropsied under the supervision of a pathologist, facility veterinarian, or veterinary designee and cause of death will be determined and recorded, if possible. Moribund animals will be euthanized by carbon dioxide (CO_2) inhalation and death confirmed by cervical dislocation.
Scheduled:	Twenty-four hours (± 2 hours) after the final dose administration, animals will be humanely euthanized by CO_2 asphyxiation with death confirmed by cervical dislocation; euthanasia will occur in the same order as they were dosed. The first four animals from each group will be euthanized on PND 69, and the second four animals from each group will be euthanized on PND 70.
Tissue Collection:	Gross observations of the tissues that are being excised for tissue weights will be recorded.
Tissue Weights:	The following tissues will be excised, trimmed of excess adhering tissue and fat, weighed, and weights recorded to the nearest 0.0001 g. 1. Ventral Prostate 2. Seminal vesicles with coagulating gland with fluid 3. Levator ani plus bulbocavernous muscle complex 4. Cowper's glands (weighed as a pair) 5. Glans penis

3.8 Statistical Analysis

Descriptive statistics (mean, standard deviation, coefficient of variance) for initial and final body weight, body weight gain, and tissue weights will be analyzed using SAS (Cary, NC). Studentized residual plots will be used to detect possible outliers and Levene's test will be used to assess homogeneity of variance. If the data is heterogeneous, then appropriate transformation will be performed and the data will be re-analyzed to assess homogeneity.

Final body weight, body weight gain, and tissue weights will be analyzed by an ANOVA followed by pair wise comparisons using a Dunnett's t test (one tailed- tissues weights, two tailed- final body weight and body weight gain). Statistically significant effects will be reported when $p<0.05$. Positive controls will be analyzed by appropriate t-tests.

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

3.9 Performance Criteria

The study should be evaluated if 1) three or more of the ten possible individual CV's in the negative control and high dose group exceed the maximum allowable CV's designated for androgenic and anti-androgenic effects listed in Table 5, or 2) if at least two of the target tissues' p values fall between 0.05 and 0.10 when compared to the negative control.

Table 3. Maximum Coefficients of Variation

Tissue	Androgen Agonist	Androgen Antagonist
Glans Penis	22%	17%
Cowper's Glands	55%	35%
LABC	30%	20%
Ventral Prostate	45%	40%
Seminal Vesicles	40%	40%

Source: U.S. EPA (2009)

REPORT

The report will include all items in the study protocol as well as a comprehensive presentation of all data collected in the study.

RECORD RETENTION

All original data [including the original signed study protocol and all amendments (if any), test substance information, animal receipt records, animal caretaker records, observations, body weight records, clinical observations, etc.] and the original final report will be transferred to the National Toxicology Program Archives following finalization of the study report.

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

REFERENCES

Blake, J. (2011). Padimate-O in Corn Oil Dose Formulation Development. RTI Project Number-ChemTask Number: 0212839.100.003.034-Chem11137. Unpublished study report prepared by Research Triangle Institute, International.

Blake, J. (2012). Homosalate in Corn Oil Dose Formulation Development. RTI Project Number-ChemTask Number: 0212839.200.003.063-Chem11139. Unpublished study report prepared by Research Triangle Institute, International.

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

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. *Environ. Health Perspect.* 111: 1559-1567.

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

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

KEY PERSONNEL

Study Director:
Study Toxicologist:
Toxicology Study Manager:
Animal Facility Operations Manager:
Necropsy Manager:
Facility Veterinarian:
Health and Safety Manager:
Dose Formulations:

Appendix VIII:

Amendments and Deviations

ILS-A-066
Last Revised: 08/02/12

Integrated Laboratory Systems, Inc.
Protocol Deviation

ILS Project No.-Study No.:	N135-249
Protocol Deviation No.:	1
Protocol Section Deviated from:	3.2
Nature of Deviation:	Relative humidity was out of range on the following date: 11 April 2012
Reason for Deviation:	Slight fluctuations in the HVAC system.
Corrective Action:	None, the HVAC system corrected the slightly lower humidity.
Impact on Study:	There is no significant impact on the study since the slightly lower humidity did not cause any abnormal clinical observations in the animals.
Protocol Section Deviated from:	3.2
Nature of Deviation:	Temperature was out of range on the following date: 17 April 2012
Reason for Deviation:	Slight fluctuations in the HVAC system.
Corrective Action:	None, the HVAC system corrected the slightly higher temperature.
Impact on Study:	There is no significant impact on the study since the slightly higher temperature did not cause any abnormal clinical observations in the animals.

Page **1** of **3**

Protocol Section Deviated from:	3.2
Nature of Deviation:	Afternoon environmental room check was not performed on following date: 10 April 2012
Reason for Deviation:	Technical staff oversight.
Corrective Action:	The research staff was verbally reminded that room checks must be performed twice daily.
Impact on Study:	There is no significant impact on the study since no animals were housed in the room at that time.

Protocol Section Deviated from:	3.2
Nature of Deviation:	Interval between cage changes was greater than 7 days during the week of animal receipt: 12 April 2012 – 20 April 2012
Reason for Deviation:	Technical staff oversight.
Corrective Action:	The research staff was verbally reminded that cage changes must be performed once weekly while animals are single housed
Impact on Study:	There is no significant impact on the study since no abnormal observations were made during room checks and deviation occurred prior to the start of the study.

Protocol Section Deviated from:	3.5, 3.7
Nature of Deviation:	Animal 001 and 009 were euthanized < 2 hours from the final time of dose administration.
Reason for Deviation:	Technical staff oversight.
Corrective Action:	The research staff was verbally reminded to accurately calculate the starting time of the necropsy according to the

study protocol.

Impact on Study:

There is no significant impact on the study because the animals were euthanized within 7 minutes of the specified timeframe.

[redacted]

8-2-12
Date

Study Director
Investigative Toxicology Division
Integrated Laboratory Systems, Inc.

ILS-A-067
Last Revised: 06/08/12

Integrated Laboratory Systems, Inc.
SOP Deviation

ILS Project No.-Study No.: N135-249

Sponsor Study No.:

SOP No.-Mod. No. Deviated: 1320-2

SOP Deviation No.: 1

SOP Section Deviated: II. A. 4

Nature of Deviation:	Research Assistant did not document dose (Padimate-O, Homosalate, or Flutamide) withdrawl on 22 April 2012. Corn Oil Control was not listed on dose withdrawl forms and thus not recorded as withdrawn for the duration of the study.
Reason for Deviation:	Technical oversight.
Corrective Action:	Research staff was verbally reminded to document dose withdrawl appropriately.
Impact on Study:	None. Animals were dosed as documented on the daily dosing form(s).

[redacted]

Study Director
Integrated Laboratory Systems, Inc.

6.13.12
Date

Page **1** of 1

ILS-A-067
Last Revised: 08/07/12

Integrated Laboratory Systems, Inc.
SOP Deviation

ILS Project No.-Study No.:	N135-249
SOP No.-Mod. No. Deviated:	1119-11
SOP Deviation No.:	2

SOP Section Deviated:	II. D. 2. b.
Nature of Deviation:	Research Assistant did not document that dosing was performed on 01 May 2012. No removal date was recorded for food barrel removal on the Feed Use Form.
Reason for Deviation:	Technical oversight.
Corrective Action:	Research staff was reminded to document all forms appropriately.
Impact on Study:	None. Time of dosing was recorded for each animal to verify that all animals were dosed for the day. None. Animals food was checked at each room check and barrel was removed and conclusion of the study for room sanitation.

[redacted]
Study Director
Integrated Laboratory Systems, Inc.

8-7-12
Date

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