FINAL REPORT

INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL (Charles River Laboratories)

EPA Contract Number 68-W-01-023
Work Assignment 5-15

May 3, 2006

Submitted to

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Work Assignment Manager
U.S. Environmental Protection Agency
Endocrine Disruptor Screening Program
Washington, D.C.

Battelle
505 King Avenue
Columbus, Ohio 43201

FINAL REPORT

Study Title

Interlaboratory Validation of the 15-Day Adult Intact Male Rat Assay with Linuron and Phenobarbital

Author

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Study Completed On

2 May 2006
(Final Report)

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Laboratory Project ID

Charles River Laboratories Preclinical Services Protocol Number: RTP00004

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentially is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10(d) (1)(A), (B), or (C).

This statement supersedes any other claims of confidentiality found in this report.

Company: Battelle

Company Agent: David P. Houchens, Ph.D.

Title: Program Manager

Date: 5/3/06

Signature: David P. Houchens

GOOD LABORATORY PRACTICE STATEMENT

This study was conducted according to U.S. Environmental Protection Agency. Federal Insecticide, Fungicide and Rodenticide Act/Toxic Substances Control Act (FIFRA/TSCA); Good Laboratory Practice Standards; Final Rule, 40 CFR Part 160/Part 792; the Ministry of Agriculture, Forestry and Fisheries, Japan (MAFF), Good laboratory practice (GLP) standards for agricultural chemicals. Agricultural Production Bureau Ref. No. 11-Nousan-No.6283, October 1, 1999; last revised June 30, 2003 Ref. No. 15-Seisan-2460 and the OECD Environmental Directorate, OECD Principles of good laboratory practices [C(97)186/Final] (1998); Environmental Health and Safety Division with the exception that stability of the Linuron provided by the Sponsor does not bracket the range of concentrations used for dosage administration. Any areas of noncompliance are documented in the study record. No deviations existed that affected the validity of the study.

Submitter: David P. Houchens 5/3/06

Sponsor's Representative: David P. Houchens 5/3/06

David P. Houchens, Ph.D. Date
Battelle

Study Director: Joseph W. Lech 5/2/06

Joseph W. Lech, B.S., LAT Date
Scientist
and Study Director

FLAGGING STATEMENT

I have applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of the attached study. This study neither meets nor exceeds any of the applicable criteria.

Company: Battelle

Company Agent: David P. Houchens, Ph.D.

Title: EDSP Program Manager

Date: _____

Signature: _____

TITLE: INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL

CHARLES RIVER LABORATORIES PRECLINICAL SERVICES
PROTOCOL NUMBER: RTP00004

SPONSOR'S WORK ASSIGNMENT: WA 5-15

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ABSTRACT

The 15-day intact male assay was developed by DuPont Haskell Laboratory as one component of a Tier I screening strategy for identifying endocrine-active compounds (EAC). The EAC test compounds for this assay were the weak antiandrogenic herbicide, Linuron, and the thyroid modulating barbiturate, Phenobarbital.

Intact adult male rats, 15 per group, were dosed between 0600 and 0900 hrs on test days 1 through 14 (TDs 1-14) and dosing began at approximately 0600 hrs on TD 15. Dosages of one of the test substances were administered for 15 consecutive days via oral gavage with aqueous 0.25% (w/v) methylcellulose (vehicle control), Linuron at 50, 100 and 150 mg/kg/day and Phenobarbital at 25, 50 and 100 mg/kg/day. Dosages were formulated using 0.25% (w/v) methylcellulose as the vehicle and administered at 5 mL/kg. Based on the results of the analyses of the prepared formulation and the lack of any dosing errors, all rats are believed to have been dosed properly at the correct concentration with homogeneous suspensions.

Rats were observed for viability at least twice each day of the study and for clinical observations and general appearance daily during the acclimation period. Observations for clinical signs and deaths were made daily before dosage administration and at approximately 6 hours after dosage administration, except on the day of sacrifice. Body weights were recorded three times during the acclimation period and daily during the dosage period. Feed consumption values were recorded once during the acclimation period and weekly during the dosage period. Necropsies were performed on TD 15 between 0800 and 1100 hrs (2 to 3 hours after the last dosage). Trunk blood was collected from anesthetized rats following decapitation, prior to necropsy, for testosterone, luteinizing hormone (LH), thyroid stimulating hormone (TSH), thyroxine (T4), triiodothyronine (T3), follicle stimulating hormone (FSH), estradiol, prolactin and dihydrotestosterone (DHT) analyses. The results of all hormone assays were considered reliable based on the assay performance results. The liver, testes (left and right), epididymides (paired), prostate (whole), seminal vesicles (with fluid and coagulating gland) and thyroid were weighed. The left and right testes were then weighed together and the accessory sex glands (ASG, entire prostate and seminal vesicles with fluid and coagulating gland combined) were weighed together. The right and left testis, right and left epididymis and the thyroid were individually identified and examined histopathologically.

- **Linuron**

Treatment with Linuron as high as 150 mg/kg/day, for 15 consecutive days, caused no mortality. Clinical observations of decreased motor activity, ataxia, comatose, lost righting reflex, unresponsive to touch, lacrimation, chromorhinorrhea, chromodacryorrhea, limited use of limbs, cold to touch, dehydration, impaired righting reflex, ptosis, perinasal substance, hunched posture, low carriage, excess salivation, perioral substance, ungroomed coat, sparse hair coat, bradypnea and scant feces were observed. Dosages of 50, 100 and 150 mg/kg/day caused significant reductions in food consumption values, body weight gains and terminal body weights. Absolute liver weights and accessory sex gland weights were significantly decreased in all treated groups; absolute weights of the epididymides and prostate were significantly decreased in the 100 and 150 mg/kg/day dosage groups; and seminal vesicles with fluid and coagulating gland weights were significantly decreased in the 150 mg/kg/day dosage group. These decreases in absolute organ weights are subject to change with body weights and thus may not signify specific endocrine-mediated effects. When the absolute organ weights were adjusted for its final body weight, mean relative right testis (119.1%, 117.4% and 124.4% of control), left testis (118.4%, 116.8% and 124.3% of control), paired testes (118.7%, 117.2% and 124.3% of control) were significantly increased in the 50, 100 and 150 mg/kg/day dosage groups, when compared to the vehicle control group values. These increases in relative testis weights, however, were likely secondary to the decreases in terminal body weights [(11)]. The relative thyroid gland (129.7%, 122.5% and 113.4% of control) weights were significantly increased in the 50 mg/kg/day dosage groups, when compared to the vehicle control group value. In addition, relative liver weights were significantly increased in the 150 mg/kg/day dosage group. Testosterone, T4, T3, and prolactin blood concentrations were significantly less than the vehicle control group in all three treated groups. LH blood concentrations in the 50 and 100 mg/kg/day dosage groups were significantly less than the vehicle control group and DHT levels in the 150 mg/kg/day were also significantly less than the vehicle control group. Estradiol levels were significantly greater than the vehicle control group in all three Linuron treated groups. No test substance-related microscopic changes were observed in the testes, epididymides or thyroids of the rats given the test substance as high as 150 mg/kg/day. All microscopic changes observed in the testes, epididymides and thyroids of rats treated with Linuron were considered to have occurred spontaneously and were not treatment-related.

- **Phenobarbital**

Treatment with 100 mg/kg/day, for 15 consecutive days, caused one death on test day 9. Clinical observations of ptosis, chromodacryorrhea, chromorhinorrhea, misaligned incisors, ataxia, impaired righting reflex, decreased motor activity, low carriage, limited use of limbs, hyperpnea, bradypnea, lost righting reflex, unresponsive to touch, comatose, lacrimation, no use of limbs, bent tail, cold to touch, dehydration, substance on penis, ungroomed coat, urine-stained abdominal fur, sparse hair coat and excess salivation (slight) were observed. Dosages of 100 mg/kg/day caused significant reductions in body weight gains, terminal body weights and food consumption. Absolute liver weights and

thyroid gland weights were significantly increased in the 25, 50 and 100 mg/kg/day dosage groups. Relative liver and relative thyroid gland weights were significantly increased in rats in the 25, 50 and 100 mg/kg/day dosage groups. In addition, relative right, left, and paired testes weights were significantly increased in the 100 mg/kg/day dosage group. LH, T4, T3 and prolactin blood concentrations were significantly less than the vehicle control group, in all three treated groups. Testosterone, FSH and DHT blood concentrations were significantly decreased in the 50 and 100 mg/kg/day Phenobarbital groups when compared to the vehicle control group values. Estradiol and TSH levels were significantly increased compared to the vehicle control group values in all three treated groups. An increased incidence and severity of hypertrophy and hyperplasia of the thyroid follicular epithelium occurred in the 100 mg/kg/day dosage group and was considered to be treatment-related. No test substance-related microscopic changes were observed in the testes or epididymides of the rats given the test substance as high as 100 mg/kg/day. All microscopic changes observed in the testes and epididymides of rats treated were considered to have occurred spontaneously and were not treatment-related.

In conclusion, Linuron did produce the expected decreased blood testosterone, dihydrotestosterone, and luteinizing hormone levels, and increased blood estradiol levels. Also consistent with other EACs, Linuron increased thyroid weights and decreased thyroid hormone levels. Phenobarbital increased relative liver and thyroid weights; decreased blood levels of testosterone, FSH, T3 and T4, with the concomitant increase in thyroid stimulating hormone; and altered reproductive hormone concentrations (decreased serum dihydrotestosterone, prolactin, and luteinizing hormone and increased levels of estradiol). Phenobarbital also increased the incidence and severity of hypertrophy and hyperplasia of the thyroid follicular epithelium in rats given 100 mg/kg/day.

1. OBJECTIVES

The purpose of this project was designed to evaluate the ability of this assay to detect endocrine active compounds by measuring body and organ weight changes, histology, and changes in circulating concentrations of hormones and to demonstrate that three contract laboratories can adopt this assay by analyzing the repeatability of the results for each endpoint across laboratories.

The EPA has selected two test chemicals for evaluation in the 15-day intact male assay, and has selected all of the target doses (in mg/kg/day) for each of them. The two test chemicals and their target/mechanism of action are as follows: (1) Linuron - an anti-androgen through competitive binding to androgen receptors and (2) phenobarbital which indirectly alters thyroid function via enhanced thyroid hormone excretion [(1)].

2. DESCRIPTION OF TEST PROCEDURES

2.1. Conduct of Study

2.1.1. Sponsor

Battelle, 505 King Avenue, Columbus, Ohio 43201-2693

2.1.2. Testing Facility

Charles River Laboratories Preclinical Services, Pennsylvania, 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1241

2.1.3. Study Number

RTP00004

2.1.4. Sponsor's Work Assignment

WA 5-15

2.1.5. Purpose of the Study

The purpose of this study was designed to evaluate the responses of the adult male rat assay to two chemicals that have known endocrine activity as detected by primarily measuring body and organ weight changes, histology and changes in circulating concentrations of hormones.

2.1.6. Regulatory Compliance

The study was conducted in compliance with Good Laboratory Practice (GLP) regulations of the EPA[2], the Japanese MAFF[3] and the OECD[4]. Quality Assurance Unit findings derived from the inspections during the conduct of this study are documented and have been provided to the Study Director and the Testing Facility Management.

2.1.7. Ownership of the Study

The U.S. Environmental Protection Agency owns the study. All raw data, analyses, reports and preserved tissues are the property of the U.S. Environmental Protection Agency.

2.1.8. Sponsor's Representative

David P. Houchens, Ph.D.
Address as cited above for Sponsor.

2.1.9. Study Director

Joseph W. Lech, B.S., LAT (Scientist)
Address as cited previously for Testing Facility.

2.1.10. Technical Performance

2.1.10.1. Charles River Laboratories Preclinical Services, Pennsylvania

John F. Barnett, Sr., B.S. (Director of Operations)
Gerard M. Zimmerman, ALAT (Study Supervisor)
Daniel E. Fisher, B.S. (Laboratory Technician)
James Maier, III, B.S. (Necropsy Laboratory Technician)
Kevin E. Cegielski (Formulation Laboratory Technician)

2.1.10.2. Charles River Laboratories Preclinical Services, Massachusetts

Dorothy Savage, B.S. (Principal Investigator) - Formulation Analysis

2.1.10.3. Battelle

Paul I. Feder Ph.D. (Principal Investigator) - Report Table Preparation and Statistical Analysis

2.1.10.4. Subcontractor Facilities

W. Ray Brown, D.V.M., Ph.D., Diplomate, ACVP (Principal Investigator, Research Pathology Services, Inc., New Britain, PA) - Histopathology
Carol D. Sloan M.S., TS, LATG (Principal Investigator, RTI International, Research Triangle Park, NC) - Hormone Analyses

2.1.11. Report Preparation

Joseph W. Lech, B.S., LAT (Scientist)
Cheryl L. Karvounis, A.S. (Data Management Specialist)
Tsai-Liang Chiang, B.S. (Senior Report Administrator)

2.1.12. Report Review

Alan M. Hoberman, Ph.D., DABT (Director of Research)
Valerie A. Sharper, M.S. (Principal Research Scientist)

2.1.13. Date Protocol Signed

7 October 2005

2.1.14. Dates of Technical Performance

Rat Arrival	18 OCT 05
Dosage Period (TD[a] 1 through 15)	
Replicate 1	25 OCT 05 - 08 NOV 05
Replicate 2	26 OCT 05 - 09 NOV 05
Replicate 3	27 OCT 05 - 10 NOV 05
Scheduled Sacrifice and Necropsy (TD 15)	
Replicate 1	08 NOV05
Replicate 2	09 NOV 05
Replicate 3	10 NOV 05
Experimental Start Date	25 OCT 05
Experimental Completion Date	27 MAR 06

2.1.15. Records Maintained

The original report, raw data and reserve samples of the bulk test substances, the prepared control substance and all data and records (including the Final report) that is a result of the hormonal analyses performed at RTI are retained in the archives of the Testing Facility. Any preserved tissues are retained in the archives of the Testing Facility for one year after the mailing of the draft final report, after which time the Sponsor will decide their final disposition. All residual formulations were discarded at the Testing Facility. Backup samples will be discarded at the Testing Facility following issue of the final report. The remaining unused bulk test substances were returned to the Sponsor on 14 November 2005.

2.2. Test Substances Information

NOTE: The Sponsor provided the test substances. Except for chemistry formulation and analyses, all tests, analyses and measurements were conducted by individuals without knowledge of the identity of the test substances. A key code for the dosage levels and concentrations were provided to the formulation and Quality Assurance personnel for the purpose of formulation preparation and auditing of critical phases, respectively. The identities of the test substances, dosage levels and concentrations were added to the protocol by amendment following the completion of the in-life phases of the study.

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a. TD is used as an abbreviation for Test Day.

2.2.1. Descriptions, Dates Received, Storage Conditions, Lot Numbers and Expiration Dates

Test Substance (CAS No.)	Description	Dates Received	Storage Conditions	Lot Number	Expiration Date
Linuron (330-55-2)	White Powder	13 SEP 05 28 SEP 05	Room Temperature	348-8A	AUG 2008
Phenobarbital (50-06-6)	White Powder	13 SEP 05 28 SEP 05	Room Temperature	104K2600	FEB 2010

2.2.2. Special Handling Instructions

Standard safety precautions (use of protective clothing, gloves, Tyvek® sleeves, dust-mist/HEPA-filtered mask, safety goggles or safety glasses with side shields) were taken during formulation preparation and dosage. The bulk test substances were handled in a chemical fume hood.

2.2.3. Analysis of Activity

The test substances are marketed products. Therefore, appropriate documentation of the method of synthesis, fabrication or derivation of each of the test substances is on file and is available to the appropriate regulatory agencies should it be requested. Information to document or certify the identity, composition, strength and activity of each test substance was generated on study EDSP.515-01 and provided by the Sponsor to the Testing Facility. The results from these analyses are available in APPENDIX 4. A Certificate of Analysis for each test substance is available in APPENDIX 4, APPENDIX A. The expiration date of the Linuron and Phenobarbital is August 2008 and February 2010, respectively.

2.3. Control Substance Information

2.3.1. Description

Aqueous 0.25% (w/v) methylcellulose

2.3.2. Lot Numbers

Sponsor/Manufacturer's bulk lot number for prepared control substance: 062K0144

2.3.3. Dates Received and Storage Conditions

The prepared control substance, a clear, colorless liquid, was received from Sigma-Aldrich Co., St. Louis, Missouri, on 13 September 2005 and 28 September 2005 and stored refrigerated (2°C to 8°C).

2.3.4. Special Handling Instructions

Standard safety precautions (use of protective clothing, gloves, Tyvek® sleeves, dust-mist/HEPA-filtered mask, safety goggles or safety glasses with side shields) were taken when handling the prepared control substance.

2.3.5. Analysis of Purity

Neither the Sponsor nor the Study Director was aware of any potential contaminants likely to have been present in the prepared control substance that would have interfered with the results of this study.

2.4. Test Substance Preparation and Storage Conditions

Suspensions were prepared at the Testing Facility once for a prestudy preparation and twice for administration. Prepared suspensions were stored refrigerated.

All dose levels, including the prepared control substance, were brought to room temperature while being stirred for approximately one hour before dosing. Each dosage level was stirred continuously using a magnetic stir bar and stir plate during sample collection and dosage administration.

Prior to study start, the Testing Facility performed a pre-study preparation and analysis of the test substance formulations in order to validate the transfer of information provided by the Sponsor regarding preparation and analysis of the test substance formulations.

2.4.1. Sample Information

Sample Type	Size	Date Retained	Storage Conditions	Shipped To/Shipping Conditions	Date Shipped
Concentration and Homogeneity[a] (all levels	1 mL	07 OCT 05 18 OCT 05	Refrigerated	Charles River Laboratories, Preclinical Services, Massachusetts[b]/On cold packs	07 OCT 05 18 OCT 05
Bulk Test Substance Reserves	1 g	11 NOV 05	Room temperature	Testing Facility Archives	23 NOV 05
Control Substance Reserve	5 mL	11 NOV 05	Room temperature	Testing Facility Archives	23 NOV 05

a. Quadruplicate samples were taken for each test substance from the top, middle and bottom of each concentration on the day prepared from both the prestudy preparation and from the first preparation of the formulations used for dosage administration in order to: 1) validate the transfer of information provided by the Sponsor from the pre-study preparation and 2) verify the concentration of the test substances in the control substance from the dosing formulations. Two samples from each quadruplicate set were shipped for analysis; the remaining samples are retained at the Testing Facility as backup samples and will be discarded following the issue of the final report.

b. Charles River Laboratories, Preclinical Services, Worcester, Massachusetts.

2.4.2. Formulation Analyses

Prepared formulations (Linuron and Phenobarbital) used for dosage administration were analyzed for concentration and homogeneity. The results of these analyses found the samples to be within the acceptable limits of ± 15% and ≤5% RSD. The results of the concentration and homogeneity analyses for the pre-study preparation and the prepared formulations used on study are available in APPENDIX 5. Based on the results of these analyses and the lack of any dosing errors, all rats are believed to have been dosed properly at the correct concentration with homogeneous suspensions. Results of the homogeneity and concentration analyses from the first preparation of the test substances that were used for administration during the study were approved by the Study Director before administration. Information to document the stability of the prepared formulations bracketing the range of concentrations used in this study was provided by the Sponsor and is available in APPENDIX 4.

2.5. Test System

2.5.1. Species

Rat

2.5.2. Strain

Crl:CD(SD)

2.5.3. Supplier (Source)

Charles River Laboratories, Inc., Raleigh, NC

2.5.4. Sex

Male

2.5.5. Rationale for Test System

The Crl:CD(SD) rat was selected as the Test System because of known response to toxic effects on reproductive capacity and history of use as a rodent species in these evaluations[5-7].

2.5.6. Test System Data

Number of Rats	115
Approximate Date of Birth	17 AUG 05
Approximate Age at Arrival	63 days
Weight (g) the Day after Arrival	220.9 - 301.0
Weight (g) at Study Assignment	285.8 - 351.2

2.5.7. Method of Randomization

Upon arrival, rats were assigned to individual housing on the basis of computer-generated random units.

After a minimum of one week of acclimation, in which the rats were monitored for general health daily, rats were selected for study on the basis of physical appearance and body weights recorded during acclimation. During the acclimation period all rats were examined by the laboratory veterinarian for release on study. The rats were assigned to dosage groups based on computer-generated (weight-ordered) randomization procedures.

In order to accommodate the necropsy schedule, rats were assigned to three replicates that began dosing and were sacrificed on consecutive days.

2.5.8. System of Identification

Rats were assigned temporary numbers at receipt and given unique permanent identification numbers when assigned to the study. Rats were permanently identified using Monel® self-piercing ear tags (No. MSPT 20101, Gey Band and Tag Co., Inc., Norristown, PA). Cage tags were marked with the study number, permanent rat number, sex, generation and group number.

2.6. Husbandry

2.6.1. Research Facility Registration

USDA Registration No. 14-R-0144 under the Animal Welfare Act, 7 U.S.C. 2131 *et seq.*

2.6.2. Study Room

The study room was maintained under conditions of positive airflow relative to a hallway and independently supplied with a minimum of ten changes per hour of 100% fresh air that had been passed through 99.97% HEPA filters. Room temperature and humidity were monitored constantly throughout the study. Room temperature was targeted at 64°F to 79°F (18°C to 26°C); relative humidity was targeted at 30% to 70%[a].

2.6.3. Housing

During the acclimation and study periods, the rats will be individually housed in stainless steel, wire-bottomed cages. All cage sizes and housing conditions were in compliance with the *Guide for the Care and Use of Laboratory Animals*[8].

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a. See APPENDIX 6 (ENVIRONMENTAL AND HUSBANDRY REPORTS).

2.6.4. Light

An automatically controlled 12-hour light:12-hour dark fluorescent light cycle was maintained. Each dark period began at 1800 hours. The lights were turned on 5 to 10 minutes early on the days of sacrifice in order to facilitate dosing, blood collection and/or the necropsy schedule.

2.6.5. Sanitization

Cage pan liners were changed at least three times weekly. Cages were changed approximately every other week.

2.6.6. Diet

Rats were given Harlan's Teklad 2018CM meal feed (a low phytoestrogen diet), available *ad libitum* from individual feeders.

2.6.7. Diet Analysis

Analyses were performed by NP Analytical Laboratories, St. Louis, MO. No contaminants at levels exceeding the maximum concentration limits for certified feed or deviations from expected nutritional requirements were detected by these analyses.

The concentrations of genistein equivalents (genistein plus 0.8 x daidzein) were [115 + (0.8 x 120) = 211 ppm] which is $\leq$ 300 ppm per lot. The diet was analyzed by separating the conjugated and unconjugated (aglycone forms) of genistein, daidzein, and glycitein in the diet using high-pressure liquid chromatography (HPLC). Each of those forms was then converted into aglycone equivalents[9]. Copies of the results of the feed analyses are available in the raw data and in APPENDIX 6.

Neither the Sponsor nor the Study Director was aware of any potential contaminants likely to have been present in the feed that would have interfered with the results of this study.

2.6.8. Water

Local water that had been processed by passage through a reverse osmosis membrane (R.O. water) was available to the rats *ad libitum* from an automatic watering access system and/or individual water bottles attached to the cages. The water bottles used for this study are composed Fortiflex® B53-35H-100, which is a high density polyethylene copolymer developed for injection blow molding. The stoppers used for the bottles were composed of either black rubber or neoprene and the sipper tubes were composed of stainless steel. Chlorine was added to the processed water as a bacteriostat.

2.6.9. Water Analysis

The processed water is analyzed twice annually for possible chemical contamination (Lancaster Laboratories, Lancaster, PA) and monthly for possible bacterial contamination (QC Laboratories, Southampton, PA). Copies of the results of the water analyses are available in the raw data and in APPENDIX 6.

Neither the Sponsor nor the Study Director was aware of any potential contaminants likely to have been present in the water that would have interfered with the results of this study.

2.7. Methods

2.7.1. Dosage Administration

Dosage Group	Number of Rats	Test Substance/()[a]	Dosage (mg/kg/day)[b]	Concentration (mg/mL)	Dosage Volume (mL/kg)	Assigned Rat Numbers
1	15	Aqueous 0.25% (w/v) Methylcellulose (A)	0 (Vehicle)	0	5	10301 - 10315
2	15	Linuron (B)	50	10	5	10316 - 10330
3	15	Linuron (C)	100	20	5	10331 - 10345
4	15	Linuron (D)	150	30	5	10346 - 10360
5	15	Phenobarbital (E)	25	5	5	10361 - 10375
6	15	Phenobarbital (F)	50	10	5	10376 - 10390
7	15	Phenobarbital (G)	100	20	5	10391 - 10405

a. Assigned Group Letter
b. The test substances were considered 100% active for the purpose of dosage calculations.

2.7.2. Rationale for Dosage Selection

Chemicals selected for this phase of validation were chosen to represent a couple of different modes of action. Each of the test chemicals has previously been run in the adult male assay with results documented in a review publication[10]. Based on the results of these studies, the high dosage level is not expected to exceed the maximum tolerated dose (MTD; body weight at necropsy within approximately 10% of controls). The lower dosage levels were selected to assess dose-response relationships.

2.7.3. Route and Rationale for Route of Administration

The oral (gavage) route was selected for use because: 1) in comparison with the dietary route, the exact dosage can be accurately administered; and 2) it is one possible route of human exposure.

2.7.4. Frequency of Administration

Male rats were administered one of the test substances and/or control substance once daily for 15 days. The first day of dosage for each replicate was designated Test Day 1

(TD 1) of the study. Rats were sacrificed on the day of the last dosage (TD 15), 2 to 3 hours after the last dosage.

Daily dosages were based on the daily body weight, except on TD 15, which used the previous day's body weight. On TDs 1 through 14, dosing of rats was between 0600 and 0900 hrs. On TD 15, dosing of the rats started at approximately 0600 hrs so that rats could have blood collected and be necropsied between 0800 and 1100 hrs.

2.7.5. Method of Study Performance

NOTE: Test substances provided by the Sponsor were identified by code. Except for chemistry formulation and prepared formulation analyses, all tests, analyses and measurements were conducted by individuals without knowledge of the identity of the test substances.

Rats were observed for viability at least twice each day of the study and for clinical observations and general appearance daily during the acclimation period. Observations for clinical signs and deaths were made daily before dosage administration and at approximately 6 hours after dosage administration, except on the day of sacrifice[a].

Body weights were recorded three times during the acclimation period and daily during the dosage period. Feed consumption values were recorded once during the acclimation period and weekly during the dosage period.

2.7.6. Gross Necropsy

Gross lesions were retained in neutral buffered 10% formalin. Unless specifically cited below, all other tissues were discarded. Representative photographs of gross lesions are available in the raw data.

All rats were moved from the study room to the necropsy area and held for at least one hour prior to necropsy to minimize potential stress-induced changes in hormone levels related to cage transport. On TD 15 rats were anesthetized by exposure to carbon dioxide for no more than one minute and sacrificed by decapitation[b]. Rats were sacrificed between 0800 and 1100 hrs (2 to 3 hours after the last dosage).

Rats were sacrificed and examined for gross lesions. Gross necropsy included an initial physical examination of external surfaces and all orifices, as well as an internal examination of tissues and organs *in situ*. In addition, the cranial, thoracic and abdominal cavities were examined. Tissue trimming and histopathology were performed under the supervision of or by a Board-Certified Veterinary Pathologist.

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a. See PROTOCOL DEVIATIONS, item 1.
b. See PROTOCOL DEVIATIONS, item 2.

The liver, testes (left and right), epididymides (paired), prostate (whole), seminal vesicles (with fluid and coagulating gland) and thyroid were weighed (to the nearest 0.0001 g). The left and right testes were then weighed together and the accessory sex glands (ASG, entire prostate and seminal vesicles with fluid and coagulating gland combined) were weighed together. The right and left testes were fixed in Bouin's solution for 24 hours before being rinsed and retained in 70% alcohol[a]. The right and left epididymides, prostate and liver were retained in neutral buffered 10% formalin. The thyroid and the surrounding tissue were removed from the neutral buffered 10% formalin after at least 48 hours of fixation[b]. The thyroid was then dissected under a dissecting microscope by one individual in order to reduce the variability of the dissection procedure and hence, reduce the variability of the thyroid weights[c].

The dose groups but not the compounds were known to the pathologist during evaluation. Histopathological examination was performed on all control and high dose rats of each test substance. The right testis and left testis, the right epididymis and left epididymis and the thyroid were individually identified and examined histopathologically and were routinely processed, embedded in paraffin, sectioned at 5 microns and stained with hematoxylin and eosin[d]. Summaries of the histological findings are available in APPENDIX 7.

The rat that died was examined for cause of death on the day the observation was made. The rat was necropsied and examined to the extent possible as described above, but the tissues were histologically examined[e].

2.7.6.1. Hormone Analysis

Blood samples (approximately 9 mL) for evaluation of serum hormones were collected from trunk blood immediately following sacrifice. The time of sample collection was documented in the raw data. Blood was collected and immediately placed into serum separator tubes and allowed to clot at room temperature in order to yield approximately 4500 mcL of serum, to be aliquotted into nine vials of approximately 500 mcL each[f]. The sequence in which the hormones were assayed was testosterone, luteinizing hormone (LH), thyroid stimulating hormone (TSH), thyroxine (T4), triiodothyronine (T3), follicle stimulating hormone (FSH), estradiol, prolactin and dihydrotestosterone (DHT). Serum samples were immediately frozen on dry ice and maintained frozen (-68°C to -78°C) until analysis by RTI International, Research Triangle Park, North Carolina. Assay performance criteria and results of serum sample analyses are presented in APPENDIX 8.

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a. See PROTOCOL DEVIATIONS, item 3.
b. See PROTOCOL DEVIATIONS, item 4.
c. See PROTOCOL DEVIATIONS, item 5.
d. See PROTOCOL DEVIATIONS, item 6.
e See PROTOCOL DEVIATIONS, item 7.
f. See PROTOCOL DEVIATIONS, item 8.

2.7.7. Data Collection and Statistical Analyses

Data generated during the course of this study were recorded either by hand or using the *Argus Automated Data Collection and Management System* and the *Vivarium Temperature and Relative Humidity Monitoring System*. All data were tabulated and summarized using the *Argus Automated Data Collection and Management System*, the *Vivarium Temperature and Relative Humidity Monitoring System, Microsoft® Excel* (part of Microsoft® Office 97/2000/XP), Quattro Pro 8 and/or *The SAS System* (version 6.12).

2.7.8. Statistics

Individual clinical observation and necropsy/mortality tables were generated by the Testing Facility. This information was not summarized or statistically analyzed.

The Sponsor prepared the report tables and performed the statistical analyses (daily body weight and body weight change, feed consumption, hormonal analysis and organ weights). For the results of these analyses see APPENDIX 9. The Sponsor's QAU was responsible for auditing the report tables and statistical analysis generated by Battelle and that all applicable GLP regulations were followed in the conduct.

3. RESULTS

3.1. Mortality and Clinical Observations (Individual Data - APPENDICES 1 and 2)

3.1.1. Mortality

One rat in the 100 mg/kg/day Phenobarbital group was found dead during morning examinations for viabilities on test day 9 (TD 9). Clinical observations observed for this rat included bradypnea, dehydration, limited use of hindlimbs, ptosis, lacrimation, ataxia, impaired righting reflex, lost righting reflex, decreased motor activity and low carriage. Dehydration and lacrimation were confirmed at necropsy. This death was considered treatment-related.

All other Linuron and Phenobarbital treated rats survived until terminal sacrifice.

3.1.2. Clinical Observations

3.1.2.1. Vehicle

There were no treatment-related clinical observations observed in the rats given the vehicle (0.25% methylcellulose). A missing/broken incisor, sparse hair coat and localized alopecia on the limbs occurred for two different rats.

3.1.2.2. Linuron

Sparse hair coat was observed in one rat in each of the 50, 100 and 150 mg/kg/day dosage groups. Ungroomed coat was observed in one rat in the 100 mg/kg/day dosage group. Decreased motor activity, ataxia, lost righting reflex, unresponsive to touch, lacrimation, chromorhinorrhea, chromodacryorrhea, limited use of limbs, cold to touch, and dehydration were observed in one or more than one rat in the 100 and/or 150 mg/kg/day dosage groups of rats treated with Linuron. In addition, impaired righting reflex, comatose, ptosis, perinasal substance, hunched posture, low carriage, excess salivation, perioral substance, bradypnea and scant feces were observed in one or more than one rat in the 150 mg/kg/day dosage group.

3.1.2.3. Phenobarbital

Ptosis was observed in one or more rats in the 25, 50 and 100 mg/kg/day dosage group rats treated with Phenobarbital. Chromodacryorrhea and chromorhinorrhea were observed in one or more rats in the 25 and 100 mg/kg/day dosage group rats treated with Phenobarbital. Incisor(s) misaligned was observed in one rat in the 25 mg/kg/day dosage group. Ataxia, impaired righting reflex, decreased motor activity, low carriage, limited use of limbs, and bradypnea were observed in one or more than one rat in the 50 and/or 100 mg/kg/day dosage groups treated with Phenobarbital. In addition, lost righting reflex, unresponsive to touch, comatose, lacrimation, no use of limbs, bent tail, cold to

touch, dehydration, substance on penis, hyperpnea, ungroomed coat, urine-stained abdominal fur and sparse hair coat were observed in one or more than one rat in the 100 mg/kg/day dosage group. Excess salivation (slight) was observed in two and one rats in the 25 and 50 mg/kg/day dosage groups, respectively.

3.2. Body Weight Gains (APPENDIX 9 - Figures 1 through 5, Summaries - Tables 1 through 3)

3.2.1. Linuron

Body weight gains were significantly decreased ($p \leq 0.05/8$)[a] at 50, 100 and 150 mg/kg/day in rats treated with Linuron over test days (TDs) 1 to 8, 8 to 15, and overall TDs 1 to 15.

3.2.2. Phenobarbital

Body weight gains were significantly decreased ($p \leq 0.05/8$[a]) at 100 mg/kg/day in rats treated with Phenobarbital over test days TDs 1 to 8, and overall TDs 1 to 15. Body weight gains in the 25 and 50 mg/kg/day dosage groups were not significantly different when compared to the vehicle control group values during any tabulated intervals.

3.3. Terminal Body Weights and Organ Weights (APPENDIX 9 - Figures 6 and 10 through 27, Summaries - Tables 2 through 5)

3.3.1. Linuron

Average final body weights were 403.4g, 353.1g, 344.5g and 321.2g for the vehicle control group and the 50, 100 and 150 mg/kg/day Linuron groups, respectively. Terminal body weights in the 50, 100 and 150 mg/kg/day dosage groups were 87.5%, 85.4% and 79.6%, respectively, of the vehicle control final body weights and were significantly decreased ($p \leq 0.05/8$[a]) in the 50, 100 and 150 mg/kg/day Linuron groups.

Absolute liver weights (85.0%, 87.8% and 87.1% of control) and accessory sex glands (88.4%, 85.3% and 72.2% of control) were significantly decreased ($p \leq 0.05$ or $p \leq 0.05/8$[a]) in the 50, 100 and 150 mg/kg/day dosage groups, respectively; absolute weights of the paired epididymides (94.5%, 92.9% and 86.5% of control) and prostate (90.6%, 83.9% and 74.0% of control) were significantly decreased ($p \leq 0.05$ or $p \leq 0.05/8$[a]) in the 100 and 150 mg/kg/day dosage groups, respectively; and seminal vesicles with fluid and coagulating gland (86.6%, 86.4% and 70.7% of control) were significantly decreased ($p \leq 0.05/8$[a]) in the 150 mg/kg/day dosage group of Linuron, when compared to the vehicle control group values. Absolute weights of the right testis (104.2%, 100.6% and 99.3% of control), left testis (103.5%, 100.0% and 99.2% of control), paired testes (103.8%, 100.3% and 99.2% of control) and thyroid gland (114.2%, 105.4% and 90.2% of control)

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a. A Bonferroni adjusted *p*-level.

weights were not significantly different when compared to the vehicle control group values.

When the absolute organ weight of a rat was adjusted for its terminal body weights, mean relative right testis (119.1%, 117.4% and 124.4% of control), left testis (118.4%, 116.8% and 124.3% of control), paired testes (118.7%, 117.2% and 124.3% of control) were significantly increased ($p \leq 0.05/8^{a}$) in the 50, 100 and 150 mg/kg/day dosage groups, when compared to the vehicle control group values. The relative thyroid gland (129.7%, 122.5% and 113.4% of control) weights were significantly increased ($p \leq 0.05$) in the 50 mg/kg/day dosage groups, when compared to the vehicle control group value. In addition, relative liver weights (97.3%, 102.8% and 109.3% of control) were significantly increased ($p \leq 0.05/8^{a}$) in the 150 mg/kg/day dosage group only when compared to the vehicle control group values. Relative weights of the paired epididymides (107.9%, 108.4% and 108.1% of control), prostate (102.7%, 96.8% and 91.1% of control), seminal vesicles with fluid and coagulating gland (98.1%, 100.0% and 88.0% of control) and accessory sex glands (100.2%, 98.5% and 89.4% of control) were not significantly different when compared to the vehicle control group values.

3.3.2. Phenobarbital

Average final body weights were 403.4g, 412.0g, 407.5g and 381.4g for the vehicle control group and the 25, 50 and 100 mg/kg/day Phenobarbital groups, respectively. Terminal body weights in the 25, 50 and 100 mg/kg/day dosage groups were 102.1%, 101.0% and 94.5%, respectively, of the vehicle control final body weights and were significantly decreased ($p \leq 0.05$) in rats in the 100 mg/kg/day dosage group when compared to the vehicle control group value. There were no significant differences in terminal body weights between the 25 and 50 mg/kg/day Phenobarbital treated groups and the vehicle control group.

Absolute liver weights (129.4%, 134.5% and 145.4% of control) and thyroid gland weights (131.2%, 123.2% and 124.5% of control) were significantly increased ($p \leq 0.05$ and/or $p \leq 0.05/8^{a}$) in rats in the 25, 50 and 100 mg/kg/day dosage group. Absolute weights of the right testis (101.2%, 103.3% and 103.4% of control), left testis (101.6%, 100.6% and 103.2% of control), paired testes (101.4%, 101.9% and 103.3% of control), paired epididymides (97.3%, 101.0% and 102.6% of control), prostate (103.0%, 109.0% and 94.2% of control), seminal vesicles with fluid and coagulating gland (102.3%, 105.5% and 100.0% of control) and accessory sex glands (102.6%, 107.1% and 97.4% of control) were not significantly different when compared to the vehicle control group values.

When the absolute organ weight of a rat was adjusted for its terminal body weight, mean relative liver weights (126.7%, 133.2% and 153.7% of control) and relative thyroid gland weights (128.2%, 121.6% and 130.8% of control) were significantly increased ($p \leq 0.05$ or $p \leq 0.05/8^{a}$) in rats in the 25, 50 and 100 mg/kg/day dosage groups, respectively, when

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a. A Bonferroni adjusted p-level.

compared to the vehicle control group values. In addition, relative right testis weights (99.1%, 102.1% and 109.1% of control), left testis weights (99.6%, 99.4% and 109.0% of control) and paired testes weights (99.3%, 100.7% and 109.0% of control) were significantly increased ($p \leq 0.05$) in the 100 mg/kg/day dosage group only when compared to the vehicle control group values. Relative weights of the paired epididymides (95.4%, 99.9% and 108.4% of control), prostate (100.4%, 107.3% and 99.0% of control), seminal vesicles with fluid and coagulating gland (100.7%, 104.7% and 104.6% of control) and accessory sex glands (100.6%, 105.8% and 102.1% of control) were not significantly different when compared to the vehicle control group values.

3.4. Food Consumption (APPENDIX 9 - Figures 7 through 9, Summaries - Tables 1 through 3)

3.4.1. Linuron

Food consumption values were significantly decreased ($p \leq 0.05$ or $p \leq 0.05/8$[a]) at 50, 100 and 150 mg/kg/day in rats treated with Linuron over test days (TDs) 1 to 8, 8 to 15, and overall TDs 1 to 15, when compared to the vehicle control group values.

3.4.2. Phenobarbital

Food consumption values were significantly decreased ($p \leq 0.05$ or $p \leq 0.05/8$[a]) at 100 mg/kg/day in rats treated with Phenobarbital over test days TDs 1 to 8, and overall TDs 1 to 15 when compared to the vehicle control group values. Feed consumption values in the 25 and 50 mg/kg/day dosage groups were not significantly different when compared to the vehicle control group values during any tabulated intervals.

3.5. Reproductive Hormone Analyses (APPENDIX 9 - Figures 28 through 36, Summary - Tables 6 and 7)

3.5.1. Linuron

Mean testosterone blood concentrations were 9.93, 4.83, 3.98 and 3.28 ng/mL (48.6%, 40.1% and 33.0% of control) for the vehicle control, 50, 100 and 150 mg/kg/day Linuron groups, respectively. All three dosage groups treated with Linuron were significantly ($p \leq 0.05$ or $p \leq 0.05/8$[a]) decreased when compared to the vehicle control group value.

Mean luteinizing hormone (LH) blood concentrations were 2.18, 1.75, 1.78 and 1.86 ng/mL (80.5%, 81.8% and 85.6% of control) for the vehicle control and the 50, 100 and 150 mg/kg/day Linuron groups, respectively. Only the 50 and 100 mg/kg/day dosage groups treated with Linuron were significantly ($p \leq 0.05$) decreased when compared to the vehicle control group value.

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a. A Bonferroni adjusted p-level.

Mean thyroid stimulating hormone (TSH) blood concentrations were 13.10, 9.84, 12.21 and 10.46 ng/mL (75.2%, 93.2% and 79.9% of control) for the vehicle control and the 50, 100 and 150 mg/kg/day Linuron groups, respectively. There were no significant differences in the blood levels between the vehicle control and the 50, 100 and 150 mg/kg/day Linuron groups.

Mean thyroxine (T4) blood concentrations were 4.73, 3.10, 1.82 and 1.54 µg/dL (65.5%, 38.5% and 32.5% of control) for the vehicle control and the 50, 100 and 150 mg/kg/day Linuron groups, respectively. All three dosage groups treated with Linuron were significantly ($p \leq 0.05/8$[a]) decreased when compared to the vehicle control group value.

Mean triiodothyronine (T3) blood concentrations were 81.65, 68.20, 65.62 and 64.47 ng/dL (83.5%, 80.4% and 79.0% of control) for the vehicle control and the 50, 100 and 150 mg/kg/day Linuron groups, respectively. All three dosage groups treated with Linuron were significantly ($p \leq 0.05/8$[a]) decreased when compared to the vehicle control group value.

Mean follicle stimulating hormone (FSH) blood concentrations were 14.81, 14.27, 15.62 and 15.91 ng/mL (96.4%, 105.5% and 107.4% of control) for the vehicle control and the 50, 100 and 150 mg/kg/day Linuron groups, respectively. There were no significant differences in the blood levels between the vehicle control and the 50, 100 and 150 mg/kg/day Linuron groups.

Mean estradiol blood concentrations were 25.40, 33.20, 40.95 and 37.74 pg/mL (130.7%, 161.2% and 148.6% of control) for the vehicle control, 50, 100 and 150 mg/kg/day Linuron groups, respectively. All three dosage groups treated with Linuron were significantly ($p \leq 0.05/8$[a]) increased when compared to the vehicle control group value.

Mean prolactin blood concentrations were 36.48, 4.74, 5.57 and 2.02 ng/mL (13.0%, 15.2% and 5.5% of control) for the vehicle control, 50, 100 and 150 mg/kg/day Linuron groups, respectively. All three dosage groups treated with Linuron were significantly ($p \leq 0.05/8$[a]) decreased when compared to the vehicle control group value.

Dihydrotestosterone (DHT) concentrations were 487.73, 345.97, 357.77 and 299.87 pg/mL for the vehicle control, 50, 100 and 150 mg/kg/day Linuron groups, respectively; DHT levels were decreased to 70.9%, 73.4% and 61.5% of control values in the 50, 100 and 150 mg/kg/day Linuron groups and were significantly ($p \leq 0.05$) decreased in the 150 mg/kg/day Linuron group.

The results of all hormone assays (testosterone, LH, TSH, T4, T3, FSH, estradiol, prolactin and DHT) were considered reliable based on the assay performance results which are available in APPENDIX 8.

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a. A Bonferroni adjusted p-level.

3.5.2. Phenobarbital

Mean testosterone blood concentrations were 9.93, 6.07, 3.50 and 2.19 ng/mL (61.2%, 35.2% and 22.1% of control) for the vehicle control, 25, 50 and 100 mg/kg/day Phenobarbital groups, respectively. The 50 and 100 mg/kg/day Phenobarbital dosage groups were significantly ($p \leq 0.05/8^a$) decreased when compared to the vehicle control group value.

Mean luteinizing hormone (LH) blood concentrations were 2.18, 1.81, 1.44 and 1.56 ng/mL (83.1%, 65.9% and 71.4% of control) for the vehicle control and the 25, 50 and 100 mg/kg/day Phenobarbital groups, respectively. All three dosage groups treated with Phenobarbital were significantly ($p \leq 0.05$ or $p \leq 0.05/8^a$) decreased when compared to the vehicle control group value.

Mean thyroid stimulating hormone (TSH) blood concentrations were 13.10, 23.35, 25.74 and 29.73 ng/mL (178.3%, 196.6% and 227.0% of control) for the vehicle control and the 25, 50 and 100 mg/kg/day Phenobarbital groups, respectively. All three dosage groups treated with Phenobarbital were significantly ($p \leq 0.05/8^a$) increased when compared to the vehicle control group value.

Mean thyroxine (T4) blood concentrations were 4.73, 3.75, 3.64 and 2.62 µg/dL (79.3%, 77.0% and 55.4 % of control) for the vehicle control and the 25, 50 and 100 mg/kg/day Phenobarbital groups, respectively. All three dosage groups treated with Phenobarbital were significantly ($p \leq 0.05/8^a$) decreased when compared to the vehicle control group value.

Mean triiodothyronine (T3) blood concentrations were 81.65, 64.85, 65.41 and 56.15 ng/dL (79.4%, 80.1% and 68.8% of control) for the vehicle control and the 25, 50 and 100 mg/kg/day Phenobarbital groups, respectively. All three dosage groups treated with Phenobarbital were significantly ($p \leq 0.05/8^a$) decreased when compared to the vehicle control group value.

Mean follicle stimulating hormone (FSH) blood concentrations were 14.81, 13.84, 12.46 and 12.28 ng/mL (93.5%, 84.1% and 82.9% of control) for the vehicle control and the 25, 50 and 100 mg/kg/day Phenobarbital groups, respectively. The 50 and 100 mg/kg/day dosage groups treated with Phenobarbital were significantly ($p \leq 0.05/8^a$) decreased when compared to the vehicle control group value

Mean estradiol blood concentrations were 25.40, 33.71, 36.52 and 38.52 pg/mL (132.7%, 143.8% and 151.6% of control) for the vehicle control, 25, 50 and 100 mg/kg/day Phenobarbital groups, respectively. All three dosage groups treated with Phenobarbital were significantly ($p \leq 0.05/8^a$) increased when compared to the vehicle control group value.

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a. A Bonferroni adjusted *p*-level.

Mean prolactin blood concentrations were 36.48, 14.05, 8.12 and 4.22 ng/mL (38.5%, 22.3% and 11.6% of control) for the vehicle control, 25, 50 and 100 mg/kg/day Phenobarbital groups, respectively. All three dosage groups treated with Phenobarbital were significantly ($p \leq 0.05$ or $p \leq 0.05/8^{a}$) decreased when compared to the vehicle control group value.

Dihydrotestosterone (DHT) blood concentrations were 487.73, 389.62, 301.38 and 248.85 pg/mL (79.9%, 61.8% and 51.0% of control) for the vehicle control, 25, 50 and 100 mg/kg/day Phenobarbital groups, respectively; DHT blood levels were significantly ($p \leq 0.05$ or $p \leq 0.05/8^{a}$) decreased in the 50 and 100 mg/kg/day Phenobarbital groups when compared to the vehicle control group value.

The results of all hormone assays (testosterone, LH, TSH, T4, T3, FSH, estradiol, prolactin and DHT) were considered reliable based on the assay performance results which are available in APPENDIX 8.

3.6. Gross Necropsy (Individual Data - APPENDIX 2)

3.6.1. Vehicle

All tissues appeared normal at terminal necropsy in the vehicle control group rats.

3.6.2. Linuron

Small seminal vesicles occurred in one, three, and six rats in the 50, 100 and 150 mg/kg/day dosages of Linuron, respectively. The right lobe of the thymus appeared red once in each of the 100 and 150 mg/kg/day dosage group rats and the spleen appeared black in one 150 mg/kg/day group rat. In addition, the accessory sex glands and prostrate appeared small in one 150 mg/kg/day group rat. All other tissues appeared normal at terminal necropsy.

3.6.3. Phenobarbital

Small seminal vesicles occurred in two rats in the 100 mg/kg/day dosage of Phenobarbital. All other tissues appeared normal at terminal necropsy.

3.7. Histopathology (APPENDIX 7)

3.7.1. Vehicle

There were no treatment-related microscopic changes observed in the testes, epididymides or thyroids of the rats given the vehicle (0.25% methylcellulose). Two control rats had minimal or mild hypertrophy of the thyroid follicular epithelium, which does occasionally occur spontaneously in male rats.

3.7.2. Linuron

There were no treatment-related microscopic changes observed in the testes, epididymides or thyroids of the rats given 150 mg/kg/day of Linuron.

3.7.3. Phenobarbital

Microscopic examination of the thyroid of the rats given 100 mg/kg/day of Phenobarbital revealed an increased incidence and severity (minimal to moderate) of hypertrophy and hyperplasia of the thyroid follicular epithelium. Histomorphologically, the change in the thyroid was characterized by increased size of the follicular epithelium (hypertrophy) and an increase in the amount of follicles and cellularity of the follicles (hyperplasia). There were no treatment-related microscopic changes observed in the testes or epididymides of the rats given the 100 mg/kg/day of Phenobarbital.

4. DISCUSSION

The 15-day intact male assay was developed by DuPont Haskell Laboratory[11, 12] as one component of a Tier I screening strategy for identifying endocrine-active compounds (EAC). In this report, two EACs (Linuron and Phenobarbital), each at three dose levels, were examined in order to evaluate their sensitivity in the assay. The EAC test compounds for this assay were the weak antiandrogenic herbicide, Linuron, and the thyroid modulating barbiturate, Phenobarbital, that works by enhancing thyroid hormone excretion. The endpoints evaluated included final body weight and organ weights (liver, thyroid gland, testes, epididymides, prostate, seminal vesicles with fluid, accessory sex gland), serum hormone concentrations (testosterone, estradiol, dihydrotestosterone, luteinizing hormone, follicle stimulating hormone, prolactin, T3, T4, TSH), and histopathology (testis, epididymis, and thyroid gland). For each compound, the results were compared to the expected pattern of responses based on the known mechanism of action.

4.1. General Toxicity

All dosages of Linuron used in these studies caused significant reductions in food consumption and body weight gains and terminal body weights, albeit treatment with Linuron as high as 150 mg/kg/day, for 15 consecutive days, caused no mortality. The

low dosage of Linuron (50 mg/kg/day) did not cause severe or significant adverse clinical observations. The decrease in absolute liver weight with Linuron treatment was likely secondary to body weight changes given that the relative liver weights were significantly increased at 150 mg/kg/day Linuron

Treatment with Phenobarbital at 100 mg/kg/day, for 15 consecutive days, caused one death, severe adverse clinical observations, significant reductions in body weight gains, terminal body weights and food consumption values. Altered clinical observations were seen at the middle dose level, 50 mg/kg/day, but there were no effects on body weight, body weight gains or food consumption values. Absolute liver weights and thyroid gland weights were significantly increased in rats in the 25, 50 and 100 mg/kg/day Phenobarbital dosage groups. Relative liver and relative thyroid gland weights were significantly increased in rats in the 25, 50 and 100 mg/kg/day Phenobarbital dosage groups. In addition, relative right, left, and paired testes weights were significantly increased in the 100 mg/kg/day Phenobarbital dosage group. Increases in relative weights of these organs were not surprising given that the weights of these organs are conserved with minor body weight changes.

Table A depicts the number/percentage of rats with a specific percent decrease in body weight of each rat compared to the control group mean body weights. The number of rats with larger decreases in body weight increased with dose for Linuron, demonstrating a dose response to decreases in body weight. All fifteen rats exposed to 150 mg/kg/day Linuron experienced greater than 11% reduction in total body weight, compared to the control group.

This decrease in body weight dose response does not occur for Phenobarbital. Approximately 73% of the 15 rats exposed to 100 mg/kg/day Phenobarbital experienced a total body weight loss of less than 10%.

Table A. Body Weight (BW) Decreases from the Control Group Mean at TD 15

Group Number	Treatment	Total Number in Group	≤10% Decrease in BW from Control Group n (%)	11-15% Decrease in BW from Control Group n (%)	16-20% Decrease in BW from Control Group n (%)	>20 Decrease in BW from Control Group n (%)
2	Linuron 50 MKD	15	5 (33.3%)	7 (46.7%)	1 (6.7%)	2 (13.3%)
3	Linuron 100 MKD	15	3 (20%)	4 (26.7%)	7 (46.7%)	1 (6.7%)
4	Linuron 150 MKD	15	0 (0%)	5 (33.3%)	3 (20%)	7 (46.7%)
5	Phenobarbital 25 MKD	15	13 (86.7%)	2 (13.3%)	0 (0%)	0 (0%)
6	Phenobarbital 50 MKD	15	14 (93.3%)	1 (6.7%)	0 (0%)	0 (0%)
7	Phenobarbital 100 MKD	14[a]	11 (78.6%)	2 (14.3%)	0 (0%)	1 (7.1%)

a. Male rat 10396 was found dead on test day 9.

4.2. Endocrinological and Thyroid Modulation Effects

The two compounds selected were both endocrine-active compounds (EACs). Linuron is a weak antiandrogen and Phenobarbital enhances thyroid hormone excretion.

Table B depicts the organ weight averages normalized by the control group mean organ weights. As expected, the accessory sex gland absolute weights were significantly decreased in all Linuron treatment groups but the relative accessory sex gland weights were not statistically different from control values. When the relative accessory sex gland weights of the rats exposed to Linuron were normalized to control values, only values for the rats in the 150 mg/kg/day Linuron exposure group were reduced (89.4% of controls). Phenobarbital exposure had no effect on normalized relative accessory sex gland weights (all groups were 100-106% of controls).

Table B. Organ Weight Averages - Percent (Absolute/Relative) of the Vehicle Control Group

Group	Treatment	Liver (Relative)	Paired Testes (Absolute)	Paired Epididymides (Absolute)	Prostate (Relative)	Seminal Vesicles with Fluid and Coagulating gland (Relative)	Accessory Sex Gland (Relative)	Thyroid (Relative)
2	Linuron 50 MKD	97.3	103.8	94.5	102.7	98.1	100.2	129.7
3	Linuron 100 MKD	102.8	100.3	92.9	96.8	100.0	98.5	122.5
4	Linuron 150 MKD	109.3	99.2	86.5	91.1	88.3	89.4	113.4
5	Pheno-barbital 25 MKD	126.7	101.4	97.3	100.4	100.7	100.6	128.2
6	Pheno-barbital 50 MKD	133.2	101.9	101.0	107.3	104.7	105. 8	121.6
7	Pheno-barbital 100 MKD	153.7	103.3	102.6	99.0	104.6	102.1	130.8

In general, Linuron exposure only slightly increased the absolute or relative thyroid gland weights (only the 50 mg/mg/day level was significant), whereas, Phenobarbital exposure, as expected, significantly increased both absolute and relative thyroid gland weights. When the relative thyroid gland weights of the rats exposed to Linuron and Phenobarbital were normalized to control values, the rats exposed to Linuron displayed a thyroid weight gain inversely related to dose and the rats exposed to Phenobarbital displayed a thyroid weight gain that was neither inversely related to dose or increased related to dose.

Phenobarbital did increase liver weight (127-154% of control values), an effect observed in a similar study [11].

As expected, no test substance-related microscopic changes were observed in the testes, epididymides or thyroids of the rats given the 150 mg/kg/day of Linuron, a weak antiandrogen. All microscopic changes observed in the testes, epididymides and thyroids of rats treated with Linuron were considered to have occurred spontaneously and were not treatment-related.

An increased incidence and severity of hypertrophy and hyperplasia of the thyroid follicular epithelium occurred in the rats given 100 mg/kg/day of Phenobarbital, an effect consistent with thyroid modulators. No test substance-related microscopic changes were observed in the testes or epididymides of the rats given 100 mg/kg/day of Phenobarbital. The testes and epididymides of rats in the 25 and 50 mg/kg/day levels were not examined microscopically. All microscopic changes observed in the testes and epididymides of rats treated with Phenobarbital were considered to have occurred spontaneously and were not treatment-related.

Table C depicts the selected hormone levels of each dosage group normalized by the control group mean hormone level. As expected, all doses of Linuron decreased testosterone (more than 50% below control levels); decreased dihydrotestosterone (more than 25% below control levels); decreased luteinizing hormone (15-20% below control levels); and increased estradiol (30-61% above control levels).

In addition, T4 (34-67% below control levels), T3 (16-21% below control levels), and prolactin (85-95% below control levels) blood concentrations were significantly less than the vehicle control group in all three Linuron treated groups, however, there was not a dose- dependent change in TSH level observed. The follicle stimulating hormone remained essentially unchanged.

As expected, Phenobarbital exposure increased thyroid hormone excretion. All doses decreased blood levels of T3 (20-31% below control levels) and T4 (23-45% below control levels); and increased thyroid stimulating hormone release (78-127% above control levels).

In addition, serum values for luteinizing hormone (17-34% below control levels) and prolactin (62-88% below control levels) concentrations were significantly less than the vehicle control group, in all three Phenobarbital treated groups. Testosterone (39-78% below control levels), follicle stimulating hormone (6-17% below control levels) and dihydrotestosterone (20-49% below control levels) blood concentrations were significantly decreased in the 50 and 100 mg/kg/day Phenobarbital groups when compared to the vehicle control group values. Estradiol (33-52% above control levels) was significantly increased compared to the vehicle control group values in all three Phenobarbital treated groups. These findings were also observed in a similar study [11].

Table C. Serum Hormone Averages - Percent of the Vehicle Control Group

Group	Treatment	TEST [a]	DHT	LH	Estra-diol	FSH	Pro-lactin	TSH	T4	T3
2	Linuron 50 mg/kg/day	48.6	70.9	80.5	130.7	96.4	13.0	75.2	65.5	83.5
3	Linuron 100 mg/kg/day	40.1	73.4	81.8	161.2	105.5	15.2	93.2	38.5	80.4
4	Linuron 150 mg/kg/day	33.0	61.5	85.6	148.6	107.4	5.5	79.9	32.5	79.0
5	Phenobarbital 25 mg/kg/day	61.2	79.9	83.1	132.7	93.5	38.5	178.3	79.3	79.4
6	Phenobarbital 50 mg/kg/day	35.2	61.8	65.9	143.9	84.1	22.3	196.6	77.0	80.1
7	Phenobarbital 100 mg/kg/day	22.1	51.0	71.4	151.6	82.9	11.6	227.0	55.4	68.8

a. TEST is used as an abbreviation for testosterone.

5. CONCLUSION

In conclusion, the 15-day intact male assay was able to identify Linuron and Phenobarbital as EACs at the dosages tested.

Linuron caused adverse clinical observations, significant decreases in body weight gains, terminal body weights and feed consumption values at all levels tested. Consistent with the mode of action for a weak androgen receptor antagonist Linuron did produced the expected decreases in absolute epididymal weights and absolute accessory sex gland weights, but only at doses producing a greater than 15% change in terminal body weights. Consistent with these organ weight changes, decreased blood testosterone, dihydrotestosterone, and luteinizing hormone levels, and increased blood estradiol levels were observed. The effects on thyroid weights are more difficult to interpret given that relative thyroid weights were statistically increased only at 50 mg/kg/day and the effects did not follow a dose-response relationship.

Phenobarbital caused one death in the 100 mg/kg/day dosage group and adverse clinical observations of varying severity, at all levels tested. The effects seen at 25 mg/kg/day were minimal compared to one death and the accompanying observations seen at 100 mg/kg/day. Significant decreases in body weight gains, terminal body weights and feed consumption were also observed at 100 mg/kg/day. Consistent with the mode of action of a thyroid modulator that acts by increasing the excretion of thyroid hormones, Phenobarbital increased relative liver and thyroid weights; decreased blood levels of T3 and T4, with the concomitant increase in estradiol and thyroid stimulating hormone; and altered reproductive hormone concentrations (decreased serum dihydrotestosterone, FSH, luteinizing hormone, prolactin and testosterone). Phenobarbital also increased the incidence and severity of hypertrophy and hyperplasia of the thyroid follicular epithelium in rats given 100 mg/kg/day.

Christopher J. Sanders FOR 02 MAY 2006

Raymond G. York, Ph.D., DABT Date
Principal Scientist
Associate Director of Research

Joseph W. Lech 5/2/06

Joseph W. Lech, B.S., LAT Date
Scientist
Study Director

6. REFERENCES

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7. PROTOCOL DEVIATIONS

1. The 6 hour postdose clinical observations were performed 3 to 37 minutes outside (late) of the protocol specified range for all rats dosed on 27 October 2005. This deviation did not adversely affect the outcome or interpretation of the study because the postchecks were ultimately performed and all data was captured.

2. The end time of exposure to carbon dioxide was not recorded for rats 10327 and 10381 in the 50 mg/kg/day Linuron and phenobarbital dosage groups, respectively, on 09 and 10 October 2005, TD 15. This deviation did not adversely affect the outcome or interpretation of the study because the exposure to the carbon dioxide did not cause mortality and the blood sample collections were performed according to the protocol.

3. The transfer of testes was not documented for rat 10375, in the 25 mg/kg/day Phenobarbital dosage group. This deviation did not adversely affect the outcome or interpretation of the study because the testes were transferred from Bouins solution to alcohol.

4. The date that the thyroid was trimmed was not recorded for rat 13070, in the 25 mg/kg/day Phenobarbital dosage group. This deviation did not adversely affect the outcome or interpretation of the study because the thyroid was processed and trimmed accordingly.

5. The date that the thyroid was weighed was not recorded for rat 10396, in the 100 mg/kg/day Phenobarbital dosage group. This deviation did not adversely affect the outcome or interpretation of the study because the thyroid was fixed in 10% NBF for 48 hours, trimmed and a weight was recorded according to protocol.

6. The epididymides were not individually identified for histopathological evaluation. This deviation did not adversely affect the outcome or interpretation of the study because there were no treatment-related microscopic changes observed histopathologically.

7. The tissues from the rat which was found dead, 10396, in the 100 mg/kg/day Phenobarbital dosage group, were sent for histopathological evaluation. This deviation did not adversely affect the outcome or interpretation of the study because this did not result in any loss of data.

8. The blood samples from the following rats were placed on wet ice and allowed to clot prior to centrifugation.

Animal Number	Test Substance	Dosage Level (mg/kg /day)	Animal Number	Test Substance	Dosage Level (mg/kg/day)
10301	Aqueous 0.25% (w/v) Methylcellulose	0	10347	Linuron	150
10302	Aqueous 0.25% (w/v) Methylcellulose	0	10348	Linuron	150
10303	Aqueous 0.25% (w/v) Methylcellulose	0	10361	Phenobarbital	25
10304	Aqueous 0.25% (w/v) Methylcellulose	0	10362	Phenobarbital	25
10316	Linuron	50	10363	Phenobarbital	25
10317	Linuron	50	10376	Phenobarbital	50
10318	Linuron	50	10377	Phenobarbital	50
10331	Linuron	100	10378	Phenobarbital	50
10332	Linuron	100	10391	Phenobarbital	100
10333	Linuron	100	10392	Phenobarbital	100
10346	Linuron	150	10393	Phenobarbital	100

This deviation did not adversely affect the outcome or interpretation of the study because the samples were collected and the serum was harvested.

All deviations are documented in the raw data.

Joseph W. Lech, B.S., LAT — Date
Scientist and Study Director

SUPPORTING DATA

APPENDICES 1 AND 2 - INDIVIDUAL DATA

PROTOCOL RTP00004: INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
(SPONSOR'S WORK ASSIGNMENT: WA 5-15)

APPENDIX 1 (PAGE 1): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

RAT #		DESCRIPTION	
DOSAGE GROUP 1		0.25% METHYLCELLULOSE	0 (VEHICLE) MG/KG/DAY
10301		NO ADVERSE FINDINGS	
10302		NO ADVERSE FINDINGS	
10303		NO ADVERSE FINDINGS	
10304		NO ADVERSE FINDINGS	
10305		NO ADVERSE FINDINGS	
10306		NO ADVERSE FINDINGS	
10307		NO ADVERSE FINDINGS	
10308		NO ADVERSE FINDINGS	
10309		NO ADVERSE FINDINGS	
10310		NO ADVERSE FINDINGS	
10311		NO ADVERSE FINDINGS	
10312	TD(5- 8)	SPARSE HAIR COAT	
	TD(9- 15)	LOCALIZED ALOPECIA: LIMB(S)a	
10313		NO ADVERSE FINDINGS	
10314		NO ADVERSE FINDINGS	
10315	TD(14- 15)	INCISOR(S): MISSING/BROKEN a	
DOSAGE GROUP 2		LINURON	50 MG/KG/DAY
10316		NO ADVERSE FINDINGS	
10317		NO ADVERSE FINDINGS	
10318	TD(6- 15)	SPARSE HAIR COAT a	
10319		NO ADVERSE FINDINGS	
10320		NO ADVERSE FINDINGS	
10321		NO ADVERSE FINDINGS	
10322		NO ADVERSE FINDINGS	
10323		NO ADVERSE FINDINGS	
10324		NO ADVERSE FINDINGS	
10325		NO ADVERSE FINDINGS	
10326		NO ADVERSE FINDINGS	
10327		NO ADVERSE FINDINGS	
10328		NO ADVERSE FINDINGS	
10329		NO ADVERSE FINDINGS	
10330		NO ADVERSE FINDINGS	

DS = DAY OF STUDY
a. Observation confirmed at necropsy.

PROTOCOL RTP00004: INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
(SPONSOR'S WORK ASSIGNMENT: WA 5-15)

APPENDIX 1 (PAGE 2): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

RAT #		DESCRIPTION
DOSAGE GROUP 3		LINURON 100 MG/KG/DAY
10331	TD(7- 15)	DEHYDRATION a
	TD(9)	CHROMODACRYORRHEA
	TD(14)	CHROMORHINORRHEA
	TD(14)	CHROMODACRYORRHEA
10332	TD(4- 5)	ATAXIA
	TD(6- 10)	DEHYDRATION
10333	TD(3- 6)	CHROMORHINORRHEA
	TD(4- 5)	ATAXIA
	TD(7- 11)	DEHYDRATION
10334	TD(2- 3)	LACRIMATION
	TD(2- 4)	DECREASED MOTOR ACTIVITY
	TD(2- 4)	LOST RIGHTING REFLEX
	TD(3)	UNRESPONSIVE TO TOUCH
	TD(3)	CHROMORHINORRHEA
	TD(3)	DEHYDRATION
	TD(3)	UNGROOMED COAT
	TD(3- 4)	CHROMODACRYORRHEA
	TD(3- 4)	COLD TO TOUCH
	TD(7)	DEHYDRATION
10335		NO ADVERSE FINDINGS
10336	TD(2- 4)	ATAXIA
	TD(3)	CHROMORHINORRHEA
	TD(4- 5)	LIMITED USE OF BOTH HINDLIMBS
	TD(6- 7)	DEHYDRATION
	TD(10- 13)	DEHYDRATION
10337	TD(3)	CHROMORHINORRHEA
10338		NO ADVERSE FINDINGS
10339	TD(2)	ATAXIA
	TD(4)	ATAXIA
	TD(6- 9)	SPARSE HAIR COAT
	TD(10- 15)	LOCALIZED ALOPECIA: LIMB(S)a
10340	TD(5)	ATAXIA
10341	TD(3)	ATAXIA
10342	TD(2)	ATAXIA
	TD(3)	CHROMORHINORRHEA
	TD(9- 10)	DEHYDRATION
10343		NO ADVERSE FINDINGS
10344	TD(2)	ATAXIA
10345		NO ADVERSE FINDINGS

TD = TEST DAY
a. Observation confirmed at necropsy.

PROTOCOL RTP00004: INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
(SPONSOR'S WORK ASSIGNMENT: WA 5-15)

APPENDIX 1 (PAGE 3): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

RAT #		DESCRIPTION	
DOSAGE GROUP 4		LINURON	150 MG/KG/DAY
10346	TD(4)	CHROMORHINORRHEA	
	TD(5)	IMPAIRED RIGHTING REFLEX	
	TD(6)	ATAXIA	
	TD(6)	DEHYDRATION	
	TD(11)	RED PERINASAL SUBSTANCE	
	TD(11- 12)	EXCESS SALIVATION - EXTREME	
	TD(12)	RED PERIORAL SUBSTANCE	
	TD(14)	CHROMORHINORRHEA	
	TD(14)	DEHYDRATION	
10347	TD(2- 3)	DECREASED MOTOR ACTIVITY	
	TD(2- 8)	ATAXIA	
	TD(3- 5)	LIMITED USE OF RIGHT HINDLIMB AND/OR BOTH HINDLIMBS	
	TD(4- 5)	CHROMORHINORRHEA	
	TD(5)	IMPAIRED RIGHTING REFLEX	
	TD(5- 15)	DEHYDRATION a	
	TD(11)	CHROMORHINORRHEA	
10348	TD(2- 5)	ATAXIA	
	TD(4- 5)	CHROMORHINORRHEA	
	TD(5)	IMPAIRED RIGHTING REFLEX	
	TD(5- 7)	DEHYDRATION	
	TD(6)	HUNCHED POSTURE	
	TD(9- 15)	DEHYDRATION a	
10349	TD(2)	IMPAIRED RIGHTING REFLEX	
	TD(2)	LACRIMATION	
	TD(2- 3)	DECREASED MOTOR ACTIVITY	
	TD(5- 12)	DEHYDRATION	
	TD(12)	CHROMORHINORRHEA	
	TD(14)	DEHYDRATION	

TD = TEST DAY
a. Observation confirmed at necropsy.

PROTOCOL RTP00004: INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
(SPONSOR'S WORK ASSIGNMENT: WA 5-15)

APPENDIX 1 (PAGE 4): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

RAT #		DESCRIPTION	
DOSAGE GROUP 4		LINURON	150 MG/KG/DAY
10350	TD(2)	CHROMORHINORRHEA	
	TD(2- 6)	DECREASED MOTOR ACTIVITY	
	TD(3- 4)	IMPAIRED RIGHTING REFLEX	
	TD(5)	ATAXIA	
	TD(5)	LACRIMATION	
	TD(5)	BRADYPNEA	
	TD(5- 6)	LOST RIGHTING REFLEX	
	TD(5- 7)	COLD TO TOUCH	
	TD(5- 15)	DEHYDRATION	
	TD(6)	LIMITED USE OF BOTH HINDLIMBS AND FORELIMBS	
	TD(7- 9)	ATAXIA	
	TD(10- 11)	DECREASED MOTOR ACTIVITY	
	TD(10- 11)	IMPAIRED RIGHTING REFLEX	
10351	TD(2)	IMPAIRED RIGHTING REFLEX	
	TD(2- 3)	DECREASED MOTOR ACTIVITY	
	TD(3)	LOST RIGHTING REFLEX	
	TD(3)	LACRIMATION	
	TD(3)	PTOSIS	
	TD(3- 10)	ATAXIA	
	TD(4- 9)	DEHYDRATION	
	TD(5)	DECREASED MOTOR ACTIVITY	
	TD(6- 9)	IMPAIRED RIGHTING REFLEX	
	TD(7)	COLD TO TOUCH	
	TD(7)	LOW CARRIAGE	
	TD(7- 9)	CHROMORHINORRHEA	
	TD(7- 9)	LIMITED USE OF BOTH HINDLIMBS	
	TD(7- 10)	DECREASED MOTOR ACTIVITY	
10352	TD(3)	ATAXIA	
	TD(4)	HUNCHED POSTURE	
	TD(4- 14)	DEHYDRATION	

TD = TEST DAY

PROTOCOL RTP00004: INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
(SPONSOR'S WORK ASSIGNMENT: WA 5-15)

APPENDIX 1 (PAGE 5): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

RAT #		DESCRIPTION	
DOSAGE GROUP 4		LINURON	150 MG/KG/DAY
10353	TD(4)	ATAXIA	
	TD(4)	IMPAIRED RIGHTING REFLEX	
	TD(4)	LACRIMATION	
	TD(4- 14)	DEHYDRATION	
	TD(5)	CHROMODACRYORRHEA	
	TD(5- 6)	SCANT FECES	
	TD(6- 15)	LOCALIZED ALOPECIA: UNDERSIDE a	
	TD(6- 15)	LOCALIZED ALOPECIA: LIMB(S)a	
	TD(9)	CHROMODACRYORRHEA	
	TD(12)	CHROMODACRYORRHEA	
10354	TD(2)	DECREASED MOTOR ACTIVITY	
	TD(2)	IMPAIRED RIGHTING REFLEX	
	TD(2- 3)	COLD TO TOUCH	
	TD(3)	UNRESPONSIVE TO TOUCH	
	TD(3)	COMATOSE	
	TD(3)	LACRIMATION	
	TD(3)	PTOSIS	
	TD(3)	BRADYPNEA	
	TD(3- 4)	LOST RIGHTING REFLEX	
	TD(4)	DECREASED MOTOR ACTIVITY	
	TD(4)	ATAXIA	
	TD(4- 15)	DEHYDRATION a	
	TD(5- 10)	SCANT FECES	
	TD(9)	IMPAIRED RIGHTING REFLEX	
	TD(9- 10)	DECREASED MOTOR ACTIVITY	
	TD(9- 10)	ATAXIA	
10355	TD(2- 15)	SPARSE HAIR COAT a	
	TD(3- 5)	ATAXIA	
	TD(5)	LIMITED USE OF BOTH HINDLIMBS	
	TD(6- 13)	DEHYDRATION	

TD = TEST DAY
a. Observation confirmed at necropsy.

PROTOCOL RTP00004: INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
(SPONSOR'S WORK ASSIGNMENT: WA 5-15)

APPENDIX 1 (PAGE 6): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

RAT #		DESCRIPTION
DOSAGE GROUP 4		LINURON 150 MG/KG/DAY
10356	TD(2)	DECREASED MOTOR ACTIVITY
	TD(2)	LOST RIGHTING REFLEX
	TD(2)	BRADYPNEA
	TD(3- 4)	IMPAIRED RIGHTING REFLEX
	TD(4)	HUNCHED POSTURE
	TD(4- 5)	DECREASED MOTOR ACTIVITY
	TD(5)	LOST RIGHTING REFLEX
	TD(5)	LIMITED USE OF BOTH HINDLIMBS AND FORELIMBS
	TD(5)	DEHYDRATION
	TD(5)	BRADYPNEA
	TD(9)	IMPAIRED RIGHTING REFLEX
	TD(9- 14)	DECREASED MOTOR ACTIVITY
	TD(9- 15)	DEHYDRATION a
	TD(11- 14)	IMPAIRED RIGHTING REFLEX
	TD(14)	CHROMORHINORRHEA
10357	TD(3)	ATAXIA
	TD(4)	IMPAIRED RIGHTING REFLEX
	TD(4- 5)	DEHYDRATION
	TD(5)	DECREASED MOTOR ACTIVITY
	TD(9)	DEHYDRATION
	TD(12)	DEHYDRATION
10358	TD(3)	ATAXIA
	TD(4- 15)	DEHYDRATION a
	TD(5)	ATAXIA
10359	TD(2)	DECREASED MOTOR ACTIVITY
	TD(2)	LOST RIGHTING REFLEX
	TD(2)	LACRIMATION
	TD(2)	COLD TO TOUCH
	TD(2)	BRADYPNEA
	TD(2- 3)	PTOSIS
	TD(3)	IMPAIRED RIGHTING REFLEX
	TD(3- 5)	DEHYDRATION
	TD(5)	DECREASED MOTOR ACTIVITY
	TD(5)	IMPAIRED RIGHTING REFLEX
	TD(5)	LIMITED USE OF BOTH HINDLIMBS
	TD(5)	BRADYPNEA
	TD(5- 6)	ATAXIA
	TD(8- 9)	IMPAIRED RIGHTING REFLEX
	TD(12- 13)	DEHYDRATION

TD = TEST DAY
a. Observation confirmed at necropsy.

PROTOCOL RTP00004: INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
(SPONSOR'S WORK ASSIGNMENT: WA 5-15)

APPENDIX 1 (PAGE 7): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

RAT #		DESCRIPTION	
DOSAGE GROUP 4		LINURON	150 MG/KG/DAY
10360	TD(2- 3)	ATAXIA	
	TD(2- 4)	CHROMORHINORRHEA	
	TD(3- 6)	DECREASED MOTOR ACTIVITY	
	TD(3- 15)	DEHYDRATION a	
	TD(4- 6)	IMPAIRED RIGHTING REFLEX	
	TD(5)	BRADYPNEA	
	TD(5- 6)	ATAXIA	

TD = TEST DAY
a. Observation confirmed at necropsy.

PROTOCOL RTP00004: INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
(SPONSOR'S WORK ASSIGNMENT: WA 5-15)

APPENDIX 1 (PAGE 8): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

RAT #		DESCRIPTION	
DOSAGE GROUP 5		PHENOBARBITAL	25 MG/KG/DAY
10361		NO ADVERSE FINDINGS	
10362		NO ADVERSE FINDINGS	
10363		NO ADVERSE FINDINGS	
10364	TD(7)	EXCESS SALIVATION - SLIGHT	
10365		NO ADVERSE FINDINGS	
10366		NO ADVERSE FINDINGS	
10367		NO ADVERSE FINDINGS	
10368		NO ADVERSE FINDINGS	
10369		NO ADVERSE FINDINGS	
10370		NO ADVERSE FINDINGS	
10371		NO ADVERSE FINDINGS	
10372		NO ADVERSE FINDINGS	
10373	TD(4)	PTOSIS	
	TD(10)	CHROMORHINORRHEA	
	TD(10- 13)	CHROMODACRYORRHEA	
	TD(10- 15)	INCISOR(S): MISALIGNED a	
	TD(12)	CHROMORHINORRHEA	
10374		NO ADVERSE FINDINGS	
10375	TD(9)	EXCESS SALIVATION - SLIGHT	

TD = TEST DAY
a. Observation confirmed at necropsy.

PROTOCOL RTP00004: INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
(SPONSOR'S WORK ASSIGNMENT: WA 5-15)

APPENDIX 1 (PAGE 9): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

RAT #		DESCRIPTION
DOSAGE GROUP 6		PHENOBARBITAL 50 MG/KG/DAY
10376	TD(4)	ATAXIA
10377	TD(1- 7)	ATAXIA
	TD(3- 4)	DECREASED MOTOR ACTIVITY
	TD(3- 5)	IMPAIRED RIGHTING REFLEX
	TD(4)	LOW CARRIAGE
	TD(6- 7)	PTOSIS
10378	TD(2- 5)	ATAXIA
	TD(3)	DECREASED MOTOR ACTIVITY
	TD(3- 4)	IMPAIRED RIGHTING REFLEX
	TD(7)	ATAXIA
10379	TD(2- 7)	ATAXIA
	TD(4)	IMPAIRED RIGHTING REFLEX
	TD(11)	EXCESS SALIVATION - SLIGHT
10380	TD(2)	ATAXIA
	TD(2- 4)	IMPAIRED RIGHTING REFLEX
10381	TD(2)	DECREASED MOTOR ACTIVITY
	TD(2- 3)	IMPAIRED RIGHTING REFLEX
	TD(2- 5)	ATAXIA
	TD(4)	PTOSIS
	TD(7- 10)	ATAXIA
	TD(13)	ATAXIA
10382	TD(2)	ATAXIA
	TD(2)	DECREASED MOTOR ACTIVITY
	TD(2- 3)	IMPAIRED RIGHTING REFLEX
	TD(4)	ATAXIA
	TD(6)	BRADYPNEA
	TD(6- 7)	ATAXIA
10383	TD(2)	ATAXIA
	TD(2)	IMPAIRED RIGHTING REFLEX
	TD(2)	DECREASED MOTOR ACTIVITY
	TD(2)	LIMITED USE OF BOTH HINDLIMBS
	TD(4- 5)	ATAXIA
10384		NO ADVERSE FINDINGS

TD = TEST DAY

PROTOCOL RTP00004: INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
(SPONSOR'S WORK ASSIGNMENT: WA 5-15)

APPENDIX 1 (PAGE 10): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

RAT #		DESCRIPTION	
DOSAGE GROUP 6		PHENOBARBITAL	50 MG/KG/DAY
10385	TD(2)	DECREASED MOTOR ACTIVITY	
	TD(2- 3)	IMPAIRED RIGHTING REFLEX	
	TD(2- 5)	ATAXIA	
	TD(9)	ATAXIA	
	TD(13)	ATAXIA	
10386	TD(2- 5)	PTOSIS	
	TD(3- 5)	ATAXIA	
10387	TD(7- 8)	ATAXIA	
	TD(7- 8)	PTOSIS	
10388		NO ADVERSE FINDINGS	
10389	TD(4)	ATAXIA	
10390	TD(10)	ATAXIA	

TD = TEST DAY

PROTOCOL RTP00004: INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
(SPONSOR'S WORK ASSIGNMENT: WA 5-15)

APPENDIX 1 (PAGE 11): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

RAT #		DESCRIPTION
DOSAGE GROUP 7		PHENOBARBITAL 100 MG/KG/DAY
10391	TD(1- 14)	ATAXIA
	TD(2- 4)	IMPAIRED RIGHTING REFLEX
	TD(3)	DECREASED MOTOR ACTIVITY
	TD(3- 14)	PTOSIS
	TD(4)	LACRIMATION
10392	TD(1- 2)	ATAXIA
	TD(2- 4)	LIMITED USE OF BOTH HINDLIMBS
	TD(2- 6)	LOST RIGHTING REFLEX
	TD(2- 6)	LACRIMATION
	TD(3- 4)	DECREASED MOTOR ACTIVITY
	TD(3- 14)	PTOSIS
	TD(5)	UNRESPONSIVE TO TOUCH
	TD(5- 6)	COMATOSE
	TD(5- 6)	COLD TO TOUCH
	TD(5- 8)	BRADYPNEA
	TD(6- 7)	CHROMODACRYORRHEA
	TD(6- 7)	URINE-STAINED ABDOMINAL FUR
	TD(6- 8)	DECREASED MOTOR ACTIVITY
	TD(6- 8)	LIMITED USE OF BOTH HINDLIMBS AND/OR BOTH FORELIMBS
	TD(7- 11)	IMPAIRED RIGHTING REFLEX
	TD(7- 15)	DEHYDRATION a
	TD(10- 14)	ATAXIA
	TD(11)	LIMITED USE OF BOTH HINDLIMBS
	TD(13- 14)	IMPAIRED RIGHTING REFLEX
	TD(13- 14)	LIMITED USE OF BOTH HINDLIMBS
10393	TD(1- 3)	ATAXIA
	TD(1- 14)	PTOSIS
	TD(2- 4)	LOST RIGHTING REFLEX
	TD(2- 5)	LIMITED USE OF BOTH HINDLIMBS
	TD(2- 10)	DECREASED MOTOR ACTIVITY
	TD(3)	IMPAIRED RIGHTING REFLEX
	TD(4- 9)	BRADYPNEA
	TD(5- 7)	IMPAIRED RIGHTING REFLEX
	TD(5- 14)	ATAXIA
	TD(7)	LOW CARRIAGE
	TD(8- 12)	DEHYDRATION
	TD(9- 13)	IMPAIRED RIGHTING REFLEX

TD = TEST DAY
a. Observation confirmed at necropsy.

PROTOCOL RTP00004: INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
(SPONSOR'S WORK ASSIGNMENT: WA 5-15)

APPENDIX 1 (PAGE 12): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

RAT #		DESCRIPTION	
DOSAGE GROUP 7		PHENOBARBITAL	100 MG/KG/DAY
10394	TD(1)	PTOSIS	
	TD(1- 3)	ATAXIA	
	TD(1- 15)	TAIL BENT a	
	TD(2- 4)	LOST RIGHTING REFLEX	
	TD(2- 5)	DECREASED MOTOR ACTIVITY	
	TD(3- 5)	PTOSIS	
	TD(5)	LOW CARRIAGE	
	TD(5- 14)	ATAXIA	
	TD(14)	IMPAIRED RIGHTING REFLEX	
	TD(14)	LOW CARRIAGE	
10395	TD(1)	ATAXIA	
	TD(2- 4)	LACRIMATION	
	TD(2- 6)	LOST RIGHTING REFLEX	
	TD(2- 7)	DECREASED MOTOR ACTIVITY	
	TD(3)	LIMITED USE OF BOTH HINDLIMBS	
	TD(3)	HYPERPNEA	
	TD(3- 5)	PTOSIS	
	TD(3- 7)	NO USE OF BOTH HINDLIMBS AND FORELIMBS	
	TD(4- 5)	BRADYPNEA	
	TD(5)	CHROMORHINORRHEA	
	TD(6)	CHROMODACRYORRHEA	
	TD(6- 7)	IMPAIRED RIGHTING REFLEX	
	TD(6- 15)	DEHYDRATION a	
	TD(7)	BRADYPNEA	
	TD(8- 14)	ATAXIA	
	TD(9- 14)	IMPAIRED RIGHTING REFLEX	
	TD(9- 14)	PTOSIS	
	TD(10- 14)	BRADYPNEA	
	TD(11- 14)	SPARSE HAIR COAT	

TD = TEST DAY
a. Observation confirmed at necropsy.

PROTOCOL RTP00004: INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
(SPONSOR'S WORK ASSIGNMENT: WA 5-15)

APPENDIX 1 (PAGE 13): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

RAT #		DESCRIPTION	
DOSAGE GROUP 7		PHENOBARBITAL	100 MG/KG/DAY
10396	TD(1- 2)	ATAXIA	
	TD(2)	IMPAIRED RIGHTING REFLEX	
	TD(2- 8)	DECREASED MOTOR ACTIVITY	
	TD(2- 8)	PTOSIS	
	TD(3)	LOST RIGHTING REFLEX	
	TD(3)	LACRIMATION	
	TD(3- 5)	LOW CARRIAGE	
	TD(3- 5)	LIMITED USE OF BOTH HINDLIMBS	
	TD(4- 5)	BRADYPNEA	
	TD(5- 8)	DEHYDRATION a	
	TD(6)	IMPAIRED RIGHTING REFLEX	
	TD(6- 8)	ATAXIA	
	TD(7- 8)	LIMITED USE OF BOTH HINDLIMBS	
	TD(7- 8)	LOW CARRIAGE	
	TD(7- 8)	BRADYPNEA	
	TD(8)	LACRIMATION a	
	TD(9)	FOUND DEAD	
10397	TD(2- 5)	LOST RIGHTING REFLEX	
	TD(2- 14)	DECREASED MOTOR ACTIVITY	
	TD(2- 14)	PTOSIS	
	TD(3)	ATAXIA	
	TD(3)	LOW CARRIAGE	
	TD(3- 8)	CHROMODACRYORRHEA	
	TD(4- 6)	NO USE OF BOTH HINDLIMBS AND FORELIMBS	
	TD(5)	LACRIMATION	
	TD(5- 6)	IMPAIRED RIGHTING REFLEX	
	TD(5- 10)	BRADYPNEA	
	TD(6- 15)	DEHYDRATION a	
	TD(7)	COMATOSE	
	TD(7- 8)	LACRIMATION	
	TD(8- 9)	NO USE OF BOTH HINDLIMBS	
	TD(8- 10)	LOST RIGHTING REFLEX	
	TD(8- 10)	LIMITED USE OF BOTH FORELIMBS	
	TD(8- 14)	ATAXIA	
	TD(9- 14)	LOW CARRIAGE	
	TD(10)	CHROMODACRYORRHEA	
	TD(10- 14)	IMPAIRED RIGHTING REFLEX	
	TD(14)	LACRIMATION	
	TD(14)	CHROMODACRYORRHEA	

TD = TEST DAY
a. Observation confirmed at necropsy.

PROTOCOL RTP00004: INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
(SPONSOR'S WORK ASSIGNMENT: WA 5-15)

APPENDIX 1 (PAGE 14): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

RAT #		DESCRIPTION	
DOSAGE GROUP 7		PHENOBARBITAL	100 MG/KG/DAY
10398	TD(1- 2)	LOST RIGHTING REFLEX	
	TD(1- 14)	DECREASED MOTOR ACTIVITY	
	TD(2)	UNRESPONSIVE TO TOUCH	
	TD(2)	LACRIMATION	
	TD(2)	LIMITED USE OF BOTH FORELIMBS	
	TD(2)	NO USE OF BOTH HINDLIMBS	
	TD(2- 3)	CHROMODACRYORRHEA	
	TD(2- 3)	COLD TO TOUCH	
	TD(2- 3)	UNGROOMED COAT	
	TD(2- 10)	IMPAIRED RIGHTING REFLEX	
	TD(2- 13)	PTOSIS	
	TD(2- 14)	ATAXIA	
	TD(4)	LIMITED USE OF BOTH HINDLIMBS	
	TD(5- 13)	DEHYDRATION	
	TD(6- 14)	LOW CARRIAGE	
	TD(8- 9)	LIMITED USE OF BOTH HINDLIMBS	
	TD(10- 11)	BRADYPNEA	
	TD(14)	IMPAIRED RIGHTING REFLEX	
	TD(14)	LIMITED USE OF BOTH HINDLIMBS	
10399	TD(1- 4)	ATAXIA	
	TD(2)	LOST RIGHTING REFLEX	
	TD(2)	DECREASED MOTOR ACTIVITY	
	TD(2)	PTOSIS	
	TD(3- 4)	LOW CARRIAGE	
	TD(3- 8)	IMPAIRED RIGHTING REFLEX	
	TD(4)	PTOSIS	
	TD(5)	LIMITED USE OF BOTH HINDLIMBS	
	TD(6- 9)	DECREASED MOTOR ACTIVITY	
	TD(6- 14)	ATAXIA	
	TD(7- 8)	LOW CARRIAGE	
	TD(9- 11)	PTOSIS	
	TD(10- 15)	DEHYDRATION a	
	TD(13- 14)	PTOSIS	

TD = TEST DAY
a. Observation confirmed at necropsy.

PROTOCOL RTP00004: INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
(SPONSOR'S WORK ASSIGNMENT: WA 5-15)

APPENDIX 1 (PAGE 15): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

RAT #		DESCRIPTION	
DOSAGE GROUP 7		PHENOBARBITAL	100 MG/KG/DAY
10400	TD(1- 5)	ATAXIA	
	TD(2)	LOST RIGHTING REFLEX	
	TD(2)	DECREASED MOTOR ACTIVITY	
	TD(2- 6)	PTOSIS	
	TD(3- 6)	IMPAIRED RIGHTING REFLEX	
	TD(4- 15)	DEHYDRATION a	
	TD(5- 9)	LIMITED USE OF BOTH HINDLIMBS	
	TD(5- 10)	DECREASED MOTOR ACTIVITY	
	TD(6)	LOW CARRIAGE	
	TD(6)	BRADYPNEA	
	TD(7- 14)	ATAXIA	
	TD(11)	IMPAIRED RIGHTING REFLEX	
	TD(13- 14)	PTOSIS	
10401	TD(1)	ATAXIA	
	TD(1)	IMPAIRED RIGHTING REFLEX	
	TD(2)	LOST RIGHTING REFLEX	
	TD(2)	DECREASED MOTOR ACTIVITY	
	TD(2)	LIMITED USE OF BOTH HINDLIMBS AND FORELIMBS	
	TD(2- 3)	BRADYPNEA	
	TD(3)	LOW CARRIAGE	
	TD(3)	CHROMODACRYORRHEA	
	TD(3- 9)	DEHYDRATION	
	TD(3- 13)	PTOSIS	
	TD(3- 14)	ATAXIA	
	TD(4- 5)	DECREASED MOTOR ACTIVITY	
	TD(5)	LOW CARRIAGE	
	TD(5- 9)	BRADYPNEA	
	TD(5- 12)	IMPAIRED RIGHTING REFLEX	
	TD(7- 9)	LIMITED USE OF BOTH HINDLIMBS	
	TD(12)	DECREASED MOTOR ACTIVITY	
	TD(14)	IMPAIRED RIGHTING REFLEX	

TD = TEST DAY
a. Observation confirmed at necropsy.

PROTOCOL RTP00004: INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
(SPONSOR'S WORK ASSIGNMENT: WA 5-15)

APPENDIX 1 (PAGE 16): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

RAT #		DESCRIPTION
DOSAGE GROUP 7		PHENOBARBITAL 100 MG/KG/DAY
10402	TD(1)	DECREASED MOTOR ACTIVITY
	TD(1- 14)	ATAXIA
	TD(2)	IMPAIRED RIGHTING REFLEX
	TD(2)	LOW CARRIAGE
	TD(3- 12)	PTOSIS
	TD(4- 5)	LOW CARRIAGE
	TD(5)	BRADYPNEA
	TD(9- 10)	LIMITED USE OF BOTH HINDLIMBS
	TD(10- 12)	IMPAIRED RIGHTING REFLEX
	TD(14)	IMPAIRED RIGHTING REFLEX
	TD(14)	PTOSIS
10403	TD(1)	LOST RIGHTING REFLEX
	TD(1- 2)	DECREASED MOTOR ACTIVITY
	TD(1- 12)	ATAXIA
	TD(2)	CHROMODACRYORRHEA
	TD(2- 4)	PTOSIS
	TD(4- 5)	IMPAIRED RIGHTING REFLEX
	TD(4- 5)	LOW CARRIAGE
	TD(4- 5)	BRADYPNEA
	TD(8)	LOW CARRIAGE
	TD(8- 9)	BRADYPNEA
	TD(8- 12)	PTOSIS
	TD(10- 12)	LIMITED USE OF BOTH HINDLIMBS
	TD(11)	IMPAIRED RIGHTING REFLEX
	TD(12- 13)	DEHYDRATION
	TD(14)	ATAXIA
10404	TD(1)	IMPAIRED RIGHTING REFLEX
	TD(1- 4)	ATAXIA
	TD(3)	IMPAIRED RIGHTING REFLEX
	TD(3- 5)	DECREASED MOTOR ACTIVITY
	TD(3- 9)	PTOSIS
	TD(4)	PENIS: RED SUBSTANCE
	TD(5)	IMPAIRED RIGHTING REFLEX
	TD(7- 8)	IMPAIRED RIGHTING REFLEX
	TD(7- 8)	LOW CARRIAGE
	TD(7- 9)	BRADYPNEA
	TD(7- 13)	ATAXIA
	TD(8- 12)	DECREASED MOTOR ACTIVITY
	TD(12)	IMPAIRED RIGHTING REFLEX

TD = TEST DAY

PROTOCOL RTP00004: INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
(SPONSOR'S WORK ASSIGNMENT: WA 5-15)

APPENDIX 1 (PAGE 17): CLINICAL OBSERVATIONS - INDIVIDUAL DATA

RAT #		DESCRIPTION	
DOSAGE GROUP 7		PHENOBARBITAL	100 MG/KG/DAY
10405	TD(1)	DECREASED MOTOR ACTIVITY	
	TD(1- 5)	IMPAIRED RIGHTING REFLEX	
	TD(1- 12)	PTOSIS	
	TD(1- 14)	ATAXIA	
	TD(2)	LOW CARRIAGE	
	TD(2)	LACRIMATION	
	TD(4)	LACRIMATION	
	TD(4- 5)	LOW CARRIAGE	
	TD(4- 9)	BRADYPNEA	
	TD(7- 11)	IMPAIRED RIGHTING REFLEX	

TD = TEST DAY

PROTOCOL RTP00004: INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
(SPONSOR'S WORK ASSIGNMENT: WA 5-15)

APPENDIX 2 (PAGE 1): NECROPSY OBSERVATIONS - INDIVIDUAL DATA

DOSAGE GROUP DESCRIPTOR DOSAGE (MG/KG/DAY)	RAT NUMBER	DAY OF NECROPSY	DOSES ADMINISTERED	OBSERVATIONS a	TYPE OF DEATH
1					
25% METHYLCELLULOSE	10301	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
0 (VEHICLE)	10302	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10303	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10304	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10305	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10306	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10307	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10308	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10309	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10310	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10311	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10312	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10313	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10314	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10315	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED

TD = TEST DAY
a. Refer to the individual clinical observations table (Appendix 1) for external observations confirmed at necropsy.

PROTOCOL RTP00004: INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
(SPONSOR'S WORK ASSIGNMENT: WA 5-15)

APPENDIX 2 (PAGE 2): NECROPSY OBSERVATIONS - INDIVIDUAL DATA

DOSAGE GROUP DESCRIPTOR DOSAGE (MG/KG/DAY)	RAT NUMBER	DAY OF NECROPSY	DOSES ADMINISTERED	OBSERVATIONS a	TYPE OF DEATH
2 LINURON 50	10316	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10317	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10318	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10319	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10320	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10321	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10322	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10323	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10324	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10325	TD 15	15	SEMINAL VESICLES WITH FLUID: SMALL. ALL OTHER TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10326	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10327	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10328	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10329	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10330	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED

TD = TEST DAY
a. Refer to the individual clinical observations table (Appendix 1) for external observations confirmed at necropsy.

PROTOCOL RTP00004: INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
(SPONSOR'S WORK ASSIGNMENT: WA 5-15)

APPENDIX 2 (PAGE 3): NECROPSY OBSERVATIONS - INDIVIDUAL DATA

DOSAGE GROUP DOSAGE (MG/KG/DAY)	RAT NUMBER	DAY OF NECROPSY	DOSES ADMINISTERED	OBSERVATIONS a	TYPE OF DEATH
3					
LINURON	10331	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
100	10332	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10333	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10334	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10335	TD 15	15	SEMINAL VESICLES WITH FLUID: SMALL. ALL OTHER TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10336	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10337	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10338	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10339	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10340	TD 15	15	SEMINAL VESICLES WITH FLUID: SMALL. ALL OTHER TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10341	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10342	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10343	TD 15	15	THYMUS: RIGHT LOBE, RED (1.8 CM X 0.7 CM) ALL OTHER TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10344	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10345	TD 15	15	SEMINAL VESICLES WITH FLUID: SMALL. ALL OTHER TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED

TD = TEST DAY
a. Refer to the individual clinical observations table (Appendix 1) for external observations confirmed at necropsy.

PROTOCOL RTP00004: INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
(SPONSOR'S WORK ASSIGNMENT: WA 5-15)

APPENDIX 2 (PAGE 4): NECROPSY OBSERVATIONS - INDIVIDUAL DATA

DOSAGE GROUP DOSAGE (MG/KG/DAY)	RAT NUMBER	DAY OF NECROPSY	DOSES ADMINISTERED	OBSERVATIONS a	TYPE OF DEATH
4 LINURON 150	10346	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10347	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10348	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10349	TD 15	15	SEMINAL VESICLES WITH FLUID: SMALL. ALL OTHER TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10350	TD 15	15	SPLEEN: BLACK. SEMINAL VESICLES WITH FLUID: SMALL. ALL OTHER TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10351	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10352	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10353	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10354	TD 15	15	ACCESSORY SEX GLAND (ASG): SMALL. SEMINAL VESICLES WITH FLUID: SMALL. PROSTATE: SMALL. ALL OTHER TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10355	TD 15	15	SEMINAL VESICLES WITH FLUID: RIGHT LOBE, SMALL. ALL OTHER TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10356	TD 15	15	THYMUS: RIGHT LOBE, RED (0.5 CM X 0.6 CM). SEMINAL VESICLES WITH FLUID: SMALL. ALL OTHER TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10357	TD 15	15	SEMINAL VESICLES WITH FLUID: SMALL. ALL OTHER TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10358	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10359	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10360	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED

TD = TEST DAY
a. Refer to the individual clinical observations table (Appendix 1) for external observations confirmed at necropsy.

PROTOCOL RTP00004: INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
(SPONSOR'S WORK ASSIGNMENT: WA 5-15)

APPENDIX 2 (PAGE 5): NECROPSY OBSERVATIONS - INDIVIDUAL DATA

DOSAGE GROUP DOSAGE (MG/KG/DAY)	RAT NUMBER	DAY OF NECROPSY	DOSES ADMINISTERED	OBSERVATIONS a	TYPE OF DEATH
5					
PHENOBARBITAL	10361	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
25	10362	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10363	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10364	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10365	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10366	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10367	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10368	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10369	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10370	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10371	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10372	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10373	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10374	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10375	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
6					
PHENOBARBITAL	10376	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
50	10377	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10378	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10379	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10380	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10381	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10382	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10383	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10384	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10385	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10386	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10387	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10388	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10389	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10390	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED

TD = TEST DAY
a. Refer to the individual clinical observations table (Appendix 1) for external observations confirmed at necropsy.

PROTOCOL RTP00004: INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
(SPONSOR'S WORK ASSIGNMENT: WA 5-15)

APPENDIX 2 (PAGE 6): NECROPSY OBSERVATIONS - INDIVIDUAL DATA

DOSAGE GROUP DOSAGE (MG/KG/DAY)	RAT NUMBER	DAY OF NECROPSY	DOSES ADMINISTERED	OBSERVATIONS a	TYPE OF DEATH
7					
PHENOBARBITAL	10391	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
100	10392	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10393	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10394	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10395	TD 15	15	SEMINAL VESICLES WITH FLUID: RIGHT LOBE, SMALL. ALL OTHER TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10396	TD 9	8	FOUND DEAD ON TEST DAY 9. ALL TISSUES APPEARED NORMAL.	FOUND DEAD
	10397	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10398	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10399	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10400	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10401	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10402	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10403	TD 15	15	SEMINAL VESICLES WITH FLUID: LEFT LOBE, SMALL. ALL OTHER TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10404	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED
	10405	TD 15	15	ALL TISSUES APPEARED NORMAL.	SCHEDULED SACRIFICED

TD = TEST DAY
a. Refer to the individual clinical observations table (Appendix 1) for external observations confirmed at necropsy.

APPENDIX 3 - PROTOCOL

CHARLES RIVER
LABORATORIES
Preclinical Services

PROTOCOL NUMBER RTP00004

SPONSOR'S WORK ASSIGNMENT: WA 5-15

STUDY TITLE

Interlaboratory Validation of the 15-Day Adult Intact Male Rat Assay with Linuron and Phenobarbital

PURPOSE

The purpose of this study is to evaluate the responses of the adult male rat assay to two chemicals that have known endocrine activity as detected by primarily measuring body and organ weight changes, histology and changes in circulating concentrations of hormones.

TESTING FACILITY

Charles River Laboratories
Preclinical Services, Pennsylvania
905 Sheehy Drive, Building A
Horsham, Pennsylvania 19044-1241
USA
Telephone: (215) 443-8710
Telefax: (215) 443-8587

STUDY DIRECTOR

Joseph W. Lech, B.S., LAT
Scientist
E-mail: joseph.lech@us.crl.com
Address as cited above for Testing Facility.

PRINCIPAL SCIENTIST

Raymond G. York, Ph.D., DABT
Associate Director of Research
Address as cited above for Testing Facility.
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SPONSOR

Battelle
505 King Avenue
Columbus, Ohio 43201-2693
USA

SPONSOR'S REPRESENTATIVE

David P. Houchens, Ph.D.
Address as cited previously for Sponsor.
Telephone: (614) 424-3564
Telefax: (614) 458-3564
E-mail: houchensd@BATTELLE.ORG

REGULATORY CITATIONS

U.S. Environmental Protection Agency. Federal Insecticide, Fungicide and Rodenticide Act/Toxic Substances Control Act (FIFRA/TSCA); Good Laboratory Practice Standards; Final Rule. 40 CFR Part 160/Part 792.

Ministry of Agriculture, Forestry and Fisheries, Japan (MAFF). Good laboratory practice (GLP) standards for agricultural chemicals. Agricultural Production Bureau Ref. No. 11-Nousan-No.6283. October 1, 1999; last revised June 30, 2003 Ref. No. 15-Seisan-2460.

OECD Environmental Directorate. OECD Principles of good laboratory practices [C(97)186/Final] (1998); Environmental Health and Safety Division.

REGULATORY COMPLIANCE

This study will be conducted in compliance with the Good Laboratory Practice (GLP) regulations cited above.

All changes or revisions of this protocol shall be documented, signed by the Study Director and the Sponsor, dated and maintained with the protocol.

The Testing Facility's Quality Assurance Unit (QAU) will audit the protocol, the raw data and the report, and will inspect critical phases of those portions of the study conducted at the Testing Facility in accordance with the Standard Operating Procedures of the Testing Facility.

The Sponsor will prepare the report tables and perform the statistical analyses (daily body weight and body weight change, feed consumption, hormonal analysis and organ weights). The Sponsor's QAU will be responsible for auditing the report tables and statistical analysis generated by Battelle and that all applicable GLP regulations were followed in the conduct.

The final report will include a compliance statement signed by the Study Director that the report accurately reflects the raw data obtained during the performance of the study and that all applicable GLP regulations were followed in the conduct of the study. Should significant deviations from GLP regulations occur, each will be described in detail, together with how the deviation might affect the quality or integrity of the study.

Should any portion of the study be conducted by a subcontractor identified in this protocol or by the Sponsor, the Study Director will ensure that a qualified Principal Investigator is identified by the facility conducting that portion of the study. The QAU for that facility will conduct critical phase inspections and audit respective results and reports for that study portion according to the SOPs of that facility. Such critical phase inspection reports and report audits will be submitted by the subcontractor facilities identified in this protocol to the Principal Investigator and the Study Director. The dates of the inspections and report submissions will be incorporated into a QAU Statement generated by that facility and provided to the Testing Facility for inclusion in the final report. In addition, the subcontractor facilities identified in this protocol will provide a statement of GLP compliance, as described above, signed by the Principal Investigator for inclusion in the final report.

SCHEMATIC OF STUDY DESIGN AND STUDY SCHEDULE

See ATTACHMENT 1 to the protocol.

TEST AND CONTROL SUBSTANCES

NOTE: The Sponsor will provide the test substances. Except for chemistry formulation and analyses, all tests, analyses and measurements will be conducted by individuals without knowledge of the identity of the test substances. A key code for the dosage levels and concentrations will be provided to the formulation and Quality Assurance personnel for the purpose of formulation preparation and auditing of critical phases, respectively. The identities of the test substances, dosage levels and concentrations will be added to the protocol by amendment following the completion of the in-life phases of the study.

Identification

Test Substances

Test Substance (CAS No.)	Lot Number	Manufacturer/Location	Purity Mfg.%	Prepared Formulations Storage Conditions	Formulation Type/Concentration (mg/ml)
Linuron (330-55-2)	348-8A	ChemService, West Chester, Pennsylvania, USA	99.5	Refrigerated (2°C to 8°C)	Suspension/ 10, 20 and 30
Phenobarbital (50-06-6)	104K2600	Sigma-Aldrich, St. Louis, Missouri, USA	99.1	Refrigerated (2°C to 8°C)	Suspension/ 5, 10 and 20

The Sponsor will provide documentation for the identity, composition, strength and activity/purity of the test substances and stability of the bulk test substances. This documentation will be included in the final report. The test substances are marketed products and therefore the method of synthesis information has been documented.

Control Substance

Control Substance (Bulk CAS No.)	Bulk Lot Number	Bulk Manufacturer/Location	Prepared Formulations Storage Conditions	Formulation Type
Aqueous 0.25% (w/v) methylcellulose (9004-67-5)	062K0144[a]	Sigma-Aldrich, St. Louis, Missouri, USA	Refrigerated (2°C to 8°C)	Suspension

a. Manufacturer's lot number for bulk methylcellulose. The prepared control substance was assigned Lot Number 14601TC by the Sponsor prior to shipment.

Neither the Sponsor nor the Study Director is aware of any potential contaminants likely to be present in the control substance that would interfere with the results of this study. Therefore, no analyses other than those mentioned in this protocol will be conducted.

Safety Precautions

Gloves, dust-mist/HEPA-filtered mask, appropriate eye protection, uniform/lab coat and tyvek® sleeves to be worn during formulation preparation and dosage. Bulk test substances will be handled in a chemical fume hood. The Material Safety Data Sheet (MSDS) for each test substance is attached to the protocol (ATTACHMENT 2).

Storage

Bulk Test Substances:	Room temperature
Prepared Control Substance:	Refrigerated (2°C to 8°C)
Prepared Formulations:	Refrigerated (2°C to 8°C)

All test substance shipments should be addressed to the attention of Mark Coker, Manager of Formulation Laboratory, at the previously cited Testing Facility address and telephone number.

Shipments should include information concerning storage conditions and shipping cartons should be labeled appropriately. The recipient should be notified in advance of shipment.

FORMULATION

Frequency of Preparation

Formulations (suspensions) will be prepared at the Testing Facility once for a prestudy formulation analysis and once for the formulations that will be used during the dosage period.

Prior to use, all dose levels, including the control substance, will be brought to room temperature while being stirred for approximately one hour before dosing. Each dosage level will be stirred continuously using a magnetic stir bar and stir plate during sample collection and dosage administration.

Detailed preparation procedures are attached to this protocol (ATTACHMENT 3).

Adjustment for Activity/Purity

The test substances will be considered 100% active/pure for the purpose of dosage calculations.

Testing Facility Reserve Samples

The Testing Facility will reserve a sample of each lot of bulk test substance (approximately 1 g each) and prepared control substance (5 mL) used during the course of the study. Samples will be stored under the previously cited conditions.

ANALYSES

Results of required analyses will be provided to the Testing Facility for inclusion in the study report.

Samples additional to those described below may be taken if deemed necessary during the course of the study. Additional analyses, if required, will be documented by protocol amendment.

Prior to study start, the Testing Facility will perform a prestudy preparation and analysis of the test substance formulations in order to validate the transfer of information provided by the Sponsor regarding preparation and analysis of the test substance formulations.

Results of the homogeneity and concentration analyses of the test substances to be used during the study will be approved by the Study Director before administration.

Acceptance Criteria

Acceptance criteria for analytical results for each group are defined as follows: 1) concentration results will be considered acceptable if the difference between the actual mean value and the targeted concentration is ≤15%; and 2) homogeneity results for a group will be considered acceptable if the relative standard deviation (RSD) for the formulation, calculated as the RSD for the grand mean of the average values for top, middle and bottom locations, is ≤5%.

Analyses of Prepared Formulations

Concentration and Homogeneity

Concentration and homogeneity of the prepared formulations will be verified during the course of this study. Quadruplicate samples (1 mL each) will be taken from the top, middle and bottom of each concentration on the day prepared for both the prestudy and formulations used for dosage administration. Two samples from each quadruplicate set will be shipped for analysis; the remaining samples will be retained at the Testing Facility as backup samples. Quadruplicate samples (1 mL each) will be taken from each concentration of the formulations used for dosage administration on the last day of the dosage period. Two samples from each quadruplicate set will be shipped for analysis; the remaining samples will be retained as backup samples. Backup samples will be stored under the previously cited conditions and discarded at the Testing Facility following issue of the final report.

Stability

Stability data of the bulk test substances and of the prepared formulations bracketing the range of concentrations in this study are on file with the Sponsor and will not be determined during the conduct of this study; this information will be provided to the Study Director and included in the final report.

Shipping Instructions

Samples to be analyzed will be shipped (on cold packs) to:

Principal Investigator: Kim Barnard
Charles River Laboratories
Preclinical Services, Massachusetts
57 Union Street
Worcester, Massachusetts 01608
USA
Telephone: (508) 890-0100
Telefax: (508) 753-1834
E-mail: kim.barnard@us.crl.com

The recipient will be notified in advance of sample shipment.

DISPOSITION

Residual formulations will be discarded at the Testing Facility. Backup samples will be discarded at the Testing Facility following issue of the final report. The remaining bulk test substances will be will be sent to (ambient conditions):

Michael E. Cobb
Batelle Marine Sciences Laboratory
1529 West Sequim Bay Road
Sequim, Washington 98382
USA
Telephone: (360) 681-4580
Telefax: (360) 681-3699
E-mail: michael.cobb@pnl.gov

The recipient will be notified in advance of sample shipment.

TEST SYSTEM

Species/Strain and Reason for Selection

The Crl:CD(SD) rat was selected as the Test System because of known response to toxic effects on reproductive capacity and history of use as a rodent species in these evaluations[1-3].

Number

Initial population acclimated:	115 male rats.
Population selected for study:	105 male rats (15 per dosage group).

Sex

Male

Body Weight and Age

Male rats will be ordered to be approximately 63 days (9 weeks) of age at receipt and approximately 70 days (10 weeks) of age at the initiation of dosage. The rats will be expected to weigh from 225 g to 350 g each at randomization. Actual body weights will be recorded the day after receipt and will be documented in the raw data. The weight ranges will be included in the final report.

Source

Charles River Laboratories, Inc.

The rats will be shipped in filtered cartons by air freight and/or truck from Charles River Laboratories, Inc., to the Testing Facility.

Identification

Rats are permanently identified using Monel® self-piercing ear tags (Gey Band and Tag Co., Inc., No. MSPT 20101). Rats are assigned temporary numbers at receipt and given unique permanent identification numbers when assigned to the study before administration of the first dosage.

ANIMAL HUSBANDRY

All cage sizes and housing conditions are in compliance with the *Guide for the Care and Use of Laboratory Animals*[4].

Housing

During the acclimation and study periods, the rats will be individually housed in stainless steel wire-bottomed cages.

Room Air, Temperature and Humidity

The animal room is independently supplied with at least ten changes per hour of 100% fresh air that has been passed through 99.97% HEPA filters. Room temperature will be maintained at 64°F to 79°F (18°C to 26°C) and monitored constantly. Room humidity will also be monitored constantly and maintained at 30% to 70%.

Light

An automatically controlled 12-hour light:12-hour dark fluorescent light cycle will be maintained. Each dark period will begin at 1800 hours. The light cycle may be adjusted by the Study Director or designee if deemed necessary to accommodate scheduled laboratory activities. Any such adjustment will be documented in the raw data.

Diet

Rats will be given Harlan's Teklad 2018c meal feed, available *ad libitum* from individual feeders.

The concentrations of genistein equivalents (aglycone) or daidzein will be ≤ 300 ppm per lot. Approval by the Sponsor will be required in order to use a lot of Harlan's Teklad 2018c meal feed that has a genistein or daidzein equivalent content greater than 300 ppm per lot.

Water:

Water will be available *ad libitum* from individual bottles attached to the cages or from an automatic watering access system. All water will be from a local source and passed through a reverse osmosis membrane before use. Chlorine will be added to the processed water as a bacteriostat; processed water is expected to contain no more than 1.2 ppm chlorine at the time of analysis. Water is analyzed monthly for possible bacterial contamination and twice annually for possible chemical contamination.

Contaminants

Neither the Sponsor nor the Study Director is aware of any potential contaminants likely to be present in the certified diet or in the drinking water at levels that would interfere with the results of this study. Therefore, no analyses other than those routinely performed by the feed supplier or those mentioned in this protocol will be conducted.

RANDOMIZATION AND ACCLIMATION

Upon arrival, rats will be assigned to individual housing on the basis of computer-generated random units.

After a minimum of one week of acclimation, in which the rats were monitored for general health daily, rats will be selected for study on the basis of physical appearance and body weights recorded during acclimation. During the acclimation period all rats are to be examined by the laboratory veterinarian for release on study. The rats will be assigned to dosage groups based on computer-generated (weight-ordered) randomization procedures.

In order to accommodate the necropsy schedule, rats will be assigned to three replicates that will begin dosage and be sacrificed on consecutive days.

ADMINISTRATION

Route and Reason for Choice

The oral (gavage) route was selected for use because: 1) in comparison with the dietary route, the exact dosage can be accurately administered; and 2) it is one possible route of human exposure.

Method and Frequency

Each dosage level will be stirred continuously using a magnetic stir bar and stir plate during sample collection and dosage administration.

Male rats will be administered one of the test substances and/or control substance once daily for 15 days. The first day of dosage for each replicate will be Test Day 1 (TD 1) of the study. Rats will be sacrificed on the day of the last dosage (TD15), 2 to 3 hours after the last dosage.

Daily dosages will be based on the daily body weight, except on TD 15, which will use the previous day's body weight. On TDs 1 through 14, dosing of rats will be between 0600 and 0900 hrs. On TD 15, dosing of rats will start at approximately 0600 hrs so that rats can have blood collected and be necropsied between 0800 and 1100 hrs on TD 15.

Rationale for Dosage Selection

Chemicals selected for this phase of validation were chosen to represent a couple of different modes of action. Each of the test chemicals has previously been run in the adult male assay with results documented in a review publication[5]. Based on the results of these studies, the high dosage level is not expected to exceed the maximum tolerated dose (MTD; body weight at necropsy within 10% of controls). The lower dosage levels were selected to assess dose-response relationships.

Dosage Groups, Levels and Volumes

Dosage Group	Number of Rats	Dosage (mg/kg/day)[a]	Concentration (mg/mL)	Dosage Volume (mL/kg)	Batch Number
1	15	TBA	TBA	5	B-RTP00004-A(Day.Month.Year)
2	15	TBA	TBA	5	B-RTP00004-B(Day.Month.Year)
3	15	TBA	TBA	5	B-RTP00004-C(Day.Month.Year)
4	15	TBA	TBA	5	B-RTP00004-D(Day.Month.Year)
5	15	TBA	TBA	5	B-RTP00004-E(Day.Month.Year)
6	15	TBA	TBA	5	B-RTP00004-F(Day.Month.Year)
7	15	TBA	TBA	5	B-RTP00004-G(Day.Month.Year)

The test substances will be considered 100% active/pure for the purpose of dosage calculations.
TBA - To be added to the protocol by amendment.

a. Test substances will be provided by the Sponsor. The Testing Facility will identify the test substances by key code. All tests, analyses and measurements will be conducted by individuals without knowledge of the identity or dosage level of the test substances except for formulation preparation and analysis.

TESTS, ANALYSES AND MEASUREMENTS

Viability

All Periods: At least twice daily.

Clinical Observations and/or General Appearance

Acclimation Period: Daily.

Dosage Period: Daily before dosage and approximately 6 hrs post dose.

Clinical observations may be recorded more frequently than cited above.

Body Weights

Acclimation Period: At least three times (not tabulated).

Dosage Period: Daily.

Sacrifice: Terminal weight following dosage administration.

Feed Consumption Values

Acclimation Period: At least once (not tabulated).

Dosage Period: Weekly (TD 1, 8 and 15)

Day of Sacrifice: Feed left recorded.

Feed consumption values may be recorded more frequently if it is necessary to replenish the feed. These intervals will not be tabulated.

SACRIFICE

Method of Sacrifice

All rats that survive until scheduled sacrifice will be anesthetized by exposure to carbon dioxide for no more than 60 seconds and then sacrificed by decapitation. Rats sacrificed in moribund condition will be sacrificed by asphyxiation with carbon dioxide.

Scheduled Sacrifice

Male rats will be sacrificed on the day of the last administration of the test substance (TD 15) and necropsied as described below. All rats will be moved from the study room to the necropsy area and held for at least one hour prior to necropsy to minimize potential stress-induced changes in hormone levels related to cage transport.

GROSS NECROPSY, HORMONE ANALYSIS AND HISTOPATHOLOGY

On TD 15, rats will be necropsied and examined for gross lesions. Rats will be sacrificed between 0800 and 1100 hrs (2 to 3 hours after the last dosage).

Blood samples (at least 8 mL) for evaluation of serum hormones will be collected from trunk blood immediately following sacrifice. The time of sample collection will be documented in the raw data. Blood will be collected and immediately placed into serum separator tubes in wet ice until serum is prepared. Enough blood should be collected in order to yield approximately 3600 mcL of serum, to be aliquotted into nine vials (duplicate) of approximately 200 mcL each. The sequence in which the hormones should be assayed is testosterone, luteinizing hormone (LH), thyroid stimulating hormone (TSH), thyroxine (T4), triiodothyronine (T3), follicle stimulating hormone (FSH), estradiol and prolactin. Only when relative liver weights are significantly increased should dihydrotestosterone (DHT) levels be measured. Serum samples will be immediately frozen on dry ice and maintained frozen (-68°C to -78°C) until analysis.

Hormone analysis will be conducted at CTBR Bio-Research Inc. utilizing radioimmunoassay (RIA) kits. Each sample will be run in duplicate and include the low and high quality control serum samples. Each assay will include all samples from the control group and each dose level for both chemicals. For the QC samples, the kit-supplied zero standard or the medium in which the standards are prepared can be spiked with respective hormones at concentrations that are expected to encompass 70% (+/-10%) B/B_0, for the low, and 30% (+/-10%) B/B_0, for the high. The results for the quality control samples will be used to assess within- and between-assay variability for each laboratory.

Gross necropsy will include an initial physical examination of external surfaces and all orifices, as well as an internal examination of tissues and organs *in situ*. In addition, the cranial, thoracic and abdominal cavities will be examined.

Rats will be sacrificed and examined for gross lesions. Gross lesions associated with the testes, epididymides and thyroid will be retained in neutral buffered 10% formalin and examined histologically, all other gross lesions will be retained for possible histological evaluation. Gross lesions associated with the liver (only if the relative weights are significantly increased) will be shipped for histological examination. Tissue trimming and histopathology will be performed under the supervision of or by a Board-Certified Veterinary Pathologist.

See ATTACHMENT 4 for tissues to be weighed and retained and histological evaluations to be conducted. All other tissues will be discarded.

Shipping Instructions

Serum samples for analysis will be shipped (frozen on dry ice) to:

Principal Investigator: Stephane Besner
Laboratory Sciences
CTBR Bio-Research Inc.
87 Senneville Road
Senneville Quebec, Canada, H9X3R3
Telephone: 514-630-8200 ext 8970
E-mail: stephane.besner@ca.crl.com

The recipient will be notified in advance of sample shipment.

Rats Found Dead or Unscheduled Sacrifice

All rats accidentally killed during the acclimation period will be discarded without further evaluation. Rats that die or are unscheduled sacrificed because of condition during the acclimation period or the dosage period will be examined for the cause of death or condition on the day the observation is made. The rats will be necropsied and examined to the extent possible as described above, but the tissues will not be histologically examined. All rats unscheduled sacrificed because of condition will euthanized by asphyxiation with carbon dioxide (CO_2).

Data Presentation and Statistics

Individual clinical observation and necropsy/mortality tables will be generated by the Testing Facility. This information will not be summarized or statistically analyzed.

The four categories of data to be evaluated are as follows:

1. Growth - body weights and food consumption – (7 endpoints)
   Daily body weight (TD1 – TD15)
   Body weight change (TD8 – TD1)
   Body weight change (TD15 – TD8)
   Body weight change (TD15 – TD1)
   Food consumption (TD8 - TD1)
   Food consumption (TD15 - TD8)
   Food consumption (TD15 - TD1)

TD15 weight will be the live weight before sacrifice.
Body weights will be reported in grams (g). Body weight changes will be reported in g/day. Body weight responses will be reported to the nearest 0.1g or 0.1 g/day.
Food consumption will be reported in g/kg/day. Responses will be reported to the nearest 0.1 g/kg/day.

2. Hormonal analysis - (8 - 9 hormones)
   Testosterone (ng/ml)
   LH (ng/ml)
   TSH (ng/ml)
   T4 (µg/dl)
   T3 (ng/dl)
   FSH (ng/ml)
   Estradiol (pg/ml)

Prolactin (ng/ml)
*DHT (pg/ml)

*Only if relative liver weights are significantly increased should DHT levels be measured.

3. Organ weights – (9 organs)
   - Liver
   - Right testis
   - Left testis
   - Testes paired (sum of left and right testis weights)
   - Epididymides (paired weight)
   - Entire prostate
   - Seminal vesicles with fluid and coagulating gland
   - Accessory sex gland (ASG) (sum of entire prostate and seminal vesicles with fluid and coagulating gland weights)
   - Thyroid

Organ weights will be reported in grams (g). Organ weights will be reported wet to the nearest 0.0001 g.
Organ weights will be analyzed in two ways:
- Unadjusted
- Organ to final body weight ratio (expressed as percent)

4. Histology – (5 - 6 organs)
   - *Liver
   - Right testis
   - Left testis
   - Right Epididymus
   - Left Epididymus
   - Thyroid

*Liver will be evaluated microscopically at the discretion of the pathologist, the study director and sponsor.

Microscopic evaluations will be performed on control and high dose rats for all compounds. The dose groups but not the compounds will be known to the pathologist during evaluation. After evaluation, the nature of the compounds will be known to the pathologist for report writing. Compounds which show effects in the high dose group will have the remaining groups evaluated and this will be added by amendment.

Histology data will not be analyzed statistically.

The test method specifies that all rats will be sacrificed on TD 15.

If rats died prior to necropsy their body weights will be included in summaries and displays up to the time of death, but will not be imputed beyond date of death nor will they be included in the final body weight gain summaries (in either the initial or final weight average). The number of deaths per group prior to necropsy will be reported for each group.

All data values that are reported by a laboratory as being associated with a test or clerical error, and which the laboratory states should be excluded, will be omitted from all summaries, displays, and analyses. All data that enter into the statistical analyses will be *a priori* valid data.

Outlier Detection

Outlier screens will be carried out prior to analysis. Screens will be carried out separately for each endpoint, based on untransformed data. When both unadjusted and body weight adjusted values are called for in the statistical analysis plan (organ weights), the outlier screens will be carried out based on the unadjusted values.

For each endpoint a one way analysis of variance model will be fitted to the data. For the growth data the body weight change from TD1 to TD15 will be used. The data will include seven groups with n=15 rats per group, less any data omitted due to deaths or procedural errors. The model will assume separate standard deviations within each group. Studentized residuals will be determined based on the analysis of variance fit and ordered in absolute value. Assuming no data were omitted, there will be 105 values. A procedure which generalizes Grubbs[6] procedure to accommodate heterogeneous variances will be used. The absolute studentized residuals will be compared to a cutoff value corresponding to a 2.5% significance level (for a two-sided test) of the maximum of seven component maximum studentized residuals, each component maximum studentized residual based on 15 observations. This cutoff value is 2.84. Any studentized residual in excess of 2.84 in absolute value will be flagged. Just a single iteration of the outlier screening procedure will be carried out.

Normal probability plots of the studentized residuals will be prepared. If the flagged values appear to be outliers in the probability plots, in that they depart from the trend in the body of the residuals, they will be treated as potential outliers. If the trend observed in the tails of the normal probability plot is continuous but is heavily skewed or is considerably heavier tailed than normal, a data transformation (e.g. square root, (natural)

logarithm) might be attempted to improve agreement with normal distribution assumptions. The outlier screen would be repeated on the transformed data. However, if the tails of the normal probability plot depart just slightly to moderately from straight line behavior, the data will be analyzed without transformation.

Subsequent statistical analyses will be carried out both including and excluding the flagged values that are identified as potential outliers. The subsets of flagged values will be response specific.

Heterogeneity of Residual Variance Across Laboratories and Treatments

Tests for heterogeneity of variance will be carried out on the data excluding the values flagged by the outlier screen and identified as potential outliers. The transform of the variable (or none) used for the variance heterogeneity comparisons will be that decided upon in the outlier screen.

For each endpoint extent of heterogeneity of variability will be assessed across treatment groups. A one-way analysis of variance model will be fitted to the data, including the factor treatment (fixed). The factors in the analysis of variance will be:

Source	df
Treatment	6
Residual ≡ Replicate (Treatment)	14×7=98
	104

Three versions of the model will be fitted to test for heterogeneity of residual variance.

1. Separate variances for each treatment group (7 variances)
2. Separate variances for each chemical (or control) (3 variances)
3. Common variances across all groups

These models will be compared by likelihood ratio tests.

Data Summaries

Data summaries will consist of tables and figures. Summary tables will be prepared including all the data and excluding the values screened as possible outliers. There will be a set of eight tables for each case, for a total of 16 tables. Summary figures will only be prepared including all the data.

Tables

Tables 1 and 2 will display summary values for the seven body weight and food consumption endpoints. These will be TD15 body weight, 3 body weight change variables as shown in the data section, and 3 food consumption variables as shown in the data section. There will be one table per chemical.

For each endpoint and each dose group the following statistics will be reported:

- Number of rats on which the statistic is based
- Mean ± standard error
- Coefficient of variation
- Mean as a percent of control group mean ± standard error[1]

In addition the linear trend slope contrast will be estimated for each chemical based on the control group and the three graded dose groups, treating the control group and the three dose groups as equally spaced[2]. The estimated treatment slope and its standard error will be reported

Tables 3 to 6 will display summary values for the nine organ weights endpoints specified in the test method Tables 3 and 4 will correspond to unadjusted organ weights and organ to body weight ratios respectively for Chemical #1. Tables 5 and 6 will correspond to unadjusted organ weights and organ weight to body weight ratios respectively for Chemical #2.

The tables will include the same summary statistics as specified for Tables 1 and 2.

Tables 7 and 8 will display summary values for the nine hormonal analysis endpoints specified in the test method. There will be one table per chemical. The tables will include the same summary statistics as specified for Tables 1 and 2.

---

1. If X, Y denote the control group least squares mean and the dose group least squares mean respectively, with variance-covariance matrix (S_X^2, S_Y^2, S_{XY}), an approximate standard error for $R \equiv Y/X$ is

$$Se[R(X, Y)] \approx |1/X| [(Y/X)^2 S_X^2 + S_Y^2 - 2(Y/X) S_{XY}]^{1/2}$$

2 If X_0, X_1, X_2, X_3 denote the least squares means for the control group "0" and (equally spaced) dose groups "1", "2", "3" then the linear contrast among these is defined to be

$$\text{Linear Contrast} \equiv [-3X_0 - X_1 + X_2 + 3X_3]/[20]^{1/2}$$

Tables 1 - 8 will be based on all the data. Tables 9 – 16, to the extent needed, will be a repetition of Tables 1- 8, but based on the data excluding the flagged potential outliers. Tables 9-16 need only include the subset of responses for which potential outliers were flagged.

Figures

The figures will include mean daily body weights and figures to compare the various endpoints across chemicals and dose groups. The figures will include all the data. For organ weights the figures will be based only on the unadjusted weights.

Figures 1-2 will display mean body weight ± 2 standard errors for each day from TD1 to TD15 for the control group and for each dose group. Each figure will correspond to a single chemical.

For the 7 body weight and food consumption measures, the 9 unadjusted organ weights, and the 8 - 9 hormone concentrations (25 endpoints) summarized in Tables 1-8 a figure will be prepared that displays the (least squares) means ± 2 standard errors for each of the seven dose groups (control group + three dose groups x 2 chemicals). Each figure will contain seven bars, corresponding to a control group or chemical and dose group. Each bar will be centered at the (least squares mean) with width 2 standard errors above and below the least squares mean.

Analysis of Variance

For each of the 34 endpoints summarized in Tables 1-8 analysis of variance models will be fitted to the data to estimate treatment effects. For the nine organ weight responses the unadjusted responses will be analyzed as well as the organ to final body weight ratio (percent) responses.

Analyses will be carried out based on all the data and after omitting responses flagged as potential outliers. The (possibly heterogeneous) residual variance structure assumed in these analyses will be that arrived at as discussed in the section - "Heterogeneity of Residual Variance Across Laboratories and Treatments". If a transformation was decided on during the outlier screening process, the analyses will be carried out on transformed variables. Otherwise analyses will be carried out on the untransformed data, using the simplest variance structure compatible with the data.

For each (possibly transformed) response the following one-way analysis of variance model will be fitted to the combined data across laboratories and chemicals. The factors in the analysis of variance model are as shown below.

Source	df
Treatment	6
Residual = Replicate (Treatment)	14×7=98
	104

Least squares means for individual treatment groups and for differences between dose groups and control group and associated standard errors and ± 2 standard error intervals will be calculated based on the above model. In addition linear trend contrasts among the control group and the three dose groups within a chemical will be calculated, treating the control group and the three dose groups as equally spaced (using the linear contrast shown in footnote 2). For each chemical separately, least squares means will be compared between the treatment groups and the control group by means of two-sample t-tests. Linear trend statistics will be compared to 0 trend by means of one-sample t-tests.

Two-tailed unadjusted significance levels will be reported. If the unadjusted significance levels are less than 0.05, they will be indicated with a single asterisk, '*'. If they are less than 0.006 they will be indicated with two asterisks, '**'. A significance level of 0.006 (≈ 0.05/8) corresponds to Bonferroni's simultaneity adjusted significance level 0.05, adjusting for eight inferences (6 comparisons of dose groups with control and 2 linear trend statistics). The least squares means, standard errors, CVs, and ± 2 standard error intervals will be back transformed to the original scale, if necessary, for purposes of display.

The Principal Investigator assigned to preparing the report tables and performing the statistical analysis (daily body weight and body weight change, feed consumption, hormonal analysis and organ weights) is Paul Feder of Battelle.

DATA ACQUISITION, VERIFICATION AND STORAGE

Data generated during the course of this study will be recorded either by hand or using the *Argus Automated Data Collection and Management System* and the *Vivarium Temperature and Relative Humidity Monitoring System*. All data will be tabulated, summarized and/or statistically analyzed using the *Argus Automated Data Collection and Management System*, the *Vivarium Temperature and Relative Humidity Monitoring System, Microsoft® Excel* (part of Microsoft® Office 97/2000/XP), Quattro Pro 8 and/or *The SAS System* (version 6.12).

Records will be reviewed by the Study Director and/or appropriate management personnel within 21 days after generation. All original records will be stored in the archives at the Testing Facility. All raw data will be bound and indexed following finalization of the study report. The archived raw data will be scanned, paginated electronically and retained as an Adobe® Acrobat PDF file. A copy of all raw data will be supplied to the Sponsor upon request. Preserved tissues will be stored at the Testing Facility at no additional charge for one year after mailing of the draft final report, after which time the Sponsor will be contacted to determine the disposition of these materials.

RECORDS TO BE MAINTAINED

Protocol and Amendments.
Test Substance, Control Substance and/or Reagent Receipt, Preparation and Use.
Animal Acquisition.
Randomization Schedules.
Treatment (if prescribed by Staff Veterinarian).
General Comments.
Clinical Observations and/or General Appearance.
Body Weights.
Feed Consumption Values.
Blood Sample Collection, Processing and Shipment.
Gross Necropsy Observations.
Organ Weights.
Tissue Sample Collection, Processing and Shipment.
Photographs (if required).
Study Maintenance (room and environmental records).
Feed and Water Analyses.
Packing and/or Shipment Lists.

KEY PERSONNEL

Director of Research: Alan M. Hoberman, Ph.D., DABT
Scientist and Study Director: Joseph W. Lech, B.S., LAT
Principal Scientist and Associate Director of Research: Raymond G. York, Ph.D., DABT
Director of Operations: John F. Barnett, B.S.
Senior Manager, Study Management: Jo Anne M. Vico, B.S.
Senior Manager, Regulatory Compliance: Nancy A. Catricks, M.S.
Attending Veterinarian: Dena C. Lebo, V.M.D., Division Veterinarian
Chair, Institutional Animal Care and Use Committee: Douglas B. Learn, Ph.D.
Consultant, Veterinary Pathology: W. Ray Brown, D.V.M., Ph.D., Diplomate, ACVP
Consultant, Veterinary Pathology: Charles River Laboratories Preclinical Services Pathology Associates Division

FINAL REPORT

The Study Director will provide periodic updates of study progress to the Sponsor. Draft summary tables of unaudited computer-recorded data may accompany these updates. Statistical analyses will not be performed on these interim data.

A comprehensive draft final report will be prepared on completion of the study and will be finalized following consultation with the Sponsor. The report will include the following:

- Executive Summary (describing the number and strain of rats used in the study, the dose levels and chemicals tested, and the effects with levels of statistical significance for all endpoints).
- Experimental Design and Method.
- Evaluation of Test Results.
- Conclusion.
- Appendices: Figures, Summary and Individual Tables Summarizing the Above Data, Protocol and Associated Amendments and Deviations, Study Director's GLP Compliance Statement, Reports of Supporting Data (if appropriate) and QAU Statement.

The Sponsor will receive one copy of the draft report. A copy of the final report will be provided on CD-ROM in Adobe Acrobat PDF format. The PDF document will be created from native electronic files to the extent possible, including text and tables generated by the Testing Facility. Report components not available in native electronic files and/or original signature pages will be scanned and converted to PDF image files for incorporation. A hard copy printed from the electronic file will accompany the final report on CD-ROM. The hard copy of the report with original signatures retained at the Testing Facility will be considered the GLP-compliant original.

The Sponsor will also receive an electronic copy (SAS Transport files or SAS dataset-compatible *Microsoft® Excel* files) of clinical observations, body weights, feed consumption values and organ weights within one month of completion of in-life. Audited raw data for histopathological observations and hormone level analyses will be provided electronically within two months of completion of in-life.

Study reports should be finalized within six months of submission of the audited draft final report. Two Sponsor-requested revisions to the draft report will be addressed by the Testing Facility at no charge. Additional revisions to the draft report or amendments to the final report may incur additional costs. If the Sponsor has not provided comments to the report within six months of draft submission, the report will be finalized by the Testing Facility.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE STATEMENT

The procedures described in this protocol have been reviewed by the Testing Facility's Institutional Animal Care and Use Committee. All procedures described in this protocol that involve study rats will be conducted in a manner to avoid or minimize discomfort, distress or pain to the rats.

The signature of the Sponsor's representative below is assurance that the study is not an unnecessary duplication of previous work. Documentation for the necessity of this study may be obtained from the Sponsor. No alternative procedures were available to meet the stated purposes of the study.

REFERENCES

(1) Christian.MS., Voytek.PE. In Vivo reproductive and mutagenicity tests. A guide to general toxicology. Basel (CH): S. Karger; 1982. p. 295-325.

(2) Christian.MS. Reproductive toxicity and teratology evaluations of naltrexone. J Clin Psych 1984; 45 (9 Sec 2):7-10.

(3) Lang.PL.Embryo and fetal developmental toxicity (Teratology) control data in the Charles River Crl:CD®BR rat. (Database provided by Argus Research Laboratories, Inc.). Wilmington (MA): Charles River Laboratories, Inc. 1988.

(4) Guide for the care and use of laboratory animals. Institute of Laboratory Animal Resources Commission on Life Sciences and the National Research Council. Washington (D.C.): National Academy Press; 1996.

(5) O'Connor JC, Cook JC, Marty.M.S., Davis LG, Kaplan AM, and Carney EW. Evaluation of Tier I screening approaches for detecting endocrine-active compounds (EACs).

(6) Grubbs FE. Procedures for detecting outlying observations in samples. Technometrics 1969; 11(1):1-21.

PROTOCOL APPROVAL

FOR THE TESTING FACILITY

Alan M. Hoberman, Ph.D., DABT
Director of Research

7-Oct-05
Date

Joseph W. Lech, B.S., LAT
Scientist
Study Director

07 oct 2005
Date

Mathew B. Carlson, B.A. FOR
Member, Institutional Animal Care and
Use Committee

07 OCT 2005
Date

FOR THE SPONSOR

Sponsor approval received via E-mail on 06 OCT 05
Date

David P. Houchens, Ph.D
Program Manager
Battelle

10/10/05
Date

Terri Pollock
Quality Assurance
Battelle

10-10-05
Date

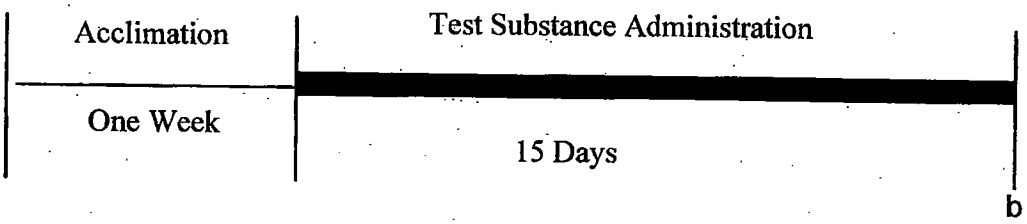
ATTACHMENT 1

SCHEMATIC OF STUDY DESIGN AND STUDY SCHEDULE

ATTACHMENT 1

SCHEMATIC OF STUDY DESIGN

15-DAY INTACT ADULT MALE ASSAY IN RATS[a]



[redacted] Dosage Period.

a. For additional details, see "Tests, Analyses and Measurements" section of the protocol.

b. All rats sacrificed.

ATTACHMENT 1

STUDY SCHEDULE[a]

11 OCT 05	Animal Receipt.
18 OCT 05	Proposed Experimental Start Date
18 OCT 05 - 01 NOV 05	Dosage Administration (replicate1).
19 OCT 05 - 02 NOV 05	Dosage Administration (replicate2).
20 OCT 05 - 03 NOV 05	Dosage Administration (replicate3).
01 NOV 05	Sacrifice and Necropsy (replicate 1).
02 NOV 05	Sacrifice and Necropsy (replicate 2).
03 NOV 05	Sacrifice and Necropsy (replicate 3).
01 DEC 05	SAS Transport and EXCEL Files.
08 DEC 05	Proposed Experimental Termination Date
15 DEC 05	Draft Final Report.

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a. The study initiation date is the date the Study Director signs the protocol.

ATTACHMENT 2

MATERIAL SAFETY DATA SHEETS

AND

CERTIFICATES OF ANALYSIS

Chem Service, Inc.
MATERIAL SAFETY DATA SHEET PS-372

Invoice: CS264916 PO: 19293

Printed: 08/11/2005
Last Revised: May 26, 2005

SECTION 1 - CHEMICAL PRODUCT and COMPANY IDENTIFICATION

Catalog Number: PS-372
Description: Linuron
Other Name(s): 3-(3,4-Dichlorophenyl)-1-methoxy-1-methylurea

Supplied by CHEM SERVICE, Inc. PO BOX 599, WEST CHESTER, PA 19381 (610)-692-3026
EMERGENCY PHONE: 1-610-692-3026

SECTION 2 - COMPOSITION, INFORMATION ON INGREDIENTS

CAS No.: 330-55-2
Description: Linuron
EINECS No.: 206-356-5
Hazard Symbols: Xn

SECTION 3 - HAZARDS IDENTIFICATION

Contact lenses should not be worn in the laboratory.
All chemicals should be considered hazardous - Avoid direct physical contact!
May cause eye irritation. Can cause skin irritation.
Dust and/or vapors can cause irritation to respiratory tract.
Can be irritating to mucous membranes. May be harmful if absorbed through the skin.
May be harmful if inhaled. May be harmful if swallowed.

SECTION 4 - FIRST AID MEASURES

An antidote is a substance intended to counteract the effect of a poison. It should be administered only by a physician or trained emergency personnel. Medical advice can be obtained from a POISON CONTROL CENTER.

In case of contact: Flush eyes continuously with water for 15-20 minutes. Flush skin with water for 15-20 minutes. If no burns have occurred-use soap and water to cleanse skin.
If inhaled remove patient to fresh air. Administer oxygen if patient is having difficulty breathing. If patient has stopped breathing administer artificial respirations.
If patient is in cardiac arrest administer CPR.
Continue life supporting measures until medical assistance has arrived.
Remove and wash contaminated clothing.
If patient is exhibiting signs of shock - Keep warm and quiet.
Contact Poison Control Center immediately if necessary. Induce vomiting if swallowed.
Do not administer liquids or induce vomiting to an unconscious or convulsing person.
If patient is vomiting-watch closely to make sure airway does not become obstructed by vomit.
Get medical attention if necessary.

SECTION 5 - FIRE AND EXPLOSION DATA

Flash Point: Not Available

Cat No.: PS-372
Page: 2

SECTION 5 - FIRE AND EXPLOSION DATA CONTINUED

rtinguishing Media:
Carbon dioxide, dry chemical powder or spray.
Upper Explosion Limit: Not Available
Lower Explosion Limit: Not Available
Autoignition Temperature: Not Available
NFPA Hazard Rating: Not Available

SECTION 6 - ACCIDENTAL RELEASE MEASURES

Spills or leaks: Evacuate area. Wear appropriate OSHA regulated equipment. Ventilate area.
Sweep up and place in an appropriate container. Hold for disposal.
Wash contaminated surfaces to remove any residues.
Remove contaminated clothing and wash before reuse.

SECTION 7 - HANDLING AND STORAGE

Handling:
This chemical should be handled only in a hood. Eye shields should be worn.
Use appropriate OSHA/MSHA approved safety equipment.
Avoid contact with skin, eyes and clothing. Avoid ingestion and inhalation
Wash thoroughly after handling.

Storage:
Store in a cool dry place. Store only with compatible chemicals.
Keep tightly closed.

SECTION 8 - EXPOSURE CONTROLS/PERSONAL PROTECTION

OSHA PEL (TWA): Not Available
ACGIH TLV (TWA): Not Available
ACGIH TLV (STEL): Not Available

Personal Protective Equipment
Eyes: Wear Safety Glasses.
Skin: Wear appropriate protective gloves to prevent skin exposure.
Clothing: Wear appropriate protective clothing to minimize contact with skin.
Respirators: A respiratory protection program that meets OSHA's 29 CFR 1910.134 requirements must be followed whenever workplace conditions warrant a respirator's use.

SECTION 9 - PHYSICAL AND CHEMICAL PROPERTIES

Color:	Colorless
Phase:	Crystalline solid
Melting Point:	93-94 C
Boiling Point:	Not Available
Specific Gravity:	Not Available
Vapor Pressure:	0.05mPa@20 C
Vapor Density:	Not Available

Solubility in Water:	Very slightly soluble
Odor:	Not Available
Vaporation Rate (Butyl acetate=1):	Not Available
Molecular Weight	249.11
Molecular Formula	C9H10Cl2N2O2

SECTION 10 - STABILITY AND REACTIVITY

Sensitive to light - dark color does not affect purity. Sensitive to heat. Decomposes under alkaline conditions. Decomposes under acidic conditions.

SECTION 11 - TOXICOLOGY INFORMATION

RTECS: YS9100000
Oral Rat or Mouse LD50: 4000ag/kg
Dermal Rat or Mouse LD50: >2000 mg/kg
Rat or Mouse LC50: >6.15 mg/l air(4h)

Carcinogenicity
- OSHA: No
- IARC: No
- NTP: No
- ACGIH: No
- NIOSH: No
- Other: No

SECTION 12 - ECOLOGICAL INFORMATION

Ecotoxicity: Not Available
Environmental Fate: Not Available

SECTION 13 - DISPOSAL CONSIDERATIONS

DISPOSAL: Burn in a chemicals incinerator equipped with an afterburner and scrubber.

SECTION 14 - TRANSPORTATION INFORMATION

Not regulated as a hazardous material.

SECTION 15 - REGULATORY INFORMATION

European Labeling in Accordance with EC Directives
Hazard Symbols: Xn
Risk Phrases
- R40
  - Possible risk of irreversible effects.

Safety Phrases
- S36/37
  - Wear suitable protective clothing and gloves.

SECTION 16 - OTHER INFORMATION

The above information is believed to be correct on the date it is published and must not be considered all inclusive. The information has been obtained only by a search of available literature and is only a guide for handling the chemicals. OSHA regulations require that if other hazards become evident, an upgraded MSDS must be made avalible to the employee within three months. RESPONSIBILITY for updates lies with the employer and not with CHEM SERVICE, Inc.

Persons not specifically and properly trained should not handle this chemical or its container. This MSDS is provided without any warranty expressed or implied, including merchantability or fitness for any particular purpose.

This product is furnished FOR LABORATORY USE ONLY! Our products may NOT BE USED as drugs, cosmetics, agricultural or pesticidal products, food additives or as household chemicals.

[redacted]
660 Tower Lane • P.O. Box 599 • West Chester, PA 19381-0599
1-800-452-9994 • 1-610-692-3026 • Fax 1-610-692-8729
info@chemservice.com • www.chemservice.com

CERTIFICATE OF ANALYSIS

INVOICE #: CS264916
PO #: 19293

CATALOG #: PS-372

CAS #: 330-55-2

DESCRIPTION: Linuron

LOT #: 348-8A

PURITY: 99.5%

EXPIRATION DATE: 08/08

Chem Service, Inc. guarantees the purity of this chemical ± 0.5% deviation prior to the expiration date shown on the label and exclusive of any customer contamination.

Two or more of the following methods of analysis are used to determine purity: Melting point, refractive index, titration, IR, TLC, GC/FID, GC/TCD, GC/ECD, GC/MS, HPLC or DSC.

Our standards are suitable for use with all EPA methods.

Certified By:

John Conrad

John Conrad
CSM/TC

ISO 9001
Certificate Number: 31610

SIGMA-ALDRICH

MATERIAL SAFETY DATA SHEET

Date Printed: 09/12/2005
Date Updated: 07/25/2005
Version 1.4

Section 1 - Product and Company Information

Product Name	PHENOBARBITAL FREE ACID—DEA SCHEDULE IV ITEM
Product Number	P1636
Brand	SIGMA
Company	Sigma-Aldrich
Street Address	3050 Spruce Street
City, State, Zip, Country	SAINT LOUIS MO 63103 US
Technical Phone:	314 771 5765
Emergency Phone:	414 273 3850 Ext. 5996
Fax:	800 325 5052

Section 2 - Composition/Information on Ingredient

Substance Name	CAS #	SARA 313
5-ETHYL-5-PHENYLBARBITURIC ACID	50-06-6	No

Formula: C12H12N2O3

Synonyms: Acido 5-fenil-5-etilbarbiturico (Italian) * Adonal * Aephenal * Agrypnal * Amylofene * Aphenylbarbit * Aphenyletten * Austrominal * Barbenyl * Barbiphenyl * Barbipil * Barbita * Barbivis * Barbonal * Barbophen * Bialminal * Blu-phen * Cardenal * Cratecil * Dormiral * Doscalun * Duneryl * Eskabarb * 5-Ethyl-5-phenylbarbituric acid * 5-Ethyl-5-phenyl-2,4,6-(1H,3H,5H)pyrimidinetrione * Etilfen * Euneryl * Fenemal * Fenobarbital * Gardenal * Gardepanyl * Helional * Hysteps * Lepinal * Lepinaletten * Liquital * Lixophen * Lubergal * Lubrokal * Luminal * Lumofridetten * Luphenil * Luramin * Molinal * Neurobarb * Nirvonal * Noptil * Nova-pheno * Nunol * Parkotal * Pharmetten * Phenaemal * Phen-Bar * Phenemal * Phenobal * Phenobarbital * Phenobarbitone * Phenobarbituric acid * Phenobarbyl * Phenoluric * Phenolurio * Phenomet * Phenonyl * Phenoturic * Phenylethylbarbiturate * Phenyl-ethyl-barbituric acid * 5-Phenyl-5-ethylbarbituric acid * Phenylethylmalonylurea * Phenyletten * Phenyral * PHOB * 2,4,6(1H,3H,5H)-Pyrimidinetrione, 5-ethyl-5-phenyl- * Sedabar * Seda-Tablinen *

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Toxic.

May cause harm to the unborn child. Toxic if swallowed. Limited evidence of a carcinogenic effect. May cause sensitization by skin contact.

Possible Carcinogen (US). Target organ(s): Heart. Liver. Calif.

Prop. 65 carcinogen.

HMIS RATING
HEALTH: 3*
FLAMMABILITY: 0
REACTIVITY: 0

NFPA RATING
HEALTH: 3
FLAMMABILITY: 0
REACTIVITY: 0

*additional chronic hazards present.

For additional information on toxicity, please refer to Section 11.

Section 4 - First Aid Measures

ORAL EXPOSURE
If swallowed, wash out mouth with water provided person is conscious. Call a physician immediately.

INHALATION EXPOSURE
If inhaled, remove to fresh air. If not breathing give artificial respiration. If breathing is difficult, give oxygen.

DERMAL EXPOSURE
In case of skin contact, flush with copious amounts of water for at least 15 minutes. Remove contaminated clothing and shoes. Call a physician.

EYE EXPOSURE
In case of contact with eyes, flush with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers. Call a physician.

Section 5 - Fire Fighting Measures

FLASH POINT
N/A

AUTOIGNITION TEMP
N/A

FLAMMABILITY
N/A

EXTINGUISHING MEDIA
Suitable: Water spray. Carbon dioxide, dry chemical powder, or appropriate foam.

FIREFIGHTING
Protective Equipment: Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.
Specific Hazard(s): Emits toxic fumes under fire conditions.

Section 6 - Accidental Release Measures

PROCEDURE TO BE FOLLOWED IN CASE OF LEAK OR SPILL
Evacuate area.

PROCEDURE(S) OF PERSONAL PRECAUTION(S)

Wear self-contained breathing apparatus, rubber boots, and heavy rubber gloves.

METHODS FOR CLEANING UP
Sweep up, place in a bag and hold for waste disposal. Avoid raising dust. Ventilate area and wash spill site after material pickup is complete.

Section 7 - Handling and Storage

HANDLING
User Exposure: Do not breathe dust. Do not get in eyes, on skin, on clothing. Avoid prolonged or repeated exposure.

STORAGE
Suitable: Keep tightly closed.

Section 8 - Exposure Controls / PPE

ENGINEERING CONTROLS
Use only in a chemical fume hood. Safety shower and eye bath.

PERSONAL PROTECTIVE EQUIPMENT
Respiratory: Government approved respirator.
Hand: Compatible chemical-resistant gloves.
Eye: Chemical safety goggles.

GENERAL HYGIENE MEASURES
Wash contaminated clothing before reuse. Wash thoroughly after handling.

Section 9 - Physical/Chemical Properties

Appearance — Physical State: Solid
Color: White

Property	Value	At Temperature or Pressure
Molecular Weight	232.2 AMU	
pH	N/A	
BP/BP Range	N/A	
MP/MP Range	174 °C	
Freezing Point	N/A	
Vapor Pressure	N/A	
Vapor Density	N/A	
Saturated Vapor Conc.	N/A	
SG/Density	N/A	
Bulk Density	N/A	
Odor Threshold	N/A	
Volatile%	N/A	
VOC Content	N/A	
Water Content	N/A	
Solvent Content	N/A	
Evaporation Rate	N/A	
Viscosity	N/A	
Surface Tension	N/A	
Partition Coefficient	N/A	
Decomposition Temp.	N/A	
Flash Point	N/A	
Explosion Limits	N/A	
Flammability	N/A	
Autoignition Temp	N/A	

Refractive Index	N/A
Optical Rotation	N/A
Miscellaneous Data	N/A
Solubility	N/A

N/A = not available

Section 10 - Stability and Reactivity

STABILITY
Stable: Stable.
Materials to Avoid: Strong oxidizing agents.

HAZARDOUS DECOMPOSITION PRODUCTS
Hazardous Decomposition Products: Nitrogen oxides Carbon monoxide, Carbon dioxide.

HAZARDOUS POLYMERIZATION
Hazardous Polymerization: Will not occur

Section 11 - Toxicological Information

ROUTE OF EXPOSURE
Skin Contact: May cause skin irritation.
Skin Absorption: May be harmful if absorbed through the skin.
Eye Contact: May cause eye irritation.
Inhalation: Material may be irritating to mucous membranes and upper respiratory tract. May be harmful if inhaled.
Ingestion: Toxic if swallowed.

SENSITIZATION
Skin: May cause allergic skin reaction.

TARGET ORGAN(S) OR SYSTEM(S)
Central nervous system. Kidneys. Liver. Heart.

SIGNS AND SYMPTOMS OF EXPOSURE
May cause respiratory depression, constipation, nausea, anorexia, vomiting, headache, drowsiness, depression, and skin effects. Exposure to and/or consumption of alcohol may increase toxic effects. Prolonged or repeated exposure can lead to habituation or addiction. To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

TOXICITY DATA

Oral
Woman
25.272 mg/kg
LDLO
Remarks: Nutritional and Gross Metabolic:Changes in:Body temperature increase. Behavioral:Coma. Skin and Appendages:Skin: After systemic exposure: Dermatitis, allergic.

Oral
Man
6.485 mg/kg
LDLO
Remarks: Nutritional and Gross Metabolic:Changes in:Body temperature increase. Skin and Appendages:Skin: After systemic exposure: Dermatitis, allergic.

Oral
Rat
162 mg/kg
LD50

Intraperitoneal
Rat
110 MG/KG
LD50

Subcutaneous
Rat
200 MG/KG
LD50

Intravenous
Rat
209 MG/KG
LD50

Rectal
Rat
284 MG/KG
LD50
Remarks: Behavioral:General anesthetic. Behavioral:Change in motor activity (specific assay). Nutritional and Gross Metabolic:Changes in:Body temperature decrease.

Oral
Mouse
137 mg/kg
LD50

Intraperitoneal
Mouse
88 MG/KG
LD50

Subcutaneous
Mouse
228 MG/KG
LD50

Intravenous
Mouse
218 MG/KG
LD50
Remarks: Peripheral Nerve and Sensation:Local anesthetic.

Intramuscular
Mouse
175 MG/KG
LD50

Oral
Dog
150 mg/kg
LD50
Remarks: Behavioral:Somnolence (general depressed activity).

Oral

Rabbit
185 mg/kg
LD50

Intravenous
Rabbit
187 MG/KG
LD50
Remarks: Behavioral:Convulsions or effect on seizure threshold. Lungs, Thorax, or Respiration:Respiratory stimulation.

Oral
Guinea pig
130 mg/kg
LD50

CHRONIC EXPOSURE - CARCINOGEN
Result: This product is or contains a component that has been reported to be possibly carcinogenic based on its IARC, ACGIH, NTP, or EPA classification.

Species: Rat
Route of Application: Oral
Dose: 7560 MG/KG
Exposure Time: 36W
Frequency: C
Result: Tumorigenic:Cells (cultured) transformed. Liver:Tumors. Tumorigenic:Equivocal tumorigenic agent by RTECS criteria.

Species: Mouse
Route of Application: Oral
Dose: 22 GM/KG
Exposure Time: 1Y
Frequency: C
Result: Tumorigenic:Neoplastic by RTECS criteria. Liver:Tumors.

Species: Mouse
Route of Application: Oral
Dose: 38 GM/KG
Exposure Time: 90W
Frequency: C
Result: Tumorigenic:Neoplastic by RTECS criteria. Liver:Tumors. Lungs, Thorax, or Respiration:Tumors.

Species: Rat
Route of Application: Oral
Dose: 4200 MG/KG
Exposure Time: 20W
Frequency: C
Result: Tumorigenic:Equivocal tumorigenic agent by RTECS criteria. Tumorigenic:Cells (cultured) transformed. Liver:Tumors.

Species: Rat
Route of Application: Oral
Dose: 30 MG/KG
Exposure Time: 78W
Frequency: C
Result: Tumorigenic:Equivocal tumorigenic agent by RTECS criteria. Endocrine:Thyroid tumors. Liver:Tumors.

Species: Rat
Route of Application: Oral

Dose: 3990 MG/KG
Exposure Time: 19W
Frequency: C
Result: Tumorigenic:Carcinogenic by RTECS criteria. Tumorigenic:Cells (cultured) transformed. Endocrine:Thyroid tumors.

Species: Rat
Route of Application: Oral
Dose: 2520 MG/KG
Exposure Time: 12W
Frequency: C
Result: Tumorigenic:Neoplastic by RTECS criteria. Tumorigenic:Cells (cultured) transformed. Endocrine:Thyroid tumors.

Species: Rat
Route of Application: Oral
Dose: 2100 MG/KG
Exposure Time: 12W
Frequency: C
Result: Tumorigenic:Carcinogenic by RTECS criteria. Endocrine:Thyroid tumors. Tumorigenic:Cells (cultured) transformed.

Species: Mouse
Route of Application: Oral
Dose: 5200 MG/KG
Exposure Time: 52W
Frequency: C
Result: Tumorigenic:Equivocal tumorigenic agent by RTECS criteria. Liver:Tumors.

IARC CARCINOGEN LIST

Rating: Group 2B

CHRONIC EXPOSURE - TERATOGEN
Result: May cause congenital malformation in the fetus.

Species: Woman
Dose: 151 MG/KG
Route of Application: Oral
Exposure Time: (27-39W PREG)
Result: Specific Developmental Abnormalities: Musculoskeletal system. Effects on Newborn: Drug dependence.

Species: Woman
Dose: 3600 UG/KG
Route of Application: Oral
Exposure Time: (26W PREG)
Result: Specific Developmental Abnormalities: Hepatobiliary system. Effects on Newborn: Biochemical and metabolic.

Species: Woman
Dose: 491 MG/KG
Route of Application: Unreported
Exposure Time: (1-39W PREG)
Result: Specific Developmental Abnormalities: Musculoskeletal system. Specific Developmental Abnormalities: Respiratory system. Specific Developmental Abnormalities: Body wall.

Species: Woman
Dose: 907 MG/KG
Route of Application: Unreported
Exposure Time: (1-36W PREG)
Result: Specific Developmental Abnormalities: Central nervous system. Specific Developmental Abnormalities: Urogenital system. Effects on Newborn: Other postnatal measures or effects.

Species: Woman
Dose: 529 MG/KG
Route of Application: Unreported
Exposure Time: (1-42W PREG)
Result: Specific Developmental Abnormalities: Gastrointestinal system.

Species: Woman
Dose: 454 MG/KG
Route of Application: Unreported
Exposure Time: (1-36W PREG)
Result: Specific Developmental Abnormalities: Central nervous system.

Species: Rat
Dose: 880 MG/KG
Route of Application: Oral
Exposure Time: (7-17D PREG)
Result: Effects on Embryo or Fetus: Fetotoxicity (except death, e.g., stunted fetus). Specific Developmental Abnormalities: Blood and lymphatic system (including spleen and marrow).

Species: Rat
Dose: 440 MG/KG
Route of Application: Oral
Exposure Time: (7-17D PREG)
Result: Specific Developmental Abnormalities: Musculoskeletal system.

Species: Rat
Dose: 800 MG/KG
Route of Application: Oral
Exposure Time: (6-13D PREG)
Result: Effects on Embryo or Fetus: Fetal death.

Species: Rat
Dose: 1 GM/KG
Route of Application: Intraperitoneal
Exposure Time: (1-20D PREG)
Result: Specific Developmental Abnormalities: Other developmental abnormalities. Effects on Embryo or Fetus: Fetal death. Effects on Embryo or Fetus: Extra embryonic structures (e.g., placenta, umbilical cord).

Species: Rat
Dose: 180 MG/KG
Route of Application: Intraperitoneal
Exposure Time: (19-21D PREG)
Result: Effects on Embryo or Fetus: Other effects to embryo.

Species: Rat
Dose: 320 MG/KG
Route of Application: Subcutaneous
Exposure Time: (12-19D PREG)

Result: Specific Developmental Abnormalities: Urogenital system. Effects on Newborn: Physical. Effects on Newborn: Delayed effects.

Species: Rat
Dose: 200 MG/KG
Route of Application: Intramuscular
Exposure Time: (18-20D PREG/15D POST)
Result: Specific Developmental Abnormalities: Central nervous system. Effects on Embryo or Fetus: Cytological changes (including somatic cell genetic material).

Species: Mouse
Dose: 400 MG/KG
Route of Application: Oral
Exposure Time: (6-15D PREG)
Result: Specific Developmental Abnormalities: Craniofacial (including nose and tongue).

Species: Mouse
Dose: 1080 MG/KG
Route of Application: Oral
Exposure Time: (16-18D PREG)
Result: Specific Developmental Abnormalities: Central nervous system.

Species: Mouse
Dose: 63 GM/KG
Route of Application: Oral
Exposure Time: (1-21D PREG)
Result: Effects on Embryo or Fetus: Cytological changes (including somatic cell genetic material).

Species: Mouse
Dose: 30 GM/KG
Route of Application: Oral
Exposure Time: (9-18D PREG)
Result: Specific Developmental Abnormalities: Central nervous system.

Species: Mouse
Dose: 150 MG/KG
Route of Application: Intraperitoneal
Exposure Time: (8-10D PREG)
Result: Effects on Embryo or Fetus: Fetotoxicity (except death, e.g., stunted fetus).

Species: Rabbit
Dose: 450 MG/KG
Route of Application: Oral
Exposure Time: (8-16D PREG)
Result: Specific Developmental Abnormalities: Musculoskeletal system. Specific Developmental Abnormalities: Cardiovascular (circulatory) system.

Species: Rabbit
Dose: 536 MG/KG
Route of Application: Oral
Exposure Time: (3-31D PREG)
Result: Effects on Embryo or Fetus: Cytological changes (including somatic cell genetic material). Effects on Newborn: Biochemical and metabolic.

CHRONIC EXPOSURE - MUTAGEN
Result: Laboratory experiments have shown mutagenic effects.

Species: Human
Dose: 400 MG/L
Exposure Time: 90M
Cell Type: leukocyte
Mutation test: Cytogenetic analysis

Species: Human
Dose: 10 MG/L
Cell Type: lymphocyte
Mutation test: Cytogenetic analysis

Species: Human
Dose: 1 GM/L
Cell Type: lymphocyte
Mutation test: Mutation in mammalian somatic cells.

Species: Rat
Route: Oral
Dose: 1260 MG/KG
Exposure Time: 6W
Mutation test: Morphological transformation.

Species: Rat
Dose: 1 MMOL/L
Cell Type: liver
Mutation test: DNA damage

Species: Rat
Route: Oral
Dose: 4410 MG/KG
Exposure Time: 21W
Mutation test: Unscheduled DNA synthesis

Species: Rat
Dose: 100 PMOL/L
Cell Type: liver
Mutation test: Unscheduled DNA synthesis

Species: Rat
Dose: 1500 UMOL/L
Cell Type: liver
Mutation test: DNA inhibition

Species: Rat
Route: Intraperitoneal
Dose: 240 MG/KG
Exposure Time: 3D
Mutation test: Phage inhibition capacity

Species: Mouse
Dose: 2 GM/L (+S9)
Cell Type: lymphocyte
Mutation test: Mutation in microorganisms

Species: Mouse
Dose: 667 MG/L
Cell Type: Embryo
Mutation test: Morphological transformation.

Species: Mouse
Route: Oral
Dose: 400 MG/KG
Exposure Time: 4D
Mutation test: Unscheduled DNA synthesis

Species: Mouse
Dose: 3 MMOL/L
Cell Type: liver
Mutation test: Other mutation test systems

Species: Mouse
Route: Oral
Dose: 3332 UG/KG
Mutation test: Cytogenetic analysis

Species: Mouse
Route: Oral
Dose: 210 MG/KG
Exposure Time: 5D
Mutation test: Dominant lethal test

Species: Mouse
Dose: 500 MG/L
Cell Type: lymphocyte
Mutation test: Mutation in mammalian somatic cells.

Species: Hamster
Dose: 100 MG/L
Cell Type: Embryo
Mutation test: Morphological transformation.

Species: Hamster
Dose: 15 MMOL/L
Cell Type: ovary
Mutation test: Cytogenetic analysis

Species: Hamster
Dose: 2 GM/L
Exposure Time: 48H
Cell Type: lung
Mutation test: Cytogenetic analysis

Species: Hamster
Dose: 100 MG/L
Cell Type: liver
Mutation test: Cytogenetic analysis

Species: Hamster
Dose: 15 MMOL/L
Cell Type: ovary
Mutation test: Sister chromatid exchange

Species: Hamster
Dose: 10 MG/L
Cell Type: lung
Mutation test: Sister chromatid exchange

CHRONIC EXPOSURE - REPRODUCTIVE HAZARD

Species: Rat

Dose: 300 MG/KG
Route of Application: Oral
Exposure Time: (4-8D PREG)
Result: Effects on Fertility: Abortion. Effects on Fertility: Post-implantation mortality (e.g., dead and/or resorbed implants per total number of implants).

Species: Rat
Dose: 320 MG/KG
Route of Application: Oral
Exposure Time: (7-10D PREG)
Result: Effects on Newborn: Weaning or lactation index (e.g., # alive at weaning per # alive at day 4). Effects on Newborn: Behavioral.

Species: Rat
Dose: 280 MG/KG
Route of Application: Oral
Exposure Time: (1-7D POST)
Result: Effects on Newborn: Biochemical and metabolic.

Species: Rat
Dose: 60 MG/KG
Route of Application: Oral
Exposure Time: (7-18D PREG)
Result: Effects on Newborn: Viability index (e.g., # alive at day 4 per # born alive).

Species: Rat
Dose: 320 MG/KG
Route of Application: Subcutaneous
Exposure Time: (MULTIGENERATIONS)
Result: Effects on Fertility: Male fertility index (e.g., # males impregnating females per # males exposed to fertile nonpregnant females).

Species: Rat
Dose: 750 MG/KG
Route of Application: Subcutaneous
Exposure Time: (1D PRE)
Result: Effects on Fertility: Other measures of fertility

Species: Rat
Dose: 20 MG/KG
Route of Application: Subcutaneous
Exposure Time: (5-8D PREG)
Result: Effects on Newborn: Behavioral.

Species: Rat
Dose: 160 MG/KG
Route of Application: Subcutaneous
Exposure Time: (17-20D PREG)
Result: Effects on Newborn: Delayed effects. Effects on Newborn: Physical.

Species: Rat
Dose: 40 MG/KG
Route of Application: Unreported
Exposure Time: (17D PREG)
Result: Effects on Newborn: Other postnatal measures or effects.

Species: Rat

Dose: 340 MG/KG
Route of Application: Multiple
Exposure Time: (20-22D PREG/7D POST)
Result: Effects on Newborn: Other postnatal measures or effects.

Species: Mouse
Dose: 210 MG/KG
Route of Application: Oral
Exposure Time: (5D MALE)
Result: Effects on Fertility: Post-implantation mortality (e.g., dead and/or resorbed implants per total number of implants). Effects on Fertility: Pre-implantation mortality (e.g., reduction in number of implants per female; total number of implants per corpora lutea).

Species: Mouse
Dose: 33 MG/KG
Route of Application: Oral
Exposure Time: (9-19D PREG)
Result: Effects on Newborn: Physical.

Species: Mouse
Dose: 3600 MG/KG
Route of Application: Oral
Exposure Time: (9-18D PREG)
Result: Effects on Newborn: Behavioral. Effects on Newborn: Growth statistics (e.g., reduced weight gain).

Species: Mouse
Dose: 560 MG/KG
Route of Application: Intraperitoneal
Exposure Time: (8-14D PREG)
Result: Effects on Fertility: Post-implantation mortality (e.g., dead and/or resorbed implants per total number of implants).

Species: Mouse
Dose: 300 MG/KG
Route of Application: Intraperitoneal
Exposure Time: (17-19D PREG)
Result: Effects on Newborn: Biochemical and metabolic.

Species: Mouse
Dose: 280 MG/KG
Route of Application: Subcutaneous
Exposure Time: (15-21D PREG)
Result: Effects on Newborn: Behavioral.

Species: Mouse
Dose: 60 UG/KG
Route of Application: Parenteral
Exposure Time: (1D PRE)
Result: Maternal Effects: Uterus, cervix, vagina.

Species: Mouse
Dose: 40 MG/KG
Route of Application: Unreported
Exposure Time: (1D PRE)
Result: Effects on Fertility: Other measures of fertility

Species: Rabbit
Dose: 450 MG/KG
Route of Application: Oral

Exposure Time: (8-16D PREG)
Result: Effects on Fertility: Litter size (e.g.; # fetuses per litter; measured before birth). Effects on Embryo or Fetus: Fetal death. Effects on Fertility: Post-implantation mortality (e.g., dead and/or resorbed implants per total number of implants).

Species: Rabbit
Dose: 175 GM/KG
Route of Application: Intramuscular
Exposure Time: (20-27D PREG)
Result: Effects on Newborn: Biochemical and metabolic.

Species: Hamster
Dose: 140 MG/KG
Route of Application: Intraperitoneal
Exposure Time: (1D PRE)
Result: Effects on Fertility: Other measures of fertility

Section 12 - Ecological Information

ACUTE ECOTOXICITY TESTS

Test Type: LC50 Fish
Species: Pimephales promelas (Fathead minnow)
Time: 96 h
Value: 484 mg/l

Section 13 - Disposal Considerations

APPROPRIATE METHOD OF DISPOSAL OF SUBSTANCE OR PREPARATION
Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber. Observe all federal, state, and local environmental regulations.

Section 14 - Transport Information

DOT
Proper Shipping Name: Toxic solids, organic, n.o.s.
UN#: 2811
Class: 6.1
Packing Group: Packing Group III
Hazard Label: Toxic substances.
PIH: Not PIH

IATA
Proper Shipping Name: Toxic solid, organic, n.o.s.
IATA UN Number: 2811
Hazard Class: 6.1
Packing Group: III

Section 15 - Regulatory Information

EU ADDITIONAL CLASSIFICATION
Symbol of Danger: T
Indication of Danger: Toxic.
R: 61-25-40-43
Risk Statements: May cause harm to the unborn child. Toxic if swallowed. Limited evidence of a carcinogenic effect. May cause sensitization by skin contact.

S: 53-36/37-45
Safety Statements: Avoid exposure - obtain special instructions before use. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

US CLASSIFICATION AND LABEL TEXT
Indication of Danger: Toxic.
Risk Statements: May cause harm to the unborn child. Toxic if swallowed. Limited evidence of a carcinogenic effect. May cause sensitization by skin contact.
Safety Statements: Avoid exposure - obtain special instructions before use. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
US Statements: Possible Carcinogen (US). Target organ(s): Heart. Liver. Calif. Prop. 65 carcinogen.

UNITED STATES REGULATORY INFORMATION
SARA LISTED: No

UNITED STATES - STATE REGULATORY INFORMATION

CALIFORNIA PROP - 65
California Prop - 65: This product is or contains chemical(s) known to the state of California to cause cancer.

CANADA REGULATORY INFORMATION
WHMIS Classification: This product has been classified in accordance with the hazard criteria of the CPR, and the MSDS contains all the information required by the CPR.
DSL: Yes
NDSL: No

Section 16 - Other Information

DISCLAIMER
For R&D use only. Not for drug, household or other uses.

WARRANTY
The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Inc., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.

SIGMA-ALDRICH

Certificate of Analysis

Product Name	Phenobarbital,
Product Number	P1636
Product Brand	Sigma
CAS Number	50-06-6
Molecular Formula	$C_{12}H_{12}N_2O_3$
Molecular Weight	232.24

TEST	SPECIFICATION	LOT 104K2600 RESULTS
APPEARANCE	WHITE POWDER	WHITE POWDER
SOLUBILITY	CLEAR COLORLESS SOLUTION AT 50MG/ML IN ETHANOL	CLEAR COLORLESS SOLUTION
IR SPECTRUM	CONSISTENT WITH STRUCTURE	CONFORMS
PURITY BY NAOH TITRATION	NLT 99%	99.1%
PURITY BY THIN LAYER CHROMATOGRAPHY	NLT 99%	GREATER THAN 99%
SHELF LIFE	5 YEARS	FEBRUARY 2010
QC ACCEPTANCE DATE		FEBRUARY 2005

Lori Schulz, Manager
Analytical Services
St. Louis, Missouri USA

SIGMA-ALDRICH

Certificate of Analysis

Product Name	Methyl cellulose, viscosity 4,000 cP (2% aqueous solution, 20 Â°C) (lit.)
Product Number	M0512
Product Brand	Sigma
CAS Number	9004-67-5
Molecular Formula	
Molecular Weight	

TEST	SPECIFICATION	LOT 062K0144 RESULTS
APPEARANCE	OFF-WHITE POWDER	CONFORMS
SOLUBILITY	CLEAR TO HAZY COLORLESS TO LIGHT YELLOW VISCOUS SOLUTION AT 20MG/ML IN WATER	VERY SLIGHTLY HAZY VERY FAINT YELLOW
VISCOSITY OF A 2% AQUEOUS SOLUTION	3500 TO 5600 CPS (20DEGC)	4,202 CPS (SUPPLIER TEST RESULT)
QC ACCEPTANCE DATE		JULY 2002
PRODUCT CROSS REFERENCE INFORMATION		REPLACEMENT FOR ALDRICH #274410

Lori Schulz, Manager
Analytical Services
St Louis, Missouri USA

ATTACHMENT 3

TEST SUBSTANCE PREPARATION PROCEDURES

ATTACHMENT 3

TEST SUBSTANCE PREPARATION PROCEDURE

Test Substances: Linuron and Phenobarbital

Vehicle: Aqueous 0.25% (w/v) methylcellulose

A. Purpose:

The purpose of this procedure is to provide a method for the preparation of dosage formulations of the test substances for oral (gavage) administration to rats on Protocol RTP00004.

B. General Information:

1. All formulation containers will be labeled and color-coded. Each label will specify the protocol number, coded identification as indicated by the formulation key, batch number, dosage group, preparation date, expiration date and storage conditions.

2. Formulations (suspensions) will be prepared at least once prior to the initiation of the study in order to validate the transfer of information provided by the Sponsor and once for the formulations that will be used during the dosage period. Formulations (suspensions) will be prepared at the Testing Facility.

3. Formulations will be administered at a final dosage volume of 5 mL/kg.

4. Safety:
   - X Gloves, uniform/lab coat, goggles or safety glasses with side shields
   - X Dust-Mist/HEPA-filtered Mask
   - ___ Half-Face Respirator
   - ___ Full-Face Respirator/Positive Pressure Hood
   - X Tyvek® Sleeves
   - ___ Full Face Shield
   - X Bulk TA/S will be handled in a chemical fume hood

5. The test substances will be considered 100% active/pure for the purpose of

TEST SUBSTANCE PREPARATION PROCEDURE

dosage calculations.

6. Sampling requirements: Cited in protocol.

7. Storage: Cited in protocol.

C. Dosage Formulation Preparation:

1. Weigh the required amount of test substance* (see TA/S PREPARATION CALCULATIONS) onto a piece of weigh paper or into an appropriately sized and labeled, amber glass bottle. If weigh paper is used, quantitatively transfer the test substance into an appropriately sized and labeled amber glass bottle.

   * - Prior to weighing, each test substance will be screened so that a uniform suspension can be prepared. A round (at least six inches in diameter) 180 micron screen is set up with a collection pan and cover. Each test substance is placed on a separate screen and the screen is shaken to push the test substance through the screen.

2. Measure the required amount of vehicle (see TA/S PREPARATION CALCULATIONS) in an appropriately sized graduated cylinder. Add the vehicle to the bottle containing the test substance.

3. Add an appropriately sized magnetic stir bar to the bottle. Place the bottle on a magnetic stir plate and mix thoroughly the suspension for at least 60 minutes prior to and during sampling and/or aliquotting. Visual inspection should show an evenly distributed suspension.

4. Aliquot the formulation into an appropriate number of appropriately sized and labeled amber glass bottles. The aliquots will be stored refrigerated until use.

5. On each day of dosage, the required number of aliquots will be removed from the refrigerator. A magnetic stir bar will be added to each aliquot. The aliquots will be placed on a magnetic stir plate and the suspension will

ATTACHMENT 3

TEST SUBSTANCE PREPARATION PROCEDURE

be stirred to suspend the test substance and warm the suspension. Stir the suspensions for at least 60 minutes prior to sampling and/or dosage administration. The suspensions should be stirred vigorously enough to show a slight vortex only in order to avoid introduction of excessive air into the suspension and the production of foam. Visual inspection should show an evenly distributed suspension.

6. Repeat steps C.1. through C.5. for each concentration of each test substance.

Written by: Michael D. Brennan

Approved by: Joseph Lech Date: 10-7-05

Clarification: X No ___ Yes (See attached clarification form.)

Initials/Date: Joseph Lech 1.16.06

ATTACHMENT 4

TISSUES TO BE WEIGHED AND RETAINED FOR POSSIBLE HISTOPATHOLOGICAL EVALUATION

ATTACHMENT 4

TISSUES TO BE WEIGHED AND RETAINED FOR POSSIBLE HISTOPATHOLOGICAL EVALUATION

Tissues to be Weighed

NOTE: All organ weights will be recorded to the nearest 0.0001 g.

The following organs will be weighed as soon as possible after excision to avoid drying:

Liver	Prostate (whole)
Right Testis	Seminal Vesicles (with fluid and coagulating gland)
Left Testis	Thyroid*
Epididymides (paired)	

* The thyroid gland and the surrounding tissue will be removed from neutral buffered 10% formalin after at least 48 hours fixation prior to trimming and weighing. Following fixation, final dissection will be performed under a dissecting microscope by one individual in order to reduce the variability of the dissection procedure and hence, reduce the variability of the thyroid weights.

The following organ weights will be calculated.

Testes (paired, left and right testis weights combined	Accessory Sex Gland (ASG, entire prostate and seminal vesicles with fluid and coagulating gland combined)

Tissues to be Retained

The following tissues will be collected from all rats at necropsy and retained in neutral buffered 10% formalin.

Right Testis*	Prostate
Left Testis*	Liver
Right Epididymis	Thyroid
Left Epididymis	

* Testes will be fixed in Bouin's solution for approximately 24 hours before being transferred to and retained in 70% alcohol.

ATTACHMENT 4

Histopathological Evaluation

The dose groups but not the compounds will be known to the pathologist during evaluation. After evaluation, the nature of the compounds will be known to the pathologist for report writing.

The right testis and left testis, the right epididymis and left epididymis and the thyroid will be individually identified and examined histopathologically and will be routinely processed, embedded in paraffin, sectioned at 5 microns and stained with hematoxylin and eosin. The liver will also be examined hisologically if the weights are significantly increased.

Only gross lesions associated with the testes, epididymides and thyroid will be examined histopathologically. Gross lesions associated with the liver (only if the relative weights are significantly increased) will be examined histologically.

Histopathological examination will be performed on all control and high dose rats of each test substance. If lesions attributed to the test substance are observed by the Study Director and/or Veterinary Pathologist in the rats exposed to the high test substance concentration, the same organs will be examined histopathologically in the rats exposed to the lower test substance concentrations by amendment. Should results from the control and high dosage groups warrant examination of the lower dosage groups and conduct of the quantitative evaluation, scheduled report dates will be adjusted accordingly. Additional costs will be incurred should these evaluations be required. Histopathological findings will be presented in a Contributing Scientist Report.

Shipping Instructions:

Retained tissues will be shipped (ambient conditions) to:

Principal Investigator: W. Ray Brown, D.V.M., Ph.D., Diplomate, ACVP
Veterinary Pathologist
Research Pathology Services, Inc.
438 E. Butler Avenue
New Britain, Pennsylvania 18901
USA
Telephone: (215) 345-7070
Telefax: (215) 345-4326
E-mail: WRBRPS@concentric.net

The recipient will be notified in advance of sample shipment

CHARLES RIVER
LABORATORIES
Preclinical Services

PROTOCOL RTP00004

INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL

SPONSOR'S WORK ASSIGNMENT: WA 5-15

Amendment 1 - 02 November 2005

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1. Formulation - Frequency of Preparation (page 5 of the protocol):

   [Effective Date: 11 October 2005] Phenobarbital (suspensions) to be used during the dosing period will be prepared twice rather than once.

   Reason for Change:

   It was determined that the Phenobarbital suspensions were not stable at 21 days. The maximum stability for the Phenobarbital is 14 days.

2. Study Schedule (page 2 of 2 ATTACHMENT 1 of the protocol):

   [Effective Date: 11 October 2005] The study schedule will be revised as follows:

18 OCT 05	Animal Receipt.
25 OCT 05	Proposed Experimental Start Date
25 OCT 05 - 08 NOV 05	Dosage Administration (replicate1).
26 OCT 05 - 09 NOV 05	Dosage Administration (replicate2).
27 OCT 05 - 10 NOV 05	Dosage Administration (replicate3).

Any revisions to this finalized amendment must be made by subsequent amendment.

905 Sheehy Drive, Bldg. A, Horsham, PA 19044 • 215.443.8710 • FAX 215.443.8587

08 NOV 05 Sacrifice and Necropsy (replicate 1).

09 NOV 05 Sacrifice and Necropsy (replicate 2).

10 NOV 05 Sacrifice and Necropsy (replicate 3).

Reason for Change:

Animals to be received for study have been delayed one week.

3. Analyses of Prepared Formulations - Shipping Instructions (pages 6 and 7 of the protocol):

[Effective Date: 25 October 2005] The Principal Investigator assigned to the analysis of the prepared formulations has been changed to Dorothy Savage. Samples to be analyzed will be shipped to:

Principal Investigator: Dorothy Savage, B.S.
Charles River Laboratories
Preclinical Services Massachusetts
57 Union Street
Worcester, MA 01608
USA
Telephone: 508.890.0100
Telefax: 508.791.9713
E-mail: dorothy.savage@us.crl.com

Reason for Change:

The original Principal Investigator assigned to this project is no longer an employee of Charles River Laboratories.

4. Formulation - Frequency of Preparation (page 5 of the protocol):

[Effective Date: 25 October 2005] Linuron (suspensions) to be used during the dosing period will be prepared twice rather than once.

Reason for Change:

This change is being made in order to have the results of the concentration and homogeneity analyses prior to the start of administration.

Any revisions to this finalized amendment must be made by subsequent amendment.

5. Analyses of Prepared Formulations (page 6 of the protocol):

[Effective Date: 25 October 2005] Quadruplicate samples (1 mL each) will be taken from the top, middle and bottom of each concentration on the day prepared for both the prestudy and from the first preparation of each test substance used for dosage administration. Samples will not be taken from the second preparation of either test substance.

Reason for Change:

This change is being made in order to clarify the intentions of the protocol.

6. Gross Necropsy, Hormone Analysis and Histopathology (page 13 and page 2 of 2 of ATTACHMENT 4 of the protocol):

[Effective Date: 25 October 2005] The wording indicated below describing the criteria before assay of dihydrotestosterone (DHT) and the evaluation of the liver histologically will replace the original wording in the protocol.

Original wording

Only if relative liver weights are significantly increased should DHT levels be measured.

Revised wording

Determination of serum DHT concentrations or the evaluation of the livers histologically will be done on a group basis if there is a statistically significant mean decrease in one or more androgen-dependent organ weights (relative weight for ASG, seminal vesicles and prostate and absolute paired weights for the testes and epididymides) and no corresponding decrease in serum testosterone concentrations in the treated groups compared to the control group. If one or more of these conditions is seen, the Study Director will contact the Sponsor to verify the need to assay DHT.

Reason for Change:

This change is being made at the request of the Sponsor in order to clarify the criteria to be used in regards to evaluating DHT hormone levels and the livers histologically from all control and high dose rats of each test substance.

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7. Gross Necropsy, Hormone Analysis and Histopathology (page 13 of the protocol):

[Effective Date: 25 October 2005] The volume of blood collected for evaluation of serum hormones should be approximately 9mL rather than at least 8 mL. Enough blood should be collected in order to yield approximately 4500 mcL of serum, to be aliquotted into nine vials of approximately 500 mcL each.

Reason for Change:

This change is being made in order to assure enough serum is harvested in order to perform all of the hormone assays.

8. Gross Necropsy, Hormone Analysis and Histopathology and Shipping Instructions (page 13 and 14 of the protocol):

[Effective Date: 25 October 2005] The Principal Investigator assigned to the analysis of the serum hormones has been changed to Carol D. Sloan at RTI International. Samples to be analyzed will be shipped to:

Principal Investigator: Carol D. Sloan
RTI International
3040 Cornwallis Rd.
PO Box 12194
Research Triangle Park, NC 27709-2194
USA
Telephone: 919.541.6337
Telefax: 919.541.6499
E-mail: css@rti.org

Reason for Change:

This change is being made in order to have the hormone analyses performed in the timeframe requested by the Sponsor.

9. Gross Necropsy, Hormone Analysis and Histopathology (page 13 of the protocol):

[Effective Date: 25 October 2005] Blood will be collected and immediately placed into serum separator tubes and allowed to clot at room temperature rather than being placed in wet ice prior to the serum being prepared.

Reason for Change:

This change is being made at the request of the Principal Investigator assigned to the hormone analysis in order to yield more serum for the hormone analyses.

11-2-05
Alan M. Hoberman, Ph.D., DABT Date
Director of Research

11-2-05
Joseph W. Lech, B.S., LAT Date
Scientist
Study Director

02Nov05
Mathew B. Carlson, B.A. Date
Member, Institutional Animal Care
and Use Committee

11/3/05
David P. Houchens, Ph.D. Date
Program Manager
Battelle

11-3-05
Terri Pollock Date
Quality Assurance
Battelle

Any revisions to this finalized amendment must be made by subsequent amendment.

CHARLES RIVER
LABORATORIES
Preclinical Services

PROTOCOL RTP00004

INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL

SPONSOR'S WORK ASSIGNMENT: WA 5-15

Amendment 2 - 18 November 2005

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1. Gross Necropsy, Hormone Analysis and Histopathology (page 13 of the protocol and point 6 of Amendment 1):

   [Effective Date: 15 November 2005] Determination of serum DHT concentrations will be performed for all dosage groups as a hormonal end point that will be assayed in sequence along with the other hormones after testosterone.

   Reason for Change:

   This change is being made at the request of the Sponsor.

11-18-05

Alan M. Hoberman, Ph.D., DABT — Date
Director of Research

11-18-05

Joseph W. Lech, B.S., LAT — Date
Scientist
Study Director

18 NOV 05

Mathew B. Carlson, B.A. — Date
Member, Institutional Animal Care
and Use Committee

11/21/05

David P. Houchens, Ph.D. — Date
Program Manager
Battelle

11-21-05

Terri Pollock — Date
Quality Assurance
Battelle

Any revisions to this finalized amendment must be made by subsequent amendment.

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CHARLES RIVER
LABORATORIES
Preclinical Services

PROTOCOL RTP00004

INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL

SPONSOR'S WORK ASSIGNMENT: WA 5-15

Amendment 3 - 28 December 2005

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1. Test and Control Substances and Dosage Groups, Levels and Volumes (pages 3 and 11 of the protocol):

   [Effective Date: 21 December 2005] The dosage levels and concentration for each test substance are as follows:

Test Substance	Dosage Group	Chemical Name	Dosage (mg/kg/day)	Concentration (mg/ml)
A	1	0.25% Methylcellulose	0 (Vehicle)	0
B	2	Linuron	50	10
C	3	Linuron	100	20
D	4	Linuron	150	30
E	5	Phenobarbital	25	5
F	6	Phenobarbital	50	10
G	7	Phenobarbital	100	20

Reason for Change:

This table is being added in order to identify dosage levels and concentrations for the test substances as per protocol.

12/28/05 — 12/28/05

Alan M. Hoberman, Ph.D., DABT Date
Director of Research

Joseph W. Lech, B.S., LAT Date
Scientist
Study Director

Any revisions to this finalized amendment must be made by subsequent amendment.

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28 DEC 2005 1/3/06

Mathew B. Carlson, B.A. FOR Date
Member, Institutional Animal Care
and Use Committee

David P. Houchens, Ph.D. Date
Program Manager
Battelle

1-3-06

Terri Pollock Date
Quality Assurance
Battelle

Finalization of this draft amendment will occur upon receipt of the Sponsor's approval.

CHARLES RIVER
LABORATORIES
Preclinical Services

PROTOCOL RTP00004

INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL

SPONSOR'S WORK ASSIGNMENT: WA 5-15

Amendment 4 - 07 February 2006

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1. Test and Control Substances - Identification (pages 4 of the protocol):

   [Effective Date: 27 January 2006] The lot number of the prepared control substance used for this study was 062K0144 rather than 14601TC.

   Reason for Change:

   The Sponsor supplied this information in order to clarify the protocol.

2. Data Presentation and Statistics (pages 18 through 20 of the protocol):

   [Effective Date: 27 January 2006] Clarification of the tables and figures supplied by Battelle are listed below. Summary tables 1 through 5 included all values, including the possible outlier values, based on judgment of the Study Director. Summary tables 6 and 7 included all values with the exception of possible outlier values, based on judgment of the Study Director.

   In the statistical analysis carried out in December, 2005 – January, 2006:

   •Table 1 displays the statistics underlying the decisions that were made concerning the degree of variance pooling (if any) in the subsequent analyses for each endpoint.
   •Tables 2 and 3 contain the summary values for the seven body weight and food consumption endpoints, for Linuron (Table 2) and Phenobarbitol (Table 3) respectively.
   •Tables 4 and 5 contain the summary values for the nine absolute organ weight endpoints and the nine organ to body weight ratio endpoints, for Linuron (Table 4) and Phenobarbitol (Table 5) respectively.

Any revisions to this finalized amendment must be made by subsequent amendment.

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•Tables 6 and 7 contain the summary values for the nine hormonal assay endpoints, for Linuron (Table 6) and Phenobarbitol (Table 7) respectively.

•Figures 1 and 2 display the mean body weights for each day from TD1 to TD15, for Linuron (Figure 1) and Phenobarbitol (Figure 2), respectively.
•Figures 3 to 9 display the means ± 2 standard errors for the seven body weight and food consumption endpoints, one figure per endpoint.
•Figures 10 to 18 display the means ± 2 standard errors for the nine absolute organ weight endpoints, one figure per endpoint.
•Figures 19 to 27 display the means ± 2 standard errors for the nine organ to body weight ratio endpoints, one figure per endpoint.
•Figures 28 to 36 display the means ± 2 standard errors for the nine hormonal assay endpoints, one figure per endpoint.

Reason for Change:

The Sponsor supplied this information in order to clarify the protocol.

7-Feb-06
Alan M. Hoberman, Ph.D., DABT Date
Director of Research

2/7/06
Joseph W. Lech, B.S., LAT Date
Scientist
Study Director

07FEB06
Mathew B. Carlson, B.A. Date
Member, Institutional Animal Care
and Use Committee

2/9/06
David P. Houchens, Ph.D. Date
Program Manager
Battelle

2-9-06
Terri Pollock Date
Quality Assurance
Battelle

Any revisions to this finalized amendment must be made by subsequent amendment.

APPENDIX 4 - ANALYTICAL REPORT - BULK TEST SUBSTANCE

Battelle
The Business of Innovation

Chemical Repository Services for the EDSP
EPA Contract No. 68-W-01-023

1.0 TITLE PAGE

Study Title: Analysis of Test Substances for Work Assignment 5-15

Authors: Tim Fortman, Michael Cobb

Study Initiation Date: 8/26/05

Study Completion Date: January 12, 2006

Performing Lab: EDSP Chemical Repository,
Battelle Marine Sciences Laboratory,
1529 West Sequim Bay Road,
Sequim, WA 98382

Study Number: EDSP.515-01

Data Requirement: 40 CFR Part 160.105, 160.113

Submitted To: Dr. David P. Houchens,
EDSP Program Manager
Battelle Columbus Operations,
505 King Avenue,
Columbus, OH, 43201-2693

Total Number of Pages: 52

2.0 STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentially is made for any information contained in this study on the basis of its falling within the scope of the United States Environmental Protection Agency Federal Insecticide, Fungicide, and Rodenticide Act Section 10(d) (1)(A), (B), or (C).

Company: Battelle

Company Agent: David P. Houchens, Ph.D.

Title: EDSP Program Manager

Signature: David P. Houchens Date: 11/2/06

3.0 STATEMENT OF COMPLIANCE

This study meets the requirements for 40 CFR Part 160, EPA FIFRA Good Laboratory Practices:

Note: Protocol, and any amendments and deviations are provided in Appendix B of this report. Method deviations are described in Appendix F of this report.

Study Director: _____ 1/12/06
Michael Cobb — Date
Battelle – Marine Sciences Laboratory

Sponsor's Representative: _____ 1/6/06
David Houchens, Ph.D. — Date
Battelle Columbus Operations

Submitter: _____ 1/12/06
Date

4.0 QUALITY ASSURANCE

This study was examined for compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160, 17 August 1989. The dates of all audits and inspections and the dates of any findings were reported to the Study Director and Test Facility Management as follows:

ACTIVITY	DATE CONDUCTED	DATE REPORTED TO:	
		STUDY DIRECTOR	MANAGEMENT
Technical Systems Audit, Analysis of Day 7 Phenobarbital Samples	September 22, 2005	September 22, 2005	September 22, 2005
Audit of Data Quality, Stability/Purity Data and Draft Report	December 30, 2005 January 9, 2006	January 9, 2006	January 9, 2006
Final Report	January 12, 2006	January 12, 2006	January 12, 2006

Mary E. Lynn
Quality Assurance

1/12/06
Date

5.0 APPROVALS PAGE

Study Title: Analysis of Test Substances for Work Assignment 5-15

Submitted by: Battelle Marine Sciences Laboratory
Address: 1529 West Sequim Bay Road
Sequim, WA 98382

Prepared by:

Eric Crecelius for Fortman 1-12-06

Timothy Fortman — Date
Senior Chemistry Analyst
Battelle – Marine Sciences Laboratory

Approved by:

1-12-06

Michael E. Cobb — Date
EDSP Chemical Repository Study Director
Battelle – Marine Sciences Laboratory

Approved by:

Eric Crecelius 1-12-06

Eric Crecelius — Date
Manager, EDSP Chemical Repository
Battelle – Marine Sciences Laboratory

Personnel participating in this study:

Analysts: Linda Bingler, Timothy Fortman

Chemical Repository Study Director: Michael Cobb

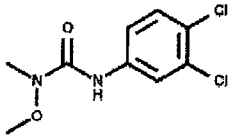
Experimental Start: September 14, 2005

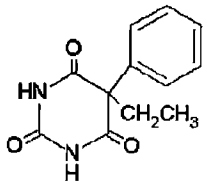
Experimental Termination: October 6, 2005

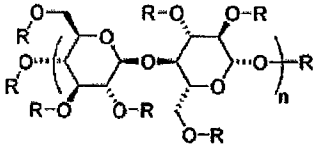
6.0 EXECUTIVE SUMMARY

Analysis of Test Substances for Work Assignment 5-15

Table 1. Study Test and Reference Substances and Vehicle

Parameter	Test & Reference Substance	Linuron
Compound Name	Linuron	
CAS #	330-55-2	
Central File No.	2463-1	
Initial Receipt Date	08/24/2005	
Expiration Date	August 2008	
Supplier	Chem Service	
Lot Number	348-8A	
Method	EDSP.H4-033	

Parameter	Test & Reference Substance	Phenobarbital
Compound Name	Phenobarbital	
CAS #	50-06-6	
Central File No.	2461-1	
Initial Receipt Date	08/16/2005	
Expiration Date	February 2010	
Manufacturer	Sigma	
Lot Number	104K2600	
Method	EDSP.H4-034	

Parameter	Test Substance	Methylcellulose
Compound Name	Methylcellulose	
CAS #	9004-67-5	
Central File No.	2462-1	
Initial Receipt Date	08/24/05	
Expiration Date	August 2010	
Supplier	Sigma	
Lot Number	14601TC	
Method	N/A*	R = CH_3 or H

*Not applicable

Executive Summary

Work Assignment (WA) 5-15 of the Environmental Protection Agency's (EPA) Endocrine Disruptor Screening Program (EDSP) describes an *Inter-laboratory Validation of the 15-Day Intact Adult Male Rat Assay*. The Chemical Repository (CR) has the responsibility for carrying out the purity, formulation preparation, method development, method validation, and formulation stability determinations of selected study test substances for EDSP studies. The chemistry formulation (in a methylcellulose carrier), purity determination, and stability studies for the test substance phenobarbital, and the formulation, homogeneity, and purity of the test substance linuron (also formulated in methylcellulose) are documented in the present report.

The EPA limited the study for linuron to determination of homogeneity and purity (stability was done during WA 2-28). The test substance purities as determined by the supplier and confirmed by the CR are provided in Table 2.

Table 2. Test and Reference Substance Purity

TEST SUBSTANCE	REPORTED PURITY	LOT NUMBER	CR DETERMINED PURITY[1]
Linuron	99.5%	348-8A	97.69%
Phenobarbital	99.1%	104K2600	99.98%

The formulation preparation procedures developed for the test substance linuron produced a suspension with actual concentrations measured in the top 1/3 and the bottom 1/3 of the container that were within 10 percent of the target concentrations for linuron per specifications. Determinations for both levels were carried out in triplicate. The protocol specified that recoveries at the two levels would agree within 10%. The linuron values met this specification. The phenobarbital formulation yielded concentrations that were within the formulation accuracy specification but fell out of the 10% agreement (homogeneity) specification (for the 5 mg/mL day 1 determination and the 20 mg/mL day 7 determination). The formulation concentrations that were analyzed for both test substances are summarized in Tables 3 and 4.

Table 3. Formulation Homogeneity - Linuron

Test Substance	Position of Measurement	Recovery	Agreement
Linuron	Top 1/3	90.58%	3.16%
	Bottom 1/3	93.49%	

Table 4. Formulation Homogeneity – Phenobarbital 5 mg/mL

Test Substance	Position of Measurement	Recovery (day 1)	Agreement (day 1)	Recovery (day 7)	Agreement (day 7)	Recovery (day 14)	Agreement (day 14)
Phenobarbital 5 mg/mL	Top 1/3	91.32%	13.17%	96.96%	2.84%	97.32%	0.59%
	Bottom 1/3	104.19%		99.75%		96.75%	

Table 5. Formulation Homogeneity – Phenobarbital 20 mg/mL

Test Substance	Position of Measurement	Recovery (day 0)	Agreement (day 0)	Recovery (day 7)	Agreement (day 7)	Recovery (day 14)	Agreement (day 14)
Phenobarbital 20 mg/mL	Top 1/3	96.90%	0.63%	91.97%	10.95%	97.13%	0.93%
	Bottom 1/3	97.51%		102.62%		98.04%	

As determined in WA 2-28, linuron (at 5 mg/mL in 0.25% methylcellulose): demonstrated stability performance at ≥ 90% of the target concentration for the testing period of 21 days. The stability evaluation for the phenobarbital formulations was specified as a 28 day study with sample analyses to be carried out on days 0, 7, 14, 21, and 28. Due to poor performance of the day zero 5 mg/mL phenobarbital result, the day zero determination was repeated the following day resulting in test intervals[2] for the 5 mg/mL suspension of 1, 7, 14, and 21. Results at day 14 (Table 4) indicated the 5 mg/mL suspension of phenobarbital was still within ±10% of the nominal concentration, but by day 21 had fallen to a 68% recovery. Recovery for the 20 mg/mL suspension of phenobarbital at day 14 (Table 5) was still within ±10% of the nominal concentration, but by day 21 had fallen to 71%. The study for the two suspensions was stopped after day 21.

[1]Calculations for purity are: area of compound of interest divided by the total area where the total area is adjusted by subtracting a blank area.

[2]A protocol deviation (EDSP.515-01-D1) was generated to document this change in stability test intervals for the 5 mg/mL phenobarbital suspension (see Appendix B).

7.0 TABLE OF CONTENTS

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8.0 INTRODUCTION

The goal of the Battelle-Sequim, Marine Sciences Laboratory (MSL) Chemical Repository for the Endocrine Disruptor Screening Program (EDSP) is to provide the participating laboratory or laboratories with requested chemicals of documented quality and if required, at concentrations in a matrix appropriate for different toxicological tests. The EDSP Chemical Repository (CR) provides supplier information regarding purity, the material safety data sheet (MSDS) chemical information, and independent analysis of purity, formulation preparation, method development, method validation, and stability in a matrix specified by the Study Protocol: *Analysis of Test Substances for Work Assignment 5-15 [EDSP Study Number: EDSP.515-01]*, made in collaboration with the requesting Study Director. Under Work Assignment (WA) 5-15, the Environmental Protection Agency (EPA) contracted with the CR for purity characterization of the test substances (Table 6), linuron and phenobarbital. The CR was charged with carrying out method development and validation, formulation preparation (in a 0.25% methylcellulose carrier), homogeneity determination, and purity testing on both test substances. In addition, a 28 day stability study was scheduled for phenobarbital as formulated in the carrier at two concentrations. Both test substances were suspensions in the carrier at the study concentrations.

9.0 GENERAL METHODS

Methods of standard operation of the CR are currently addressed in MSL SOPs numbered R-001 through R-017. These procedures address chemical procurement including procurement of controlled substances, when applicable, which have unique permitting, ordering, handling, inventory, and storage requirements; chemical receipt and chain of custody, chemical log-in and labeling, inventory, chemical storage, stock solution preparation, documentation and archiving, test solution preparation, documentation and shipping, chemical disposal, and CR maintenance over time. The quality assurance (QA) requirements for procurement of chemicals for use in the CR are addressed in the Quality Assurance Project Plan (QAPP) for EDSP CR.

9.1 TEST SUBSTANCE PROCUREMENT

As requested by EPA linuron, (CAS No. 330-55-2), phenobarbital (CAS No. 50-06-6), and the carrier methylcellulose (CAS No. 9004-67-5), formulated in water at 0.25%, were purchased from two suppliers as outlined in Table 6. The two test substances were used for purity, method development, method validation (phenobarbital only), formulation preparation, and stability analysis (phenobarbital only), as specified in section 8.0 above, and shipped to the participating laboratories for use in the *Inter-laboratory Validation of the 15-Day Intact Adult Male Rat Assay*. The chemicals were logged into the Chemical Management System (CMS) and each given a unique CMS barcode and log-in (central file) number as per the QAPP for the EDSP CR. The chemicals were stored in the CR at conditions specified in the material safety data sheets and documented in test substance specific Chemical Acquisition Task Notebooks.

Table 6. Study Test and Reference Substances and Vehicle

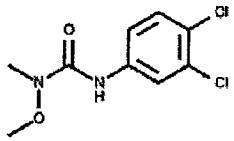
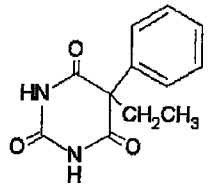
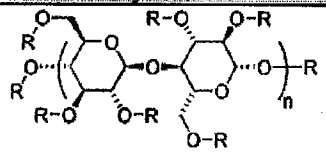
Parameter	Test Substance	Linuron
Compound Name	Linuron	
CAS #	330-55-2	
Central File No.	2463-1	
Initial Receipt Date	08/24/2005	
Expiration Date	August 2008	
Supplier	Chem Service	
Lot Number	348-8A	
Method	EDSP.H4-033	

Table 6. Study Test and Reference Substances (continued)

Parameter	Test Substance	Phenobarbital
Compound Name	Phenobarbital	
CAS #	50-06-6	
Central File No.	2461-1	
Initial Receipt Date	08/16/2005	
Expiration Date	February 2010	
Manufacturer	Sigma	
Lot Number	104K2600	
Method	EDSP.H4-034	

Parameter	Test Substance	Methylcellulose*
Compound Name	Methylcellulose	
CAS #	9004-67-5	
Central File No.	2462-1	
Initial Receipt Date	08/24/05	
Expiration Date	August 2010	
Supplier	Sigma	
Lot Number	14601TC	
Method	N/A	

R = CH_3 or H

* structure for sucrose shown, structure for a singe chain of methylcellulose will be similar

9.2 TEST SUBSTANCE PURITY

Test substance purity for linuron was determined using high performance liquid chromatography (HPLC) with ultraviolet/visible (UV/VIS) detection. Purity verification for this test substance was conducted by making a solution of about 5.0 µg/ml of the substance in 60% acetonitrile and 40% water. This matrix was then run on an HPLC with a UV/VIS diode array detector. A 60% acetonitrile and 40% water blank was also analyzed on the system. The purity was determined by comparing the area of the peak associated with the substance of interest with the total area of all the peaks in the chromatogram. The areas associated with peaks common to the blank were eliminated by subtraction. The percentage associated with the largest peak represented the purity of the test substance. This result was compared to the supplier's certificate of analysis/purity (Appendix A). The HPLC was optimized with a Phenomenex SYNERGI 4µ Hydro-RP 80A 250 X 4.6 mm 4µ HPLC column. Pressure limit on the column was 250 BAR. The system employs a UV/VIS diode array detector set to a collection wavelength of 250 nm. The run time was set to 12 minutes. A single replicate was analyzed for linuron.

Test substance purity for phenobarbital was determined using (HPLC) with UV/VIS detection. Purity verification for this test substance was conducted by making a solution of about 200 µg/mL of the substance in 50% acetonitrile and 50% water. This matrix was then run on an HPLC with a UV/VIS diode array detector. A 50% acetonitrile and 50% water blank was also analyzed on the system. The purity was determined by comparing the area of the peak associated with the substance of interest with the total area of all the peaks in the chromatogram. The areas associated with peaks common to the blank were eliminated by subtraction. The percentage associated with the largest peak represented the purity of the test substance. This result was compared to the supplier's certificate of analysis/purity (Appendix A). The HPLC was set up with a Phenomenex SYNERGI 4µ Hydro-RP 80A 250 X 4.6 mm 4µ HPLC column. Pressure limit on the column was 3000 PSI. The detector is a diode array detector set to a collection wavelength of 225 nm. The run time was set to 8 minutes. A single replicate was analyzed for phenobarbital.

9.3 STUDY VEHICLE

Methylcellulose was dissolved at 0.25% W/V in deionized water and used as the vehicle (carrier) for the test substance formulations.

9.4 FORMULATION PREPARATION AND STABILITY DETERMINATIONS

The study plan for formulation preparation and analysis development and validation, and stability testing, based on the *Technical Work Plan* for WA 5-15, was developed and documented in the Study Protocol: *Analysis of Test Substances for Work Assignment 5-15, EDSP Study Number: EDSP.515-01.* This protocol with amendments and deviations is presented in Appendix B.

The stability evaluation of linuron was not repeated for this study as it was previously evaluated in a 0.25% methylcellulose vehicle for WA 2-28. Stock and diluter formulation concentrations for phenobarbital were prepared in the 0.25% methylcellulose vehicle for determining stability (Table 7). Formulations were analyzed in triplicate for calculation of a mean concentration and relative standard deviation (RSD).

A 2.5 g/L (0.25%) methylcellulose solution was prepared by adding 700 mL of deionized water to a one liter flask. The flask was placed on a hot plate and stirred while adding 2.5 grams of methylcellulose. The solution was then carefully brought to a boil. The solution was allowed to cool and then allowed to stir for 2 hours. The solution was then transferred to a one liter volumetric flask and diluted to the mark with deionized water. The solution was stored at 2-8°C.

Formulations for phenobarbital were prepared on 9/15/2005 for testing. Briefly, for the stock solution, an amount of the test substance was passed through a six inch round 180 µm screen to insure a small particle size to maximize dissolution properties. Two phenobarbital suspensions were made up (phenobarbital is not soluble in 0.25% methylcellulose at 5 and 20 mg/mL). The 5 mg/mL suspension was made by weighing 1 gram of the sized phenobarbital into a 250 mL amber bottle with 200 mL of the methylcellulose solution (described above). A 20 mg/mL suspension of phenobarbital was prepared by weighing 4 grams of the sized phenobarbital into a 250 mL amber bottle and adding 200 mL of the 0.25% methylcellulose. The stability solutions were stored at 2-8°C.

For phenobarbital, sampling and analysis of the stability solutions was scheduled to be carried out on days 0, 7, 14, 21, and 28 of storage.

Table 7. Formulations Prepared for Phenobarbital Stability Testing

Target Conc.	Nominal Conc.	Sample ID	Stock Matrix
20 mg/ml	20.03 mg/ml	Phenobarb 20 mg/ml	0.25% methylcellulose in DI water
5 mg/ml	5.01 mg/ml	Phenobarb 5 mg/ml	0.25% methylcellulose in DI water

9.5 ANALYTICAL METHODS

Formulation stability, purity, homogeneity, and accuracy of phenobarbital were evaluated using the method described below (and provided in Appendix E). Purity, formulation accuracy, and homogeneity of linuron were evaluated using the method described below (and provided in Appendix E). The frequency of determinations and the duration of testing were selected by the Work Assignment Leader (WAL) and the chemists based on *a priori* knowledge of the stability of these chemicals in the vehicle (carrier) and usage schedule required for the dosing formulations to conduct the study.

9.5.1 Test Formulation Sampling

Prior to sampling for analysis, the phenobarbital formulations were removed from the refrigerator and allowed to come to room temperature (approximately 1 hr). The formulations were placed on stir plates and stirred to maximize dispersion uniformity of the phenobarbital. Sampling was done at 2 vertical levels in the bottles. The 1st triplicate sampling was collected at a level about 1/3 below the top of the solution. The second triplicate sampling was collected at a level about 2/3 below the top of the solution. For each sampling, 1 mL was taken, using a 3 mL syringe fitted with a 3.5 inch needle. Each 1 mL aliquot was dispensed into an individually tared 25 mL volumetric flask, weighed and the weight recorded. Each flask was then filled to the mark with acetonitrile. The flasks were agitated and 0.1 mL was removed from each and placed into individual 1.8 mL autosampler vials with 0.9 mL of the mobile phase (50% water:50% acetonitrile). The vials were capped and mixed by agitation. All solutions were then run on the HPLC. The same process was followed with the linuron sample except the sample was placed into a 100 ml volumetric flask, and the final dilution utilized 0.01 mL of the diluted suspension and 0.99 mL of the mobile phase (40% water:60% acetonitrile) into a 1.8 mL autosampler vial.

9.5.2 Analysis of Test Substances with HPLC with UV/VIS Detection

All sample analysis employed HPLC with UV/VIS detection. Conditions employed are described in Tables 8 and 9.

Table 8. Phenobarbital HPLC Conditions

HPLC System	Agilent 1100 HPLC (Palo Alto, CA)
Column	SYNERGI 4µ Hydro-RP 80A 250 X 4.6 mm 4µ HPLC column
Detector	Diode array UV/Vis, set to collect at a wavelength, 225 nm
Column Pressure Limit	250 BAR
Run Time	8 minutes
Injection Volume	5 µl
Eluent; flow pattern	50% water:50% acetonitrile, Isocratic (eluent also called mobile phase)

Table 9. Linuron HPLC Conditions

HPLC System	Agilent 1100 HPLC (Palo Alto, CA)
Column	SYNERGI 4µ Hydro-RP 80A 250 X 4.6 mm 4µ HPLC column
Detector	Diode array UV/Vis, set to collect at a wavelength, 250 nm
Column Pressure Limit	250 BAR
Run Time	12 minutes
Injection Volume	100 µl
Eluent; flow pattern	40% water:60% acetonitrile, Isocratic (eluent also called mobile phase)

Calibration of the HPLC was done individually using 5 calibration standards for each of the analytes. To start, a stock is made at a concentration of about 1000 µg/mL for each analyte. Approximately 0.0500 grams of the analyte is weighed into a 50 mL volumetric flask and diluted to the mark with acetonitrile. The phenobarbital stock is serially diluted to make standards ranging from about 1 µg/mL to 200 µg/mL using a solution that will mimic the eluent, 50% acetonitrile:50% water. For the linuron, the stock is serially diluted to make standards ranging from about 0.05 µg/mL to 5 µg/mL using a solution that will mimic the eluent, 60% acetonitrile:40% water.

9.5.3 Calibration Performance and Quality Control for both Phenobarbital and Linuron

Calibration linearity specifications for both test substances were an R^2 value of greater than or equal to 0.995. Initial and continuing calibration verification standards for both test substances (ICV and CCV) were run where each of the ICVs consisted of a solution made from an independent standard and diluted to be within the calibration range of the standards. The CCVs were mid-point calibration standards run every 10 samples to verify the analytical

system remained calibrated for the entire run. Both ICV and CCV performance standards were specified to be within 10% of target concentrations for the test substances. The purpose of an ICV is to verify that the calibration standards were properly made.

Matrix spikes and blanks were run for method validation and with each sampling for phenobarbital. A matrix spike was prepared prior to the start of the tests and was made at concentrations similar to the low dose formulation concentrations. For linuron, since homogeneity and formulation verification were the only samples run, a matrix spike would have been the same as the actual sampling, therefore, matrix spikes were deemed unnecessary.

10.0 RESULTS

10.1 TEST SUBSTANCE PURITY

The purities of linuron and phenobarbital determined by the CR were 97.69% and 99.98% respectively (Table 10), both within the protocol set accuracy window of ±3% of the values provided on the suppliers' certificates of analysis.

Table 10. Summary of Test Substance Purity

TEST SUBSTANCE	SUPPLIER REPORTED PURITY	LOT NUMBER	CR DETERMINED PURITY
Linuron	99.5%	348-8A	97.69%
Phenobarbital	99.1%	104K2600	99.98%

10.2 FORMULATION ANALYSIS RESULTS

The formulation preparation procedures developed for the test substance linuron produced a suspension with a measured concentration within 10% of the nominal concentration per protocol specifications (Table 11). The actual concentrations measured in the top 1/3 and the bottom 1/3 of the container were also within the 10 percent homogeneity (agreement) specification (Table 12). Triplicate determinations were carried out for both levels.

The phenobarbital formulation yielded concentrations that met the formulation accuracy specification (Table 11). The phenobarbital homogeneity specification was met for 4 of the 6 determinations[3] carried out (Tables 13 and 14). The chemist deduced that the issue for phenobarbital was in method precision, not suspension homogeneity.

Table 11. Nominal & Measured (Day 0) Formulation Concentration Comparisons

Test Substance	Nominal Conc (µg/mL)	Avg Measured Conc (µg/mL)	% Deviation = nominal versus measured
Linuron 30 mg/L	29940.0	27554.40	7.97%
Phenobarbital 5 mg/L	5010.5	4897.94	2.25%
Phenobarbital 20 mg/L	20025.5	19465.32	2.80%

Table 12. Formulation Homogeneity - Linuron

Test Substance	Position of Measurement	Recovery	Agreement
Linuron	Top 1/3	90.58%	3.16%
	Bottom 1/3	93.49%	

[3]A protocol deviation (EDSP.515-01-D1) was generated to document this sub-specification performance in homogeneity for the phenobarbital suspension (see Appendix B).

Table 13. Formulation Homogeneity – Phenobarbital 5 mg/mL

Test Substance	Position of Measurement	Recovery (day 1)	Agreement (day 1)	Recovery (day 7)	Agreement (day 7)	Recovery (day 14)	Agreement (day 14)
Phenobarbital 5 mg/mL	Top 1/3	91.32%	13.17%	96.96%	2.84%	97.32%	0.59%
	Bottom 1/3	104.19%		99.75%		96.75%	

Table 14. Formulation Homogeneity – Phenobarbital 20 mg/mL

Test Substance	Position of Measurement	Recovery (day 0)	Agreement (day 0)	Recovery (day 7)	Agreement (day 7)	Recovery (day 14)	Agreement (day 14)
Phenobarbital 20 mg/mL	Top 1/3	96.90%	0.63%	91.97%	10.95%	97.13%	0.93%
	Bottom 1/3	97.51%		102.62%		98.04%	

10.3 FORMULATION STABILITY RESULTS

Stability for linuron was determined in a previous study (WA 2-28 at 5 mg/mL in 0.25% methylcellulose). The results from this earlier evaluation demonstrated stability performance at ≥ 90% of the linuron target concentration for the testing period of 21 days. Dosing formulation stability for phenobarbital as a percent of nominal values is tabulated in Table 15 and plotted in Figure 1. Typical chromatograms for phenobarbital and linuron are provided in Figures 2 and 3.

Table 15. Formulation Stability Results

Test Substance	Test Duration*	Calculated Nominal Conc. ug/ml	Percent of Nominal
Phenobarbital 5 mg/ml	14 days	5010.5	91.32% to 104.2%
Phenobarbital 20 mg/ml	14 days	20025.5	91.97% to 102.6%

* Test originally scheduled to run for 28 days, test fell below recovery spec at 21 days – test terminated.

Method detection limits (MDL) and ICV/CCV recovery ranges for the two test substances are provided in Table 16. The analytical and quality control (QC) results are presented in Appendix C.

Table 16. MDL and ICV/CCV Recovery Ranges

Test Substance	Method Detection Limit	ICV/CCV Recoveries
Phenobarbital	77.76 ug/ml	99.3% to 103.6%
Linuron	Not Done[4]	97.7% to 109.3%

Calibration curves all met the R^2 criteria of 0.995, see table 17. Blanks and matrix spikes were analyzed with every batch for QC purposes. All blanks were less than 3 times the detection limit for all the compounds.

Table 17. Calibration Acceptance

Calibration Curve Date	Test Substance	R^2 Value
9/21/05	Linuron	0.99999
9/14/05	Phenobarbital	0.99998
9/15/05	Phenobarbital	0.99999
9/16/05	Phenobarbital	0.99999
9/22/05	Phenobarbital	0.99999
9/29/05	Phenobarbital	0.99999
10/6/05	Phenobarbital	0.99999

[4] The method validation, which includes MDL, was not done for linuron, a protocol deviation (EDSP.515-01-D1) was generated (see Appendix B).

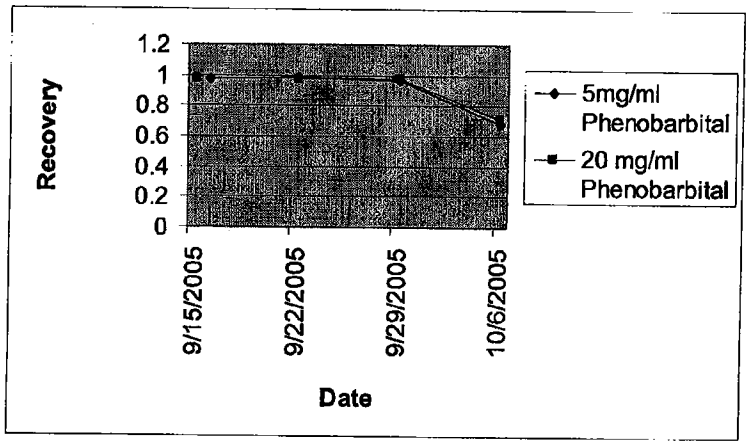


Figure 1. Recoveries of Phenobarbital Plotted Against Time

11.0 CONCLUSIONS

11.1 TEST SUBSTANCE PURITY

Purity determinations for phenobarbital and linuron, carried out by the CR, compared favorably (within 2%) to the supplier's reported results.

11.2 FORMULATION ANALYSIS

Linuron met the suspension homogeneity specification, while phenobarbital met the homogeneity specification for 4 of the 6 determinations carried out. Comparisons of the nominal and actual concentrations of the linuron formulation prepared revealed a 92.04% accuracy at 30 mg/ml. Phenobarbital formulation accuracy was 97.76% at 5 mg/mL and 97.21% at 20 mg/mL, using T=0 concentrations of the stability study. All formulations met the specification of ± 10% of nominal value.

11.3 FORMULATION STABILITY

Stability of the phenobarbital suspensions remained within 90% of the nominal concentration for the first 14 days of the 28 day stability study for both the 5 and 20 mg/mL concentrations. The study was terminated at day 21 when the recovery dropped below the 90% specification for both the 5 and 20 mg/mL suspensions.

11.4 ARCHIVING

Archive samples of the test substance employed in this study will be maintained in the EDSP Chemical Repository for the shelf life indicated on the chemical label.

The protocol, any amendments, all records and the final report generated as a result of this study will be transported to and maintained for archival purposes at the following address:

PNNL Records Management
540 Fifth Street
Richland, WA 99352
PH: 509.375.2340

```
Data File D:\CHEM32\1\DATA\PHEN5\phen5000020.D
Sample Name: phen20 T 1 R1

==========================================================================
Injection Date  : 9/15/2005 4:21:32 PM          Seq. Line :  20
Sample Name     : phen20 T 1 R1                  Location : Vial 20
Acq. Operator   : timothy                           Inj :  1
Acq. Instrument : Instrument 1                  Inj Volume : 5 µl
Sequence File   : D:\CHEM32\1\SEQUENCE\PHEN5.S
Method          : D:\CHEM32\1\METHODS\PHEN5.M
Last changed    : 9/15/2005 2:20:39 PM by timothy
phenobarbital method
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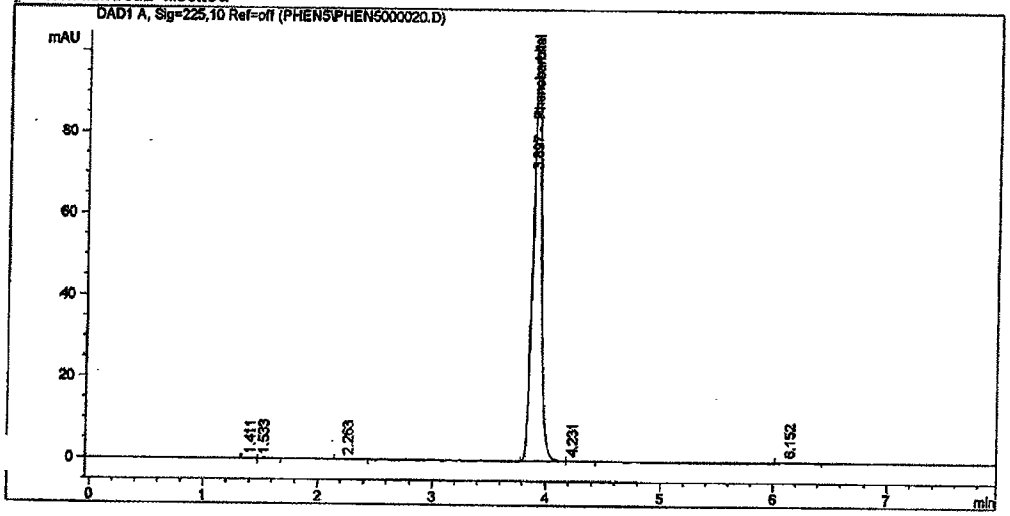


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==========================================================================
                        External Standard Report
==========================================================================

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Calib. Data Modified  :      Thursday, September 15, 2005 2:20:39 PM
Multiplier            :    238.0950
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=225,10 Ref=off
Uncalibrated Peaks    :      compound name not specified

RetTime Sig Type     Area      Amt/Area    Amount    Grp   Name
 [min]              [mAU*s]                [ug/l]
-------|--|------|----------|----------|----------|--|---------------
  1.411  1 BV     4.13544e-1    0.00000    0.00000    ?
  1.533  1 VB     7.24195e-1    0.00000    0.00000    ?
  2.263  1 BB     1.74869e-1    0.00000    0.00000    ?
  3.897  1 BV      507.10126 1.63494e-1 1.97400e4   * Phenobarbital
  4.231  1 VB        1.21614    0.00000    0.00000    ?
  6.152  1 BB        1.71290    0.00000    0.00000    ?

Totals :                                 1.97400e4

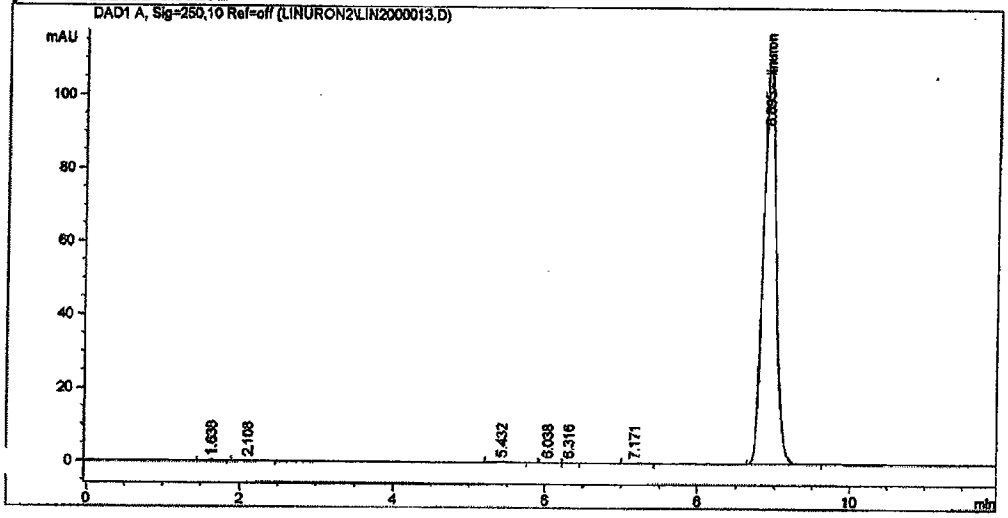
==========================================================================

Instrument 1 9/15/2005 5:24:57 PM timothy                    Page  40 of 54
```

Figure 2. Typical Chromatogram for WA 5-15 HPLC Analysis of Phenobarbital

```
Data File D:\CHEM32\1\DATA\LINURON2\lin2000013.D
Sample Name: Lin 30 top R-1

==========================================================================
Injection Date  : 9/21/2005 2:27:39 PM          Seq. Line :  13
Sample Name     : Lin 30 top R-1                 Location : Vial 13
Acq. Operator   : timothy                           Inj :   1
Acq. Instrument : Instrument 1                Inj Volume : 100 µl
Sequence File   : D:\CHEM32\1\SEQUENCE\LINURON2.S
Method          : D:\CHEM32\1\METHODS\LINURON2.M
Last changed    : 9/21/2005 1:00:46 PM by timothy
phenobarbital method
```

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==========================================================================
                      External Standard Report
==========================================================================

Sorted By             :      Retention Time
Calib. Data Modified  :      Wednesday, September 21, 2005 1:00:46 PM
Multiplier            :    9.259e3
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,10 Ref=off

RetTime Sig Type     Area      Amt/Area    Amount   Grp   Name
 [min]              [mAU*s]                [ug/ml]
-------|--|------|----------|----------|----------|--|----------------
  8.895  1 BB     1285.47363 2.10147e-3 2.50128e4     linuron

Totals :                                 2.50128e4

==========================================================================

Instrument 1 9/21/2005 4:04:34 PM timothy                Page  26 of 40
```

Figure 3. Typical Chromatogram for WA 5-15 HPLC Analysis of Linuron

APPENDIX A

SUPPLIER'S CERTIFICATES OF TEST SUBSTANCE ANALYSIS/PURITY

[redacted]

660 Tower Lane • P.O. Box 599 • West Chester, PA 19381-0599
1-800-452-9994 • 1-610-692-3026 • Fax 1-610-692-8729
info@chemservice.com • www.chemservice.com

CERTIFICATE OF ANALYSIS

INVOICE #: CS264916
PO #: 19293

CATALOG #: PS-372

CAS #: 330-55-2

DESCRIPTION: Linuron

LOT #: 348-8A

PURITY: 99.5%

EXPIRATION DATE: 08/08

Chem Service, Inc. guarantees the purity of this chemical ± 0.5% deviation prior to the expiration date shown on the label and exclusive of any customer contamination.

Two or more of the following methods of analysis are used to determine purity: Melting point, refractive index, titration, IR, TLC, GC/FID, GC/TCD, GC/ECD, GC/MS, HPLC or DSC.

Our standards are suitable for use with all EPA methods.

Certified By:

John Conrad

John Conrad
CSM/TC

ISO 9001
Certificate Number: 31610

SIGMA-ALDRICH

Received 8/16/05 mt
CF 2461-1

Certificate of Analysis

Product Name	Phenobarbital,
Product Number	P1636
Product Brand	Sigma
CAS Number	50-06-6
Molecular Formula	$C_{12}H_{12}N_2O_3$
Molecular Weight	232.24

TEST	SPECIFICATION	LOT 104K2600 RESULTS
APPEARANCE	WHITE POWDER	WHITE POWDER
SOLUBILITY	CLEAR COLORLESS SOLUTION AT 50MG/ML IN ETHANOL	CLEAR COLORLESS SOLUTION
IR SPECTRUM	CONSISTENT WITH STRUCTURE	CONFORMS
PURITY BY NAOH TITRATION	NLT 99%	99.1%
PURITY BY THIN LAYER CHROMATOGRAPHY	NLT 99%	GREATER THAN 99%
SHELF LIFE	5 YEARS	FEBRUARY 2010
QC ACCEPTANCE DATE		FEBRUARY 2005

Lori Schulz

Lori Schulz, Manager
Analytical Services
St. Louis, Missouri USA

SIGMA-ALDRICH

3050 Spruce Street
Saint Louis, Missouri 63105 USA
Telephone (800) 521-8956 • (314) 771-5765
Fax (800) 325-5052 • (314) 77-5757
Visit Us At www.sigma-aldrich.com

Certificate of Analysis

BATTELLE NORTHWEST
11372928MEC
MARINE SCIENCES LAB
1529 W SEQUIM BAY RD
SEQUIM WA 98382

PO NBR: CC/Smith

PRODUCT NUMBER: 274429-100G
LOT NUMBER: 14601TC

PRODUCT NAME: METHYL CELLULOSE, AVERAGE MN CA. 41,000

FORMULA: C99
FORMULA WEIGHT: 0.00

APPEARANCE	WHITE POWDER
INFRARED SPECTRUM	CONFORMS TO STRUCTURE.
MISCELLANEOUS ASSAYS	29.8% METHOXYL *
LOSS ON DRYING	1.9% LOSS *
VISCOSITY	APPARENT VISCOSITY: 504 CPS (2%, H2O) *
	* SUPPLIER DATA
QUALITY CONTROL ACCEPTANCE DATE	DECEMBER 2004

ALDRICH CHEMICAL COMPANY
RONNIE MARTIN
AUGUST 9, 2005

We are Committed to the success of our Customers, Employees and Shareholders through leadership in Life Science, High Technology and Service.

APPENDIX B

STUDY PROTOCOL, AMENDMENTS, AND DEVIATIONS

Study Protocol:
Analysis of Test Substances for Work Assignment 5-15
EDSP Study Number: EDSP.515-01

Study Objective:

The following tasks will be carried out for the (2) two test *Chemicals* as specified in Table 2:

1. Prepare and validate an analytical method as required for each of the test substances over the concentration range needed to measure the target stock concentration and the low exposure concentration (if sensitivity allows).
2. Demonstrate a viable and accurate formulation for each of the test substances, at the *Stock Solution Concentrations* listed in Table 2, in the specified carrier (methylcellulose).
3. Determine the homogeneity of any test substance that forms a suspension as described in the experimental design below.
4. Determine the stability of phenobarbital dissolved in methylcellulose (at the concentrations specified in Table 2), over a 28 day period.
5. Provide a report documenting the results on the above tasks.
6. Provide documented and validated methods, for procedures cited in 1, 2, and 3 above and identified by method number in Table 2 below, to the test laboratories specified by the EPA for the follow-on in-life studies for this work assignment.

This study is in support of EPA contract number 68-W-01-023, MSL Work Assignment Number 5-15, *Inter-laboratory Validation of the 15-Day Intact Adult Male Rat Assay.*

Address of Testing Facility:	**Address of Sponsor's Representative**
Battelle – Marine Research Operations 1529 West Sequim Bay Road Sequim, Washington 98382 Ph: (360) 681-4580 FAX (360) 681-3699 Email: michael.cobb@pnl.gov	Battelle 550 King Avenue Columbus, Ohio 43201-2693 Ph: (614) 424-3564 FAX (614) 458-3564 Email: houchensd@battelle.org

Proposed experimental start and termination dates:

Start Date – August 25, 2005
Termination Date – November 15, 2005

Definitions:

Test Substances: The test substances are the 2 chemicals listed in Table 2. The test substances are the subject chemicals of the tasks described in this protocol.

Reference Substance: The reference substances are identical chemicals to the test substances and may be from the same manufacturer and lot, or purchased as different lots and/or possibly from separate manufacturers than the test substances. The source, purity, and lot number of reference substances will be documented in the data and reported. Regardless of the source, the reference substance solutions will be made up separately from the test substance solutions. The reference substances (Table 1) are used for the calibration standards

in the analytical methods referenced in Table 2. A reference substance can also be a material used to facilitate the analysis of the test substance, such as an internal standard.

TABLE 1
Test Substance Abbreviations:

Chemical	Abbreviation
Linuron	Lin
Phenobarbital	φBarb

TABLE 2
Test Substance Specifications:

Chemical Name	Lin	φBarb
Manufacturer	Chem Service, Inc.	Sigma/Aldrich
CAS #	330-55-2	7601-89-0
Lot #	348-8A	104K2600
Supplier Purity requirement	≥ 97%	≥ 97%
Supplier Purity Claim	99.5%	99.1%
Target Concentration Stock Solution/Suspension	30 mg/mL	20 mg/mL
Duration Stability Study	[1]	28 Days
Concentrations for Stability Study	[1]	5 and 20 mg/mL
Carrier (Vehicle)	0.25% Methylcellulose in H_2O	0.25% Methylcellulose in H_2O
Analytical Method	EDSP.H4-033	EDSP.H4-034

[1] Will use data from previous EDSP Chemical Repository study (WA 2-28)
TBA = To Be Amended

Experimental Design:

- Analytical methods will be tested for each of the test substances.
- Purity of linuron and phenobarbital will be verified using High Performance Liquid Chromatography (HPLC). All purities determined should be within ±3% of the value provided on the Certificates of Analysis by the manufacturer. To use substances with values that fall outside this ±3% range or are less than 97% pure, written pre-approval must be secured from the designated EPA work assignment manager.
- Solubility of phenobarbital will be assessed visually in the carrier at the stock formulation concentration (see Table 2). Linuron has been demonstrated to be a suspension at 20 mg/mL of 0.25% methylcellulose. The specific method employed for preparation of the suspension of linuron will be the same as the method described on pages 3 and 4 of the *Chemistry Report for WA 2-28 (Revised March 28, 2005)*.
- Formulation accuracy and homogeneity of the linuron suspensions will be tested on samples collected at liquid levels approximately 1/3 and 2/3 down from the top of the liquid level in the container (with constant stirring during sampling) using the analytical methods referenced in Table 2. Sampling will be carried out in triplicate/level.
- The accuracy of attaining the target concentration for the formulations that form solutions will be verified in triplicate using the analytical methods referenced in Table 2.

- Stability test solutions – Stability testing of phenobarbital will be carried out at the stock concentration level and the low exposure concentration (as specified in Table 2), stored in the dark (i.e., same storage conditions of solutions employed in the in-life studies of WA 5-15) at room temperature. Nominal concentrations to be tested are delineated in Table 2 but the actual concentrations used for testing will be within ±10 percent of the target concentration.
- Storage and Labeling Requirements of Formulations – Stock formulations will be stored at room temperature. Minimally, containers will be uniquely labeled with the name of the test substance, the date of preparation, the formulation concentration, and the study number.
- Testing Schedule – Samples will be analyzed the day of collection from the test formulation.
- Replicates – 3 aliquots per sample tested at each analysis time point.
- Sampling schedule. – Samples will be collected for analysis at initiation of the stability study (on day of formulation preparation), then on days 7, 14, 21, and 28 of storage (if a test date falls on a holiday, testing scheduled for that date will be carried out on the closest work day).
- For details of the analytical methods see the substance specific method cited in Table 2.

Data Analysis:

The stability data collected on days 0, 7, 14, 21 and 28 (average of triplicate determinations) for phenobarbital will be compared to the nominal test concentration prepared for the study. Percent variation from the nominal concentration will be used to determine instability for phenobarbital.

Accuracy of phenobarbital and linuron formulations will be based on the average of triplicate analyses compared to the nominal values.

Homogeneity of the linuron suspensions will be based on comparisons of the average of triplicate analyses at each of the two levels within the suspensions.

Acceptance Criteria:

Acceptable stability for phenobarbital will be defined as the concentration not varying more than 10 percent from the nominal concentration over the 28 day stability period. The Work Assignment Leader will be consulted for a recommended course of action for any data found outside the ±10% acceptance range. If needed, more frequent preparation of stock solutions will be recommended for in-life studies and in-life sampling and testing will be coordinated to insure testing is carried out within the viable sample stability window.

Acceptable accuracy of formulation preparations will be ±10 percent of the target concentration.

The mean linuron concentrations measured at the top 1/3 and bottom 1/3 of the suspensions must be within 5% of one another (homogeneity). The overall actual concentration must be within 10% of the target concentration for all test results of this study.

Regulatory requirements:

This study will be conducted in compliance with EPA FIFRA Good Laboratory Practices (40 CFR, Part 160). An EDSP QA representative will inspect the study at least once while in-progress and will audit the data and final report.

Report:

A final report covering the following information for both chemicals (where applicable) will be issued to the Sponsor Representative (Dr. David Houchens, EDSP Program Manager), who will then forward the report to the testing laboratories:

- Title Page
- Executive Summary
- Table of Contents
- Introduction
- General Methods
  - Chemical Procurement
  - Purity
  - Formulation Preparation (Methods)
  - Solubility and Homogeneity
  - Stability Testing Plan Design and Detail
  - Analytical Method
- Results
  - Purity
  - Formulation Analysis
  - Solubility and Homogeneity
  - Analytical Method Validation
  - Formulation Stability
- Conclusions
- Appendices
  - Manufacturer's Certificates of Analysis
  - Document to the Testing Laboratories
    - Title Page
    - Table of Contents
    - Introduction
    - Neat Chemical/Vehicle Storage Recommendations
    - Dosing Formulation Preparation Procedure
    - Dosing Formulation Storage Recommendations
    - Dosing Formulation Analysis Procedure
    - Protocol
    - Protocol Amendments
    - Protocol Deviations
    - Method Documents
    - Method Deviations

Records to be maintained:

All records, including the protocol, any amendments, and the data and final reports, generated as a result of analysis of the two test substances evaluated for this study, will be transported to and maintained for archival purposes at the following address:

PNNL Records Management
540 Fifth Street
Richland, WA 99352
PH: 509.375.2340

Approval:

Chemical Repository Study Director _____ 8/26/05
Michael Cobb **Date**

Chemical Repository Manager Eric Crecelius 8/26/05
Eric Crecelius, Ph.D. **Date**

Sponsor Representative _____ 8/22/05
David Houchens, Ph. D. **Date**

PROTOCOL AMENDMENT
STUDY NUMBER: EDSP.515-01
AMENDMENT NUMBER: A-1

ENDOCRINE DISRUPTOR SCREENING PROGRAM AMENDMENT REPORT

STUDY NUMBER: EDSP.515-01				DATE: September 8, 2005	
AMENDMENT NUMBER: A-1				WAL/STUDY DIRECTOR:	
NOTEBOOK NUMBER: N/A				Dave Houchens/Michael Cobb	
TITLE OF STUDY: Analysis of Test Substances for Work Assignments 5-15					
QAPP/PROTOCOL ID: Work Assignment 5-15					
AMENDMENT RELATING TO:					
[ ]	QAPP	[ ]	QMP	[x]	Protocol
[ ]	SOP	[ ]	Method		

ORIGINAL DOCUMENT SPECIFICATIONS:

All protocol details that will be amended are indicated in bold, underlined, and in a Georgia font.

1. **Experimental Design:**

- **Solubility of phenobarbital will be assessed visually in the carrier at the stock formulation concentration (see Table 2). Linuron has been demonstrated to be a suspension at 20 mg/mL of 0.25% methylcellulose.** The specific method employed for preparation of the suspension of linuron will be the same as the method described on pages 3 and 4 of the *Chemistry Report for WA 2-28 (Revised March 28, 2005).*
- Formulation accuracy and homogeneity of the **linuron suspensions** will be tested on samples collected at liquid levels approximately 1/3 and 2/3 down from the top of the liquid level in the containers (with constant stirring during sampling) using the analytical methods referenced in Table 2. Sampling will be carried out in triplicate/level.
- **The accuracy of attaining the target concentration for the formulations that form solutions will be verified in triplicate using the analytical methods referenced in Table 2.**
- Stability test solutions – Stability testing of phenobarbital will be carried out at the stock concentration level and the low exposure concentration (as specified in Table 2), stored in the dark (i.e., same storage conditions of solutions employed in the in-life studies of WA 5-15) at **room temperature**. Nominal concentrations to be tested are delineated in Table 2 but the actual concentrations used for testing will be within ±10 percent of the target concentration.
- Storage and Labeling Requirements of Formulations – Stock formulations will be stored at **room temperature**. Minimally, containers will be uniquely labeled with the name of the test substance, the date of preparation, the formulation concentration, and the study number.

2. **Data Analysis**

Homogeneity of the **linuron suspensions** will be based on comparisons of the average of triplicate analyses at each of the two levels within the suspensions.

3. **Acceptance Criteria**

The mean **linuron concentrations measured at the top 1/3 and bottom 1/3 of the suspensions must be within 5% of one another (homogeneity).** The overall actual concentration must be within 10% of the target concentration for all test results of this study.

DATE: September 8, 2005

PROTOCOL AMENDMENT
STUDY NUMBER: EDSP.515-01
AMENDMENT NUMBER: A-1

AMENDMENT:

Changes are underlined

1. In the *Experimental Design* Section.

Experimental Design:

- Both linuron and phenobarbital are suspensions at the study concentrations in 0.25% methylcellulose. The specific method employed for preparation of the suspension of linuron will be the same as the method described on pages 3 and 4 of the *Chemistry Report for WA 2-28 (Revised March 28, 2005).*
- Formulation accuracy and homogeneity of the linuron and phenobarbital suspensions will be tested on samples collected at liquid levels approximately 1/3 and 2/3 down from the top of the liquid level in the containers (with constant stirring during sampling) using the analytical methods referenced in Table 2. Sampling will be carried out in triplicate/level.
- Stability test solutions – Stability testing of phenobarbital will be carried out at the stock concentration level and the low exposure concentration (as specified in Table 2), stored in the dark (i.e., same storage conditions of solutions employed in the in-life studies of WA 5-15) at 2 to 8 degrees C. Nominal concentrations to be tested are delineated in Table 2 but the actual concentrations used for testing will be within ±10 percent of the target concentration.
- Storage and Labeling Requirements of Formulations – Stock formulations will be stored at 2 to 8 degrees C. Minimally, containers will be uniquely labeled with the name of the test substance, the date of preparation, the formulation concentration, and the study number.

2. Data Analysis

Homogeneity of the linuron and phenobarbital suspensions will be based on comparisons of the average of triplicate analyses at each of the two levels within the suspensions.

3. Acceptance Criteria

The mean linuron and phenobarbital concentrations measured at the top 1/3 and bottom 1/3 of the suspensions must be within 10% of one another (homogeneity). The overall actual concentration must be within 10% of the target concentration for all test results of this study.

REASON FOR CHANGES:

1. During the workup of the materials for the studies, it was determined that phenobarbital was a suspension and not a solution at the study concentrations. The storage temperature of the stability solutions was incorrectly identified as room temperature and should have been specified as 2 to 8 degrees C.

2. With phenobarbital shown to be a suspension, the homogeneity of the suspension requires verification so this test was added to the data analysis section.

3. During method verification, the noise in the analytical method proved too high to allow for a 5% range of consistency across the suspensions. The value was increase to 10%.

DATE: September 8, 2005

PROTOCOL AMENDMENT
STUDY NUMBER: EDSP.515-01
AMENDMENT NUMBER: A-1

Approvals:

Work Assignment Leader	Dirk P. Vanderlaan	Date 9/8/05
Study Director		Date 9/12/05
EDSP QA Representative	Mary E Lynn	Date 9/19/05
MSL Laboratory Director	RM Ecker	Date 9/16/05
EDSP Program Management	Dirk P. Vanderlaan	Date 9/8/05
EDSP Battelle QAM	Chris Pollock	Date 9-8-05

cc: Send final approved copies to:
MSL QA Manager
EDSP Battelle QAM

DATE: September 8, 2005

PROTOCOL DEVIATION
STUDY NUMBER: EDSP.515-01
DEVIATION NUMBER: D-1
DATE: January 10, 2006
Page 1 of 3

ENDOCRINE DISRUPTOR SCREENING PROGRAM DEVIATION FORM

STUDY NUMBER: EDSP.515-01				DATE: January 10, 2006	
AMENDMENT NUMBER: D-1				WAL/STUDY DIRECTOR:	
NOTEBOOK NUMBER: N/A				David Houchens/Michael Cobb	
TITLE OF STUDY: Analysis of Test Substances for Work Assignment 5-15					
QAPP/PROTOCOL ID: Work Assignment 5-15					
AMENDMENT RELATING TO:					
[ ]	QAPP	[ ]	QMP	[x]	Protocol
[ ]	SOP	[ ]	Method		

ORIGINAL DOCUMENT SPECIFICATIONS:

1. **Experimental Design:**
   - Sampling schedule. – Samples will be collected for analysis at initiation of the stability study (on day of formulation preparation), then on days 7, 14, 21, and 28 of storage (if a test date falls on a holiday, testing scheduled for that date will be carried out on the closest work day).

2. Table 2 of the protocol listed the CAS number for phenobarbital as: 7601-89-0.

3. **Study Objective**
   1. Prepare and validate an analytical method as required for each of the test substances over the concentration range needed to measure the target stock concentration and the low exposure concentration (if sensitivity allows).

4. **Acceptance Criteria:**
   The mean linuron and phenobarbital concentrations measured at the top 1/3 and bottom 1/3 of the suspensions must be within 10% of one another (homogeneity). The overall actual concentration must be within 10% of the target concentration for all test results of this study.

DEVIATION:

1A. The phenobarbital stability study at the 20 mg/mL level was terminated after analysis of the day 21 sample.

1B. Analysis of the day zero, 5 mg/mL phenobarbital, stability study sample did not provide usable results. The day zero sample analysis was repeated on the following day with viable results. This altered the stability study monitoring intervals to 1, 7, 14, and 21 days for the 5 mg/mL sample. The analysis was terminated on day 21.

2. The correct CAS number for the phenobarbital is: 50-06-6

3. The linuron method, developed for a previous study was not validated with an MDL and spikes prior to analysis of the formulation.

4. The phenobarbital formulation yielded concentrations that fell out of the 10% agreement (homogeneity) specification (for the 5 mg/mL day 0 determination and the 20 mg/mL day 7 determination).

PROTOCOL DEVIATION
STUDY NUMBER: EDSP.515-01
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DATE: January 10, 2006
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REASON/IMPACT:

1A. The 20 mg/mL phenobarbital test solution remained within the acceptable stability recovery range at the 14 day sampling interval but fell below the acceptable stability range at 21 days. As a result of these findings, the Work Assignment Leader approved termination of the stability testing at 21 days. The 20 mg/mL phenobarbital sample in 0.25% methylcellulose was deemed stable for 14 days.

1B. Due to poor assay performance on day zero, the 5 mg/mL sample was rerun on the following day. The 5 mg/mL phenobarbital test solution remained within the acceptable stability recovery range at the 14 day sampling interval but fell below the acceptable stability range at 21 days. As a result of these findings, the Work Assignment Leader approved termination of the stability testing at 21 days. The 5 mg/mL phenobarbital sample in 0.25% methylcellulose was deemed stable for 14 days.

2. Used a previous protocol as a template for the 5-15 protocol and inadvertently left the CAS number from the previous study in place. No impact.

3. The linuron concentrations evaluated in the study were at a level where substantial dilutions were required prior to analysis. The system was not challenged from a sensitivity perspective so the MDL study was not carried out to reduce time expended on the project. The formulations were tested without a standard method validation with every expectation of good results and saving study hours. The formulation results demonstrated good recoveries so the shortcut in this case was justified. No impact.

4. Of the 6 homogeneity measurements carried out on the phenobarbital formulations, 4 of them met the 10% agreement specification and two were out (13.2% for the 5 mg/mL solution on day zero and 11.5% for the 20 mg/mL suspension on day 7). The analytical method was somewhat noisy and though these 2 homogeneity results fell out of spec, all the recovery determinations met the 90% to 110% requirement. Based on routine performance of the analytical method, the specification was set too low and should have been set at +/-15% agreement. No impact on validity of data and conclusions.

PROPOSED CORRECTIVE ACTION AND SCHEDULE FOR COMPLETION:
None, beyond this documentation.

ACTIONS TO PREVENT RECURRENCE:
None, beyond this documentation.

PROTOCOL DEVIATION
STUDY NUMBER: EDSP.515-01
DEVIATION NUMBER: D-1
DATE: January 10, 2006
Page 3 of 3

Approval:

Work Assignment Leader		Date 1/12/06
Study Director		Date 1/12/06
EDSP QA Representative		Date 1/12/06
MSL Laboratory Director		Date 1/12/06
EDSP Program Management		Date 1/12/06
EDSP Battelle QAM		Date 1-12-06

cc: Send final approved copies to:
MSL QA Manager
EDSP Battelle QAM

APPENDIX C

ANALYTICAL RESULTS OF STABILITY TESTING

(Note: All calculations were conducted at full precision in a spreadsheet.)

Table C1a. Phenobarbital Stability Results in Methylcellulose Vehicle for 5 mg/ml Suspension

Nominal Conc. (µg/ml)	Sample ID	Date	Measured Phenobarbital (µg/ml)	Average (µg/ml)	Recovery	RSD
5010.5	Phen5 T 1 R-1	9/16/2005	4928.13	4575.35	91.32%	10.73%
5010.5	Phen5 T 1 R-2	9/16/2005	4014.83			
5010.5	Phen5 T 1 R-3	9/16/2005	4783.11			
5010.5	Phen5 B 1 R-1	9/16/2005	6617.12	5220.53	104.19%	23.28%
5010.5	Phen5 B 1 R-2	9/16/2005	4641.06			
5010.5	Phen5 B 1 R-3	9/16/2005	4403.40			
5010.5	Phen5 T 2 R-1	9/22/2005	4407.82	4858.00	96.96%	13.92%
5010.5	Phen5 T 2 R-2	9/22/2005	5635.33			
5010.5	Phen5 T 2 R-3	9/22/2005	4530.84			
5010.5	Phen5 B 2 R-1	9/22/2005	4777.64	4997.89	99.75%	4.66%
5010.5	Phen5 B 2 R-2	9/22/2005	4974.10			
5010.5	Phen5 B 2 R-3	9/22/2005	5241.93			
5010.5	Phen5 T 3 R-1	9/29/2005	4799.77	4876.26	97.32%	3.74%
5010.5	Phen5 T 3 R-2	9/29/2005	5084.23			
5010.5	Phen5 T 3 R-3	9/29/2005	4744.80			
5010.5	Phen5 B 3 R-1	9/29/2005	5001.85	4847.42	96.75%	3.58%
5010.5	Phen5 B 3 R-2	9/29/2005	4880.99			
5010.5	Phen5 B 3 R-3	9/29/2005	4659.42			
5010.5	Phen5 T 4 R-1	10/6/2005	2914.95	3801.39	75.87%	28.10%
5010.5	Phen5 T 4 R-2	10/6/2005	4987.57			
5010.5	Phen5 T 4 R-3	10/6/2005	3501.65			
5010.5	Phen5 B 4 R-1	10/6/2005	3051.69	3032.34	60.52%	19.47%
5010.5	Phen5 B 4 R-2	10/6/2005	3612.76			
5010.5	Phen5 B 4 R-3	10/6/2005	2432.58			

Table C1b. Phenobarbital Stability Results in Methylcellulose Vehicle for 20 mg/ml Suspension

Nominal Conc. (µg/ml)	Sample ID	Date	Measured Phenobarbital (µg/ml)	Average (µg/ml)	Recovery	RSD
20025.5	Phen20 T 1 R-1	9/15/2005	19740.0	19404.23	96.90%	2.65%
20025.5	Phen20 T 1 R-2	9/15/2005	18811.8			
20025.5	Phen20 T 1 R-3	9/15/2005	19660.9			
20025.5	Phen20 B 1 R-1	9/15/2005	19399.3	19526.40	97.51%	1.23%
20025.5	Phen20 B 1 R-2	9/15/2005	19804.0			
20025.5	Phen20 B 1 R-3	9/15/2005	19375.9			
20025.5	Phen20 T 2 R-1	9/22/2005	19076.8	18417.27	91.97%	14.94%
20025.5	Phen20 T 2 R-2	9/22/2005	15395.7			
20025.5	Phen20 T 2 R-3	9/22/2005	20779.3			
20025.5	Phen20 B 2 R-1	9/22/2005	20305.6	20550.70	102.62%	3.36%
20025.5	Phen20 B 2 R-2	9/22/2005	21329.3			
20025.5	Phen20 B 2 R-3	9/22/2005	20017.2			

Table C1b. Phenobarbital Stability Results in Methylcellulose Vehicle for 20 mg/ml Suspension (continued)

Nominal Conc. (µg/ml)	Sample ID	Date	Measured Phenobarbital (µg/ml)	Average (µg/ml)	Recovery	RSD
20025.5	Phen20 T 3 R-1	9/29/2005	15888.2	19450.33	97.13%	16.14%
20025.5	Phen20 T 3 R-2	9/29/2005	21813.6			
20025.5	Phen20 T 3 R-3	9/29/2005	20649.2			
20025.5	Phen20 B 3 R-1	9/29/2005	19974.0	19634.00	98.04%	1.75%
20025.5	Phen20 B 3 R-2	9/29/2005	19288.2			
20025.5	Phen20 B 3 R-3	9/29/2005	19639.8			
20025.5	Phen20 T 4 R-1	10/6/2005	13205.2	13853.43	69.18%	8.08%
20025.5	Phen20 T 4 R-2	10/6/2005	13208.4			
20025.5	Phen20 T 4 R-3	10/6/2005	15146.7			
20025.5	Phen20 B 4 R-1	10/6/2005	14613.7	14806.70	73.94%	6.82%
20025.5	Phen20 B 4 R-2	10/6/2005	15899.3			
20025.5	Phen20 B 4 R-3	10/6/2005	13907.1			

Table C2. Homogeneity Results for Linuron in Methylcellulose Vehicle for 30 mg/ml Suspension

Nominal Conc. (µg/ml)	Sample ID	Date	Measured Linuron (µg/ml)	Average (µg/ml)	Recovery	RSD
29940	Lin 30 top R-1	9/21/2005	25012.8	27118.17	90.58%	6.83%
29940	Lin 30 top R-2	9/21/2005	27852.2			
29940	Lin 30 top R-3	9/21/2005	28489.5			
29940	Lin 30 bttm R-1	9/21/2005	28272.8	27990.63	93.49%	1.66%
29940	Lin 30 bttm R-2	9/21/2005	28243.2			
29940	Lin 30 bttm R-3	9/21/2005	27455.9			

Table C3. MDL and ICV/CCV Recovery Ranges

Test Substance	Method Detection Limit	ICV/CCV Recoveries
Phenobarbital	77.76 ug/ml	99.3% to 103.6%
Linuron	not done	97.7% to 109.3%

Table C4. Summary of Test Substance Purity

TEST SUBSTANCE	LOT NUMBER	CR DETERMINED PURITY
Phenobarbital	104K2600	99.98%
Linuron	348-8A	97.69%

Table C5a. Calibration Verification Data for Phenobarbital

Sample Name	Date	Expected Phenobarbital (µg/mL)	Measured Phenobarbital (µg/mL)	Recovery
WA515-phen-4 ICV	9/14/2005	20.08	20.15	100.34%
WA515-phen-2C CC	9/14/2005	20.04	19.90	99.32%
WA515-phen-2C CC	9/14/2005	20.04	20.09	100.27%
WA515-phen-2C CC	9/14/2005	20.04	20.02	99.90%
WA515-phen-2C CC	9/15/2005	20.04	19.96	99.59%
WA515-phen-2C CC	9/15/2005	20.04	20.20	100.79%
WA515-phen-4 ICV	9/15/2005	20.08	20.49	102.03%

Table C5a. Calibration Verification Data for Phenobarbital (continued)

Sample Name	Date	Expected Phenobarbital (µg/mL)	Measured Phenobarbital (µg/mL)	Recovery
WA515-phen-2C CC	9/15/2005	20.04	20.39	101.73%
WA515-phen-2C CC	9/15/2005	20.04	20.46	102.11%
WA515-phen-2C CC	9/15/2005	20.04	20.52	102.38%
WA515-phen-4 ICV	9/16/2005	20.08	20.42	101.69%
WA515-phen-2C CC	9/16/2005	20.04	20.43	101.97%
WA515-phen-2C CC	9/16/2005	20.04	20.52	102.38%
WA515-phen-4 ICV	9/22/2005	20.08	20.46	101.87%
WA515-phen-2C CC	9/22/2005	20.04	20.27	101.13%
WA515-phen-2C CC	9/22/2005	20.04	20.62	102.89%
WA515-phen-2C CC	9/22/2005	20.04	20.76	103.59%
WA515-phen-4 ICV	9/29/2005	20.08	20.14	100.30%
WA515-phen-2C CC	9/29/2005	20.04	20.49	102.24%
WA515-phen-2C CC	9/29/2005	20.04	20.58	102.70%
WA515-phen-2C CC	9/29/2005	20.04	20.69	103.22%
WA515-phen-4 ICV	10/6/2005	20.08	20.42	101.71%
WA515-phen-2C CC	10/6/2005	20.04	20.76	103.58%
WA515-phen-2C CC	10/6/2005	20.04	20.39	101.74%
WA515-phen-2C CC	10/6/2005	20.04	20.51	102.36%

Table C5b. Calibration Verification Data for Linuron

Sample Name	Date	Expected Linuron (µg/mL)	Measured Linuron (µg/mL)	Recovery
WA515-lin IVC	9/21/2005	0.503	0.550	109.26%
WA515-lin-1C CC	9/21/2005	0.502	0.491	97.72%
WA515-lin-1C CC	9/21/2005	0.502	0.494	98.37%

Table C6. Spike Recovery Data for Phenobarbital Analyses

Compound	Nominal Conc. (µg/mL)	Sample ID	Date	Measured (µg/mL)	Recovery
Phenobarbital	5014	WA515phen5 spk1	9/14/2005	4829.57	96.32%
Phenobarbital	5014	WA515phen5 spk2	9/14/2005	4782.08	95.37%
Phenobarbital	5014	WA515phen5 spk3	9/14/2005	4907.14	97.87%
Phenobarbital	5014	WA515phen5 spk4	9/14/2005	4596.80	91.68%
Phenobarbital	5014	WA515phen5 spk5	9/14/2005	4921.34	98.15%
Phenobarbital	5014	Blank Spike-6	9/15/2005	4749.35	94.72%
Phenobarbital	5014	Blank Spike-7	9/15/2005	4570.97	91.16%
Phenobarbital	5014	Blank Spike-8	9/22/2005	4781.64	95.37%
Phenobarbital	5014	Blank Spike-9	9/22/2005	4604.21	91.83%
Phenobarbital	5014	Blank Spike-10	9/29/2005	4658.95	92.92%
Phenobarbital	5014	Blank Spike-11	9/29/2005	4399.57	87.75%
Phenobarbital	5014	Blank Spike-12	10/6/2005	3109.98	62.03%
Phenobarbital	5014	Blank Spike-13	10/6/2005	2937.69	58.59%

Note: no spikes done with the linuron formulation verification analysis

APPENDIX D

NEAT CHEMICAL, VEHICLE, AND FORMULATION STORAGE RECOMMENDATIONS

1. Neat Chemical Storage
   A. Phenobarbital: Keep tightly closed, store at 2-8°C.
   B. Linuron: Keep tightly closed, store in a cool, dry, well-ventilated area – room temperature.

2. Formulation Storage
   A. All formulations are to be stored refrigerated (2-8°C).

APPENDIX E

ANALYTICAL METHODS EMPLOYED BY THE CHEMICAL REPOSITORY FOR WA 5-15

Battelle
The Business of Innovation

Marine Sciences Laboratory

EFFECTIVE DATE: 9-8-05

Method # EDSP.H4-033-00

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

ANALYSIS OF LINURON IN METHYLCELLULOSE USING HPLC WITH UV/VIS DETECTION

Approvals:		
AUTHOR: Tim Fortman	Signature	9-8-05 Date
TECHNICAL REVIEWER: Linda Bingler	Signature	9-8-05 Date
STUDY DIRECTOR: Michael Cobb	Signature	9-8-05 Date

ANALYSIS OF LINURON IN METHYLCELLULOSE USING HPLC WITH UV/VIS DETECTION

1.0 SCOPE AND APPLICATION

This method describes the determination of linuron in 0.25% water solution of methylcellulose using HPLC/UV/Vis detection. The method was developed for use in the analysis of phenobarbital for the EDSP program. The eluent used is an acetonitrile/water solution.

2.0 DEFINITIONS

Initial Calibration Verification (ICV)	A standard made from a neat material prepared separately from the calibration standards. Used to verify the calibration solutions. The neat material employed for preparation of the ICV can be from the same source material used for calibration.
Continuing Calibration Verification (CCV)	A mid level calibration standard run every after every 10 samples to ensure the instrument remains in calibration.

3.0 RESPONSIBLE STAFF

Researcher/Technician - sample preparation.
Analyst - analysis, calculations
QA Manager or Representative - data verification

4.0 ANALYSIS

4.1 Hardware and Reagents

- Balance capable of weighing to 0.0001 g
- High performance liquid chromatograph Agilent 1100 or equivalent
- Phenomenex SYNERGI 4µ Hydro-RP 80A 250 X 4.6 mm 4µ HPLC column or equivalent.
- Acetonitrile, HPLC grade or better.
- ~~Phenobarbital~~ Linuron, 98% purity or better.
- 1.8 mL vials
- 1 liter amber bottle with Teflon lined lid.
- Variable positive displacement Pipetters, to pipette 0.1 mL and 0.010 mL.
- Volumetric flasks

4.2 HPLC Mobile Phase (Eluent)

4.2.1 The mobile phase is 60% acetonitrile and 40% water. This can be made by mixing 600 ml of acetonitrile with 400 ml of water or can be mixed by the HPLC equipment.

4.3 Calibration Solution

4.3.1 A 5 point curve is used to calibrate the HPLC over a range that will bracket the concentration in the stability tests. To start, a stock is made at a concentration of about 1000 µg/mL. Approximately 0.0500 grams is weighed into a 50 mL volumetric flask and diluted to the mark with acetonitrile. Record exact information and give the solution a unique identifying label. Pour the solution into an appropriate size amber vial with a Teflon lined lid. Stability of the calibration solutions should be verified at the end of the test by the analysis of a new (freshly made) solution prepared from the neat material and compared to the calibration solutions.

4.3.2 Serially dilute the solution made in 4.3.1 to make standards ranging from 0.05 µg/ mL to 5 µg/mL using a solution that will mimic the eluent, 60% acetonitrile, 40% water.

4.4 HPLC Setup

4.4.1 The HPLC pump is set up to pump at 1.0 mL/min. The mobile phase (eluent) is degassed using either helium sparging or a vacuum degasser. The pump run time should be set to 9 minutes. 12 (1-11-06)

4.4.2 The autosampler is set up to inject 100 µL. A 500 µL loop is installed. See instrument manual for setup details. The autosampler is then set to flush the contaminated surfaces with acetonitrile.

4.4.3 The column used is a Phenomenex SYNERGI 4µ Hydro-RP 80A 250 X 4.6 mm 4µ HPLC column or equivalent. Pressure limit on the column is 3000 PSI (~210 bar).

4.4.4 The detector (either a UV/Vis or a diode array detector) set to a wavelength of 260 nm.

4.5 Analysis

4.5.1 Prior to the analysis of any samples linearity must be demonstrated. A 5 point curve is run (minimum of a 4 point curve is needed). An r^2 value of greater than 0.995 is necessary before analysis can begin. Must be or (understood, left out) 1-11-06

4.5.2 Once the calibration is done, if possible it must be verified with an initial calibration verification sample (ICV). An independent solution is made and diluted to the proper concentration so that it is within the calibration range. This sample is run and the value obtained should be within 10% of the expected value.

4.5.3 After the calibration is verified, a continuing calibration verification (CCV) sample is run. This sample is usually one of the mid-level calibrators. The value obtained should be within 10% of the expected value. A CCV should be run after every 10 samples.

4.5.4 A blank should be prepared with each sampling. The blank is the matrix diluted as the samples, for this study, ~1 ml of a 0.25% methylcellulose in water solution is placed in a 25 ml volumetric flask and diluted to the mark with acetonitrile. 0.01 ml of this is placed into a 1.8 ml autosampler vial and diluted with 0.99 ml of 60% acetonitrile, 40% water. The blank should be < 3X MDL (see 4.8.6). 4.5.5 1-11-06 typo 1-11-06

4.5.5 Method Detection Limit (MDL) is determined by preparing a sample at a low concentration, using similar techniques as used to analyze the low concentration stability sample. This is done 7 times and the MDL is the students T (3.143 for 7 replicates) times the standard deviation of the seven replicate runs. An MDL should be performed prior to the analysis of any sample for linuron. Samples with no peak or quantitating at a value less than the MDL will be reported as the MDL and flagged with a "U.

4.6 Purity

4.6.1 Purity is determined by running a sample of the material that is at or near the top of the demonstrated linearity of the system. All the peaks in the purity chromatogram are summed. The peak corresponding to the linuron is then compared to all the other peaks and the purity is the area of the linuron peak divided by the sum of the total area in the chromatogram (presented as a percentage). A blank is run prior to the purity run and the peaks in the purity run that correlate to peaks in the blank run are eliminated from the calculation. This purity should be 98% or greater and should compare favorably to the purity from the vendor. Note: the limitation of using a UV/Vis detector for purity is that one cannot be certain that the impurities will absorb at the same wavelength. This purity represents an estimation.

5.0 STABILITY

5.1 A 2.5 g/L (0.25%) methyl cellulose solution is prepared by adding 700 mL of deionized water to a 1 liter flask. This solution should be prepared at least a day in advance of use. The flask is placed on a hot plate and a stir bar added. While the solution is being stirred, add 2.5 grams of methyl cellulose and then heat the solution to boiling. This process should be closely monitored as the solution must be removed from the hot plate immediately when boiling is observed so the material doesn't boil over. Allow the hot plate to cool, then replace the methyl cellulose solution on the plate and stir the solution for about 2 hours (to attain clarity). The solution is then transferred to a 1 liter volumetric flask and diluted to the mark with deionized water. The solution may be slightly cloudy at this point but will become clear by the next day. Store the solution at 2 to 8 degrees C.

5.2 Prior to use, the linuron is screened so that a uniform suspension can be prepared. A six inch round 180 µm screen is set up with a collection pan and a cover. The linuron is placed on the screen and the screen shaken to push the linuron through the screen.

5.2 A 30 mg/mL suspension is made by weighing 6 grams of linuron into a 250 mL amber bottle with 200 mL of the methyl cellulose solution prepared in section 5.1 (use a graduated cylinder to add the methyl cellulose solution). The slurry is stored at 2 to 8 degrees C.

5.3 Linuron has limited solubility in the methyl cellulose solution and the result is the formation of a suspension. The 250 ml amber bottle is supplied with a stir bar. The suspension is removed from the refrigerator and placed on a stir plate and stirred to suspend the linuron and warm the sample. Stir suspension for about 60 minutes prior to sampling, stirring should be vigorous enough to show a slight vortex, it should not be stirred so vigorously that air is aspirated into the solution (this may cause foaming). Visual inspection should show an evenly distributed suspension. Sampling is done by taking triple 1 ml aliquots. A 3 ml syringe equipped with a 3.5 inch needle of a wide

bore (17 gauge or wider) is used to collect the sample. A 25 ml volumetric flask is tared and using the syringe about 1 ml of the stability suspension is placed into the volumetric flask and a weight determined (and recorded). Sampling is done at 2 levels in the suspension, the first triplicate is taken at a depth of about one third of the distance from the top of the suspension. A second triplicate sample is taken from about two thirds of the way down from the top of the suspension. The volumetric flask is then filled to the mark with acetontrile. The flask is agitated and 0.01 mL is removed and placed into a 1.8 mL autosampler vial with 0.99 mL of the mobile phase (see 4.2.1). Cap the vial and mix by agitating.

5.4 Slurries are stored in amber bottles at 2 to 8 degrees C.

5.5 Samples should be analyzed on the day of sampling, but if this is not possible, samples should be stored at 4° C. until analysis. If samples are not analyzed on the day of sampling, the actual analysis date and storage conditions shall be documented.

6.0 DATA ANALYSIS AND CALCULATIONS

6.1 Prior to analysis of any samples, the instrument is calibrated with a minimum of a 4 point curve. External standard calculations will be performed. All calculations are done using chromatography software supplied with the instrument. If the software allows the input of a multiplier, determine and enter a multiplier so that the output reflects the concentration in the stability sample. For the linuron suspension, about 1 mL of the stability sample is diluted with 25 mL of acetonitrile, then 0.01 mL of this solution is diluted to 1 mL with mobile phase. A density of 1 is assumed for the 0.25% methyl cellulose and the weight in grams is equal to the volume in milliliters. The multiplier is determined by dividing the dilution factor of 2500 (0.01 ml of a 25 mL solution taken to 1 mL) by the volume of the stability solution removed. Calibration curve fits can be set to either linear or non-linear (quadratic fit), past experience indicates that even though the calibration meets linearity criteria, the quantification is improved with a non-linear fit.

7.0 QUALITY CONTROL

7.1 A blank is prepared with each sampling, this blank is the methyl cellulose solution processed identically to the stability solution. If background levels are sufficiently high (i.e., greater than 5 x MDL), this value may be subtracted from the values obtained for samples analyzed with that batch. Processing of these samples is very straight forward, therefore spikes are optional.

7.2 An initial calibration verification (ICV) standard will be analyzed following the calibration curve. Continuing calibration verification standards (CCVs) will be analyzed after every 10 samples. All samples should be bracketed with a valid CCV. If a CCV fails, perform system maintenance, recalibrate and rerun the samples not bracketed with a valid CCV.

8.0 SAFETY

All analysts following this procedure should be aware of routine laboratory safety concerns, including all safety protocols regarding use of chemicals, including the following:

- Gloves, protective clothing and safety glasses should be worn when handling samples and chemicals.

9.0 TRAINING REQUIREMENTS

10.1 All staff performing this analysis should first read this procedure and conduct their first analysis under the supervision of a staff member who has had previous experience conducting this or a similar procedure. Staff should demonstrate proficiency in the process prior to performing the work.

10.2 All staff should have received training in the handling of chemicals and the use of fume hoods.

Table 1. Summary of Data Quality Objectives and Corrective Actions

Quality Control Sample Type	Data Quality Objective (DQO)	Corrective Action
Procedural Blank one/batch	Less than 3 x MDL	Re-extract and analyze sample batch. If batch can not be re-extracted and analyzed, "B" flag all samples that are in the batch. Investigate sources of blank contamination.
Calibration curve acceptability	r^2 values greater than or equal to 0.995	If r^2 value is outside of criterion, re-analyze calibration standards. If r^2 is still out, perform instrument maintenance and/or remake calibration standards and rerun calibration samples.
Initial calibration verification (ICV) standard; one/batch	+/- 10 % of true value	Re-calibrate. Must meet DQO in order to continue processing samples.
Continuing calibration verification standards; one every 10th sample analyzed	+/- 10 % of true value	Re-run CCV, if still not acceptable, re-calibrate and reanalyze affected samples.
Replicate sample precision; triplicates will be analyzed for stability, duplicate for in-life	Precision: 30% as relative standard deviation (RSD) or relative percent deviation (RPD)	If RSD or RPD is not acceptable, resample and reanalyze. If reanalysis data are still not acceptable, then "*" flag the values.
Blank or Matrix Spike and spike duplicate, one set per batch (optional)	+/- 15% of true value	If recoveries are unacceptable, check the spike solution to ensure it has not degraded, also check pipettes to ensure they are delivering accurate volumes.

[a]DQO is based on limited sample analysis as part of method development experience, and may require adjustment when more experience with the method is available.

Table 2. Data Qualifiers[a]

U	The analyte was detected below the MDL. Note: Samples with no peaks are reported as zero.
B	Samples associated with procedural blank contamination.
*	QC sample data that does not meet the DQO acceptability criterion.
Q	The data are questionable.
D	Sample diluted for analysis. (note: this procedure outlines the dilution of the samples, data will not be D flagged unless diluted other than indicated in this SOP).

[a]Additional data qualifiers may be added as necessary.

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Marine Sciences Laboratory

EFFECTIVE DATE: 10-05-05

Method # EDSP.H4-034-01

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

ANALYSIS OF PHENOBARBITAL IN METHYLCELLULOSE USING HPLC WITH UV/VIS DETECTION

Approvals:		
AUTHOR: Tim Fortman	Signature	10-5-05 Date
TECHNICAL REVIEWER: Linda Bingler	Signature	10/5/05 Date
STUDY DIRECTOR: Michael Cobb	Signature	10/05/05 Date

ANALYSIS OF PHENOBARBITAL IN METHYLCELLULOSE USING HPLC WITH UV/VIS DETECTION

1.0 SCOPE AND APPLICATION

This method describes the determination of phenobarbital in 0.25% water solution of methylcellulose using HPLC/UV/Vis detection. The method was developed for use in the analysis of phenobarbital for the EDSP program. The eluent used is an acetonitrile/water solution.

2.0 DEFINITIONS

Initial Calibration Verification (ICV)	A standard made from a neat material prepared separately from the calibration standards. Used to verify the calibration solutions. The neat material employed for preparation of the ICV can be from the same source material used for calibration.
Continuing Calibration Verification (CCV)	A mid level calibration standard run every after every 10 samples to ensure the instrument remains in calibration.

3.0 RESPONSIBLE STAFF

Researcher/Technician - sample preparation.
Analyst - analysis, calculations
QA Manager or Representative - data verification

4.0 ANALYSIS

4.1 Hardware and Reagents

- Balance capable of weighing to 0.0001 g
- High performance liquid chromatograph Agilent 1100 or equivalent
- Phenomenex SYNERGI 4µ Hydro-RP 80A 250 X 4.6 mm 4µ HPLC column or equivalent.
- Acetonitrile, HPLC grade or better.
- Phenobarbital, 98% purity or better.
- 1.8 mL vials
- 1 liter amber bottle with Teflon lined lid.
- Variable positive displacement Pipetters, to pipette 0.1 mL and 0.010 mL.
- Volumetric flasks

4.2 HPLC Mobile Phase (Eluent)

4.2.1 The mobile phase is 50% acetontrile and 50% water. This can be made by mixing equal volumes of acetonitrile and water or can be mixed by the HPLC equipment.

4.3 Calibration Solution

4.3.1 A 5 point curve is used to calibrate the HPLC over a range that will bracket the concentration in the stability tests. To start, a stock is made at a concentration of about 1000 µg/mL. Approximately 0.0500 grams is weighed into a 50 mL volumetric flask and diluted to the mark with acetonitrile. Record exact information and give the solution a unique identifying label. Pour the solution into an appropriate size amber vial with a Teflon lined lid. Stability of the calibration solutions should be verified at the end of the test by the analysis of a new (freshly made) solution prepared from the neat material and compared to the calibration solutions.

4.3.2 Serially dilute the solution made in 4.3.1 to make standards ranging from 1 µg/mL to 200 µg/mL using a solution that will mimic the eluent, 50% acetonitrile, 50% water.

4.4 HPLC Setup

4.4.1 The HPLC pump is set up to pump at 1.0 mL/min. The mobile phase (eluent) is degassed using either helium sparging or a vacuum degasser. The pump run time should be set to 8 minutes.

4.4.2 The autosampler is set up to inject 5 µL. A 100 µL loop is installed. See instrument manual for setup details. The autosampler is then set to flush the contaminated surfaces with acetonitrile.

4.4.3 The column used is a Phenomenex SYNERGI 4µ Hydro-RP 80A 250 X 4.6 mm 4µ HPLC column or equivalent. Pressure limit on the column is 3000 PSI (~210 bar).

4.4.4 The detector (either a UV/Vis or a diode array detector) set to a wavelength of 225 nm.

4.5 Analysis

4.5.1 Prior to the analysis of any samples, linearity must be demonstrated. A 5 point curve is run (minimum of a 4 point curve is needed). An r^2 value of greater than 0.995 is necessary before analysis can begin.

4.5.2 Once the calibration is done, if possible it must be verified with an initial calibration verification sample (ICV). An independent solution is made and diluted to the proper concentration so that it is within the calibration range. This sample is run and the value obtained should be within 10% of the expected value.

4.5.3 After the calibration is verified, a continuing calibration verification (CCV) sample is run. This sample is usually one of the mid-level calibrators. The value obtained should be within 10% of the expected value. A CCV should be run after every 10 samples.

4.5.4 A blank should be prepared with each sampling. The blank is the matrix diluted as the samples, for this study, ~1 ml of a 0.25% methylcellulose in water solution is placed in a 25 ml volumetric flask and diluted to the mark with acetonitrile. 0.1 ml of this is placed into a 1.8 ml autosampler vial and diluted with 0.9 ml of 50% acetonitrile, 50% water. The blank should be < 3X MDL (see 4.5.6).5

4.5.5 Method Detection Limit (MDL) is determined by preparing a sample at a low concentration, using similar techniques as used to analyze the low concentration stability sample. This is done 7 times and the MDL is the students T (3.143 for 7 replicates) times the standard deviation of the seven replicate runs. An MDL should be performed prior to the analysis of any sample for phenobarbital. Samples with no peak or quantitating at a value less than the MDL will be reported as the MDL and flagged with a "U.

4.6 Purity

4.6.1 Purity is determined by running a sample of the material that is at or near the top of the demonstrated linearity of the system. All the peaks in the purity chromatogram are summed. The peak corresponding to the phenobarbital is then compared to all the other peaks and the purity is the area of the phenobarbital peak divided by the sum of the total area in the chromatogram (presented as a percentage). A blank is run prior to the purity run and the peaks in the purity run that correlate to peaks in the blank run are eliminated from the calculation. This purity should be 98% or greater and should compare favorably to the purity from the vendor. Note: the limitation of using a UV/Vis detector for purity is that one cannot be certain that the impurities will absorb at the same wavelength. This purity represents an estimation.

5.0 STABILITY

5.1 A 2.5 g/L (0.25%) methyl cellulose solution is prepared by adding 700 mL of deionized water to a 1 liter flask. This solution should be prepared a day in advance to use. The flask is placed on a hot plate and a stir bar added. While the solution is being stirred, add 2.5 grams of methyl cellulose and then heat the solution to boiling. This process should be closely monitored as the solution must be removed from the hot plate immediately when boiling is observed so the material doesn't boil over. Allow the hot plate to cool, then replace the methyl cellulose solution on the plate and stir the solution for about 2 hours (to attain clarity). The solution is then transferred to a 1 liter volumetric flask and diluted to the mark with deionized water. The solution may be slightly cloudy at this point but will become clear by the next day. Store the solution at 2 to 8 degrees C.

5.2 Prior to use, the phenobarbital is screened so that a uniform suspension can be prepared. A six inch round 180 µm screen is set up with a collection pan and a cover. The phenobarbital is placed on the screen and the screen shaken to push the phenobarbital through the screen.

5.2 Stability for phenobarbital is to run for 28 days. Two stability suspensions are prepared. A 5 mg/mL suspension is made by weighing 1 gram of phenobarbital into a 250 mL amber bottle with 200 mL of the methyl cellulose solution prepared in section 5.1 (use a graduated cylinder to add the methyl cellulose solution). A 20 mg/mL suspension is prepared by weighing 4 grams of phenobarbital into a 250 mL amber bottle and adding 200 mL of the methyl cellulose solution (section 5.1). Stability solutions are stored at 2 to 8 degrees C.

5.3 Phenobarbital has limited solubility in the methyl cellulose solution and the result is the formation of a suspension. The 250 ml amber bottle is supplied with a stir bar. The suspension is removed from the refrigerator and placed on a stir plate and stirred to suspend the Phenobarbital and warm the sample. Stir suspension for about 60 minutes prior to sampling, stirring should be vigorous enough to show a slight vortex,

it should not be stirred so vigorously that air is aspirated into the solution (this may cause foaming). Visual inspection should show an evenly distributed suspension. Sampling is done by taking triple 1 ml aliquots. A 3 ml syringe equipped with a 3.5 inch needle of a wide bore (17 gauge or wider) is used to collect the sample. A 25 ml volumetric flask is tared and using the syringe about 1 ml of the stability suspension is placed into the volumetric flask and a weight determined (and recorded). Sampling is done at 2 levels in the suspension; the first triplicate is taken at a depth of about one third of the distance from the top of the suspension. A second triplicate sample is taken from about two thirds from the top of the suspension. The volumetric flask is then filled to the mark with acetonitrile. The flask is agitated and 0.1 ml is removed and placed into a 1.8 mL autosampler vial with 0.9 mL of the mobile phase (see 4.2.1). Cap the vial and mix by agitating.

5.4 Stability solutions are stored in amber bottles at 2 to 8 degrees C.

5.5 Samples should be analyzed on the day of sampling, but if this is not possible, samples should be stored at 4° C. until analysis. If samples are not analyzed on the day of sampling, the actual analysis date and storage conditions shall be documented.

6.0 DATA ANALYSIS AND CALCULATIONS

6.1 Prior to analysis of any samples, the instrument is calibrated with a minimum of a 4 point curve. External standard calculations will be performed. All calculations are done using chromatography software supplied with the instrument. If the software allows the input of a multiplier, determine and enter a multiplier so that the output reflects the concentration in the stability sample. For phenobarbital stability, about 1 mL of the stability sample is diluted with 25 mL of acetonitrile, then 0.1 ml of this solution is diluted to 1 ml with mobile phase. A density of 1 is assumed for the 0.25% methyl cellulose and the weight in grams is equal to the volume in milliliters. The multiplier is determined by dividing the dilution factor of 250 (0.1 ml of a 25 ml solution taken to 1 ml)by the volume of the stability solution. Calibration curve fits can be set to either linear or non-linear (quadratic fit), past experience indicates that even though the calibration meets linearity criteria, the quantification is improved with a non-linear fit.

7.0 QUALITY CONTROL

7.1 A blank is prepared with each sampling, this blank is the methyl cellulose solution processed identically to the stability solution. If background levels are sufficiently high (i.e., greater than 3 x MDL), this value may be subtracted from the values obtained for samples analyzed with that batch. Processing of these samples is very straight forward, therefore spikes are optional.

7.2 An initial calibration verification (ICV) standard will be analyzed following the calibration curve. Continuing calibration verification standards (CCVs) will be analyzed after every 10 samples. All samples should be bracketed with a valid CCV. If a CCV fails, perform system maintenance, recalibrate and rerun the samples not bracketed with a valid CCV.

8.0 SAFETY

All analysts following this procedure should be aware of routine laboratory safety concerns, including all safety protocols regarding use of chemicals, including the following:

- Gloves, protective clothing and safety glasses should be worn when handling samples and chemicals.

9.0 TRAINING REQUIREMENTS

10.1 All staff performing this analysis should first read this procedure and conduct their first analysis under the supervision of a staff member who has had previous experience conducting the procedure. Staff should demonstrate proficiency in the process prior to performing the work.

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Table 1. Summary of Data Quality Objectives and Corrective Actions

Quality Control Sample Type	Data Quality Objective (DQO)	Corrective Action
Procedural Blank one/batch	Less than 3 x MDL	Re-extract and analyze sample batch. If batch can not be re-extracted and analyzed, "B" flag all samples that are in the batch. Investigate sources of blank contamination.
Calibration curve acceptability	r^2 values greater than or equal to 0.995	If r^2 value is outside of criterion, re-analyze calibration standards, if r^2 is still out, perform instrument maintenance and/or remake calibration standards and rerun calibration samples.
Initial calibration verification (ICV) standard; one/batch	+ / - 10 % of true value	Re-calibrate. Must meet DQO in order to continue processing samples.
Continuing calibration verification standards; one every 10th sample analyzed	+/ - 10 % of true value	Re-run CCV, if still not acceptable, re-calibrate and reanalyze affected samples.
Replicate sample precision; triplicates will be analyzed for stability, duplicate for in-life	Precision: 30% as relative standard deviation (RSD) or relative percent deviation (RPD)	If RSD or RPD is not acceptable, resample and reanalyze. If reanalysis data are still not acceptable, then *** flag the values.
Blank or Matrix Spike and spike duplicate, one set per batch (optional)	+/- 15% of true value	If recoveries are unacceptable, check the spike solution to ensure it has not degraded, also check pipettes to ensure they are delivering accurate volumes.

[a]DQO is based on limited sample analysis as part of method development experience, and may require adjustment when more experience with the method is available.

Table 2. Data Qualifiers[a]

U	The analyte was detected below the MDL. Note: Samples with no peaks are reported as zero.
B	Samples associated with procedural blank contamination.
*	QC sample data that does not meet the DQO acceptability criterion.
Q	The data are questionable.
D	Sample diluted for analysis. (note: this procedure outlines the dilution of the samples, data will not be D flagged unless diluted other than indicated in this SOP).

[a]Additional data qualifiers may be added as necessary.

APPENDIX F

ANALYTICAL METHOD DEVIATIONS

The following method deviations were filed:

1. EDSP.H4-033-01 – Section 4.5.5 of the method outlines the procedure for carrying out an MDL. An MDL was not done for the Linuron method. The low calibration standard was used to determine that system sensitivity was sufficient for sample analysis. The signal levels were very high for the concentrations evaluated and the method had been used in a previous study with good results. As a result, to minimize hours expended on the project, the analyst decide to bypass the MDL determination.

APPENDIX 5 - ANALYTICAL REPORT
CONCENTRATION AND HOMOGENEITY

CHARLES RIVER
LABORATORIES
Preclinical Services

FINAL REPORT

METHOD VALIDATION AND FORMULATION SAMPLE ANALYSIS FOR CHARLES RIVER LABORATORIES PRECLINICAL SERVICES

Submitted to:

Charles River Laboratories
Preclinical Services
905 Sheehy Drive, Building A
Horsham, Pennsylvania
19044-1241

Submitted by:

Charles River Laboratories
Preclinical Services
57 Union Street
Worcester, MA 01608

Report No. RTP00004AA-05-1104

Page 1 of 61

Issue Date: March 31, 2006

TABLE OF CONTENTS

1. LIST OF TABLES, FIGURES AND APPENDICES

2. APPROVAL

The study was performed under my overall scientific guidance and management. The report provides a full and accurate record of the raw data generated.

Dorothy Savage 3/31/06

Dorothy Savage, B.S./Date
Principal Investigator
Charles River Laboratories
Preclinical Services

3. COMPLIANCE STATEMENT

This project was conducted in compliance with the following regulations:

U.S Environmental Agency. Federal Insecticide, Fungicide and Rodenticide Act / Toxic Substances Control Act (FIFRA/TSCA); Good Laboratory Practice Standards; Final Rule. 40 CFR Part 160/Part 792.

Ministry of Agriculture, Forestry and Fisheries, Japan (MAFF). Good Laboratory Practice (GLP) standards for agricultural chemicals. Agricultural Protection Bureau Ref. No. 11-Nousan-No.6283. October 1, 1999; last revised June 30, 2003 Ref. No. 15-Sesian-2460.

OECD Environmental Directorate. OECD Principles of Good laboratory practices [C(97)186/Final] (1998); Environmental Health and Safety Division.

Principal Investigator:

Dorothy Savage 3/31/06

Dorothy Savage, B.S./Date

4. QUALITY ASSURANCE STATEMENT

This study has been inspected by the Quality Assurance Unit to assure conformance with the Good Laboratory Practice (GLP) regulations promulgated by U.S. Environmental Protection Agency, the Japanese Ministry of Agriculture, Forestry and Fisheries, and the OECD Directorate. Reports were submitted in accordance with Standard Operating Procedures as follows:

QA INSPECTION DATES

			Dates Findings Submitted to:		
Dates of Inspection	Phase(s) Inspected	Principal Investigator	PI Management	Study Director	Study Director Management
10/11/2005	Laboratory Procedure	10/11/2005	10/12/2005	01/12/2006	01/12/2006
01/04-05/2005	Data	01/10/2006	01/11/2006	01/12/2006	01/12/2006
01/04-05/2005	Draft Final Report	01/10/2006	01/11/2006	01/12/2006	01/12/2006

The final report has been reviewed to assure that it accurately describes the materials and methods, and the reported results accurately reflect the raw data.

Theresa A Donegan M.S./Date 3/31/05

5. CONTRIBUTING PERSONNEL

Current Principal Investigator.....Dorothy Savage, B.S.

Former Principal Investigator .....Kim Barnard, B.S.

Associate Director, Analytical Chemistry .....Richard Norlin, M.S.

Senior Director, Analytical Chemistry ..... Stephen Guyan, M.Sc.

Report Coordinator .....Brenda L. Brooks

6. ANALYTICAL REFERENCE STANDARD CHARACTERIZATION/STABILITY

Compound Phenobarbital	
Physical Description:	White Powder
Storage Conditions:	Ambient temperature
Lot Number:	104K2600
Date Received:	15-Sep-2005
Expiration/Retest Date:	28-Feb-2010
Amount Received:	1g
Received by:	Charles River Laboratories Preclinical Services, Pennsylvania
Manufacturer:	Sigma-Aldrich
Purity:	99.1%

Compound Linuron	
Physical Description:	White Solid
Storage Conditions:	Ambient temperature
Lot Number:	348-8A
Date Received:	15-Sep-2005
Expiration/Retest Date:	31-Aug-2008
Amount Received:	1g
Received by:	Charles River Laboratories Preclinical Services, Pennsylvania
Manufacturer:	ChemService, Inc.
Purity:	99.5%

6.1. Characterization and Stability

The characterization of the analytical reference standards are the responsibility of their respective supplier, as are the methods of synthesis, fabrication or derivation and stability determinations.

7. ARCHIVAL STORAGE

The original final report and raw data will be maintained for a minimum period of one year following submission of the final report in the Charles River Laboratories Preclinical Services Archives department located in Horsham, PA. After one year, storage disposition will be negotiated with the Sponsor. The Sponsor will be notified prior to disposal of any original study data. Archival material will be indexed by Report No. RTP00004AA-05-1104.

PART A: METHOD VALIDATION AND FORMULATION SAMPLE ANALYSIS FOR LINURON IN 0.25% METHYLCELLULOSE

8. ABSTRACT

Procedures were developed and validated for the analysis of Linuron in 0.25% methylcellulose formulations. The procedures involve analysis of the compound by High Performance Liquid Chromatography (HPLC) with Ultraviolet (UV) detection.

The procedures are applicable for the analysis of dose formulations at concentrations from 0.58 to 43 mg/mL of Linuron in 0.25% methylcellulose. Validation of the formulation analysis was performed using one concentration range of matrix-matched standards spanning approximately 0.005 to 0.05 mg/mL of linuron in diluent. Linearity, accuracy and precision for the analysis were confirmed. Dose formulations were diluted into the calibration range with a 60:40, ACN:Water (v:v) (diluent).

The Lower Limit of Quantitation (LLOQ) of the method was 0.005 mg/mL linuron in diluent, the lowest calibration standard. The Limit of Detection (LOD) was estimated to be approximately 0.000014 mg/mL of linuron in diluent, calculated before any corrections for dilution factors. Analysis of replicate blank vehicle samples indicated no interference peaks.

Overall, results for this validation indicate that the assay procedures were sufficiently linear, reproducible and accurate to support dose formulation analyses.

Samples for this project were analyzed for concentration and homogeneity verification according to the method described in the Charles River Laboratories Preclinical Services, Massachusetts Laboratory Method (LM) for the "Analysis of Linuron in 0.25% Methylcellulose Dose Formulations by HPLC-UV" LM LINR00. A copy of the most recent LM revision is included in Appendix B. Results indicated that the formulations were prepared accurately.

9. INTRODUCTION

The objective of this project was to develop and validate analytical procedures for the determination of levels of linuron in 0.25% methylcellulose. This procedure was used to analyze formulation samples from Charles River Laboratories Preclinical Services, Pennsylvania study RTP00004.

9.1. Experimental Design

The procedures described here involve analysis of linuron by HPLC with UV detection. Calibration standards were prepared at known concentrations and analyzed to determine the accuracy, precision, specificity, linearity and limits of quantitation and detection for the method. Formulations received from Charles River Laboratories Preclinical Services, Pennsylvania were analyzed for concentration and homogeneity verification.

10. MATERIALS AND METHODS

10.1. Computer Software

The HPLC data were acquired utilizing PerkinElmer's TotalChrom Client/Server software Version 6.2.1. TotalChrom software was used to integrate the peak areas of the analyte. Following integration the data was exported to a verified Excel spreadsheet. The Excel spreadsheet was used to perform the regression, calculate the regression constants and calculate the concentration of the analyte in unknown samples using the peak areas of the analyte. System suitability was verified using TotalChrom software.

10.2. Instrumentation

Pump:	PerkinElmer Series 200
Autosampler:	PerkinElmer Series 200
Column:	Phenomenex Synersi, 4 µm, Hydro RP, 250 mm x 4.6 mm
Column Heater:	PerkinElmer Series 200
Detector:	PerkinElmer Series 200

10.3. Preparation of Reagents and Standards

Refer to the Laboratory Method in Appendix B for the preparation of reagents and standards. During the method validation, the lowest and highest calibration standards were prepared in quadruplicate and the blank was prepared in triplicate.

10.4. Analytical Formulations for the 0.3 mg/mL Dilutions

One stock solution of linuron was prepared at approximately 0.3 mg/mL by weighing approximately 30 mg of linuron with 1 mL of vehicle into a 100 mL volumetric flask and diluting to volume with diluent 1. Replicate dilution verification solutions were prepared by pipetting 1.0 mL aliquots of the stock into four individual 10 mL volumetric flasks.

The final solutions were brought to volume with diluent containing 1% vehicle and mixed.

Resultant concentrations were approximately 0.03 mg/mL of linuron in extract solution. Aliquots of each solution were transferred into individual autosampler vials.

10.5. Preparation of Dose Formulation Samples

Samples from Charles River Laboratories Preclinical Services, Pennsylvania study RTP00004 were received on October 8 and 19, 2005. The samples were received in individual vials, each containing approximately 1 mL, and all were in good condition. All samples were received on cold packs. Those samples which were not analyzed immediately were stored refrigerated until analysis. Refer to the Laboratory Method in Appendix B for the procedures concerning preparation of samples for analysis.

Refer to the Laboratory Method in Appendix B for chromatographic conditions and calculations.

11. RESULTS AND DISCUSSION

11.1. Method Development

The methods were developed using general methodology provided by the Sponsor.

11.2. Validation Results

Refer to Table 1 for tabulated results.

11.2.1. Recovery

Recovery was not evaluated since sample preparation did not utilize extraction or precipitation.

11.2.2. Linearity

The assay was linear within the range tested of approximately 0.005 to 0.05 mg/mL of linuron in mobile phase. Refer to the plot in Figure 1, which shows the unweighted linear regression graph with the actual calibration standard data points for the validation analysis run. Linearity was also demonstrated by the correlation coefficient obtained, which was greater than 0.999, and the lack of bias in the calculated percent error values for the calibration standards. These percent errors ranged from -1.0% to +1.0%.

11.2.3. Accuracy

Accuracy of the method was evaluated by the analysis of four replicates of the analytical formulations solutions. Mean concentrations found during the run were compared to theoretical concentrations and expressed as percent errors. A value of 0.7% was obtained. Accuracy of the method was also evaluated by the back-calculated results for

the calibration standards using the linear regression standard curve. Concentrations were compared to theoretical concentrations and expressed as percent errors. These percent errors ranged from -1.0% to +1.0%. Mean accuracy values at the low and high end of the calibration range were -0.5% and 0.1%, respectively.

11.2.4. Precision

Within the run, precision was evaluated by the analysis of four replicates dilution verification solutions. The relative standard deviations (RSD) of the replicates were calculated. A value of 0.4% RSD was obtained for the dilution verification solutions. Precision was evaluated by the analysis of replicate low and high concentration calibration standards. The RSDs of the replicates were calculated. Values of 0.1% and 0.2% were obtained for the low and high calibration standards, respectively.

11.2.5. Sensitivity

The Lower Limit of Quantitation (LLOQ) for the analysis was defined as 0.005 mg/mL of linuron in diluent, the lowest calibration standard. The RSD obtained for quadruplicate calibration standards at this level was determined to be 0.1%. The LOD for undiluted samples was estimated to be 0.000014 mg/mL, calculated as three times the standard deviation of the back-calculated concentration of the low calibration standard.

11.2.6. Specificity

Specificity was demonstrated by the lack of any significant interfering chromatographic peaks found in three blank vehicle samples. Refer to Figure 3 for an example chromatogram.

11.2.7. Summary

Overall, results for the validation indicated that the procedure was sufficiently linear, reproducible, accurate and specific to support analyses of dose formulation samples.

11.3. Concentration and Homogeneity Results

Refer to the Dose Formulation Analysis Reports in Appendix A for details. Each report consists of results and conclusions from one analysis period. Preparation and analysis dates are listed for each result along with the Charles River Laboratories Preclinical Services sample identification.

11.3.1. Concentration

Test article samples prepared on October 7 and 18, 2005, were within acceptable limits of ±15%.

11.3.2. Homogeneity

Homogeneity was determined for all dose formulation concentration levels. Mean concentration results from samples taken from the top, middle and bottom of the

formulations were calculated. Homogeneity RSD was calculated by determining the percent relative standard deviation of the three mean values. All of the pre-study results were within the acceptable range of ≤5% RSD except the 20 mg/mL formulation. The values obtained were 2.6%, 6.9% and 4.1% for the 10, 20 and 30 mg/mL formulations, respectively. All of the start of study results were within the acceptable range of ≤5% RSD. The values obtained were 1.8%, 2.1% and 3.4% RSD for the 10, 20 and 30 mg/mL formulations, respectively.

PART B: METHOD VALIDATION AND FORMULATION SAMPLE ANALYSIS FOR PHENOBARBITAL IN 0.25% METHYLCELLULOSE

12. ABSTRACT

Procedures were developed and validated for the analysis of phenobarbital in 0.25% methylcellulose formulations. The procedures involve analysis of the compound by High Performance Liquid Chromatography (HPLC) with Ultraviolet (UV) detection.

The procedures are applicable for the analysis of dose formulations at concentrations from 0.29 to 43 mg/mL of phenobarbital in 0.25% methylcellulose. Validation of the phenobarbital formulation analysis was performed using one concentration range of matrix-matched standards spanning approximately 0.01 to 0.1 mg/mL of phenobarbital in 50:50, ACN: Water, (v:v) (diluent). Linearity, accuracy and precision for the analysis were confirmed. Dose formulations were diluted into the calibration range with diluent.

The Lower Limit of Quantitation (LLOQ) of the method was 0.01 mg/mL phenobarbital in diluent, the lowest calibration standard. The Limit of Detection (LOD) was estimated to be approximately 0.000099 mg/mL of phenobarbital in diluent, calculated before any corrections for dilution factors. Analysis of replicate blank vehicle samples indicated no interference peaks.

Overall, results for this validation indicate that the assay procedures were sufficiently linear, reproducible and accurate to support dose formulation analyses.

Samples for this project were analyzed for concentration and homogeneity verification according to the method described in the Charles River Laboratories Preclinical Services, Massachusetts Laboratory Method (LM) for the "Analysis of Phenobarbital in 0.25% Methylcellulose Dose Formulations by HPLC-UV", LM PHBT00. A copy of the most recent LM revision is included in Appendix B. Results indicated that the formulations were prepared accurately.

13. INTRODUCTION

The objective of this project was to develop and validate analytical procedures for the determination of levels of phenobarbital in 0.25% methylcellulose. This procedure was used to analyze formulation samples from Charles River Laboratories Preclinical Services, Pennsylvania study RTP00004.

13.1. Experimental Design

The procedures described here involve analysis of phenobarbital by HPLC with UV detection. Calibration standards were prepared at known concentrations and analyzed to determine the accuracy, precision, specificity, linearity and limits of quantitation and detection for the method. Formulations received from Charles River Laboratories Preclinical Services, Pennsylvania were analyzed for concentration and homogeneity verification.

14. MATERIALS AND METHODS

14.1. Computer Software

The HPLC data were acquired utilizing PerkinElmer's TotalChrom Client/Server software Version 6.2.1. TotalChrom software was used to integrate the peak areas of the analyte. Following integration the data was exported to a verified Excel spreadsheet. The Excel spreadsheet was used to perform the regression, calculate the regression constants and calculate the concentration of the analyte in unknown samples using the peak areas of the analyte. System suitability was verified using TotalChrom software.

14.2. Instrumentation

Pump:	PerkinElmer Series 200
Autosampler:	PerkinElmer Series 200
Column:	Phenomenex Synergi, C18, 4 μm, 250 mm x 4.6 mm
Column Heater:	PerkinElmer Series 200
Detector:	PerkinElmer Series 200

14.3. Preparation of Reagents and Standards

Refer to the Laboratory Method in Appendix B for the preparation of reagents and standards. During the method validation, the lowest and highest calibration standards were prepared in quadruplicate and the blank was prepared in triplicate.

14.4. Analytical Formulations for the 0.8mg/mL Dilutions

One stock solution of phenobarbital was prepared at approximately 0.8 mg/mL by weighing approximately 8 mg of phenobarbital with 0.4 mL of vehicle into a 10 mL volumetric flask and diluting to volume with diluent 1. Replicate dilution verification

solutions were prepared by pipetting 1.0 mL aliquots of the stock into four individual 20 mL volumetric flasks. The final solutions were brought to volume with diluent containing 4% vehicle and mixed.

Resultant concentrations for the solutions were approximately 0.04 mg/mL of phenobarbital in extract solution. Aliquots of each solution were transferred into individual autosampler vials.

14.5. Preparation of Dose Formulation Samples

Samples from Charles River Laboratories Preclinical Services, Pennsylvania were received on October 8 and 19, 2005. The samples were received in individual vials, each containing approximately 1 mL, and all were in good condition. Those samples which were not analyzed immediately, were stored refrigerated until analysis. Refer to the Laboratory Method in Appendix B for the procedures concerning preparation of samples for analysis.

Refer to the Laboratory Method in Appendix B for chromatographic conditions and calculations.

15. RESULTS AND DISCUSSION

15.1. Method Development

The methods were developed using general methodology provided by the Sponsor.

15.2. Validation Results

Refer to Table 2 for tabulated results.

15.2.1. Recovery

Recovery was not evaluated since sample preparation did not utilize extraction or precipitation.

15.2.2. Linearity

The assay was linear within the range tested of approximately 0.01 to 0.1 mg/mL of phenobarbital in mobile phase. Refer to the plot in Figure 2, which shows the unweighted linear regression graph with the actual calibration standard data points for the validation analysis run. Linearity was also demonstrated by the correlation coefficient obtained, which was greater than 0.999, and the lack of bias in the calculated percent error values for the calibration standards. These percent errors ranged from -1.4% to +0.7%.

15.2.3. Accuracy

Accuracy of the method was evaluated by the analysis of four replicates of the low concentration dilution verification solutions. Mean concentrations found during the run were compared to theoretical concentrations and expressed as percent errors. A value of +1.0% was obtained. Accuracy of the method was also evaluated by the back-calculated results for the calibration standards using the linear regression standard curve. Concentrations were compared to theoretical concentrations and expressed as percent errors. These percent errors ranged from -1.4% to +0.7%. Mean accuracy values at the low and high end of the calibration range were -0.9% and -0.1%, respectively.

15.2.4. Precision

Within the run, precision was evaluated by the analysis of four replicates of the low and high concentration dilution verification solutions. The relative standard deviations (RSD) of the replicates were calculated. A value of 0.5% RSD was obtained for the dilution verification solutions. Precision was evaluated by the analysis of replicate low and high concentration calibration standards. The RSDs of the replicates were calculated. Values of 0.3% and 0.5% were obtained for the low and high calibration standards, respectively.

15.2.5. Sensitivity

The Lower Limit of Quantitation (LLOQ) for the analysis was defined as 0.01007 mg/mL of phenobarbital in diluent, the lowest calibration standard. The RSD obtained for quadruplicate calibration standards at this level was determined to be 0.3%. The LOD for undiluted samples was estimated to be 0.000099 mg/mL, calculated as three times the standard deviation of the back-calculated concentration of the low calibration standard.

15.2.6. Specificity

Specificity was demonstrated by the lack of any significant interfering chromatographic peaks found in three blank vehicle samples. Refer to Figure 4 for an example chromatogram.

15.2.7. Summary

Overall, results for the validation indicated that the procedure was sufficiently linear, reproducible, accurate and specific to support analyses of dose formulation samples.

15.3. Concentration and Homogeneity

Refer to the Dose Formulation Analysis Reports in Appendix A for details. Each report consists of results and conclusions from one analysis period. Preparation and analysis dates are listed for each result along with Charles River Laboratories Preclinical Services sample identification.

15.3.1. Concentration

Test article samples prepared on October 7 and 18, 2005, which were used for dosing, were within acceptable limits of ±15% error.

15.3.2. Homogeneity

Homogeneity was determined for all dose formulation concentration levels. Mean concentration results from samples taken from the top, middle and bottom of the formulations were calculated. Homogeneity RSD was calculated by determining the percent relative standard deviation of the three mean values. All of the pre-study results were within the acceptable range of ≤5% RSD. The values obtained were 2.9%, 4.2% and 2.0% for the 5, 10 and 20 mg/mL formulations, respectively. All of the start of study results were within the acceptable range of ≤5% RSD. The values obtained were 3.3%, 0.7% and 0.1% RSD for the 5, 10 and 20 mg/mL formulations, respectively.

TABLES

Table 1. Linuron Validation Results

Analyzed on October 3, 2005

Concentration in mg/mL

Calibration Standard Results:

Standard Description	Theoretical Concentration	Response Area	Concentration Found	% Error			
Blank a	0	0	ND				
Blank b	0	0	ND				
Blank c	0	0	ND			Slope:	95881000
Cal Std A1a	0.005022	485570	0.004994	-0.6%		Y-Int:	6729.6
Cal Std A1b	0.005022	486538	0.005004	-0.4%		Corr:	0.99997
Cal Std A1c	0.005022	485686	0.004995	-0.5%		n:	12
Cal Std A1d	0.005022	486098	0.005000	-0.4%			
Cal Std B1	0.009970	972415	0.01007	+1.0%			
Cal Std A2	0.02009	1936093	0.02012	+0.1%			
Cal Std B2	0.02991	2889657	0.03007	+0.5%			
Cal Std A3	0.04018	3820435	0.03978	-1.0%			
Cal Std B3a	0.04985	4778002	0.04976	-0.2%		Response	Mean %
Cal Std B3b	0.04985	4795005	0.04994	+0.2%	Std	RSD	Error
Cal Std B3c	0.04985	4793879	0.04993	+0.2%	A1	0.1%	-0.5%
Cal Std B3d	0.04985	4798683	0.04998	+0.3%	B3	0.2%	0.1%

ND = None Detected

Table 1. Linuron Validation Results (Concluded)

Analyzed on October 3, 2005

Concentration in mg/mL

Analytical Formulation / Dilution Verification Results:

Theoretical Concentration	Replicate	Response	Concentration
0.3081	A	2974925	0.3096
	B	2970223	0.3091
	C	2987788	0.3109
	D	2994404	0.3116
		Mean:	0.3103
		RSD:	0.4%
		% Error:	0.7%

Table 2. Phenobarbital Validation Results

Analyzed on September 29, 2005

Concentration in mg/mL

Calibration Standard Results:

Standard Description	Theoretical Concentration	Response Area	Concentration Found	% Error			
Blank a	0	0	ND				
Blank b	0	0	ND				
Blank c	0	0	ND			Slope:	11187000
Cal Std A1a	0.01007	115747	0.009999	-0.7%		Y-Int:	3887.8
Cal Std A1b	0.01007	115589	0.009985	-0.8%		Corr:	0.99997
Cal Std A1c	0.01007	114938	0.009927	-1.4%		n:	12
Cal Std A1d	0.01007	115656	0.009991	-0.8%			
Cal Std B1	0.02016	230920	0.02029	+0.6%			
Cal Std A2	0.04027	456512	0.04046	+0.5%			
Cal Std B2	0.06048	680727	0.06050	+0.0%			
Cal Std A3	0.08054	911070	0.08109	+0.7%			
Cal Std B3a	0.1008	1129716	0.1006	-0.2%		Response	Mean %
Cal Std B3b	0.1008	1137627	0.1013	+0.5%	Std	RSD	Error
Cal Std B3c	0.1008	1125956	0.1003	-0.5%	A1	0.3%	-0.9%
Cal Std B3d	0.1008	1127075	0.1004	-0.4%	B3	0.5%	-0.1%

ND = None Detected

Table 1. Phenobarbital Validation Results (Concluded)

Analyzed on September 29, 2005

Concentration in mg/mL

Analytical Formulation / Dilution Verification Results:

Theoretical Concentration	Replicate	Response	Concentration
0.8090	A	458577	0.8129
	B	461305	0.8178
	C	460146	0.8157
	D	464330	0.8232
		Mean:	0.8174
		RSD:	0.5%
		% Error:	1.0%

FIGURES

Figure 1. Standard Curve for Linuron Validation Run

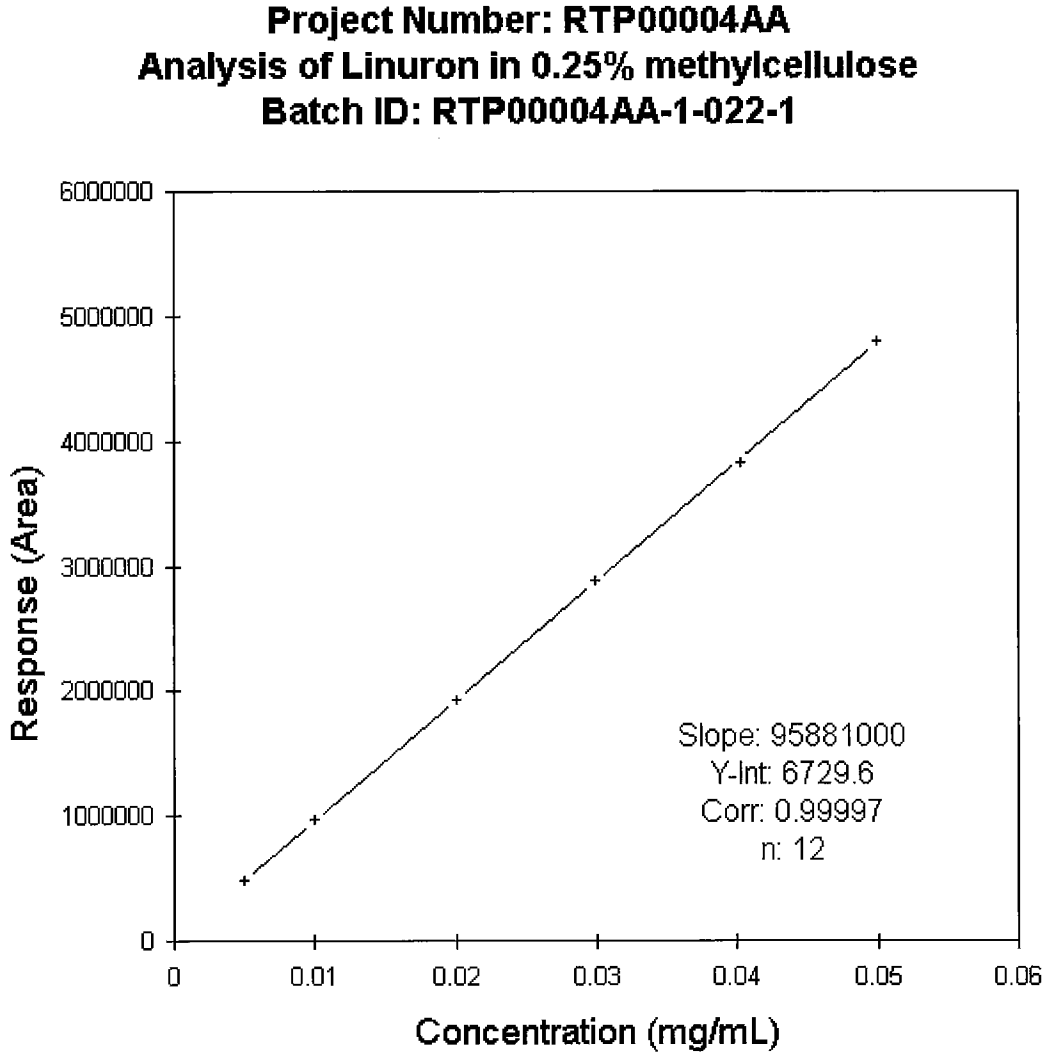


Figure 2. Standard Curve for Phenobarbital Validation Run

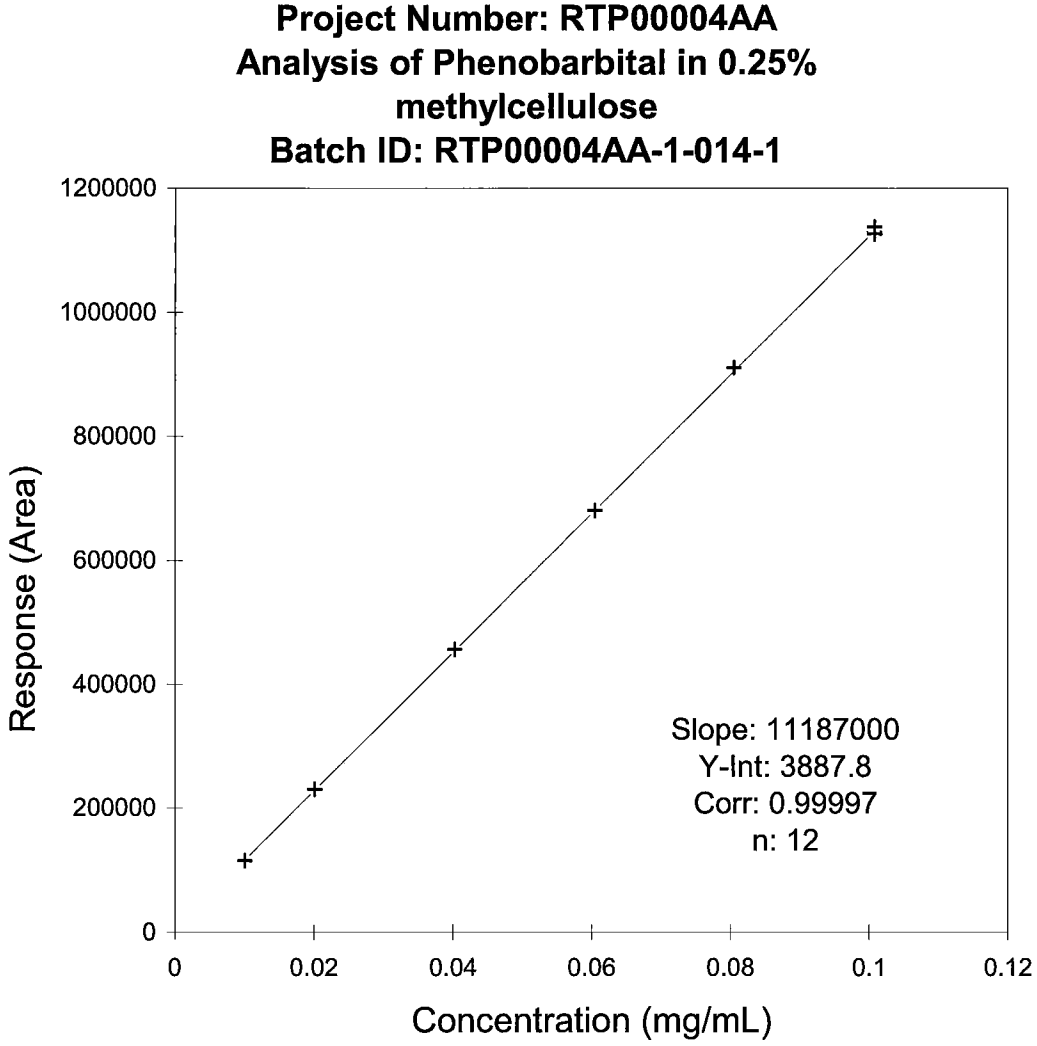


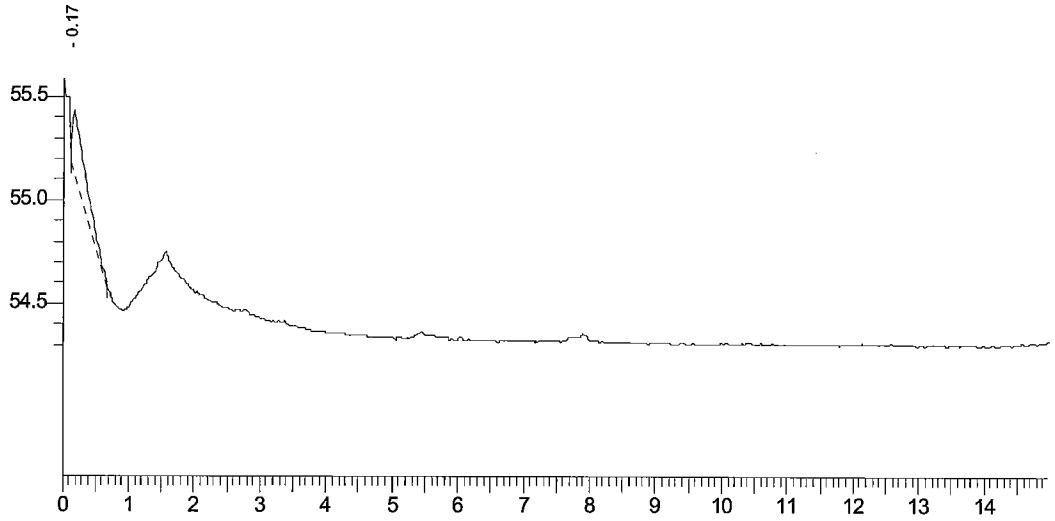
Figure 3. Linuron Example Chromatogram

Study #: RTP00004AA
Batch ID: RTP00004AA-1-022-1
Injection Volume: 20 µL
Detection: UV @ 250 nm
Column: Phenomenex, Synergi 4µ, Hydro-RP 250 x 4.6nnm 4µm

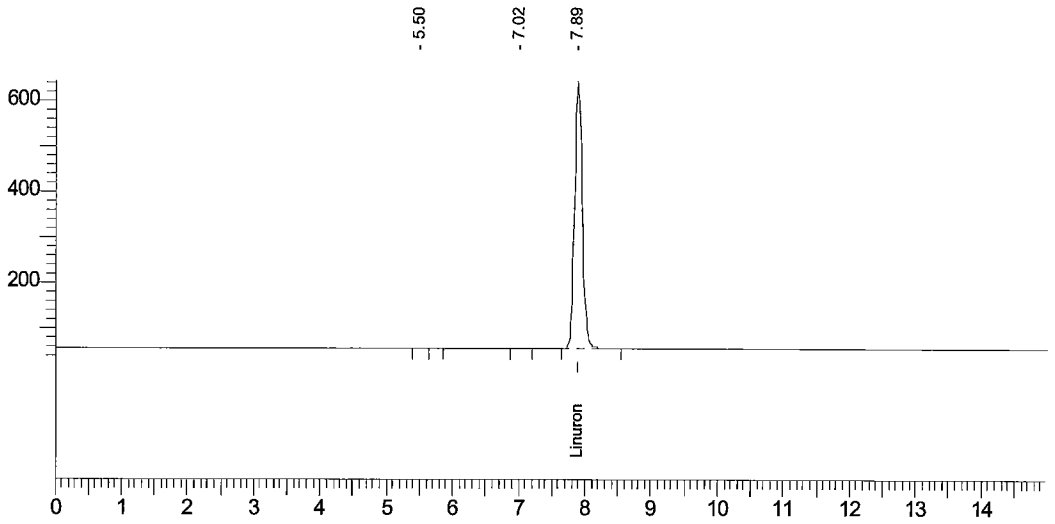
Analysis Date: October 3, 2005
Flow Rate: 1.0 mL/min
Guard Column: N/A

Linuron

Blank ID: RTP00004AA-1-022-1_054.rst (Sensitivity: 1.4 mv FS)



Standard B3a ID: RTP00004AA-1-022-1_050.rst (Sensitivity: 615.6 mv FS)



FS = Full Scale; mv = milli volts

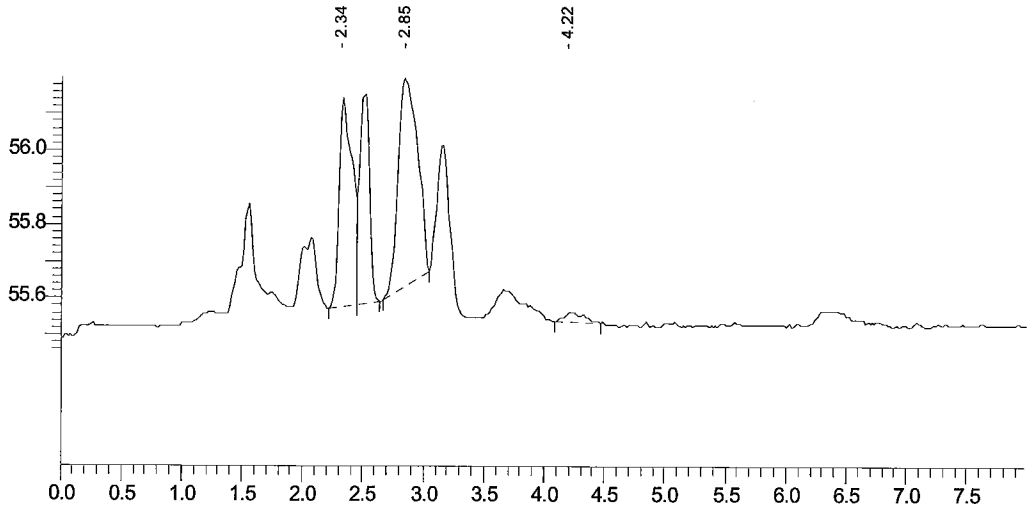
Figure 4. Phenobarbital Example Chromatogram

Study #: RTP00004AA
Batch ID: RTP00004AA-1-014-1
Injection Volume.: 10 µL
Detection: UV @ 225 nm
Column: Phenomenex, Synergi 4µ, Hydro-RP 250 x 4.6nnm 4µm

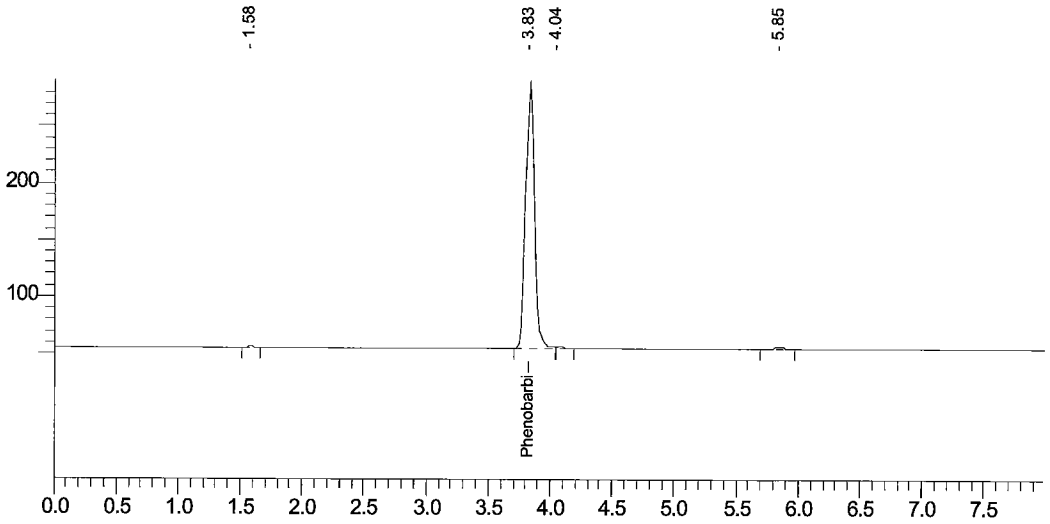
Analysis Date: September 29, 2005
Flow Rate: 1.0 mL/min
Guard Column: N/A

Phenobarbital

Blank ID: RTP00004AA-1-014-1_020.rst (Sensitivity: 0.7 mv FS)



Standard B3a ID: RTP00004AA-1-014-1_016.rst (Sensitivity: 246.9 mv FS)



FS = Full Scale; mv = milli volts

Appendix A. Dose Formulation Analysis Reports

DOSE FORMULATION ANALYSIS REPORT

Sponsor: Battelle
Study Facility: Charles River Laboratories Preclinical Services, Pennsylvania
Protocol Number: RTP00004
Analyte: Phenobarbital
Analytical Facility: Charles River Laboratories Preclinical Services, Massachusetts
Batch ID: RTP00004AA-1-032-1
Sampling Criteria: Pre-Study Homogeneity and Concentration Analysis
Vehicle: 0.25% Methylcellulose
Storage Conditions: 5±3°C
Laboratory Method: LM # PHBT00 (Draft) Revision 00
Analysis Date: October 10, 2005

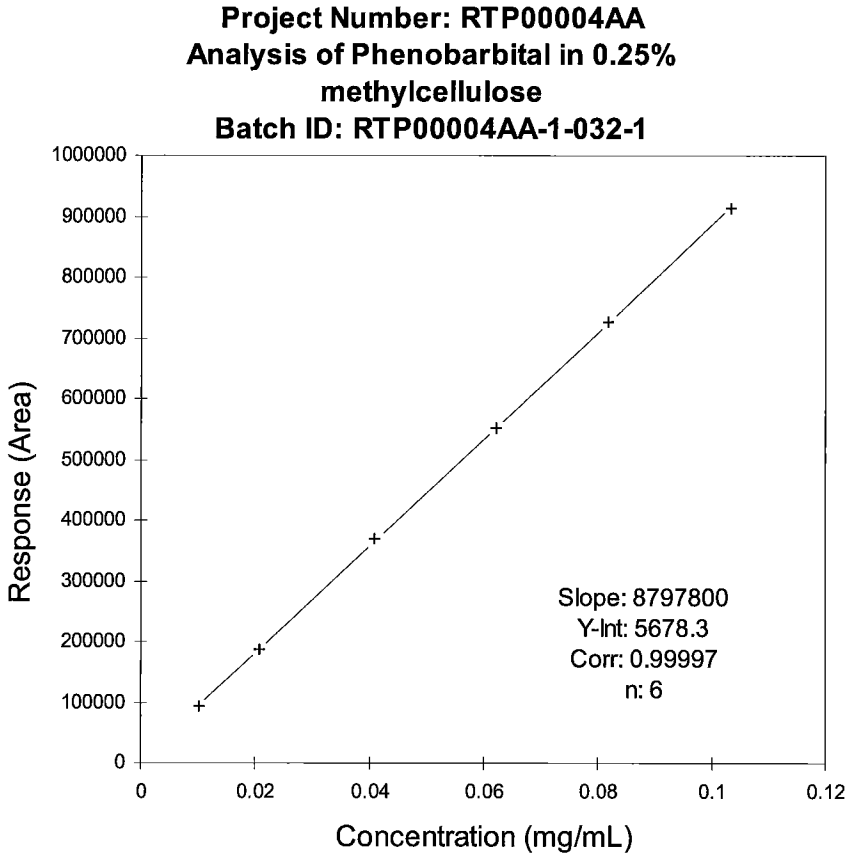
RESULTS: (Concentrations in mg/mL)

CALIBRATION STANDARDS

Standard Description	Nominal Conc.	Response Area	Calculated Conc.	% Bias	"X" = Exclude	Criteria Limit	Standard Pass/Fail
Cal Std A1	0.01022	93958	0.01003	-1.9%		5%	PASS
Cal Std B1	0.02068	186171	0.02052	-0.8%		5%	PASS
Cal Std A2	0.04088	369385	0.04134	+1.1%		5%	PASS
Cal Std B2	0.06204	550864	0.06197	-0.1%		5%	PASS
Cal Std A3	0.08176	727063	0.08200	+0.3%		5%	PASS
Cal Std B3	0.1034	912965	0.1031	-0.3%		5%	PASS

CHECK STANDARDS

Standard Description	Nominal Conc.	Response Area	Dilution Factor	Conc. Found	% Bias	Criteria Limit	Standard Pass/Fail
Check Std A3	0.08176	723715	1	0.08162	-0.2%	5.0%	PASS
Check Std A3	0.08176	724725	1	0.08173	-0.0%	5.0%	PASS


Project Number: RTP00004AA
Analysis of Phenobarbital in 0.25% methylcellulose
Batch ID: RTP00004AA-1-032-1
Response (Area)
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
0
0.02
0.04
0.06
0.08
0.1
0.12
Concentration (mg/mL)
Slope: 8797800
Y-Int: 5678.3
Corr: 0.99997
n: 6

SAMPLES

Sample Description	Prep Date	Nominal Sample Conc.	Replicate	Response Area	Total Dilution Factor	Density Corrected mg/mL	Mean mg/mL Found	% Bias
Group 5 Top	10/07/05	5	A	367715	126.5	5.207	5.132	+2.6%
			B	360860	125.2	5.056		
Group 5 Mid	10/07/05	5	A	358346	125.8	5.041	5.051	+1.0%
			B	360236	125.6	5.06		
Group 5 Bot	10/07/05	5	A	390238	123.2	5.385	5.339	+6.8%
			B	402496	117.3	5.292		
Group 6 Top	10/07/05	10	A	717387	125.4	10.14	10.72	+7.2%
			B	722456	138.5	11.29		
Group 6 Mid	10/07/05	10	A	702684	124.1	9.832	9.867	-1.3%
			B	698970	125.6	9.901		
Group 6 Bot	10/07/05	10	A	764292	121.8	10.50	10.41	+4.1%
			B	762411	120.0	10.32		
Group 7 Top	10/07/05	20	A	711663	249.1	19.99	20.03	+0.2%
			B	719920	247.3	20.07		
Group 7 Mid	10/07/05	20	A	722771	244.1	19.89	19.79	-1.1%
			B	699614	249.6	19.69		
Group 7 Bot	10/07/05	20	A	738019	243.6	20.28	20.59	+3.0%
			B	737530	251.3	20.90		

HOMOGENEITY

Sample Description	Nominal Sample Conc.	Grand Mean Conc.	RSD	% Error
Group 5	5	5.174	2.9%	3.5%
Group 6	10	10.33	4.2%	3.3%
Group 7	20	20.14	2.0%	0.7%

CONCLUSIONS: Results indicate that the formulations are within the acceptable limits of ±15% of theoretical concentrations. The formulations are also within the acceptable limits of ≤5% RSD for homogeneity.

ACTIONS TAKEN: None

DOSE FORMULATION ANALYSIS REPORT

Sponsor: Battelle
Study Facility: Charles River Laboratories Preclinical Services, Pennsylvania
Protocol Number: RTP00004
Analyte: Linuron
Analytical Facility: Charles River Laboratories Preclinical Services, Massachusetts
Batch ID: RTP00004AA-1-033-1
Sampling Criteria: Pre-Study Homogeneity and Concentration Analysis
Vehicle: 0.25% Methylcellulose
Storage Conditions: 5±3°C
Laboratory Method: LM # LINR00 Revision 00 (draft LM)
Analysis Date: October 10, 2005

RESULTS: (Concentrations in mg/mL, ND = None Detected)

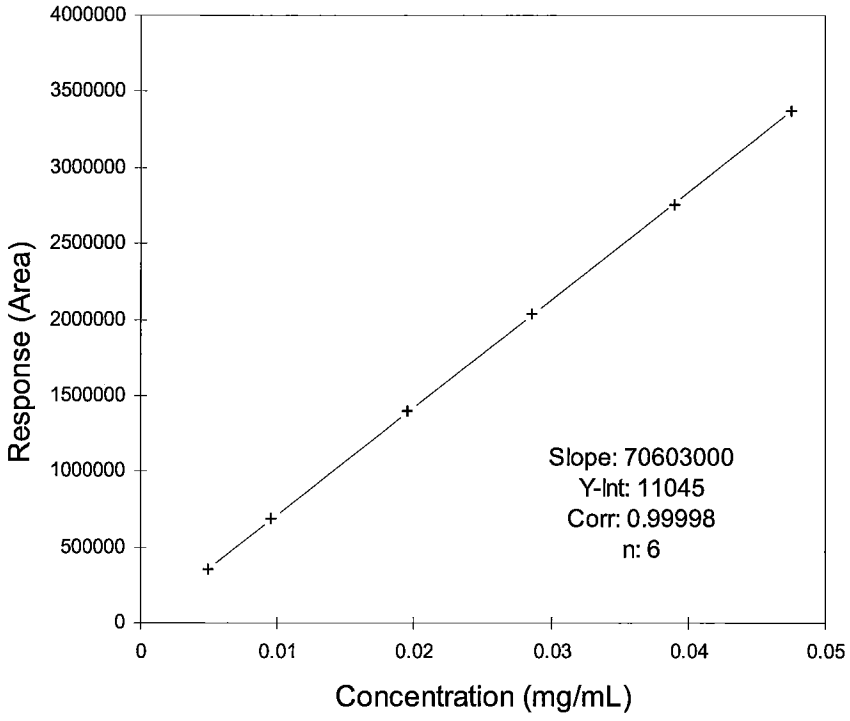
CALIBRATION STANDARDS

Standard Description	Nominal Conc.	Response Area	Calculated Conc.	% Bias	"X" = Exclude	Criteria Limit	Standard Pass/Fail
Cal Std A1	0.004880	351113	0.004817	-1.3%		5%	PASS
Cal Std B1	0.009516	684059	0.009532	+0.2%		5%	PASS
Cal Std A2	0.01952	1395700	0.01961	+0.5%		5%	PASS
Cal Std B2	0.02855	2030983	0.02861	+0.2%		5%	PASS
Cal Std A3	0.03904	2753978	0.03885	-0.5%		5%	PASS
Cal Std B3	0.04758	3376410	0.04767	+0.2%		5%	PASS

CHECK STANDARDS

Standard Description	Nominal Conc.	Response Area	Dilution Factor	Conc. Found	% Bias	Criteria Limit	Standard Pass/Fail
Check Std A3	0.03904	2757019	1	0.03889	-0.4%	5.0%	PASS
Check Std A3	0.03904	2764541	1	0.03900	-0.1%	5.0%	PASS
Check Std A3	0.03904	2775538	1	0.03916	+0.3%	5.0%	PASS
Check Std A3	0.03904	2790912	1	0.03937	+0.8%	5.0%	PASS
Check Std A3	0.03904	2788278	1	0.03934	+0.8%	5.0%	PASS

Project Number: RTP00004AA
Analysis of Linuron in 0.25% methylcellulose
Batch ID: RTP00004AA-1-033-1



SAMPLES

Sample Description	Prep Date	Nominal Sample Conc.	Replicate	Response Area	Total Dilution Factor	Density Corrected mg/mL	Mean mg/mL Found	% Bias
Group 1 Top	10/07/05	0	A	0	100.5	ND		
			B	0	100.4	ND		
Group 1 Mid	10/07/05	0	A	0	100.9	ND		
			B	0	99.57	ND		
Group 1 Bot	10/07/05	0	A	0	102.2	ND		
			B	0	100.8	ND		
Group 2 Top	10/07/05	10	A	1464407	504.8	10.39	10.35	+3.5%
			B	1450680	505.8	10.31		
Group 2 Mid	10/07/05	10	A	3005485	257.8	10.93	10.87	+8.7%
			B	1508070	509.2	10.80		
Group 2 Bot	10/07/05	10	A	1576840	507.3	11.25	10.75	+7.5%
			B	1531288	476.2	10.25		
Group 3 Top	10/07/05	20	A	2860168	501.9	20.25	19.53	-2.4%
			B	2674758	498.2	18.80		
Group 3 Mid	10/07/05	20	A	3241590	507.2	23.21	22.44	+12.2%
			B	3111265	493.2	21.66		
Group 3 Bot	10/07/05	20	A	3136945	462.2	20.47	20.96	+4.8%
			B	3128501	485.7	21.45		
Group 4 Top	10/07/05	30	A	2131269	1005	30.18	30.15	+0.5%
			B	2114646	1011	30.12		
Group 4 Mid	10/07/05	30	A	2340820	969.5	31.99	32.00	+6.7%
			B	2347053	967.0	32.00		
Group 4 Bot	10/07/05	30	A	2233400	1036	32.62	32.65	+8.8%
			B	2510058	923.1	32.67		

HOMOGENEITY

Sample Description	Nominal Sample Conc.	Grand Mean Conc.	RSD	% Error
Group 2	10	10.66	2.6%	6.6%
Group 3	20	20.98	6.9%	4.9%
Group 4	30	31.60	4.1%	5.3%

CONCLUSIONS: Results indicate that the formulations are within the acceptable limits of ±15% of theoretical concentrations. The formulations are also within the acceptable limits of ≤5% RSD for homogeneity except for the 20 mg/mL samples which were out of specification (6.9% RSD).

ACTIONS TAKEN: Notified Study Director of results.

DOSE FORMULATION ANALYSIS REPORT

Sponsor: Battelle
Study Facility: Charles River Laboratories Preclinical Services, Pennsylvania
Protocol Number: RTP00004
Analyte: Linuron
Analytical Facility: Charles River Laboratories Preclinical Services, Massachusetts
Batch ID: RTP00004AA-1-053-1
Sampling Criteria: Start of Study Homogeneity and Concentration Analysis
Vehicle: 0.25% Methylcellulose
Storage Conditions: 5±3°C
Laboratory Method: LM # LINR00 Revision 00
Analysis Date: October 19, 2005

RESULTS: (Concentrations in mg/mL, ND = None Detected)

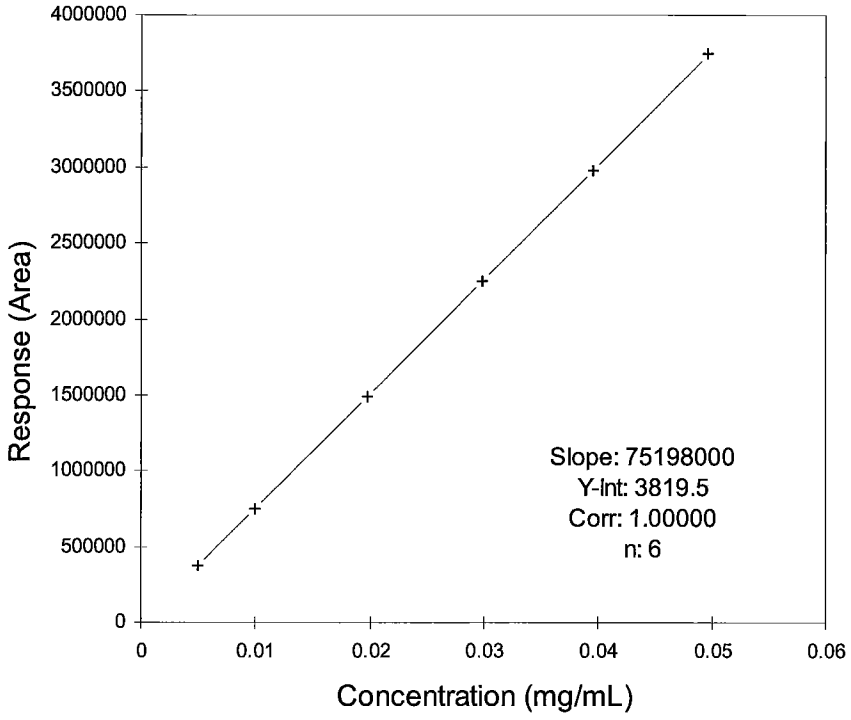
CALIBRATION STANDARDS

Standard Description	Nominal Conc.	Response Area	Calculated Conc.	% Bias	"X" = Exclude	Criteria Limit	Standard Pass/Fail
Cal Std A1	0.004950	375722	0.004946	-0.1%		5%	PASS
Cal Std B1	0.009940	753222	0.009966	+0.3%		5%	PASS
Cal Std A2	0.01980	1491205	0.01978	-0.1%		5%	PASS
Cal Std B2	0.02982	2245358	0.02981	-0.0%		5%	PASS
Cal Std A3	0.03960	2981651	0.03960	0.0%		5%	PASS
Cal Std B3	0.04970	3741897	0.04971	+0.0%		5%	PASS

CHECK STANDARDS

Standard Description	Nominal Conc.	Response Area	Dilution Factor	Conc. Found	% Bias	Criteria Limit	Standard Pass/Fail
Check Std A3	0.03960	2982515	1	0.03961	+0.0%	5.0%	PASS
Check Std A3	0.03960	2985346	1	0.03965	+0.1%	5.0%	PASS
Check Std A3	0.03960	2987738	1	0.03968	+0.2%	5.0%	PASS
Check Std A3	0.03960	2985694	1	0.03965	+0.1%	5.0%	PASS

Project Number: RTP00004AA
Analysis of Linuron in 0.25% methylcellulose
Batch ID: RTP00004AA-1-053-1



SAMPLES

Sample Description	Prep Date	Nominal Sample Conc.	Replicate	Response Area	Total Dilution Factor	Density Corrected mg/mL	Mean mg/mL Found	% Bias
Group 1 Top	10/18/05	0	A	0	104.0	ND		
			B	0	104.1	ND		
Group 1 Mid	10/18/05	0	A	0	101.0	ND		
			B	0	100.7	ND		
Group 1 Bot	10/18/05	0	A	0	98.32	ND		
			B	0	98.27	ND		
Group 2 Top	10/18/05	10	A	1595963	513.4	10.87	11.08	+10.8%
			B	1662866	511.1	11.28		
Group 2 Mid	10/18/05	10	A	1606060	508.4	10.83	10.74	+7.4%
			B	1714476	467.8	10.64		
Group 2 Bot	10/18/05	10	A	1614015	516.8	11.07	11.10	+11.0%
			B	1656195	506.3	11.13		
Group 3 Top	10/18/05	20	A	3340484	487.2	21.62	21.78	+8.9%
			B	3318620	497.7	21.94		
Group 3 Mid	10/18/05	20	A	3363732	476.8	21.30	21.58	+7.9%
			B	3270021	503.0	21.85		
Group 3 Bot	10/18/05	20	A	3486227	483.9	22.41	22.44	+12.2%
			B	3363216	503.1	22.47		
Group 4 Top	10/18/05	30	A	2768870	984.1	36.18	34.48	+14.9%
			B	2486173	993.0	32.78		
Group 4 Mid	10/18/05	30	A	2547234	964.2	32.61	32.81	+9.4%
			B	2657129	935.4	33.00		
Group 4 Bot	10/18/05	30	A	2285143	1044	31.67	32.31	+7.7%
			B	2527841	981.3	32.94		

HOMOGENEITY

Sample Description	Nominal Sample Conc.	Grand Mean Conc.	RSD	% Error
Group 2	10	10.97	1.8%	9.7%
Group 3	20	21.93	2.1%	9.7%
Group 4	30	33.20	3.4%	10.7%

CONCLUSIONS: Results indicate that the formulations are within the acceptable limits of ±15% of theoretical concentrations. The formulations are also within the acceptable limits of ≤5% RSD for homogeneity.

ACTIONS TAKEN: None.

DOSE FORMULATION ANALYSIS REPORT

Sponsor: Battelle
Study Facility: Charles River Laboratories Preclinical Services, Pennsylvania
Protocol Number: RTP00004
Analyte: Phenobarbital
Analytical Facility: Charles River Laboratories Preclinical Services, Massachusetts
Batch ID: RTP00004AA-1-064-1
Sampling Criteria: Start of Study Concentration and Homogeneity Analysis
Vehicle: 0.25% Methylcellulose
Storage Conditions: 5±3°C
Laboratory Method: LM #PHBT00 Revision 00
Analysis Date: October 19, 2005

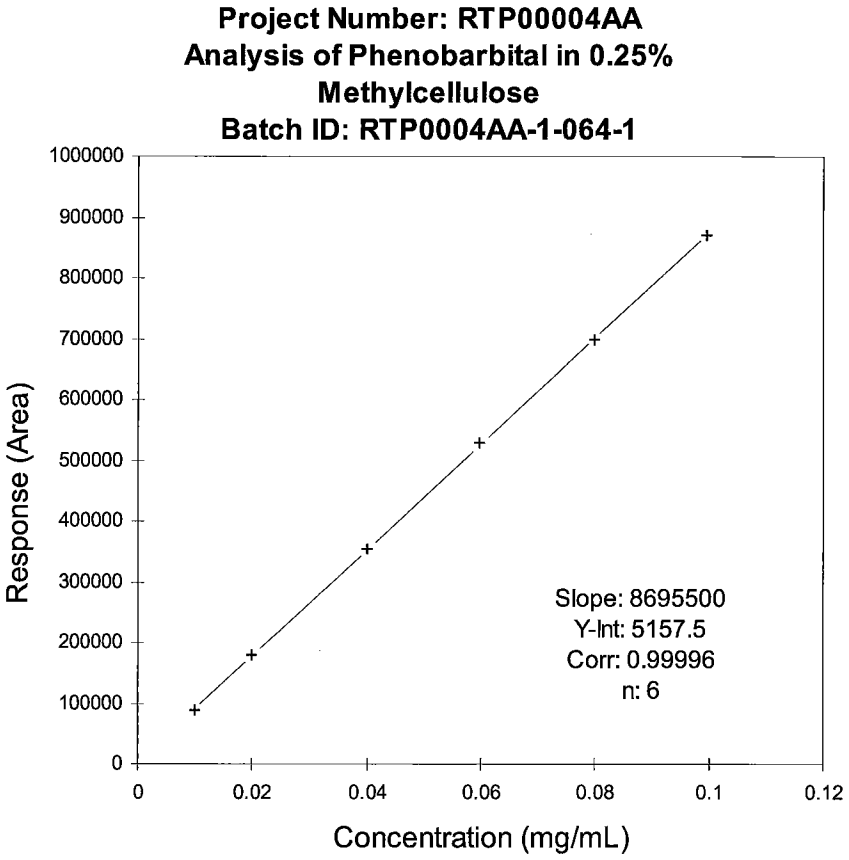
RESULTS: (Concentrations in mg/mL, ND = none detected)

CALIBRATION STANDARDS

Standard Description	Nominal Conc.	Response Area	Calculated Conc.	% Bias	"X" = Exclude	Criteria Limit	Standard Pass/Fail
Cal Std A1	0.009992	88977	0.009639	-3.5%		5%	PASS
Cal Std B1	0.01991	179311	0.02003	+0.6%		5%	PASS
Cal Std A2	0.03997	354095	0.04013	+0.4%		5%	PASS
Cal Std B2	0.05974	528424	0.06018	+0.7%		5%	PASS
Cal Std A3	0.07994	698204	0.07970	-0.3%		5%	PASS
Cal Std B3	0.09956	869811	0.09944	-0.1%		5%	PASS

CHECK STANDARDS

Standard Description	Nominal Conc.	Response Area	Dilution Factor	Conc. Found	% Bias	Criteria Limit	Standard Pass/Fail
Check Std A3	0.07994	698548	1	0.07974	-0.3%	5.0%	PASS
Check Std A3	0.07994	699379	1	0.07984	-0.1%	5.0%	PASS
Check Std A3	0.07994	709797	1	0.08103	+1.4%	5.0%	PASS
Check Std A3	0.07994	709735	1	0.08103	+1.4%	5.0%	PASS


Project Number: RTP00004AA
Analysis of Phenobarbital in 0.25% Methylcellulose
Batch ID: RTP0004AA-1-064-1
Response (Area)
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
0
0.02
0.04
0.06
0.08
0.1
0.12
Concentration (mg/mL)
Slope: 8695500
Y-Int: 5157.5
Corr: 0.99996
n: 6

SAMPLES

Sample Description	Prep Date	Nominal Sample Conc.	Replicate	Response Area	Total Dilution Factor	Density Corrected mg/mL	Mean mg/mL Found	% Bias
Group 1Top	10/18/05	0	A	0	104.0	ND		
			B	0	104.1	ND		
Group 1 Middle	10/18/05	0	A	0	101.0	ND		
			B	0	100.7	ND		
Group 1 Bottom	10/18/05	0	A	0	98.32	ND		
			B	0	98.27	ND		
Group 5 Top	10/18/05	5	A	338605	130.7	5.012	5.221	+4.4%
			B	381449	125.5	5.429		
Group 5 Middle	10/18/05	5	A	326153	127.2	4.697	4.891	-2.2%
			B	346551	129.5	5.084		
Group 5 Bottom	10/18/05	5	A	359010	127.2	5.176	4.996	-0.1%
			B	340267	125.0	4.816		
Group 6 Top	10/18/05	10	A	379977	245.5	10.58	10.73	+7.3%
			B	377524	254.1	10.88		
Group 6 Middle	10/18/05	10	A	385993	249.4	10.92	10.85	+8.5%
			B	372683	255.1	10.78		
Group 6 Bottom	10/18/05	10	A	349562	271.1	10.74	10.71	+7.1%
			B	381724	246.6	10.68		
Group 7 Top	10/18/05	20	A	367092	499.9	20.81	21.03	+5.2%
			B	377068	496.5	21.24		
Group 7 Middle	10/18/05	20	A	365420	499.3	20.68	21.04	+5.2%
			B	365320	516.7	21.40		
Group 7 Bottom	10/18/05	20	A	367932	501.9	20.94	21.08	+5.4%
			B	359641	520.3	21.21		

HOMOGENEITY

Sample Description	Nominal Sample Conc.	Grand Mean Conc.	RSD	% Error
Group 5	5	5.036	3.3%	0.7%
Group 6	10	10.76	0.7%	7.6%
Group 7	20	21.05	0.1%	5.3%

CONCLUSIONS: Results indicate that the formulations are within the acceptable limits of ±15% of theoretical concentrations. The formulations are also within the acceptable limits of ≤5% RSD for homogeneity.

ACTIONS TAKEN: None.

Appendix B. Laboratory Methods

CHARLES RIVER
LABORATORIES
Preclinical Services

LM Number:	LINR00	Revision Number:	00
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Laboratory Method for the Analysis of Linuron in 0.25% Methylcellulose Dose Formulations by HPLC-UV

Prepared By: David H. Brigham 10/17/05
David Brigham, B.S. — **Date**
Senior Laboratory Associate

Reviewed By: Kim Barnard 10/17/05
Kim Barnard, B.S. — **Date**
Associate Scientist

Authorized By 10/17/05
Stephen A. Guyan, M.Sc. — **Date**
Senior Director, Analytical Chemistry Department

LM Number:	LINR00	Revision Number:	00
Effective Date:	October 6, 2005	Page	2 Of 9

1 Purpose

The purpose of this laboratory method is to accurately determine the concentration of linuron in 0.25% methylcellulose dose formulations.

2 Scope

Analysis of linuron in dose formulation samples with limitations as stated below.

Vehicle:0.25% methylcellulose
Sample Volume: 1 mL
Volumetric Samples [ ] Gravimetric Samples [X] Both [ ]

Concentrations Covered by Laboratory Method:
NOTE: Concentrations have not been corrected for purity/salt factor.

Final Injected Concentration - mg/mL

LOD	0.000014
LLOQ to ULOQ	0.005 – 0.05

Corresponding Concentrations - mg/mL in Vehicle

	Standard Dilution (1 in 100)	Additional 1 in 5 Dilution	Additional 1 in 10 Dilution
LOD	0.0014	0.007	0.014
LLOQ to ULOQ	0.5 – 5.0	2.5 – 25	5.0 – 50
Valid Sample Range	0.58 – 4.3	2.9 - 22	5.8 - 43

3 Stability

Description	Concentration Range	Storage Conditions	Time Period
Process Stability	0.005 - 0.05 mg/mL	22 ± 5°C	24 hours

*Standards should be prepared fresh for each analysis until standard stability is established.
Note: all storage conditions are unprotected from light unless specified otherwise.

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4 Definitions/Abbreviations

HPLC:	High Performance Liquid Chromatography
ND:	None detected
N/A:	Not applicable
MP:	Mobile Phase A
LOD:	Limit of Detection
LLOQ:	Lower Limit of Quantitation
ULOQ:	Upper Limit of Quantitation
ACN	Acetonitrile

5 Correction Factors

Purity Refer to protocol

6 Materials

6.1 Chemicals

Deionized Water, Millipore, Milli-Q water, or equivalent
Acetonitrile, HPLC grade or equivalent
Methylcellulose, viscosity 4,000 cp

6.2 Supplies

Volumetric flasks and pipets
Autosampler Vials; Sun SRI Catalog # 200250 (screwtop); or equivalent
Autosampler Vial Caps; Sun SRI Catalog # 500062 (PTFE/Silicone Septa Screwcaps); or equivalent

7 Procedure

7.1 Preparation of Reagents

Other volumes may be prepared using the same proportions. Store all reagents at room temperature and use within 14 days unless noted otherwise.

7.1.1 Mobile Phase, Mobile Phase, 60:40 ACN: Milli-Q water, v:v

Combine 600 mL ACN and 400 mL milli-Q water in a suitable container, mix thoroughly.

7.1.2 Needle Rinse, 60:40 ACN: Milli-Q water, v:v

Combine 600mL ACN and 400mL milli-Q water in a suitable container, mix thoroughly.

7.1.3 Diluent 1, 60:40 ACN: Milli-Q water, v:v

Combine 600 mL ACN and 400 mL milli-Q water in a suitable container, mix thoroughly.

7.1.4 Vehicle, 0.25% methylcellulose in milli-Q water

Into approximately 80 mL of milli-Q water add 250mg of methylcellulose. Stir until all methylcellulose is completely dissolved. After methylcellulose is completely dissolved quantitatively transfer to a 100mL volumetric flask and bring to volume with milli-Q water. Transfer to a suitable container.

7.1.5 Diluent 2, 1% Vehicle in Diluent 1

Into a 100mL volumetric flask add 1mL of vehicle and bring to volume with diluent 1. Stir well, transfer to a suitable container.

7.2 Preparation of Stocks, Standards and Blanks

Stocks, standards and blanks should be stored at 5±3°C.

7.2.1 Preparation of stocks

	Linuron weight (mg)*	Volumetric Flask (mL)	Diluent
Stock A	25 ± 1.3	50	Diluent 1
Stock B	50 ± 5	50	Diluent 1

* Record weights to the nearest 0.01 mg.

7.2.2 Preparation of standards

Calibration Standards	Aliquot from Stock A (mL)	Aliquot from Stock B (mL)	Vehicle (mL)	Volumetric Flask (mL)	Diluent
A1, A2 and A3	1, 4 and 8	N/A	1	100	Diluent 1
B1, B2 and B3	N/A	1, 3 and 5	1	100	Diluent 1

7.2.3 Preparation of Blank

	Vehicle (mL)	Volumetric Flask (mL)	Diluent
Blank	1	100	Diluent 1

7.3 Sample Preparation

Store diluted samples at 5±3°C.

7.3.1 Weigh sample vials using a balance capable of reading at least 0.001 g. Transfer each sample into individual volumetric flasks as indicated in the initial dilution table below. Triple rinse the sample vial contents with diluent 1 into the appropriate volumetric flask. Bring the volumetric flask to volume with diluent 1 and mix well. The initial dilutions may be diluted further as indicated in the tables below. Transfer an aliquot of each final dilution into individual autosampler vials. Allow sample vials to dry completely and reweigh the vials.

Initial Dilution			
Sample Concentration Ranges (mg/mL)	Sample Size (mL)	Initial Dilution Volumetric Flask Size (mL)	Diluent (Triple rinse sample vial)
0, and 0.58 to 43	1	100	diluent 1

Final Dilution			
Sample Concentration Ranges (mg/mL)	Aliquot from Initial Dilution (mL)	Final Dilution Volumetric Flask Size (mL)	Diluent
From 2.9 to 22	1	5	diluent 2
From 5.8 to 43	1	10	diluent 2

7.4 Analytical Run Sequence and Composition

7.4.1 The typical run list should follow this order

2 system checks	test injections
5 replicate injections	system suitability (B3 standard)
1 injection each	six point calibration curve
1 injection	blank
≤ 10 injections	unknown samples
1 injection	check standard (A3)

7.4.2 Repeat last two lines as necessary if more then 10 samples are analyzed. A single replicate of the check standard is analyzed after the last unknown sample in the entire analysis batch.

7.5 Analytical Conditions

Use the HPLC system described below, adjusting the solvent ratio if necessary, to approximate the retention time listed below. Refer to the SOP for Chromatographic System Suitability.

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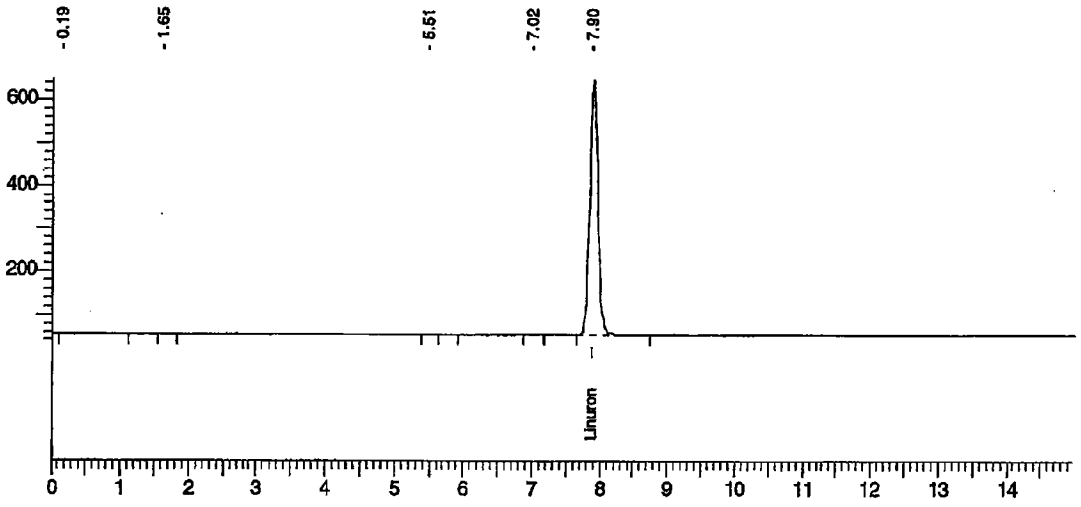
7.5.1 Instrumental

Pump:	PerkinElmer Series 200 or equivalent
Autosampler:	PerkinElmer Series 200 or equivalent
Detector:	PerkinElmer Series 200 or equivalent
Column Heater:	Perkin Elmer, Peltier Column Oven Series 200 or equivalent
Peltier Tray:	PerkinElmer Series 200 or equivalent
Degasser:	PerkinElmer Series 200 or equivalent
Analytical Column:	Phenomenex Synergi 4μ, Hydro-RP 250 x 4.6mm, 4μm
Column Temperature:	30°C
Autosampler Temp:	Ambient
Detection:	Ultraviolet @ 250nm
Sampling rate:	1 point/second
Injection Volume:	20 μL
Mobile Phase A:	60:40 Acetonitrile: Milli-Q Water
Needle Rinse:	60:40 Acetonitrile: Milli-Q Water
Flow Rate:	1.0 mL/min
Run Time:	15 minutes
Retention Time for Linuron:	7.9 ± 1.0 minutes
Run Type:	Isocratic

7.5.2 Example Chromatogram for B3 Standard



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7.6 Calculations

7.6.1 Chromatograms will be automatically integrated and visually inspected for an acceptable integration. Manual baselines will be performed when necessary.

7.6.2 Calculate the relative standard deviation (%) of the peak areas, the relative standard deviation (%) of the retention time and the mean tailing factor for five system suitability injections.

7.6.3 Calculate the concentration of the six spiked standards from the actual stock concentration, in terms of milligram of linuron per milliliter.

7.6.4 Compute the unweighted linear regression relating the peak areas of the standards to their respective linuron concentrations, without blank correction.

7.6.5 Compute the correlation coefficient for the standard curve.

7.6.6 Using the peak area of the samples and the regression equation, determine the concentration in mg/ml of linuron. Correct for the dilution factor if necessary.

7.6.7 Concentrations found to be less than the LOD will be reported as <LOD. Concentrations found to be less than the LLOQ but greater than the LOD will be reported as <LLOQ. In cases, such as blank samples, where no peak is observed, the results will be reported as none detected (N.D.).

7.6.8 Calculate mean concentrations for replicate samples. Calculate the percent error from theoretical as: (mean concentration found – theoretical concentration) / theoretical concentration x 100.

7.7 Acceptance Criteria

7.7.1 System Suitability

The linuron peaks in the five system suitability injections must meet the following acceptable limits: The mean tailing factor ≤2.0, the relative standard deviation (%) of the peak areas ≤2.0%, and the relative standard deviation (%) of the retention time ≤2.0%. If the criteria are out of the acceptable limits, make corrections to the HPLC system and repeat the suitability injections.

7.7.2 Correlation Coefficient

The correlation coefficient for the standard curve must not be less than 0.995. If the value does not exceed 0.995, repeat the preparation of the standard curve.

7.7.3 Calibration Standards

The back-calculated concentrations for calibration standards must be within ±5% of their nominal theoretical concentrations. Standards not meeting criteria can be dropped as long as no more than 20% of standards are dropped. The LLOQ or ULOQ will be redefined to the remaining lowest or highest standards if necessary.

7.7.4 Check Standards

The back- calculated concentration for the A3 check standards must be within 5.0% of nominal theoretical concentration.

7.7.5 Replication of Results

Replicate concentrations found for suspension formulations must not vary by more than 15%. Acceptance is defined as: (low value / high value) ≥ 0.85. Results that do not meet this criteria will be reviewed by the project scientist. Reason for acceptance will be documented in the raw data.

7.7.6 Samples

The mean of the back-calculated concentrations for replicate samples must be within ±15.0% of their nominal concentration.

Refer to the Standard Operating Procedure for "Resolution and Reporting of Out of Specification Dose Formulation Analysis Results" if the percent error is greater than ±15.0%.

8 Revision History

8.1 Initial Laboratory Method: Method validation performed under project RTP00004AA.

CHARLES RIVER
LABORATORIES
Preclinical Services

LM Number:	PHBT00	Revision Number:	00
Effective Date:	10/5/2005	Page	1 Of 9

Laboratory Method for the Analysis of Phenobarbital in 0.25% Methylcellulose Dose Formulations by HPLC-UV

Prepared By: David H. Brigham 10/17/05
David Brigham, B.S. **Date**
Senior Laboratory Associate

Reviewed By: Kim Barnard 10/17/05
Kim Barnard, B.S. **Date**
Associate Scientist

Authorized By 10/17/05
Stephen A. Guyan, M.Sc. **Date**
Senior Director, Analytical Chemistry Department

LM Number:	PHBT00	Revision Number:	00
Effective Date:	10/5/2005	Page	2 Of 9

1 Purpose

The purpose of this laboratory method is to accurately determine the concentration of phenobarbital in 0.25% methylcellulose dose formulations.

2 Scope

Analysis of phenobarbital in dose formulation samples with limitations as stated below.

Vehicle: 0.25% methylcellulose
Sample Volume (or Amount): 1 mL
Volumetric Samples [ ] Gravimetric Samples [X] Both [ ]

Concentrations Covered by Laboratory Method:

Final Injected Concentration - mg/mL

LOD	0.000099
LLOQ to ULOQ	0.01 – 0.1

Corresponding Concentrations - mg/mL in Vehicle

	Standard Dilution (1 in 25)	Additional 1 in 5 Dilution	Additional 1 in 10 Dilution	Additional 1 in 20 Dilution
LOD	0.0025	0.012	0.025	0.050
LLOQ to ULOQ	0.25 - 2.5	1.25 - 12.5	2.5 - 25	5.0 - 50
Valid Sample Range	0.29 - 2.1	1.4 - 10.6	2.9 - 21	5.8-43

3 Stability

Description	Concentration Range	Storage Conditions	Time Period
Process Stability	0.01 - 0.1 mg/mL	22 ± 5°C	TBD

*Standards should be prepared fresh for each analysis until standard stability is established.
Note: all storage conditions are unprotected from light unless specified otherwise.

LM Number:	PHBT00	Revision Number:	00
Effective Date:	10/5/2005	Page	3 Of 9

4 Definitions/Abbreviations

HPLC:	High Performance Liquid Chromatography
ND:	None detected
N/A:	Not applicable
MP:	Mobile Phase A
LOD:	Limit of Detection
LLOQ:	Lower Limit of Quantitation
ULOQ:	Upper Limit of Quantitation
ACN:	Acetonitrile

5 Correction Factors

Purity/Salt Factor: Refer to protocol.

6 Materials

6.1 Chemicals

Deionized Water, Millipore, Milli-Q water, or equivalent
Acetonitrile, HPLC grade or equivalent
Methylcellulose, viscosity 4,000 cp

6.2 Supplies

Volumetric flasks and pipets
Autosampler Vials; Sun SRI Catalog # 200250 (screwtop); or equivalent
Autosampler Vial Caps; Sun SRI Catalog # 500062 (PTFE/Silicone Septa Screwcaps); or equivalent

7 Procedure

7.1 Preparation of Reagents

Other volumes may be prepared using the same proportions. Store all reagents at room temperature and use within 14 days unless noted otherwise.

7.1.1 Mobile Phase, 50:50 ACN: Milli-Q water v:v

Combine 1000 mL ACN and 1000 mL milli-Q water in a suitable container, mix thoroughly.

7.1.2 Needle Rinse, 50:50 ACN: Milli-Q water v:v

Combine 500 mL ACN and 500 mL milli-Q water in a suitable container, mix thoroughly.

7.1.3 Diluent 1, 50:50 ACN: Milli-Q water v:v

Combine 1000mL ACN and 1000 mL milli-Q water in a suitable container,mix thoroughly.

7.1.4 Vehicle, 0.25% methylcellulose in milli-Q water

Into approximately 80 mL of milli-Q water add 250 mg of methylcellulose. Stir until all methylcellulose is completely dissolved. After methylcellulose is completely dissolved quantitatively transfer to a 100 mL volumetric flask and bring to volume with milli-Q water. Transfer to a suitable container.

7.1.5 Diluent 2, 4% Vehicle in Diluent 1

Into a 100mL volumetric flask add 4 mL of vehicle and bring to volume with diluent 1. Stir well, transfer to a suitable container.

7.2 Preparation of Stocks, Standards and Blanks

Stocks, standards and blanks should be stored at 5±3°C.

7.2.1 Preparation of stocks

	Phenobarbital weight (mg)*	Volumetric Flask (mL)	Diluent
Stock A	25 ± 1.3	100	Diluent 1
Stock B	25 ± 1.3	50	Diluent 1

* Record weights to the nearest 0.01 mg.

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Effective Date:	10/5/2005	Page	5 Of 9

7.2.2 Preparation of standards

Calibration Standards	Aliquot from Stock A (mL)	Aliquot from Stock B (mL)	Vehicle (mL)	Volumetric Flask (mL)	Diluent
A1, A2 and A3	1, 4 and 8	N/A	1	25	Diluent 1
B1, B2 and B3	N/A	1, 3 and 5	1	25	Diluent 1

7.2.3 Preparation of Blank

	Vehicle (mL)	Volumetric Flask (mL)	Diluent
Blank	1	25	Diluent 1

7.3 Sample Preparation

Store diluted samples at 5±3°C.

7.3.1 Weigh sample vials using a balance capable of reading at least 0.001 g. Transfer each sample into individual volumetric flasks as indicated in the initial dilution table below. Triple rinse the sample vial contents with diluent 1 into the appropriate volumetric flask. Bring the volumetric flask to volume with diluent 1 and mix well. The initial dilutions may be diluted further as indicated in the tables below. Transfer an aliquot of each final dilution into individual autosampler vials. Allow sample vials to dry completely and reweigh the vials.

Initial Dilution			
Sample Concentration Ranges (mg/mL)	Sample Size (mL)	Initial Dilution Volumetric Flask Size (mL)	Diluent (Triple rinse sample vial)
0, and 0.29 to 43	1	25	Diluent 1

Final Dilution			
Sample Concentration Ranges (mg/mL)	Aliquot from Initial Dilution (mL)	Final Dilution Volumetric Flask Size (mL)	Diluent
From 1.4 to 11	1	5	Diluent 2
From 2.9 to 21	1	10	Diluent 2
From 5.8 to 43	1	20	Diluent 2

7.4 Analytical Run Sequence and Composition

7.4.1 The typical run list should follow this order

2 system checks	test injections
5 replicate injections	system suitability (B3 standard)
1 injection each	six point calibration curve
1 injection	blank
≤ 10 injections	unknown samples
1 injection	check standard (A3)

7.4.2 Repeat last two lines as necessary if more then 10 samples are analyzed. A single replicate of the check standard is analyzed after the last unknown sample in the entire analysis batch.

7.5 Analytical Conditions

Use the HPLC system described below, adjusting the solvent ratio if necessary, to approximate the retention time listed below. Refer to the SOP for Chromatographic System Suitability.

LM Number:	PHBT00	Revision Number:	00
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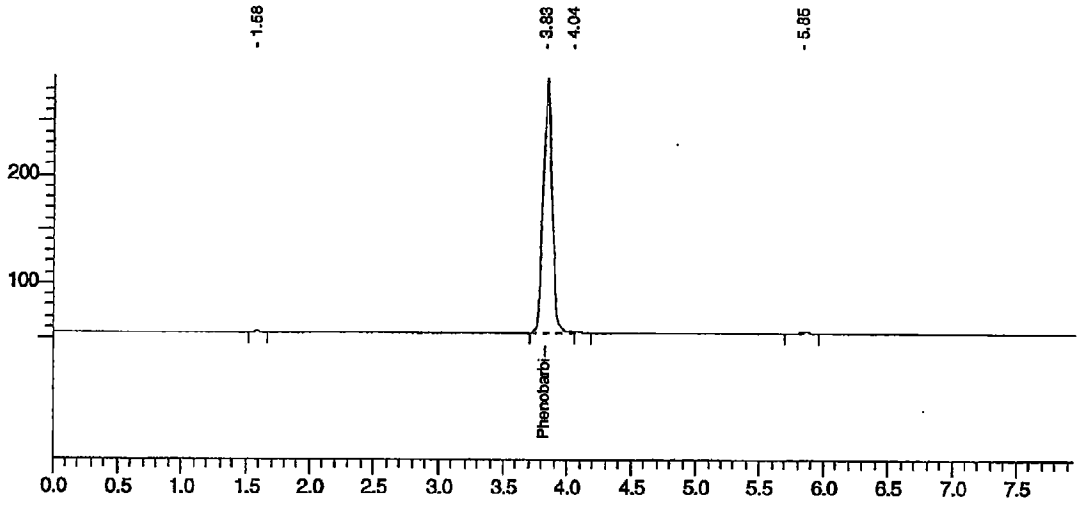
7.5.1 Instrumental

Pump:	PerkinElmer Series 200 or equivalent
Autosampler:	PerkinElmer Series 200 or equivalent
Detector:	PerkinElmer Series 200 or equivalent
Column Heater:	Perkin Elmer, Peltier Column Oven Series 200 or equivalent
Peltier Tray:	PerkinElmer Series 200 or equivalent
Degasser:	PerkinElmer Series 200 or equivalent
Analytical Column:	Phenomenex Synergi 4µ, Hydro-RP 250 x 4.6mm, 4µm
Column Temperature:	30°C
Autosampler Temp:	Ambient
Detection:	Ultraviolet @ 225nm
Sampling rate:	1 point/second
Injection Volume:	10 µL
Mobile Phase A:	50:50 Acetonitrile: Milli-Q Water (v:v)
Needle Rinse:	50:50 Acetonitrile: Milli-Q Water (v:v)
Flow Rate:	1.0 mL/min
Run Time:	8 minutes
Retention Time for Phenobarbital:	3.8 ± 1.0 minutes
Run Type:	Isocratic

7.5.2 Example Chromatogram for B3 Standard.



LM Number:	PHBT00	Revision Number:	00
Effective Date:	10/5/2005	Page	8 Of 9

7.6 Calculations

7.6.1 Chromatograms will be automatically integrated and visually inspected for an acceptable integration. Manual baselines will be performed when necessary.

7.6.2 Calculate the relative standard deviation (%) of the peak areas, the relative standard deviation (%) of the retention time and the mean tailing factor for five system suitability injections.

7.6.3 Calculate the concentration of the six spiked standards from the actual stock concentration, in terms of milligram of phenobarbital per milliliter.

7.6.4 Compute the unweighted linear regression relating the peak areas of the standards to their respective phenobarbital concentrations, without blank correction.

7.6.5 Compute the correlation coefficient for the standard curve.

7.6.6 Using the peak area of the samples and the regression equation, determine the concentration in mg/ml of phenobarbital. Correct for the dilution factor if necessary.

7.6.7 Concentrations found to be less than the LOD will be reported as <LOD. Concentrations found to be less than the LLOQ but greater than the LOD will be reported as <LLOQ. In cases, such as blank samples, where no peak is observed, the results will be reported as none detected (N.D.).

7.6.8 Calculate mean concentrations for replicate samples. Calculate the percent error from theoretical as: (mean concentration found – theoretical concentration) / theoretical concentration x 100.

7.7 Acceptance Criteria

7.7.1 System Suitability

The phenobarbital peaks in the five system suitability injections must meet the following acceptable limits: The mean tailing factor ≤2.0, the relative standard deviation (%) of the peak areas ≤2.0%, and the relative standard deviation (%) of the retention time ≤2.0%. If the criteria are out of the acceptable limits, make corrections to the HPLC system and repeat the suitability injections.

7.7.2 Correlation Coefficient

The correlation coefficient for the standard curve must not be less than 0.995. If the value does not exceed 0.995, repeat the preparation of the standard curve.

7.7.3 Calibration Standards

The back-calculated concentrations for calibration standards must be within ±5% of their nominal theoretical concentrations. Standards not meeting criteria can be dropped as long as no more than 20% of standards are dropped. The LLOQ or ULOQ will be redefined to the remaining lowest or highest standards if necessary.

7.7.4 Check Standards

The back- calculated concentration for the A3 check standards must be within 5.0% of nominal theoretical concentration.

7.7.5 Replication of Results

Replicate concentrations found for suspension formulations must not vary by more than 15%. Acceptance is defined as: (low value / high value) ≥ 0.85. Results that do not meet this criterion will be reviewed by the project scientist. Reason for acceptance will be documented in the raw data.

7.7.6 Samples

The mean of the back-calculated concentrations for replicate samples must be within ±15.0% of their nominal concentration.

Refer to the Standard Operating Procedure for "Resolution and Reporting of Out of Specification Dose Formulation Analysis Results" if the percent error is greater than ±15.0%.

8 Revision History

8.1 Initial Laboratory Method: Method validation performed under project RTP00004AA.

APPENDIX 6 - ENVIRONMENTAL AND HUSBANDRY REPORTS

TEMPERATURE AND RELATIVE HUMIDITY REPORTS

ARGUS

Temperature and Relative Humidity Report
Location: Room 35-37

Protocol Number: RTP00004

Range of Dates: 18-Oct-2005 14:01 to 10-Nov-2005 07:59

	Temperature		Relative Humidity	
Target Range:	64°F to 79°F		30% to 70%	
Species: Rat				
Total Number of Days:	24		24	
Total Number of Hours:	545.5		545.5	
Total Number of Data Points:	546		546	
Mean (± SD):	73.7	(± 0.5)	46.5	(± 1.4)
Maximum:	75.2		51.2	
Median:	73.6		46.5	
Minimum:	72.5		42.8	
Number of Points in Range (%):	546	(100.0)	546	(100.0)
Number of Points High (%):	0	(0.0)	0	(0.0)
Number of Points Low (%):	0	(0.0)	0	(0.0)

Report Generated: 28-Nov-2005 at 14:14

COMMENTS: _____

REVIEWED BY: _____ **DATE:** 11-28-05

FEED ANALYSES

N·PAL
N·P ANALYTICAL LABORATORIES

A Nestlé Purina PetCare Company
Checkerboard Square • St. Louis, MO 63164

ANALYSIS REPORT

Results are preliminary pending final approval.

To: CHARLES BENTON
HARLAN TEKLAD
2826 LATHAM DRIVE
MADISON, WI 53713
Fax: (608)277-2066

CC:

CODE
L0527403

Sample No.: L0527403-1 Receipt Date: 12/14/2005
Report Date: 12/15/2005

2018CM-091905MA

Test Code	Assay / Analyte	Result	Units	Low Lmt	Hi Lmt
IFSP	Isoflavone profile, saponification				
	Daidzin	192	ppm		
	Daidzein	3.00	ppm		
	Total Daidzein Compounds	195	ppm		
	Genistin	182	ppm		
	Genistein	1.00	ppm		
	Total Genistein Compounds	183	ppm		
	Glycitin	38.0	ppm		
	Glycitein	1.00	ppm		
	Total Glycitein Compounds	39.0	ppm		
	Total Isoflavones	417	ppm		
	Daidzin(Aglycone Units)	117	ppm		
	Daidzein(Aglycone Units)	3.00	ppm		
	Total Daidzein(Aglycone Units)	120	ppm		
	Genistin(Aglycone Units)	114	ppm		
	Genistein(Aglycone Units)	1.00	ppm		
	Total Genistein(Aglycone Units)	115	ppm		
	Glycitin(Aglycone Units)	24.0	ppm		
	Glycitein(Aglycone Units)	1.00	ppm		
	Total Glycitein(Aglycone Units)	25.0	ppm		
	Total Isoflavones (Aglycone Equiv)	260	ppm		

Person responsible for report content: Lynn Loudermilk, Director.

The test code located next to each assay is a method reference code. Results apply only to submitted samples. This report shall not be reproduced, except in its entirety, without the written permission of NP Analytical Laboratories.

For additional information, contact Customer Services at 800-423-6832 or 314-982-1310.

CH2005121509300001

The symbol "<" or the words "less than" signifies that no analyte was measured at or above the stated lower limit of quantitation of the procedure under the conditions employed. The use of the symbol "<" or the words "less than" does not imply that traces of the analyte were present. The symbol ">" or the term "greater than" signifies that the analyte was determined to be present in an amount greater than the stated level. Samples submitted to NP Analytical Laboratories for testing are retained for a minimum of thirty (30) days after the analysis report is issued when sample stability is unlimited. Requests for extended storage must be made to NP Analytical Laboratories prior to or at the time of sample submission.

TOTAL P.01

WATER ANALYSES

QC Laboratories

Analytical Report

ACCREDITED IN ACCORDANCE WITH nelac
10/12/05 07:48pm

JOE SCHWINDT
CHARLES RIVER LAB
905 SHEEHY DRIVE
HORSHAM, PA 19044

Regarding:

JOE SCHWINDT
CHARLES RIVER LAB
905 SHEEHY DRIVE
HORSHAM, PA 19044

Account No: W05899, CHARLES RIVER LAB
Project No: W05899, CHARLES RIVER LAB

P.O. No:
PWSID No:

Inv. No: 722468

Sample Number	Sample Description	Samp. Date/Time/Temp	Sampled by
L1757864-1	DRINKING WATER - ANALYTICAL Received Temp: 36°F Iced (Y/N): Y	10/07/05 10:45am NA°F	Joan Cummings Nulty, QC Laborato

Parameter	Method	Result	RLs	Test Date, Time, Analyst
COLIFORM-MF	SM 9222B	<1 col/100ml	1. col/100ml	10/07/05 05:20PM CSW
CHLORINE RESIDUAL	SM 4500-CL-G	ND mg/l	0.10 mg/l	10/07/05 10:45AM JCN

Sample Number	Sample Description	Samp. Date/Time/Temp	Sampled by
L1757864-2	DRINKING WATER - ROOM 17 Received Temp: 36°F Iced (Y/N): Y	10/07/05 10:55am NA°F	Joan Cummings Nulty, QC Laborato

Parameter	Method	Result	RLs	Test Date, Time, Analyst
COLIFORM-MF	SM 9222B	<1 col/100ml	1. col/100ml	10/07/05 05:20PM CSW
CHLORINE RESIDUAL	SM 4500-CL-G	0.90 mg/l	0.10 mg/l	10/07/05 10:55AM JCN

Sample Number	Sample Description	Samp. Date/Time/Temp	Sampled by
L1757864-3	DRINKING WATER - FILL STATION Received Temp: 36°F Iced (Y/N): Y	10/07/05 11:04am NA°F	Joan Cummings Nulty, QC Laborato

Parameter	Method	Result	RLs	Test Date, Time, Analyst
COLIFORM-MF	SM 9222B	<1 col/100ml	1. col/100ml	10/07/05 05:20PM CSW
CHLORINE RESIDUAL	SM 4500-CL-G	1.0 mg/l	0.10 mg/l	10/07/05 11:04AM JCN

Sample Number	Sample Description	Samp. Date/Time/Temp	Sampled by
L1757864-4	DRINKING WATER - G1	10/07/05 11:09am NA°F	Joan Cummings Nulty, QC Laborato

A result of "ND" indicates the concentration of the analyte tested was either not detected or below the RLs.
Definitions: ND=not detected; NEG=negative; POS=positive; COL=colonies; RLs=laboratory reporting limits; L/A=laboratory accident;
TNTC=too numerous to count
A result marked with "DRY" indicates that the result was calculated and reported on a dry weight basis.
All analysis, except field tests are conducted in Southampton, PA unless otherwise identified.
The test"pH lab"is analyzed upon receipt at the laboratory, the result will not be suitable for regulatory purposes.
Actual times of analysis for parameters reported <24 hrs are available upon request. All testing is completed within the required holding time unless otherwise noted..
QC's lab certification ID's are: Southampton (NELAP) PADEP 09-131,NJDEP PA166,Bioassay PA034.NON-NELAP labs: Wind Gap-NJ PA001, Alltest-NJ 02015,Vineland-NJ 06005;PA 68-580.
All samples are collected as "grab" samples unless otherwise identified.
MCL= is the EPA recommended "maximum contaminant level" for a parameter. PLs=customer specific permit limits.

John Bennett 11/8/05

Thomas J. Hines, President

Serial Number: 613293

1205 Industrial Blvd., P.O. Box 514, Southampton, PA 18966-0514 Phone: 215-355-3900 Fax: 215-355-7231 www.qclaboratories.com

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DEF 11/11/05

QC Laboratories

Analytical Report

10/12/05 07:48pm

Account No: W05899, CHARLES RIVER LAB
Project No: W05899, CHARLES RIVER LAB

P.O. No:
PWSID No:

Inv. No: 722468

Received Temp: 36°F Iced (Y/N): Y

Parameter	Method	Result	RLs	Test Date, Time, Analyst
COLIFORM-MF	SM 9222B	<1 col/100ml	1. col/100ml	10/07/05 05:20PM CSW
CHLORINE RESIDUAL	SM 4500-CL-G	0.40 mg/l	0.10 mg/l	10/07/05 11:09AM JCN

Sample Number	Sample Description	Samp. Date/Time/Temp	Sampled by
L1757864-5	DRINKING WATER - FORMULATION Received Temp: 36°F Iced (Y/N): Y	10/07/05 11:16am NA°F	Joan Cummings Nulty, QC Laborato

Parameter	Method	Result	RLs	Test Date, Time, Analyst
COLIFORM-MF	SM 9222B	<1 col/100ml	1. col/100ml	10/07/05 05:20PM CSW
CHLORINE RESIDUAL	SM 4500-CL-G	ND mg/l	0.10 mg/l	10/07/05 11:16AM JCN

Sample Number	Sample Description	Samp. Date/Time/Temp	Sampled by
L1757864-6	DRINKING WATER - N2 Received Temp: 36°F Iced (Y/N): Y	10/07/05 11:35am NA°F	Joan Cummings Nulty, QC Laborato

Parameter	Method	Result	RLs	Test Date, Time, Analyst
COLIFORM-MF	SM 9222B	<1 col/100ml	1. col/100ml	10/07/05 05:20PM CSW
CHLORINE RESIDUAL	SM 4500-CL-G	0.20 mg/l	0.10 mg/l	10/07/05 11:35AM JCN

L1757864-1:
1. A water supply is considered bacteriologically "SAFE" if no Coliform bacteria are detected. To be considered "SAFE" your report should indicate "<1 col/100ml" or "NEG" for the Coliform Test. If your report indicates a positive result "POS" or a value of one (1) or greater then your supply is "UNSAFE FOR DRINKING" contact your local Health Dept. or QC for advice.

L1757864-2:
1. A water supply is considered bacteriologically "SAFE" if no Coliform bacteria are detected. To be considered "SAFE" your report should indicate "<1 col/100ml" or "NEG" for the Coliform Test. If your report indicates a positive result "POS" or a value of one (1) or greater then your supply is "UNSAFE FOR DRINKING" contact your local Health Dept. or QC for advice.

L1757864-3:
1. A water supply is considered bacteriologically "SAFE" if no Coliform bacteria are detected. To be considered "SAFE" your report should indicate "<1 col/100ml" or "NEG" for the Coliform Test. If your report indicates a positive result "POS" or a value of one (1) or greater then your supply is "UNSAFE FOR DRINKING" contact your local Health Dept. or QC for advice.

L1757864-4:

A result of "ND" indicates the concentration of the analyte tested was either not detected or below the RLs.
Definitions: ND=not detected; NEG=negative; POS=positive; COL=colonies; RLs=laboratory reporting limits; L/A=laboratory accident; TNTC=too numerous to count
A result marked with "DRY" indicates that the result was calculated and reported on a dry weight basis.
All analysis, except field tests are conducted in Southampton, PA unless otherwise identified.
The test"pH lab"is analyzed upon receipt at the laboratory, the result will not be suitable for regulatory purposes.
Actual times of analysis for parameters reported <24 hrs are available upon request. All testing is completed within the required holding time unless otherwise noted..
QC's lab certification ID's are: Southampton (NELAP) PADEP 09-131,NJDEP PA166,Bioassay PA034.NON-NELAP Labs: Wind Gap-NJ PA001, Alltest-NJ 02015,Vineland-NJ 06005;PA 68-580.
All samples are collected as "grab" samples unless otherwise identified.
MCL= is the EPA recommended "maximum contaminant level" for a parameter. PLs=customer specific permit limits.

11/8/05

Thomas J. Hines, President

Serial Number: 613293

1205 Industrial Blvd., P.O. Box 514, Southampton, PA 18966-0514 Phone: 215-355-3900 Fax: 215-355-7231 www.qclaboratories.com

EXACT COPY
DEF 11/11/05

QC Laboratories

Analytical Report

10/12/05 07:48pm

Account No: W05899, CHARLES RIVER LAB | P.O. No: | Inv. No: 722468
Project No: W05899, CHARLES RIVER LAB | PWSID No:

1. A water supply is considered bacteriologically "SAFE" if no Coliform bacteria are detected. To be considered "SAFE" your report should indicate "<1 col/100ml" or "NEG" for the Coliform Test. If your report indicates a positive result "POS" or a value of one (1) or greater then your supply is "UNSAFE FOR DRINKING" contact your local Health Dept. or QC for advice.

L1757864-5:
1. A water supply is considered bacteriologically "SAFE" if no Coliform bacteria are detected. To be considered "SAFE" your report should indicate "<1 col/100ml" or "NEG" for the Coliform Test. If your report indicates a positive result "POS" or a value of one (1) or greater then your supply is "UNSAFE FOR DRINKING" contact your local Health Dept. or QC for advice.

L1757864-6:
1. A water supply is considered bacteriologically "SAFE" if no Coliform bacteria are detected. To be considered "SAFE" your report should indicate "<1 col/100ml" or "NEG" for the Coliform Test. If your report indicates a positive result "POS" or a value of one (1) or greater then your supply is "UNSAFE FOR DRINKING" contact your local Health Dept. or QC for advice.

A result of "ND" indicates the concentration of the analyte tested was either not detected or below the RLs.
Definitions: ND=not detected; NEG=negative; POS=positive; COL=colonies; RLs=laboratory reporting limits; L/A=laboratory accident; TNTC=too numerous to count
A result marked with "DRY" indicates that the result was calculated and reported on a dry weight basis.
All analysis, except field tests are conducted in Southampton, PA unless otherwise identified. .
The test"pH lab"is analyzed upon receipt at the laboratory, the result will not be suitable for regulatory purposes.
Actual times of analysis for parameters reported <24 hrs are available upon request. All testing is completed within the required holding time unless otherwise noted..
QC's lab certification ID's are: Southampton (NELAP) PADEP 09-131,NJDEP PA166,Bioassay PA034.NON-NELAP labs: Wind Gap-NJ PA001, Alltest-NJ 02015,Vineland-NJ 06005;PA 68-580.
All samples are collected as "grab" samples unless otherwise identified.
MCL= is the EPA recommended "maximum contaminant level" for a parameter. PLs=customer specific permit limits.

11/8/05

Thomas J. Hines, President

Serial Number: 613293

1205 Industrial Blvd., P.O. Box 514, Southampton, PA 18966-0514 Phone: 215-355-3900 Fax: 215-355-7231 www.qclaboratories.com

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QC Laboratories

Analytical Report

EXACT COPY
DEF 1/17/06

ACCREDITED IN ACCORDANCE WITH nelac
11/11/05 08:05pm

JOE SCHWINDT
CHARLES RIVER LAB
905 SHEEHY DRIVE
HORSHAM, PA 19044

Regarding:

JOE SCHWINDT
CHARLES RIVER LAB
905 SHEEHY DRIVE
HORSHAM, PA 19044

Account No: W05899, CHARLES RIVER LAB
Project No: W05899, CHARLES RIVER LAB

P.O. No:
PWSID No:

Inv. No: 729947

Sample Number	Sample Description	Samp. Date/Time/Temp	Sampled by
L1782812-1	DRINKING WATER-ANALYTICAL Received Temp: 37°F Iced (Y/N): Y	11/04/05 11:04am NA°F	Customer Sampled

Parameter	Method	Result	RLs	Test Date, Time, Analyst
COLIFORM-MF	SM 9222B	<1 col/100ml	1. col/100ml	11/04/05 05:00PM CSW
CHLORINE RESIDUAL	SM 4500-CL-G	ND mg/l	0.10 mg/l	11/04/05 11:04AM CU

Sample Number	Sample Description	Samp. Date/Time/Temp	Sampled by
L1782812-2	DRINKING WATER G-1 Received Temp: 37°F Iced (Y/N): Y	11/04/05 11:08am NA°F	Customer Sampled

Parameter	Method	Result	RLs	Test Date, Time, Analyst
COLIFORM-MF	SM 9222B	<1 col/100ml	1. col/100ml	11/04/05 05:00PM CSW
CHLORINE RESIDUAL	SM 4500-CL-G	0.50 mg/l	0.10 mg/l	11/04/05 11:08AM CU

Sample Number	Sample Description	Samp. Date/Time/Temp	Sampled by
[illegible]2812-3	DRINKING WATER-FORMULATION Received Temp: 37°F Iced (Y/N): Y	11/04/05 11:12am NA°F	Customer Sampled

Parameter	Method	Result	RLs	Test Date, Time, Analyst
COLIFORM-MF	SM 9222B	<1 col/100ml	1. col/100ml	11/04/05 05:00PM CSW
CHLORINE RESIDUAL	SM 4500-CL-G	ND mg/l	0.10 mg/l	11/04/05 11:12AM CU

Sample Number	Sample Description	Samp. Date/Time/Temp	Sampled by
L1782812-4	DRINKING WATER-ROOM 14	11/04/05 11:18am NA°F	Customer Sampled

A result of "ND" indicates the concentration of the analyte tested was either not detected or below the RLs.
Definitions: ND=not detected; NEG=negative; POS=positive; COL=colonies; RLs=laboratory reporting limits; L/A=laboratory accident; TNTC=too numerous to count
A result marked with "DRY" indicates that the result was calculated and reported on a dry weight basis.
All analysis, except field tests are conducted in Southampton, PA unless otherwise identified.
The test"pH lab"is analyzed upon receipt at the laboratory, the result will not be suitable for regulatory purposes.
Actual times of analysis for parameters reported <24 hrs are available upon request. All testing is completed within the required holding time unless otherwise noted..
QC certification ID's: Southampton (NELAP) PADEP 09-131,NJDEP PA166, FL E87954, Bioassay PA034.NON-NELAP labs: Wind Gap-NJ PA001, Alltest-NJ 02015,Vineland-NJ 06005;PA 68-580.
All samples are collected as "grab" samples unless otherwise identified.
NCL= is the EPA recommended "maximum contaminant level" for a parameter. PLs=customer specific permit limits.

Serial Number: 622446

Thomas J. Hines, President

1/5/06

1205 Industrial Blvd., P.O. Box 514, Southampton, PA 18966-0514 Phone: 215-355-3900 Fax: 215-355-7231 www.qclaboratories.com

QC Laboratories

Analytical Report

EXACT COPY
DEF 1/17/06

ACCREDITED IN ACCORDANCE WITH nelac
11/11/05 08:05pm

Account No: W05899, CHARLES RIVER LAB
Project No: W05899, CHARLES RIVER LAB

P.O. No:
PWSID No:

Inv. No: 729947

Received Temp: 37°F Iced (Y/N): Y

Parameter	Method	Result	RLs	Test Date, Time, Analyst
COLIFORM-MF	SM 9222B	<1 col/100ml	1. col/100ml	11/04/05 05:00PM CSW
CHLORINE RESIDUAL	SM 4500-CL-G	0.90 mg/l	0.10 mg/l	11/04/05 11:18AM CU

Sample Number L1782812-5
Sample Description: DRINKING WATER-FILLING STATION
Received Temp: 37°F Iced (Y/N): Y
Samp. Date/Time/Temp: 11/04/05 11:24am NA°F
Sampled by: Customer Sampled

Parameter	Method	Result	RLs	Test Date, Time, Analyst
COLIFORM-MF	SM 9222B	<1 col/100ml	1. col/100ml	11/04/05 05:00PM CSW
CHLORINE RESIDUAL	SM 4500-CL-G	0.90 mg/l	0.10 mg/l	11/04/05 11:24AM CU

Sample Number L1782812-6
Sample Description: DRINKING WATER H-1
Received Temp: 37°F Iced (Y/N): Y
Samp. Date/Time/Temp: 11/04/05 11:42am NA°F
Sampled by: Customer Sampled

Parameter	Method	Result	RLs	Test Date, Time, Analyst
COLIFORM-MF	SM 9222B	<1 col/100ml	1. col/100ml	11/04/05 05:00PM CSW
CHLORINE RESIDUAL	SM 4500-CL-G	0.10 mg/l	0.10 mg/l	11/04/05 11:42AM CU

L1782812-1:
1. A water supply is considered bacteriologically "SAFE" if no Coliform bacteria are detected. To be considered "SAFE" your report should indicate "<1 col/100ml" or "NEG" for the Coliform Test. If your report indicates a positive result "POS" or a value of one (1) or greater then your supply is "UNSAFE FOR DRINKING" contact your local Health Dept. or QC for advice.

L1782812-2:
1. A water supply is considered bacteriologically "SAFE" if no Coliform bacteria are detected. To be considered "SAFE" your report should indicate "<1 col/100ml" or "NEG" for the Coliform Test. If your report indicates a positive result "POS" or a value of one (1) or greater then your supply is "UNSAFE FOR DRINKING" contact your local Health Dept. or QC for advice.

L1782812-3:
1. A water supply is considered bacteriologically "SAFE" if no Coliform bacteria are detected. To be considered "SAFE" your report should indicate "<1 col/100ml" or "NEG" for the Coliform Test. If your report indicates a positive result "POS" or a value of one (1) or greater then your supply is "UNSAFE FOR DRINKING" contact your local Health Dept. or QC for advice.

L1782812-4:

A result of "ND" indicates the concentration of the analyte tested was either not detected or below the RLs.
Definitions: ND=not detected; NEG=negative; POS=positive; COL=colonies; RLs=laboratory reporting limits; L/A=laboratory accident; TNTC=too numerous to count
A result marked with "DRY" indicates that the result was calculated and reported on a dry weight basis.
All analysis, except field tests are conducted in Southampton, PA unless otherwise identified.
The test"pH lab"is analyzed upon receipt at the laboratory, the result will not be suitable for regulatory purposes.
Actual times of analysis for parameters reported <24 hrs are available upon request. All testing is completed within the required holding time unless otherwise noted..
QC certification ID's: Southampton (NELAP) PADEP 09-131,NJDEP PA166, FL E87954, Bioassay PA034.NON-NELAP labs: Wind Gap-NJ PA001, Alltest-NJ 02015,Vineland-NJ 06005;PA 68-580.
All samples are collected as "grab" samples unless otherwise identified.
MCL= is the EPA recommended "maximum contaminant level" for a parameter. PLs=customer specific permit limits.

Serial Number: 622446

Thomas J. Hines, President

1/5/6

1205 Industrial Blvd., P.O. Box 514, Southampton, PA 18966-0514 Phone: 215-355-3900 Fax: 215-355-7231 www.qclaboratories.com

QC Laboratories

Analytical Report

~~Def~~ Def. 1/17/06

ACCREDITED IN ACCORDANCE WITH nelac

11/11/05 08:05pm

Account No: W05899, CHARLES RIVER LAB
Project No: W05899, CHARLES RIVER LAB

P.O. No:
PWSID No:

Inv. No: 729947

1. A water supply is considered bacteriologically "SAFE" if no Coliform bacteria are detected. To be considered "SAFE" your report should indicate "<1 col/100ml" or "NEG" for the Coliform Test. If your report indicates a positive result "POS" or a value of one (1) or greater then your supply is "UNSAFE FOR DRINKING" contact your local Health Dept. or QC for advice.

L1782812-5:
1. A water supply is considered bacteriologically "SAFE" if no Coliform bacteria are detected. To be considered "SAFE" your report should indicate "<1 col/100ml" or "NEG" for the Coliform Test. If your report indicates a positive result "POS" or a value of one (1) or greater then your supply is "UNSAFE FOR DRINKING" contact your local Health Dept. or QC for advice.

L1782812-6:
1. A water supply is considered bacteriologically "SAFE" if no Coliform bacteria are detected. To be considered "SAFE" your report should indicate "<1 col/100ml" or "NEG" for the Coliform Test. If your report indicates a positive result "POS" or a value of one (1) or greater then your supply is "UNSAFE FOR DRINKING" contact your local Health Dept. or QC for advice.

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A result of "ND" indicates the concentration of the analyte tested was either not detected or below the RLs.
Definitions: ND=not detected; NEG=negative; POS=positive; COL=colonies; RLs=laboratory reporting limits; L/A=laboratory accident; TNTC=too numerous to count
A result marked with "DRY" indicates that the result was calculated and reported on a dry weight basis.
All analysis, except field tests are conducted in Southampton, PA unless otherwise identified.
The test"pH lab"is analyzed upon receipt at the laboratory, the result will not be suitable for regulatory purposes.
Actual times of analysis for parameters reported <24 hrs are available upon request. All testing is completed within the required holding time unless otherwise noted..
QC certification ID's: Southampton (NELAP) PADEP 09-131,NJDEP PA166, FL E87954, Bioassay PA034.NON-NELAP labs: Wind Gap-NJ PA001, Alltest-NJ 02015,Vineland-NJ 06005;PA 68-580.
All samples are collected as "grab" samples unless otherwise identified.
MCL= is the EPA recommended "maximum contaminant level" for a parameter. PLs=customer specific permit limits.

Serial Number: 622446

Thomas J. Hines, President

1/5/6

1205 Industrial Blvd., P.O. Box 514, Southampton, PA 18966-0514 Phone: 215-355-3900 Fax: 215-355-7231 www.qclaboratories.com

Analysis Report

Lancaster Laboratories

Lancaster Laboratories Sample No. WW 4552552

Sample #1 905 Analytical Lab Grab Water Sample
Semi-Annual

Collected:06/27/2005 15:14 by EA

Account Number: 02423

Submitted: 06/28/2005 15:00
Reported: 07/18/2005 at 19:49
Discard: 07/26/2005

Charles River Laboratories
57 Union Street
Worcester MA 01608

905AN

CAT No.	Analysis Name	CAS Number	As Received Result	As Received Limit of Quantitation	Units	Dilution Factor
00259	Mercury	7439-97-6	< 0.00020	0.00020	mg/l	1
07035	Arsenic	7440-38-2	< 0.0200	0.0200	mg/l	1
07036	Selenium	7782-49-2	< 0.0200	0.0200	mg/l	1
07046	Barium	7440-39-3	< 0.0050	0.0050	mg/l	1
07049	Cadmium	7440-43-9	< 0.0050	0.0050	mg/l	1
07051	Chromium	7440-47-3	< 0.0150	0.0150	mg/l	1
07055	Lead	7439-92-1	< 0.0200	0.0200	mg/l	1
07066	Silver	7440-22-4	< 0.0050	0.0050	mg/l	1
07072	Zinc	7440-66-6	< 0.0200	0.0200	mg/l	1
00224	Chloride	16887-00-6	< 2.0	2.0	mg/l	5
00226	Ortho-Phosphate as P	7723-14-0	< 0.030	0.030	mg/l	1
00228	Sulfate	14808-79-8	< 5.0	5.0	mg/l	5
00368	Nitrate Nitrogen	14797-55-8	< 0.50	0.50	mg/l	5
01504	Fluoride	16984-48-8	< 0.50	0.50	mg/l	5
01505	Bromide	24959-67-9	< 2.5	2.5	mg/l	5
01506	Nitrite Nitrogen	14797-65-0	< 0.50	0.50	mg/l	5
00178	Pesticides/PCB's in Water					
00453	Gamma BHC - Lindane	58-89-9	< 0.0096	0.0096	ug/l	1
00454	Heptachlor	76-44-8	< 0.0096	0.0096	ug/l	1
00455	Aldrin	309-00-2	< 0.0096	0.0096	ug/l	1
00469	Dieldrin	60-57-1	< 0.019	0.019	ug/l	1
00477	Endrin	72-20-8	< 0.019	0.019	ug/l	1
00478	p,p-DDT	50-29-3	< 0.024	0.024	ug/l	1
00638	Endrin Aldehyde	7421-93-4	< 0.096	0.096	ug/l	1
01902	Alpha BHC	319-84-6	< 0.0096	0.0096	ug/l	1
01903	Beta BHC	319-85-7	< 0.038	0.038	ug/l	1
01904	Delta BHC	319-86-8	< 0.0096	0.0096	ug/l	1
01905	Heptachlor Epoxide	1024-57-3	< 0.0096	0.0096	ug/l	1
01906	p,p-DDE	72-55-9	< 0.019	0.019	ug/l	1
01907	p,p-DDD	72-54-8	< 0.019	0.019	ug/l	1
01908	Chlordane	57-74-9	< 0.48	0.48	ug/l	1
01909	Toxaphene	8001-35-2	< 0.96	0.96	ug/l	1
01910	Endosulfan I	959-98-8	< 0.0096	0.0096	ug/l	1
01911	Endosulfan II	33213-65-9	< 0.040	0.040	ug/l	1
01912	Endosulfan Sulfate	1031-07-8	< 0.019	0.019	ug/l	1
01913	PCB-1016	12674-11-2	< 0.48	0.48	ug/l	1

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2425 New Holland Pike
PO Box 12425
Lancaster, PA 17605-2425
717-656-2300 Fax: 717-656-2681

D. Rhoads 7-21-05

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2216 Rev. 3/10/03

Analysis Report

Lancaster Laboratories

Lancaster Laboratories Sample No. WW 4552552

Sample #1 905 Analytical Lab Grab Water Sample
Semi-Annual

Collected:06/27/2005 15:14 by EA

Account Number: 02423

Submitted: 06/28/2005 15:00
Reported: 07/18/2005 at 19:49
Discard: 07/26/2005

Charles River Laboratories
57 Union Street
Worcester MA 01608

905AN

CAT No.	Analysis Name	CAS Number	As Received Result	As Received Limit of Quantitation	Units	Dilution Factor
01914	PCB-1221	11104-28-2	< 1.2	1.2	ug/l	1
01915	PCB-1232	11141-16-5	< 0.87	0.87	ug/l	1
01916	PCB-1242	53469-21-9	< 0.48	0.48	ug/l	1
01917	PCB-1248	12672-29-6	< 0.48	0.48	ug/l	1
01918	PCB-1254	11097-69-1	< 0.48	0.48	ug/l	1
01919	PCB-1260	11096-82-5	< 0.48	0.48	ug/l	1

The surrogate data is outside the QC limits for the LCSD. Results from the reextraction are within the limits. The hold time had expired prior to the reextraction so all results are reported from the original extract. Similar results were obtained in both extracts.

01856 Herbicides in Water

01857	2,4-D	94-75-7	< 0.50	0.50	ug/l	1
01858	2,4,5-TP	93-72-1	< 0.050	0.050	ug/l	1
05286	2,4,5-T	93-76-5	< 0.050	0.050	ug/l	1
05287	Dalapon	75-99-0	< 1.2	1.2	ug/l	1
05288	Dinoseb	88-85-7	< 0.50	0.50	ug/l	1
05289	Dicamba	1918-00-9	< 0.30	0.30	ug/l	1
05290	MCPP	93-65-2	< 200.	200.	ug/l	1
05291	MCPA	94-74-6	< 990.	990.	ug/l	1
05292	2,4-DP (Dichlorprop)	120-36-5	< 0.50	0.50	ug/l	1
05293	2,4-DB	94-82-6	< 0.99	0.99	ug/l	1
08103	Pentachlorophenol	87-86-5	< 0.050	0.050	ug/l	1

The LCS recovery for MCPA is outside the QC limits. There is no more sample to do a reextraction. The client was notified and approved reporting this data.

Commonwealth of Pennsylvania Lab Certification No. 36-037

Laboratory Chronicle

CAT No.	Analysis Name	Method	Trial#	Analysis Date and Time	Analyst	Dilution Factor
00259	Mercury	SW-846 7470A	1	07/06/2005 07:40	Damary Valentin	1
07035	Arsenic	SW-846 6010B	1	07/05/2005 15:18	Deborah A Krady	1
07036	Selenium	SW-846 6010B	1	07/15/2005 16:24	Eric L Eby	1
07046	Barium	SW-846 6010B	1	07/05/2005 15:18	Deborah A Krady	1

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D. Krady 7-21-05

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2216 Rev. 3/10/03

Analysis Report

Lancaster Laboratories

Lancaster Laboratories Sample No. WW 4552552

Sample #1 905 Analytical Lab Grab Water Sample
Semi-Annual

Collected:06/27/2005 15:14 by EA

Account Number: 02423

Submitted: 06/28/2005 15:00
Reported: 07/18/2005 at 19:49
Discard: 07/26/2005

Charles River Laboratories
57 Union Street
Worcester MA 01608

905AN

07049	Cadmium	SW-846 6010B	1	07/05/2005 15:18	Deborah A Krady	1
07051	Chromium	SW-846 6010B	1	07/05/2005 15:18	Deborah A Krady	1
07055	Lead	SW-846 6010B	1	07/05/2005 15:18	Deborah A Krady	1
07066	Silver	SW-846 6010B	1	07/05/2005 15:18	Deborah A Krady	1
07072	Zinc	SW-846 6010B	1	07/13/2005 13:41	Joanne M Gates	1
00224	Chloride	EPA 300.0	1	06/29/2005 09:28	Shannon L Phillips	5
00226	Ortho-Phosphate as P	EPA 365.3	1	06/28/2005 21:45	Daniel S Smith	1
00228	Sulfate	EPA 300.0	1	06/29/2005 09:28	Shannon L Phillips	5
00368	Nitrate Nitrogen	EPA 300.0	1	06/29/2005 09:28	Shannon L Phillips	5
01504	Fluoride	EPA 300.0	1	06/29/2005 09:28	Shannon L Phillips	5
01505	Bromide	EPA 300.0	1	06/29/2005 09:28	Shannon L Phillips	5
01506	Nitrite Nitrogen	EPA 300.0	1	06/29/2005 09:28	Shannon L Phillips	5
00178	Pesticides/PCB's in Water	EPA 608	1	07/01/2005 16:27	Richard A Shober	1
01856	Herbicides in Water	SW-846 8151A	1	06/30/2005 17:58	Michele D Hamilton	1
00816	Water Sample Herbicide Extract	SW-846 8151A	1	06/29/2005 20:30	Karen L Beyer	1
00817	Water Sample Pest. Extraction	EPA 608	1	06/30/2005 01:15	David V Hershey Jr	1
01848	WW SW846 ICP Digest (tot rec)	SW-846 3005A	1	07/04/2005 19:20	James L Mertz	1
05713	WW SW846 Hg Digest	SW-846 7470A	1	07/05/2005 20:30	Nelli S Markaryan	1

D. Lehr 7-21-05

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Analysis Report

Lancaster Laboratories

Lancaster Laboratories Sample No. WW 4552553

Sample #2 905 Formulation Lab Grab Water Sample
Semi-Annual

Collected:06/27/2005 15:03 by EA

Account Number: 02423

Submitted: 06/28/2005 15:00
Reported: 07/18/2005 at 19:49
Discard: 07/26/2005

Charles River Laboratories
57 Union Street
Worcester MA 01608

905FR

CAT No.	Analysis Name	CAS Number	As Received Result	As Received Limit of Quantitation	Units	Dilution Factor
00259	Mercury	7439-97-6	< 0.00020	0.00020	mg/l	1
07035	Arsenic	7440-38-2	< 0.0200	0.0200	mg/l	1
07036	Selenium	7782-49-2	< 0.0200	0.0200	mg/l	1
07045	Barium	7440-39-3	< 0.0050	0.0050	mg/l	1
07049	Cadmium	7440-43-9	< 0.0050	0.0050	mg/l	1
07051	Chromium	7440-47-3	< 0.0150	0.0150	mg/l	1
07055	Lead	7439-92-1	< 0.0200	0.0200	mg/l	1
07066	Silver	7440-22-4	< 0.0050	0.0050	mg/l	1
07072	Zinc	7440-66-6	< 0.0200	0.0200	mg/l	1
00224	Chloride	16887-00-6	< 2.0	2.0	mg/l	5
00226	Ortho-Phosphate as P	7723-14-0	< 0.030	0.030	mg/l	1
00228	Sulfate	14808-79-8	< 5.0	5.0	mg/l	5
00368	Nitrate Nitrogen	14797-55-8	< 0.50	0.50	mg/l	5
01504	Fluoride	16984-48-8	< 0.50	0.50	mg/l	5
01505	Bromide	24959-67-9	< 2.5	2.5	mg/l	5
01506	Nitrite Nitrogen	14797-65-0	< 0.50	0.50	mg/l	5
00178	Pesticides/PCB's in Water					
00453	Gamma BHC - Lindane	58-89-9	< 0.0095	0.0095	ug/l	1
00454	Heptachlor	76-44-8	< 0.0095	0.0095	ug/l	1
00455	Aldrin	309-00-2	< 0.0095	0.0095	ug/l	1
00469	Dieldrin	60-57-1	< 0.019	0.019	ug/l	1
00477	Endrin	72-20-8	< 0.019	0.019	ug/l	1
00478	p,p-DDT	50-29-3	< 0.024	0.024	ug/l	1
00638	Endrin Aldehyde	7421-93-4	< 0.095	0.095	ug/l	1
01902	Alpha BHC	319-84-6	< 0.0095	0.0095	ug/l	1
01903	Beta BHC	319-85-7	< 0.038	0.038	ug/l	1
01904	Delta BHC	319-86-8	< 0.0095	0.0095	ug/l	1
01905	Heptachlor Epoxide	1024-57-3	< 0.0095	0.0095	ug/l	1
01906	p,p-DDE	72-55-9	< 0.019	0.019	ug/l	1
01907	p,p-DDD	72-54-8	< 0.019	0.019	ug/l	1
01908	Chlordane	57-74-9	< 0.48	0.48	ug/l	1
01909	Toxaphene	8001-35-2	< 0.95	0.95	ug/l	1
01910	Endosulfan I	959-98-8	< 0.0095	0.0095	ug/l	1
01911	Endosulfan II	33213-65-9	< 0.040	0.040	ug/l	1
01912	Endosulfan Sulfate	1031-07-8	< 0.019	0.019	ug/l	1
01913	PCB-1016	12674-11-2	< 0.48	0.48	ug/l	1

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Analysis Report

Lancaster Laboratories

Lancaster Laboratories Sample No. WW 4552553

Sample #2 905 Formulation Lab Grab Water Sample
Semi-Annual

Collected:06/27/2005 15:03 by EA

Account Number: 02423

Submitted: 06/28/2005 15:00
Reported: 07/18/2005 at 19:49
Discard: 07/26/2005

Charles River Laboratories
57 Union Street
Worcester MA 01608

905FR

CAT No.	Analysis Name	CAS Number	As Received Result	As Received Limit of Quantitation	Units	Dilution Factor
01914	PCB-1221	11104-28-2	< 1.1	1.1	ug/l	1
01915	PCB-1232	11141-16-5	< 0.86	0.86	ug/l	1
01916	PCB-1242	53469-21-9	< 0.48	0.48	ug/l	1
01917	PCB-1248	12672-29-6	< 0.48	0.48	ug/l	1
01918	PCB-1254	11097-69-1	< 0.48	0.48	ug/l	1
01919	PCB-1260	11096-82-5	< 0.48	0.48	ug/l	1

The surrogate data is outside the QC limits for the LCSD. Results from the reextraction are within the limits. The hold time had expired prior to the reextraction so all results are reported from the original extract. Similar results were obtained in both extracts.

01856 Herbicides in Water

01857	2,4-D	94-75-7	< 0.50	0.50	ug/l	1
01858	2,4,5-TP	93-72-1	< 0.050	0.050	ug/l	1
05286	2,4,5-T	93-76-5	< 0.050	0.050	ug/l	1
05287	Dalapon	75-99-0	< 1.2	1.2	ug/l	1
05288	Dinoseb	88-85-7	< 0.50	0.50	ug/l	1
05289	Dicamba	1918-00-9	< 0.30	0.30	ug/l	1
05290	MCPP	93-65-2	< 200.	200.	ug/l	1
05291	MCPA	94-74-6	< 990.	990.	ug/l	1
05292	2,4-DP (Dichlorprop)	120-36-5	< 0.50	0.50	ug/l	1
05293	2,4-DB	94-82-6	< 0.99	0.99	ug/l	1
08103	Pentachlorophenol	87-86-5	< 0.050	0.050	ug/l	1

The LCS recovery for MCPA is outside the QC limits. There is no more sample to do a reextraction. The client was notified and approved reporting this data.

Commonwealth of Pennsylvania Lab Certification No. 36-037

Laboratory Chronicle

CAT No.	Analysis Name	Method	Trial#	Analysis Date and Time	Analyst	Dilution Factor
00259	Mercury	SW-846 7470A	1	07/06/2005 07:42	Damary Valentin	1
07035	Arsenic	SW-846 6010B	1	07/05/2005 15:23	Deborah A Krady	1
07036	Selenium	SW-846 6010B	1	07/15/2005 16:29	Eric L Eby	1
07046	Barium	SW-846 6010B	1	07/05/2005 15:23	Deborah A Krady	1

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Analysis Report

Lancaster Laboratories

Lancaster Laboratories Sample No. WW 4552553

Sample #2 905 Formulation Lab Grab Water Sample
Semi-Annual

Collected:06/27/2005 15:03 by EA

Account Number: 02423

Submitted: 06/28/2005 15:00
Reported: 07/18/2005 at 19:49
Discard: 07/26/2005

Charles River Laboratories
57 Union Street
Worcester MA 01608

905FR

07049	Cadmium	SW-846 6010B	1	07/05/2005 15:23	Deborah A Krady	1
07051	Chromium	SW-846 6010B	1	07/05/2005 15:23	Deborah A Krady	1
07055	Lead	SW-846 6010B	1	07/05/2005 15:23	Deborah A Krady	1
07066	Silver	SW-846 6010B	1	07/05/2005 15:23	Deborah A Krady	1
07072	Zinc	SW-846 6010B	1	07/13/2005 13:44	Joanne M Gates	1
00224	Chloride	EPA 300.0	1	06/29/2005 09:41	Shannon L Phillips	5
00226	Ortho-Phosphate as P	EPA 365.3	1	06/28/2005 21:45	Daniel S Smith	1
00228	Sulfate	EPA 300.0	1	06/29/2005 09:41	Shannon L Phillips	5
00368	Nitrate Nitrogen	EPA 300.0	1	06/29/2005 09:41	Shannon L Phillips	5
01504	Fluoride	EPA 300.0	1	06/29/2005 09:41	Shannon L Phillips	5
01505	Bromide	EPA 300.0	1	06/29/2005 09:41	Shannon L Phillips	5
01506	Nitrite Nitrogen	EPA 300.0	1	06/29/2005 09:41	Shannon L Phillips	5
00178	Pesticides/PCB's in Water	EPA 608	1	07/01/2005 16:46	Richard A Shober	1
01856	Herbicides in Water	SW-846 8151A	1	06/30/2005 18:26	Michele D Hamilton	1
00816	Water Sample Herbicide Extract	SW-846 8151A	1	06/29/2005 20:30	Karen L Beyer	1
00817	Water Sample Pest. Extraction	EPA 608	1	06/30/2005 01:15	David V Hershey Jr	1
01848	WW SW846 ICP Digest (tot rec)	SW-846 3005A	1	07/04/2005 19:20	James L Mertz	1
05713	WW SW846 Hg Digest	SW-846 7470A	1	07/05/2005 20:30	Nelli S Markaryan	1

D. L 7-21-05

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2216 Rev. 3/10/03

Analysis Report

Lancaster Laboratories

Lancaster Laboratories Sample No. WW 4562311

Sample #1 905 Analytical Lab Grab Water Sample (1)
Semi-Annual

Collected:07/12/2005 14:10 by EA

Account Number: 02423

Submitted: 07/13/2005 17:00
Reported: 07/21/2005 at 08:32
Discard: 07/29/2005

Charles River Laboratories
57 Union Street
Worcester MA 01608

1-905

CAT No.	Analysis Name	CAS Number	As Received Result	As Received Limit of Quantitation	Units	Dilution Factor
01856	Herbicides in Water					
01857	2,4-D	94-75-7	< 0.50	0.50	ug/l	1
01858	2,4,5-TP	93-72-1	< 0.050	0.050	ug/l	1
05286	2,4,5-T	93-76-5	< 0.050	0.050	ug/l	1
05287	Dalapon	75-99-0	< 1.2	1.2	ug/l	1
05288	Dinoseb	88-85-7	< 0.50	0.50	ug/l	1
05289	Dicamba	1918-00-9	< 0.30	0.30	ug/l	1
05290	MCPP	93-65-2	< 200.	200.	ug/l	1
05291	MCPA	94-74-6	< 990.	990.	ug/l	1
05292	2,4-DP (Dichlorprop)	120-36-5	< 0.50	0.50	ug/l	1
05293	2,4-DB	94-82-6	< 0.99	0.99	ug/l	1
08103	Pentachlorophenol	87-86-5	< 0.050	0.050	ug/l	1

Commonwealth of Pennsylvania Lab Certification No. 36-037

Laboratory Chronicle

CAT No.	Analysis Name	Method	Trial#	Analysis Date and Time	Analyst	Dilution Factor
01856	Herbicides in Water	SW-846 8151A	1	07/20/2005 02:57	Michele D Hamilton	1
00816	Water Sample Herbicide Extract	SW-846 8151A	1	07/18/2005 05:45	Danette S Blystone	1

8-1-05

(1) Repeat sample (first sampled June 2005) for herbicide analysis only based on Lancaster Laboratories' notification of possible issue with herbicide quality control summary report in June 2005.
8-1-05

MEMBER ACIL
Lancaster Laboratories, Inc.
2425 New Holland Pike
PO Box 12425
Lancaster, PA 17605-2425
717-656-2300 Fax: 717-656-2681

2216 Rev. 3/10/03

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OEE 11/4/05

Analysis Report

Lancaster Laboratories

Lancaster Laboratories Sample No. WW 4562312

Sample #2 905 Formulation Lab Grab Water Sample (1)
Semi-Annual

Collected:07/12/2005 14:15 by EA

Account Number: 02423

Submitted: 07/13/2005 17:00
Reported: 07/21/2005 at 08:32
Discard: 07/29/2005

Charles River Laboratories
57 Union Street
Worcester MA 01608

2-905

CAT No.	Analysis Name	CAS Number	As Received Result	As Received Limit of Quantitation	Units	Dilution Factor
01856	Herbicides in Water					
01857	2,4-D	94-75-7	< 0.49	0.49	ug/l	1
01858	2,4,5-TP	93-72-1	< 0.049	0.049	ug/l	1
05286	2,4,5-T	93-76-5	< 0.049	0.049	ug/l	1
05287	Dalapon	75-99-0	< 1.2	1.2	ug/l	1
05288	Dinoseb	88-85-7	< 0.49	0.49	ug/l	1
05289	Dicamba	1918-00-9	< 0.29	0.29	ug/l	1
05290	MCPP	93-65-2	< 200.	200.	ug/l	1
05291	MCPA	94-74-6	< 980.	980.	ug/l	1
05292	2,4-DP (Dichlorprop)	120-36-5	< 0.49	0.49	ug/l	1
05293	2,4-DB	94-82-6	< 0.98	0.98	ug/l	1
08103	Pentachlorophenol	87-86-5	< 0.049	0.049	ug/l	1

Commonwealth of Pennsylvania Lab Certification No. 36-037

Laboratory Chronicle

CAT No.	Analysis Name	Method	Trial#	Analysis Date and Time	Analyst	Dilution Factor
01856	Herbicides in Water	SW-846 8151A	1	07/20/2005 04:04	Michele D Hamilton	1
00816	Water Sample Herbicide Extract	SW-846 8151A	1	07/18/2005 05:45	Danette S Blystone	1

(1) Repeat sample (first sampled June 2005) for herbicide analysis only based on Lancaster Laboratories' notification of possible issue with herbicide quality control summary in June 2005. DA 8-1-05

8-1-05

MEMBER ACIL
Lancaster Laboratories, Inc.
2425 New Holland Pike
PO Box 12425
Lancaster, PA 17605-2425
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DEF 11/11/05

Lancaster Laboratories
2425 New Holland Pike • Lancaster, PA 17601

Explanation of Symbols and Abbreviations

The following defines common symbols and abbreviations used in reporting technical data:

N.D.	none detected	**BMQL**	Below Minimum Quantitation Level
TNTC	Too Numerous To Count	**MPN**	Most Probable Number
IU	International Units	**CP Units**	cobalt-chloroplatinate units
umhos/cm	micromhos/cm	**NTU**	nephelometric turbidity units
C	degrees Celsius	**F**	degrees Fahrenheit
meq	milliequivalents	**lb.**	pound(s)
g	gram(s)	**kg**	kilogram(s)
ug	microgram(s)	**mg**	milligram(s)
ml	milliliter(s)	**l**	liter(s)
m3	cubic meter(s)	**ul**	microliter(s)

< less than - The number following the sign is the limit of quantitation, the smallest amount of analyte which can be reliably determined using this specific test.

> greater than

J estimated value – The result is ≥ the Method Detection Limit (MDL) and < the Limit of Quantitation (LOQ).

ppm parts per million - One ppm is equivalent to one milligram per kilogram (mg/kg), or one gram per million grams. For aqueous liquids, ppm is usually taken to be equivalent to milligrams per liter (mg/l), because one liter of water has a weight very close to a kilogram. For gases or vapors, one ppm is equivalent to one microliter of gas per liter of gas.

ppb parts per billion

Dry weight basis Results printed under this heading have been adjusted for moisture content. This increases the analyte weight concentration to approximate the value present in a similar sample without moisture. All other results are reported on an as-received basis.

U.S. EPA CLP Data Qualifiers:

Organic Qualifiers

A	TIC is a possible aldol-condensation product
B	Analyte was also detected in the blank
C	Pesticide result confirmed by GC/MS
D	Compound quantitated on a diluted sample
E	Concentration exceeds the calibration range of the instrument
N	Presumptive evidence of a compound (TICs only)
P	Concentration difference between primary and confirmation columns >25%
U	Compound was not detected
X,Y,Z	Defined in case narrative

Inorganic Qualifiers

B	Value is <CRDL, but ≥IDL
E	Estimated due to interference
M	Duplicate injection precision not met
N	Spike sample not within control limits
S	Method of standard additions (MSA) used for calculation
U	Compound was not detected
W	Post digestion spike out of control limits
*****	Duplicate analysis not within control limits
+	Correlation coefficient for MSA <0.995

Analytical test results for methods listed on the laboratories' accreditation scope meet all requirements of NELAC unless otherwise noted under the individual analysis.

Measurement uncertainty values, as applicable, are available upon request.

Tests results relate only to the sample tested. Clients should be aware that a critical step in a chemical or microbiological analysis is the collection of the sample. Unless the sample analyzed is truly representative of the bulk of material involved, the test results will be meaningless. If you have questions regarding the proper techniques of collecting samples, please contact us. We cannot be held responsible for sample integrity, however, unless sampling has been performed by a member of our staff. This report shall not be reproduced except in full, without the written approval of the laboratory.

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APPENDIX 7 - HISTOPATHOLOGY REPORT

RESEARCH PATHOLOGY SERVICES, INC.

438 East Butler Avenue, New Britain, PA 18901
Phone: 215-345-7070 • Fax: 215-345-4326

INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
PROTOCOL NUMBER RTP00004
SPONSOR'S WORK ASSIGNMENT: WA 5-15
HISTOPATHOLOGY REPORT

Experimental Initiation Date: November 14, 2005
Experimental Completion Date: March 27, 2006

SUBMITTED TO:

Joseph W. Lech, B.S., LAT
Charles River Laboratories
Preclinical Services, Pennsylvania
905 Sheehy Drive, Building A
Horsham, PA 19044-1241

SUBMITTED BY:

W. Ray Brown mar. 27, 2006

W. Ray Brown, D.V.M., Ph.D.
Veterinary Pathologist

March 27, 2006

INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
PROTOCOL NUMBER RTP00004
SPONSOR'S WORK ASSIGNMENT: WA 5-15
HISTOPATHOLOGY REPORT

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INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
PROTOCOL NUMBER RTP00004
SPONSOR'S WORK ASSIGNMENT: WA 5-15
HISTOPATHOLOGY REPORT

METHOD:

Microscopic examination was made of sections of the specified tissues from 45 male Crl:CD(SD) rats used in a study to evaluate the responses of the adult male rat assay to two chemicals that have known endocrine activity. A brief outline of the study design is shown below.

Test Substance	Dosage Group	Number of Rats	Chemical Name	Dosage[a] (mg/kg/day)	Concentration (mg/mL)
A	1	15	0.25% Methylcellulose	0 (Vehicle)	0
B	2	15	Linuron	50	10
C	3	15	Linuron	100	20
D	4	15	Linuron	150	30
E	5	15	Phenobarbital	25	5
F	6	15	Phenobarbital	50	10
G	7	15	Phenobarbital	100	20

[a] The test substances were considered 100% active/pure for the purpose of dosage calculations.

The male rats were administered one of the test substances and/or the control substance once daily by gavage for 15 days. The first day of dosage for each replicate will be Test Day 1 (TD 1) of the study. Rats were sacrificed on the day of the last dosage (TD 15), two to three hours after the last dosage. Daily dosages were based on the daily body weight, except on TD 15, which used the previous day's body weight.

The in-life portion of the study, necropsies, and recording of the gross necropsy observations were performed by the staff of Charles River Laboratories, Preclinical Services, Pennsylvania. At necropsy, the specified tissues were collected and retained in 10% neutral buffered formalin with the exception of the testes which were fixed in Bouin's solution for approximately 24 hours before being transferred to and retained in 70% alcohol.. These preserved tissues were submitted to Research Pathology Services, Inc. for tissue processing, microscopic slide preparation and histopathologic evaluation. Samples of the left and right

testis, epididymides and thyroid of the Groups 1, 4, and 7 male rats were routinely processed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for microscopic evaluation.

Upon completion of the project, all raw data (remaining wet tissue, paraffin blocks, microscopic slides and histology records) will be returned to Charles River Laboratories, Preclinical Services, Pennsylvania for archiving.

INTERLABORATORY VALIDATION OF THE 15-DAY ADULT
INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
PROTOCOL NUMBER RTP00004
SPONSOR'S WORK ASSIGNMENT: WA 5-15
HISTOPATHOLOGY REPORT

RESULTS:

The type, incidence and degree of severity of histomorphologic changes in the specified tissues of the male rats of Groups 1, 4 and 7 are presented in Table 1. The microscopic observations in each surviving or nonsurviving rat are listed in tabular form in Table 2. A key to the histomorphologic observations is included on Table 2.

There were no treatment-related microscopic changes observed in the testes, epididymides or thyroid of the rats given the vehicle (0.25% methylcellulose) or 150 mg/kg/day of Linuron (Group 4) or in the testes and epididymides of the rats given 100 mg/kg/day of Phenobarbital (Group 7).

Microscopic examination of the thyroid of the rats given Phenobarbital (Group 7) revealed an increased incidence and severity (minimal to moderate) of hypertrophy and hyperplasia of the thyroid follicular epithelium (Table 1). Histomorphologically, the change in the thyroid was characterized by increased size of the follicular epithelium (hypertrophy) and an increase in the amount of follicles and cellularity of the follicles (hyperplasia). Two control male rats had minimal or mild hypertrophy but this does occasionally occur spontaneously in male rats. The incidence and severity was clearly increased in the rats given Phenobarbital.

All other microscopic changes observed in the tissues specified for examination were considered to be spontaneous in origin and typical of incidental changes seen in male rats of this age and strain. These changes are listed in the attached histomorphology tables.

INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
PROTOCOL NUMBER RTP00004
SPONSOR'S WORK ASSIGNMENT: WA 5-15
HISTOPATHOLOGY REPORT

SUMMARY:

Microscopic examination was made of the testes, epididymides and thyroid from 45 male Crl:CD(SD) rats used in a 15-day adult intact male rat assay with Linuron and Phenobarbital. The rats selected for histomorphologic evaluation were from 15 male rats given 150 mg/kg/day of Linuron and 15 male rats given 100 mg/kg/day of Phenobarbital. Fifteen male rats were given the vehicle (0.25% methylcellulose) alone and served as controls.

No test substance-related microscopic changes were observed in the testes, epididymides or thyroid of the rats given the vehicle alone or 150 mg/kg/day of Linuron or in the testes or epididymides of the rats given 100 mg/kg/day of Phenobarbital.

An increased incidence and severity of hypertrophy and hyperplasia of the thyroid follicular epithelium occurred in the rats given 100 mg/kg/day of Phenobarbital and was considered to be treatment-related.

All other microscopic changes observed in the testes, epididymides and thyroid were considered to have occurred spontaneously and were not treatment-related.

INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
PROTOCOL NUMBER RTP00004
SPONSOR'S WORK ASSIGNMENT: WA 5-15
HISTOPATHOLOGY REPORT

QUALITY ASSURANCE UNIT STATEMENT

All aspects of the tissue processing, microscopic slide preparation, histopathologic evaluation and report preparation for the study listed above have been performed according to the Standard Operating Procedures of Research Pathology Services, Inc. and were audited in accordance with the procedures established by the Quality Assurance Unit of Research Pathology Services, Inc. in compliance with the following Good Laboratory Practice regulations.

U.S. Environmental Protection Agency. Federal Insecticide, Fungicide and Rodenticide Act/Toxic Substances Control Art (FIFRA/TSCA); Good Laboratory Practice Standards; Final Rule. 40 CFR Part 160/Part 792.

Ministry of Agriculture, Forestry and Fisheries, Japan (MAFF). Good laboratory practice (GLP) standards for agricultural chemicals. Agricultural Production Bureau Ref. No. 11-Nousan-No. 6283. October 1, 1991; last revised June 30, 2003 Ref. No. 15-Seisan-2460.

OECD Environmental Directorate. OECD Principles of good laboratory practices [C(97)186/Final] (1998); Environmental Health and Safety Division

Quality Assurance inspections were performed as shown below. There were no deviations from the protocol, Standard Operating Procedures and/or appropriate Good Laboratory Practice regulations noted during the conduct of the study that had any impact on study integrity.

Dates of Inspection	Study Phase	Date Reported to Management	Date Reported to Study Director and Study Director Management
11/16/05	Trimming	12/19/05	12/19/05
11/22/05	Embedding	12/19/05	12/19/05
11/30/05	Microtomy	12/19/05	12/19/05
12/02/05	Staining	12/19/05	12/19/05
12/05/05	Histopathology	12/19/05	12/19/05
12/05/05	Data Entry	12/19/05	12/19/05
12/06/05	Data Verification	12/19/05	12/19/05
12/06/05	Data Processing	12/19/05	12/19/05
12/08/05	Report Preparation	12/19/05	12/19/05
12/19/05	Pre-Submission Audit	12/19/05	12/19/05
12/19/05	Draft Report	12/19/05	12/19/05
03/27/06	Final Report	03/27/06	03/27/06

Karen W Harkins, B.S. 03-27-06

Karen W. Harkins, BS — Date
Quality Assurance Unit

INTERLABORATORY VALIDATION OF THE 15-DAY ADULT
INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
PROTOCOL NUMBER RTP00004
SPONSOR'S WORK ASSIGNMENT: WA 5-15
HISTOPATHOLOGY REPORT

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

All aspects of the above-referenced study performed by Research Pathology Services, Inc. were conducted according to the Good Laboratory Practice regulations listed below:

U.S. Environmental Protection Agency. Federal Insecticide, Fungicide and Rodenticide Act/Toxic Substances Control Art (FIFRA/TSCA); Good Laboratory Practice Standards; Final Rule. 40 CFR Part 160/Part 792.

Ministry of Agriculture, Forestry and Fisheries, Japan (MAFF). Good laboratory practice (GLP) standards for agricultural chemicals. Agricultural Production Bureau Ref. No. 11-Nousan-No. 6283. October 1, 1991; last revised June 30, 2003 Ref. No. 15-Seisan-2460.

OECD Environmental Directorate. OECD Principles of good laboratory practices [C(97)186/Final] (1998); Environmental Health and Safety Division

No deviations were noted that had any significant impact on the validity of the study.

W. Ray Brown 03-27-06

W. Ray Brown, DVM, PhD, DACVP Date
Veterinary Pathologist

INTERLABORATORY VALIDATION OF THE 15-DAY ADULT
INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
PROTOCOL NUMBER RTP00004
SPONSOR'S WORK ASSIGNMENT: WA 5-15
HISTOPATHOLOGY REPORT

Table 1
Incidence and Degree of Severity of Histomorphologic Observations

Test Substance:	A	D	G
Dose Group:	1	4	7
Sex:	M	M	M
Number of Animals/Group:	15	15	15
TESTIS (LEFT):			
NO. EXAMINED	15	15	15
NO. NORMAL	14	15	15
-degeneration, seminiferous tubules, multifocal			
minimal	1	0	0
Total Incidence, All Grades	1	0	0
TESTIS (RIGHT):			
NO. EXAMINED	15	15	15
NO. NORMAL	14	15	14
-degeneration, seminiferous tubules, multifocal			
minimal	1	0	0
Total Incidence, All Grades	1	0	0
-infiltration, mononuclear-cell, tunica, focal			
minimal	0	0	1
Total Incidence, All Grades	0	0	1
EPIDIDYMIDES:			
NO. EXAMINED	15	15	15
NO. NORMAL	5	6	1
-infiltration, mononuclear-cell, focal/multifocal			
minimal	10	9	11
mild	0	0	3
Total Incidence, All Grades	10	9	14
THYROID:			
NO. EXAMINED	15	13	15
NO. NORMAL	13	13	1
Advanced autolysis precludes evaluation	0	0	1
-hypertrophy/hyperplasia, follicular epithelium			
minimal	1	0	3
mild	1	0	5
moderate	0	0	5
Total Incidence, All Grades	2	0	13

INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
PROTOCOL NUMBER RTP00004
SPONSOR'S WORK ASSIGNMENT: WA 5-15
HISTOPATHOLOGY REPORT

Table 2
Histomorphologic Observations

Test Substance:	0.25% Methylcellulose														
Dosage Group:	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Animal Number:	10301	10302	10303	10304	10305	10306	10307	10308	10309	10310	10311	10312	10313	10314	10315
Sex:	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
TESTIS (LEFT):															
-degeneration, seminiferous tubules, multifocal	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
TESTIS (RIGHT):															
-degeneration, seminiferous tubules, multifocal	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
EPIDIDYMIDES:															
-infiltration, mononuclear-cell, focal/multifocal	1	-	-	1	-	1	1	1	-	1	1	-	1	1	1
THYROID:															
-hypertrophy/hyperplasia, follicular epithelium	-	-	-	1	-	-	-	-	-	-	-	-	-	2	-

KEY: - = Not remarkable (within normal limits or indicated change not present)
1 = Minimal degree or amount of indicated change
2 = Mild degree or amount of indicated change
3 = Moderate degree or amount of indicated change
4 = Marked degree or amount of indicated change
* = Tissue not available (wet tissue submitted in vial is not thyroid)

Table 2 (Continued)
Histomorphologic Observations

Test Substance:	Linuron (150 mg/kg/day)														
Dosage Group:	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Animal Number:	10346	10347	10348	103449	10350	10351	10352	10353	10354	10355	10356	10357	10358	10359	10360
Sex:	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
TESTIS (LEFT):	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TESTIS (RIGHT):	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
EPIDIDYMIDES:															
-infiltration, mononuclear-cell, focal/multifocal	-	1	1	1	-	1	-	1	1	-	-	-	1	1	1
THYROID:	-	-	-	*	-	-	-	-	-	-	-	-	-	*	-

KEY: - = Not remarkable (within normal limits or indicated change not present)
1 = Minimal degree or amount of indicated change
2 = Mild degree or amount of indicated change
3 = Moderate degree or amount of indicated change
4 = Marked degree or amount of indicated change
* = Tissue not available (wet tissue submitted in vial is not thyroid)

INTERLABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL
PROTOCOL NUMBER RTP00004
SPONSOR'S WORK ASSIGNMENT: WA 5-15
HISTOPATHOLOGY REPORT

Table 2 (Continued)
Histomorphologic Observations

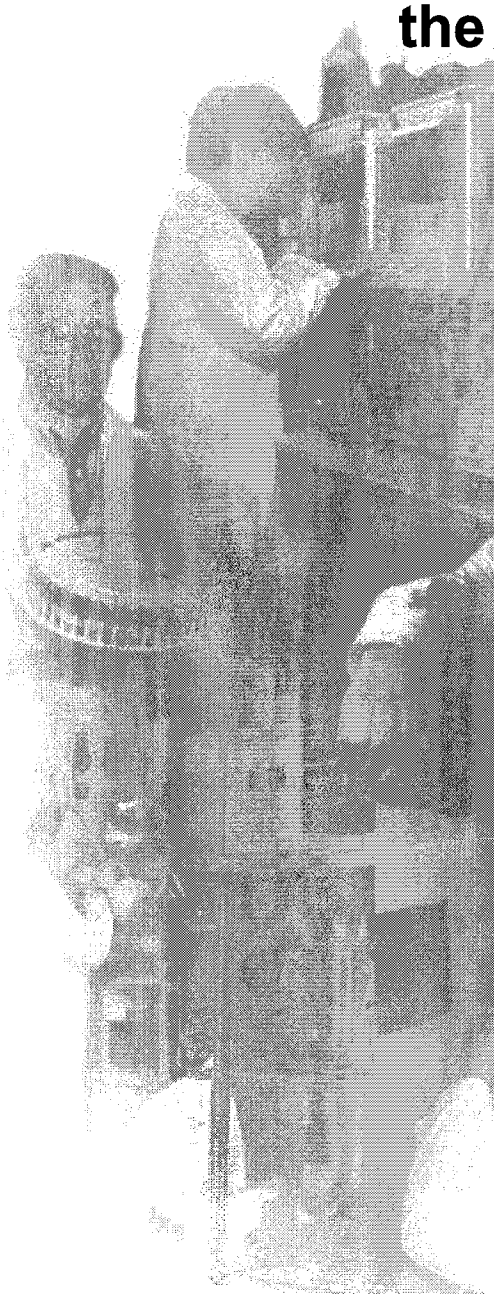
Test Substance:	Phenobarbital (100 mg/kg/day)														
Dosage Group:	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
Animal Number:	10391	10392	10393	10394	10395	10396	10397	10398	10399	10400	10401	10402	10403	10404	10405
Sex:	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
TESTIS (LEFT):	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TESTIS (RIGHT):															
-infiltration, mononuclear-cell, focal, tunica	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
EPIDIDYMIDES:															
-infiltration, mononuclear-cell, focal/multifocal	1	1	1	1	1	-	2	2	1	1	1	2	1	1	1
THYROID:															
-hypertrophy/hyperplasia, follicular epithelium	3	2	3	2	1	A	2	1	3	-	2	3	1	3	2

KEY: - = Not remarkable (within normal limits or indicated change not present)
1 = Minimal degree or amount of indicated change
2 = Mild degree or amount of indicated change
3 = Moderate degree or amount of indicated change
4 = Marked degree or amount of indicated change
* = Tissue not available (wet tissue submitted in vial is not thyroid)

APPENDIX 8 - HORMONE ANALYSES REPORT

RTI Project No. RTI-959-AN

Final Report: Hormone Concentration Determination in Rat Serum Samples for GLP Study (Inter-Laboratory Validation of the 15-Day Intact Male Rat Assay)



Center for Chemistry Services
RTI International
3040 Cornwallis Road
Research Triangle Park, NC 27709

Prepared for:
Joseph Lech, B.S., Scientist, Study Director
Charles River Laboratories
Preclinical Services
905 Sheehy Drive, Building A
Horsham, PA 19044

Dates Samples Collected: 11-08-05 to 11-10-05

Dates Samples Assayed: 11-17-05 to 12-10-05

RTP00004

RTI INTERNATIONAL

Quality Assurance Statement

Study Title: **Hormone Concentration Determination in Rat Serum Samples for GLP Study (Inter-Laboratory Validation of the 15-Day Intact Adult Male Rat Assay)**

Sponsor: **Charles River Laboratories**

Study Code: **An05-959**

Protocol Number: **RTI-959**

This study was audited by the Sciences and Engineering – 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 RTI Task Leader/ study director and Management *
Analytical Project Plan Audit	November 10, 2005	November 10, 2005
Analytical Project Plan Audit Follow-up	November 11, 2005	November 11, 2005
Sample Receipt - Process	November 15, 2005	November 15, 2005
Hormone Analysis – Process	November 28, 2005	November 30, 2005
Data Audit	November 28, 2005	November 28, 2005
Data Audit	November 29-30, 2005	November 30, 2005
Data Audit	December 9 & 12, 2005	December 12, 2005
Data Audit	December 12, 2005	December 12, 2005
Data Audit	December 13, 2005	December 13, 2005
Report Audit	December 19, 2005	December 20, 2005
Report Audit – Follow up	April 20, 2006	April 20, 2006

* The audits and inspection reports listed above were submitted to the external study director at Charles River Laboratories on January 12, 2006. The report audit issued on April 20, 2006 was also submitted to the external study director at Charles River Laboratories on April 20, 2006.

Prepared by:

Michelle Oh
Quality Assurance Specialist

04/26/06
Date

Reviewed by:

Celia Keller
Quality Assurance Assistant Manager

04/26/06
Date

Report Title: Hormone Concentration Determination in Rat Serum Samples for GLP Study (Inter-Laboratory Validation of the 15-Day Intact Male Rat Assay)

Report Prepared and Submitted by:

Carol D. Sloan
Principal Investigator
Manager, Laboratory of Reproductive and Endocrine Toxicology

4-26-06
Date

Approved by:

Brian F. Thomas, Ph.D.
Director, Center for Chemistry Services

4-26-06
Date

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List of Summary Tables

1 INTRODUCTION

One hundred and four rat serum samples with nine aliquots each (when possible) were received in the Laboratory of Reproductive and Endocrine Toxicology on November 15, 2005. The samples were required to have nine hormones determined on each one when enough serum was available. The nine hormones to be determined were Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Testosterone, Dihydrotestosterone (DHT), Prolactin, Estradiol, Thyroxine, Triiodothyronine and Thyroid Stimulating Hormone (TSH). The RTI International Laboratory of Reproductive and Endocrine Toxicology (LORET) personnel thawed out one aliquot for each individual hormone determination, so that each determination would be made on a sample that had been frozen until the time of hormone measurement. Each sample was assayed in duplicate and the average result reported, unless otherwise stated. There were 7 groups of rats with 15 animals per group. Animals were sacrificed on Test Day 15.

2 METHODS

Blood was collected from the trunk of the animal at the time of sacrifice from all animals by Charles River Laboratories staff. Serum was shipped to RTI International and stored between -65°C and -85°C until it was analyzed. The serum samples were assayed by commercially available radioimmunoassay (RIA) kits for testosterone, luteinizing hormone, thyroid stimulating hormone, thyroxine, triiodothyronine, follicle simulating hormone, estradiol, prolactin and dihydrotestosterone. Each sample was run in duplicate and assays included high and low quality control (QC) serum samples. For additional QC samples, the kit-supplied zero standards were spiked with respective hormones at concentrations that were expected to encompass 70% (± 10%) B/B_0, for the low and 30% (± 10%) B/B_0 for the high. The results for all QC samples were used to assess within- and between-assay variability for each laboratory standard. Proteinaceous rat hormones were obtained from the National Hormone and Pituitary Program and the steroids were purchased from commercial suppliers. All assays were counted in a Packard Biosciences Cobra II Series Model 5002 gamma counter using RIASMART software, version 1.0. This work was conducted according to the following regulatory guidelines:

- U.S. Environmental Protection Agency. Federal Insecticide, Fungicide and Rodenticide Act/Toxic Substances Control Act (FIFRA/TSCA); Good Laboratory Practice Standards; Final Rule. 40 CFR Part 160/Part 792.

- Ministry of Agriculture, Forestry and Fisheries, Japan (MAFF). Good laboratory practice (GLP) standards for agricultural chemicals. Agricultural Production Bureau Ref. No. 11-Nousan-No.6283. October 1, 1999; last revised June 30, 2003 Ref. No. 15-Seisan-2460.

- OECD Environmental Directorate. OECD Principles of good laboratory practices [C(97)186/Final] (1998); Environmental Health and Safety Division.

2.1 Estradiol Radioimmunoassay Procedure

The estradiol radioimmunoassay (RIA) used was a no-extraction, double antibody ^{125}I-RIA (Diagnostic Systems Laboratories [DSL], Webster, Texas) which utilized estradiol antibody, ^{125}I-estradiol, estradiol calibrators as the standard curve, and a precipitating solution consisting of goat anti-rabbit gamma globulin combined with dilute polyethylene glycol. Estradiol controls in serum were prepared in the same species/strain/sex as unknown samples by adding known concentrations of estradiol to the appropriate matrix. Estradiol controls prepared in the kit supplied zero calibrator were also assayed. From the control values, the intra-assay coefficient of variation, and percent recovery for the assay was determined (see Table 1 below). All of the samples were analyzed in one assay. The sensitivity of the assay was 0.6 pg/mL as reported by DSL. All samples read within curve range of 1.5 to 150 pg/mL. For the RIA procedure, the sample (200 µL) was pipetted into a glass culture tube and the estradiol antiserum (100 µL) was added. The tubes were vortexed and incubated at 4°C for 4 hours. The ^{125}I-estradiol (100 µL) was added, and the tubes were vortexed and incubated at 4°C for approximately 23 hours. After overnight incubation, cold precipitating solution (1mL) was added and the tubes were vortexed. After a 20 minute incubation, the tubes were centrifuged, the supernatant was decanted and the tubes containing pellets were counted in a gamma counter. Results were reported as pg/mL. The results of the assay are considered reliable based on the assay performance results, some of which are presented in Table 1.

Table 1. Parameters for Estradiol RIAs Used for Male Rat Hormone Determinations

Parameter	Rat Serum Controls[e,f]	Zero Calibrator Controls
Units	(pg/mL)	(pg/mL)
Intra-assay Variation[a]		
Mass added	0/9.2% 7.5/4.3% 25/3.9%	5/7.5% (75.8-79.27%)[d] 33.3/7.1% (31.3-34.1%)[d]
Inter-assay Variation[a]		
No. of assays	1	1
Mass added	N/A	N/A
% recovery of added mass[b]	7.5/112.0% 25/115.3%	5/100.7% 33.3/100.4%
Index of parallelism[c]	N/A	N/A

[a] Numbers are mass added/percentage variation.
[b] Numbers are mass added/percentage recovered (range of all assays).
[c] Index of parallelism = concentration of low volume ÷ concentration of high volume x 100.
[d] Range of % binding in assay.
[e] Male CD rat serum RTI Lot # 136 , Estradiol from kit calibrators
N/A = Not applicable

Table 1A. Estradiol Standard Curve Values; Assay Date 12-06-05

Defined Dose (pg/mL)	Average Calculated Dose (pg/mL)	% Bound
1.5	1.52	92.51, 94.17, 98.76, 97.36
5.0	4.83	75.83, 76.03, 80.44, 79.32
15.0	15.73	47.86, 47.40, 51.13, 51.20
50.0	47.81	24.60, 25.48, 27.40, 27.95
150.0	152.65	13.83, 12.83, 14.59, 15.11

2.2 Rat Follicle Stimulating Hormone Radioimmunoassay Procedure

The rat follicle stimulating hormone (rFSH) RIA used was a no-extraction, double antibody ^{125}I RIA (Amersham Biosciences, Piscataway, NJ) which utilized rFSH antibody, ^{125}I-rFSH, rFSH calibrators as the standard curve, and a precipitating solution consisting of donkey anti-sheep serum coated onto magnetizable polymer particles. rFSH controls in serum were prepared in the same species/strain/sex as unknown samples by adding known concentrations of rFSH to the appropriate matrix. rFSH controls in the kit supplied assay buffer were prepared using the reference preparation from the National Hormone and Pituitary Program (Torrance, CA). From the control values, the intra-assay coefficient of variation, and percent recovery for the assay was determined (see Table 2 below). All samples were analyzed in one assay. The sensitivity of the assay was 0.9 ng/mL as reported by Amersham Biosciences. All samples read within curve range of 1.6 to 100 ng/mL. For the RIA procedure, the sample

(100 µL) was pipetted into a glass culture tube and the rFSH antiserum (100 µL) was added. The tubes were vortexed and incubated at room temperature for 4 hours. The ^{125}I-rFSH (100 µL) was added, and the tubes were vortexed and incubated at room temperature for approximately 22 hours. After overnight incubation, cold precipitating solution (400 µL) was added and the tubes were vortexed. After a 10 minute incubation, the tubes were centrifuged, the supernatant was decanted and the tubes containing pellets were counted in a gamma counter. Results were reported as ng/mL. The results of the assay are considered reliable based on the assay performance results, some of which are presented in Table 2.

Table 2. Parameters for FSH RIAs Used for Male Rat Hormone Determinations

Parameter	Rat Serum Controls[e]	Zero Calibrator Controls[f]
Units	(ng/mL)	(ng/mL)
Intra-assay Variation[a]		
Mass added	0/10.1% 6.25/4.1% 25/2.6%	7.5/4.0% (63.4-66.5%)[d] 30/5.1% (26.2-28.6%)[d]
Inter-assay Variation[a]		
No. of assays	1	1
Mass added	N/A	N/A
% recovery of added mass[b]	6.25/71.3% 25/83.1%	7.5/187.3% 30/167.6%
Index of parallelism[c]	N/A	N/A

[a] Numbers are mass added/percentage variation.
[b] Numbers are mass added/percentage recovered (range of all assays).
[c] Index of parallelism = concentration of low volume ÷ concentration of high volume x 100.
[d] Range of % binding in assay.
[e] Male CD rat serum RTI Lot # 136, FSH from kit standards
[f] NIDDK-rFSH-RP-2 from the National Hormone and Pituitary Program (Torrance, CA).
N/A = Not applicable

Table 2A. Rat FSH Standard Curve Values; Assay Date 12-02-05

Defined Dose (ng/mL)	Average Calculated Dose (ng/mL)	% Bound
1.6	1.68	98.28, 97.19, 97.29, 101.4
3.1	3.00	93.17, 95.63, 96.40, 95.96
6.2	6.21	84.80, 86.47, 85.49, 87.46
12.5	12.30	67.96, 70.86, 66.85, 71.05
25.0	25.90	44.52, 46.93, 43.73, 44.21
50.0	49.06	28.12, 28.13, 27.48, 26.61
100.0	98.12	16.14, 16.63, 16.79, 18.23

2.3 Rat Luteinizing Hormone Radioimmunoassay Procedure

The rat luteinizing hormone (rLH) RIA used was a no-extraction, double antibody ^{125}I-RIA (Amersham Biosciences, Piscataway, NJ) which utilized rLH antibody, ^{125}I-rLH, rLH calibrators as the standard curve, and a precipitating solution consisting of donkey anti-rabbit serum coated onto magnetizable polymer particles. rLH controls in serum were prepared in the same species/strain/sex as unknown samples by adding known concentrations of rLH to the appropriate matrix. rLH controls in the kit supplied assay buffer were prepared using the reference preparation from the National Hormone and Pituitary Program (Torrance, CA). From the control values, the intra-assay coefficient of variation, and percent recovery for the assay was determined (see Table 3 below). All samples were analyzed in one assay. The sensitivity of the assay was 0.9 ng/mL as reported by Amersham Biosciences. All samples read within curve range of 0.8 to 50 ng/mL. For the RIA procedure, the sample (100 µL) was pipetted into a glass culture tube, the rLH antiserum (100 µL) was added, followed by the ^{125}I-rLH (100 µL), and the tubes were vortexed and incubated at room temperature for approximately 23 hours. After overnight incubation, cold precipitating solution (400 µL) was added and the tubes were vortexed. After a ten minute incubation, the tubes were centrifuged, the supernatant was decanted and the tubes containing pellets were counted in a gamma counter. Results were reported as ng/mL. The results of the assay are considered reliable based on the assay performance results, some of which are presented in Table 3.

Table 3. Parameters for LH RIAs Used for Male Rat Hormone Determinations

Parameter	Rat Serum Controls[e]	Zero Calibrator Controls[f]
Units	(ng/mL)	(ng/mL)
Intra-assay Variation[a]		
Mass added	0/8.8% 3.1/5.5% 12.5/4.5%	3.75/4.1% (70.2-72.9%)[d] 15/4.1% (25.3-27.5%)[d]
Inter-assay Variation[a]		
No. of assays	1	1
Mass added	N/A	N/A
% recovery of added mass[b]	3.1/70.2% 12.5/91.8%	3.75/130.3% 15/128.6%
Index of parallelism[c]	N/A	N/A

[a] Numbers are mass added/percentage variation.
[b] Numbers are mass added/percentage recovered (range of all assays).
[c] Index of parallelism = concentration of low volume ÷ concentration of high volume x 100.
[d] Range of % binding in assay.
[e] Male CD Rat Serum RTI Lot # 136 , LH from kit standards.
[f] NIDDK-rLH-RP-3 from the National Hormone and Pituitary Program (Torrance, CA).
N/A = Not applicable

Table 3A. Rat LH Standard Curve Values; Assay Date 11-29-05

Defined Dose (ng/mL)	Average Calculated Dose (ng/mL)	% Bound
0.8	0.79	98.30, 98.58, 98.41, 97.67
1.6	1.64	93.48, 93.84, 93.09, 93.13
3.1	3.04	83.55, 86.21, 82.87, 82.98
6.2	6.22	62.41, 64.98, 63.39, 64.96
12.5	12.59	38.62, 39.37, 38.56, 39.93
25.0	24.81	20.44, 21.80, 20.21, 20.34
50.0	49.88	10.11, 11.36, 10.19, 11.25

2.4 Rat Prolactin Radioimmunoassay Procedure

The rat prolactin (rPRL) RIA used was a no-extraction, double antibody ^{125}I RIA (Amersham Biosciences, Piscataway, NJ) which utilized rPRL antibody, ^{125}I-rPRL, rPRL calibrators as the standard curve, and a precipitating solution consisting of donkey anti-sheep serum coated onto magnetizable polymer particles. rPRL controls in serum were prepared in the same species/strain/sex as unknown samples by adding known concentrations of rPRL to the appropriate matrix. rPRL controls in the kit supplied assay buffer were prepared using the reference preparation from the National Hormone and Pituitary Program (Torrance, CA). From the control values, the intra- and inter-assay coefficient of variation, percent recovery, and index

of parallelism for the assays was determined (see Table 4 below). All samples were analyzed in two assays. The sensitivity of the assay was 0.7 ng/mL as reported by Amersham Biosciences. All samples read within curve range of 0.8 to 50 ng/mL, once diluted 10-fold with assay buffer. For the RIA procedure, the sample (100 µL) was pipetted into a glass culture tube, the rPRL antiserum (100µL) was added, followed by the ^{125}I-rPRL (100µL), and the tubes were vortexed and incubated at room temperature for 22-24 hours. After overnight incubation, cold precipitating solution (400µL) was added and the tubes vortexed. After a 10 minute incubation, the tubes were centrifuged, the supernatant was decanted and the tubes containing pellets were counted in a gamma counter. Results were reported as ng/mL. The results of the assay are considered reliable based on the assay performance results, some of which are presented in Table 4.

Table 4. Parameters for Prolactin RIAs Used for Male Rat Hormone Determinations

Parameter	Rat Serum Controls[e]	Zero Calibrator Controls[f]
Units	(ng/mL)	(ng/mL)
Intra-assay Variation[a]		
Mass added	0/3.4% and 9.6% 3.1/4.9% and 6.5% 12.5/4.9% and 0.5%	2.5/13.1% and 9.1% (61.9-69.6%)[d] 10/7.6% and 5.7% (28.4-34.4%)[d]
Inter-assay Variation[a]		
No. of assays	2	2
Mass added	0/3.4% 3.1/0.5% 12.5/6.0%	2.5/0.7% 10/0.5%
% recovery of added mass[b]	3.1/112.4-132.9% 12.5/95.5-121.9%	2.5/209.4-211.4% 10/205.8-207.2%
Index of parallelism[c]	75.1%	N/A

[a] Numbers are mass added/percentage variation.
[b] Numbers are mass added/percentage recovered (range of all assays).
[c] Index of parallelism = concentration of low volume ÷ concentration of high volume x 100.
[d] Range of % binding in assay.
[e] Male CD rat serum RTI Lot # 136, prolactin from kit standards
[f] NIDDK-rPRL-RP-3 from the National Hormone and Pituitary Program (Torrance, CA).
N/A = Not applicable

Table 4A. Rat Prolactin Standard Curve Values; Assay Date 12-07-05

Defined Dose (ng/mL)	Average Calculated Dose (ng/mL)	% Bound
0.8	0.75	96.43, 97.86, 97.76, 95.97
1.6	1.71	87.60, 90.27, 87.54, 88.61
3.1	2.95	77.90, 82.85, 76.14, 79.99
6.2	6.25	58.11, 62.93, 62.44, 61.49
12.5	12.94	39.09, 42.64, 42.28, 41.67
25.0	24.57	25.55, 27.27, 24.31, 27.93
50.0	49.04	14.02, 16.14, 14.51, 14.55

2.5 Rat Thyroid Stimulating Hormone Radioimmunoassay Procedure

The rat thyroid stimulating hormone (rTSH) RIA used was a no-extraction, double antibody ^{125}I RIA (Amersham Biosciences, Piscataway, NJ) which utilized rTSH antibody, ^{125}I-rTSH, rTSH calibrators as the standard curve, and a precipitating solution consisting of donkey anti-rabbit serum coated onto magnetizable polymer particles. rTSH controls in serum were prepared in the same species/strain/sex as unknown samples by adding known concentrations of rTSH to the appropriate matrix. rTSH controls in the kit supplied assay buffer were prepared using the reference preparation from the National Hormone and Pituitary Program (Torrance, CA). From the control values, the intra-assay coefficient of variation, and percent recovery for the assay was determined (see Table 5 below). All samples were analyzed in one assay. The sensitivity of the assay was 0.5 ng/mL as reported by Amersham Biosciences. All samples read within curve range of 1 to 64 ng/mL. For the RIA procedure, the sample (100 µL) was pipetted into a glass culture tube, the rTSH antiserum (100µL) was added, followed by the ^{125}I-rTSH (100µL), and the tubes were vortexed and incubated at room temperature for approximately 21 hours. After overnight incubation, cold precipitating solution (400µL) was added and the tubes were vortexed. After a ten minute incubation, the tubes were centrifuged, the supernatant was decanted, and the tubes containing pellets were counted in a gamma counter. Results were reported as ng/mL. The results of the assay are considered reliable based on the assay performance results, some of which are presented in Table 5.

Table 5. Parameters for TSH RIAs Used for Male Rat Hormone Determinations

Parameter	Rat Serum Controls[e]	Zero Calibrator Controls[f]
Units	(ng/mL)	(ng/mL)
Intra-assay Variation[a]		
Mass added	0/4.9% 4/4.9% 16/4.2%	2.5/3.8% (69.3-71.7%)[d] 10/8.8% (29.1-33.8%)[d]
Inter-assay Variation[a]		
No. of assays	1	1
Mass added	N/A	N/A
% recovery of added mass[b]	4/119.5% 16/126.4%	2.5/237.1% 10/207.3%
Index of parallelism[c]	N/A	N/A

[a] Numbers are mass added/percentage variation.
[b] Numbers are mass added/percentage recovered (range of all assays).
[c] Index of parallelism = concentration of low volume ÷ concentration of high volume x 100.
[d] Range of % binding in assay.
[e] Male CD Rat Serum RTI Lot # 136 , TSH from kit standards.
[f] NIDDK-rTSH-RP-3 from the National Hormone and Pituitary Program (Torrance, CA).

N/A = Not applicable

Table 5A. Rat TSH Standard Curve Values; Assay Date 12-05-05

Defined Dose (ng/mL)	Average Calculated Dose (ng/mL)	% Bound
1.0	1.03	96.22, 97.93, 98.75, 96.04
2.0	1.98	90.58, 92.23, 90.13, 94.07
4.0	3.91	79.95, 80.82, 78.65, 83.49
8.0	8.17	60.01, 62.29, 59.67, 62.54
16.0	15.98	38.43, 43.13, 37.45, 40.78
32.0	31.75	21.50, 24.44, 21.02, 21.20
64.0	64.07	11.39, 12.33, 9.29, 10.75

2.6 Total Testosterone Radioimmunoassay Procedure

The total testosterone (T) RIA used was a no-extraction, solid-phase ^{125}I-RIA which utilized T-specific antibody-coated tubes; ^{125}I-T and T calibrators as the standard curve (Diagnostic Products Corporation [DPC], Los Angeles, CA). T controls in serum were prepared in the same species/strain/sex as unknown samples by adding known concentrations of T to the appropriate matrix. From the control values, the intra- and inter-assay coefficient of variation, percent recovery, and index of parallelism for the assays was determined (see Table 6 below). All samples were analyzed in two assays. The sensitivity of the assay was 0.04 ng/mL as reported by DPC. Not all samples read within curve range of 0.2 to 16 ng/mL. For the RIA

procedure, the sample (50 µL) was pipetted into the antibody-coated tube and the ^{125}I-T (1 mL) was added. The tubes were vortexed and incubated in a 37°C water bath for three hours. After incubation, the supernatant was decanted and the tubes were counted in a gamma counter. Results were reported as ng/mL. The results of the assay are considered reliable based on the assay performance results, some of which are presented in Table 6.

Table 6. Parameters for Testosterone RIAs Used for Male Rat Hormone Determinations

Parameter	Rat Serum Controls[e,f]	Zero Calibrator Controls
Units	(ng/mL)	(ng/mL)
Intra-assay Variation[a]		
Mass added	0.5/4.8% and 6.9% 4/7.7% and 3.4% 8/5.0% and 8.0%	0.5/12.3% and 12.0% (67.7-74.6%)[d] 4/4.2% and 0.8% (31.1-34.2%)[d]
Inter-assay Variation[a]		
No. of assays	2	2
Mass added	0.5/1.4% 4/2.4% 8/2.5%	0.5/6.3% 4/3.7%
% recovery of added mass[b]	0.5/97.4-101.0% 4/99.7-103.1% 8/94.1-97.4%	0.5/109.0-117.3% 4/102.5-107.9%
Index of parallelism[c]	117.3%	N/A

[a] Numbers are mass added/percentage variation.
[b] Numbers are mass added/percentage recovered (range of all assays).
[c] Index of parallelism = concentration of low volume ÷ concentration of high volume x 100.
[d] Range of % binding in assay.
[e] Male CD rat serum RTI Lot # 135 (pooled from 10 rats)
[f] Testosterone from kit calibrators
N/A = Not applicable

Table 6A. Total Testosterone Standard Curve Values; Assay Date 11-17-05

Defined Dose (ng/mL)	Average Calculated Dose (ng/mL)	% Bound
0.2	0.19	88.43, 85.81, 88.33, 87.41
1.0	1.07	59.71, 60.74, 57.28, 59.97
4.0	4.07	32.50, 34.45, 33.07, 33.73
8.0	7.76	22.95, 22.74, 23.22, 23.06
16.0	15.58	14.04, 13.53, 15.03, 14.49

2.7 Total Triiodothyronine Radioimmunoassay Procedure

The total triiodothyronine (T3) RIA used was a no-extraction, solid-phase ^{125}I RIA which utilized T3-specific antibody-coated tubes, ^{125}I-T3, and T3 calibrators for the standard curve (DPC, Los Angeles, CA). T3 controls in serum were prepared in the same species/strain/sex as unknown samples by adding known concentrations of T3 to the appropriate matrix. From the control values, the intra-assay coefficient of variation and percent recovery for the assay was determined (see Table 7 below). All samples were analyzed in one assay. The sensitivity of the assay was 7 ng/dL as reported by DPC. All samples read within curve range of 20 to 600 ng/dL. For the RIA procedure, the sample (100 µL) was pipetted into the antibody-coated tube and the ^{125}I-T3 (1mL) was added. The tubes were vortexed and incubated in a 37°C water bath for two hours. After incubation, the supernatant was decanted and the tubes were counted in a gamma counter. Results were reported as ng/dL. The results of the assay are considered reliable based on the assay performance results, some of which are presented in Table 7.

Table 7. Parameters for Triiodothyronine (T3) RIAs Used for Male Rat Hormone Determinations

Parameter	Rat Serum Controls[e]	Zero Calibrator Controls
Units	(ng/dL)	(ng/dL)
Intra-assay Variation[a]		
Mass added	0/7.1% 50/4.8% 300/1.5%	50/4.0% (70.6-72.4%)[d] 300/5.0% (26.6-28.6%)[d]
Inter-assay Variation[a]		
No. of assays	1	1
Mass added	N/A	N/A
% recovery of added mass[b]	50/108.8% 300/113.0%	50/99.3% 300/99.1%
Index of parallelism[c]	N/A	N/A

[a] Numbers are mass added/percentage variation.
[b] Numbers are mass added/percentage recovered (range of all assays).
[c] Index of parallelism = concentration of low volume ÷ concentration of high volume x 100.
[d] Range of % binding in assay.
[e] Male CD Rat Serum RTI Lot # 136 , T3 from kit calibrators.
N/A = Not applicable

Table 7A. Total Triiodothyronine Standard Curve Values; Assay Date 11-28-05

Defined Dose (ng/dL)	Average Calculated Dose (ng/dL)	% Bound
20.0	19.56	85.34, 83.87, 84.77, 87.12
50.0	49.96	71.27, 71.06, 69.89, 73.00
100.0	100.54	52.28, 55.43, 54.42, 56.60
200.0	204.24	35.09, 35.68, 36.18, 37.63
600.0	580.73	16.12, 16.15, 16.44, 16.22

2.8 Total Thyroxine Radioimmunoassay Procedure

The total thyroxine (T4) RIA used was a no-extraction, solid-phase ^{125}I RIA which utilized T4-specific antibody-coated tubes, ^{125}I-T4, and T4 calibrators for the standard curve (DPC, Los Angeles, CA). T4 controls in serum were prepared in the same species/strain/sex as unknown samples by adding known concentrations of T4 to the appropriate matrix. From the control values, the intra- and inter-assay coefficient of variation and percent recovery for the assays was determined (see Table 8 below). All samples were analyzed in two assays. The sensitivity of the assay was 0.25 µg/dL as reported by DPC. Not all samples read within curve range of 1 to 24 µg/dL. For the RIA procedure, the sample (25 µL) was pipetted into the antibody-coated tube and the ^{125}I-T4 (1 mL) was added. The tubes were vortexed and incubated in a 37°C water bath for one hour. After incubation, the supernatant was decanted and the tubes were counted in a gamma counter. Results were reported as µg/dL. The results of the assay are considered reliable based on the assay performance results, some of which are presented in Table 8.

Table 8. Parameters for Thyroxine (T4) RIAs Used for Male Rat Hormone Determinations

Parameter	Rat Serum Controls[e]	Zero Calibrator Controls
Units	(µg/dL)	(µg/dL)
Intra-assay Variation[a]		
Mass added	0/9.1% and 14.6% 5/10.2% and 11.4% 12/5.2% and 7.6%	2.67/8.6% and 8.1% (65.4-70.4%)[d] 16/3.4% and 4.5% (27.8-31.0%)[d]
Inter-assay Variation[a]		
No. of assays	2	2
Mass added	0/6.3% 5/0.9% 12/5.2%	2.67/1.4% 16/1.7%
% recovery of added mass[b]	5/96.0-103.6% 12/90.0-101.2%	2.67/112.4-114.5% 16/99.1-101.5%
Index of parallelism[c]	N/A	N/A

[a] Numbers are mass added/percentage variation.
[b] Numbers are mass added/percentage recovered (range of all assays).
[c] Index of parallelism = concentration of low volume ÷ concentration of high volume x 100.
[d] Range of % binding in assay.
[e] Male CD Rat Serum RTI Lot # 136 , T4 from kit standards.
N/A = Not applicable

Table 8A. Total Thyroxine Standard Curve Values; Assay Date 11-18-05

Defined Dose (µg/dL)	Average Calculated Dose (µg/dL)	% Bound
1.0	0.95	88.30, 90.54, 86.30, 88.90
4.0	4.13	59.71, 62.61, 60.41, 61.15
10.0	10.35	37.72, 40.71, 37.03, 39.89
16.0	15.87	28.72, 28.93, 27.74, 31.63
24.0	23.07	21.17, 23.38, 19.62, 23.24

2.9 Dihydrotestosterone Radioimmunoassay Procedure

The dihydrotestosterone (DHT) RIA used had a sample oxidation/extraction procedure followed by a solid-phase ^{125}I RIA which utilized DHT-specific antibody-coated tubes and ^{125}I-DHT (DSL, Webster, Texas). Also included in the kit were reagents for the oxidation/extraction procedure to remove most of the testosterone which will cross-react with the DHT antiserum. These reagents were an oxidation solution and DHT sample buffer. Also needed but not included in the kit were the organic solvents for extraction, n-hexane (95% minimum) purchased from EM Science, and absolute ethanol which was purchased from AAPER Alcohol and Chemical Company. The DHT (Sigma, St. Louis, MO) curve was prepared in the zero calibrator provided in the kit. DHT controls in serum were prepared in the same species/strain/sex as unknown samples by adding known concentrations of DHT to the

appropriate matrix. From the control values, the intra-assay coefficient of variation and percent recovery was determined (see Table 9 below). All samples were analyzed in two assays. The sensitivity of the assay was 4 pg/mL as reported by DSL. All samples read within curve range of 12.5 to 800 pg/mL, once diluted 4-fold with the zero calibrator. For the RIA procedure, the sample (400 µL) was oxidized and extracted and reconstituted in 250 µL of kit supplied zero calibrator. For the RIA procedure, the sample (100 µL) was pipetted into the antibody-coated tube and the ^{125}I-DHT (500µL) was added. The tubes were vortexed and incubated at room temperature on a shaker (180 rpm) for two hours. After incubation, the supernatant was decanted and the tubes were counted in a gamma counter. Results were reported as pg/mL. The results of the assay are considered reliable based on the assay performance results, some of which are presented in Table 9.

Table 9. Parameters for Dihydrotestosterone (DHT) RIAs Used for Male Rat Hormone Determinations

Parameter	Rat Serum Controls[e]	Zero Calibrator Controls
Units	(pg/mL)	(pg/mL)
Intra-assay Variation[a]		
Mass added	0/7.8% and 4.0% 100/12.6% and 8.8% 400/2.9% and 11.1%	64/15.7% and 4.2% (64.6-72.9%)[d] 320/12.7% and 0.7% (24.8-31.3%)[d]
Inter-assay Variation[a]		
No. of assays	2	2
Mass added	0/31.4% 100/24.5% 400/26.7	64/6.4% 320/10.2%
% recovery of added mass[b]	100/110.1-134.4% 400/95.1-135.6%	64/99.1-108.4% 320/95.1-109.9%
Index of parallelism[c]	80.8%	N/A

[a] Numbers are mass added/percentage variation.
[b] Numbers are mass added/percentage recovered (range of all assays).
[c] Index of parallelism = concentration of low volume ÷ concentration of high volume x 100.
[d] Range of % binding in assay.
[e] Male CD Rat Serum RTI Lot # 136 , DHT from Sigma Aldrich.
N/A = Not applicable

Table 9A. Dihydrotestosterone Standard Curve Values; Assay Date 12-04-05

Defined Dose (pg/mL)	Average Calculated Dose (pg/mL)	% Bound
12.5	13.19	90.71, 97.03, 89.65, 90.97
25.0	23.97	86.82, 88.00, 83.98, 88.11
50.0	48.96	73.13, 79.27, 75.03, 75.91
100.0	102.44	57.04, 61.81, 56.42, 60.39
200.0	202.52	42.48, 41.35, 38.44, 40.91
400.0	392.24	25.75, 25.79, 24.44, 25.01
800.0	799.64	13.91, 15.36, 12.93, 12.16

3 RESULTS

The assays for FSH, LH, Total Testosterone, Estradiol, Thyroxine, Triiodothyronine, Thyroid Stimulating Hormone, Prolactin and Dihydrotestosterone were performed in the Laboratory of Reproductive and Endocrine Toxicology (LORET) at RTI International on rat serum samples sent by Charles River Laboratory for analysis by radioimmunoassay.

The assay parameters are presented in Text Tables 1-9. The results for the assays performed are presented in the summary Table 1. Any sample value that was based on a single determination is identified.

All data and records will be sent to the client for archiving.

SUMMARY TABLE

Table 1. Summary of Individual Hormone Results (page 1 of 4)

Animal Number	TSH (ng/mL)	DHT (pg/mL)	Estradiol (pg/mL)	FSH (ng/mL)	LH (ng/mL)	Prolactin (ng/mL)	Testosterone (ng/mL)	Thyroxine (µg/dL)	Triiodothyronine (ng/dL)
10301	10.96	495.98	26.40	14.58	1.85	53.40	6.79	4.91	72.11
10302	7.86	312.15	19.01	12.59	1.57	40.43	3.97	4.64	89.01
10303	20.99	483.20	26.59	14.75	1.64	1.69*	10.27	4.31	82.80
10304	23.88	277.76	24.05	12.84	1.70	2.76	4.29	3.54	63.86
10305	11.56	569.30	26.46	16.78	2.12	25.14	13.57	4.77	60.36
10316	9.82	202.52	28.91	15.90	1.58	1.56	1.29	3.32	73.83
10317	11.39	448.63	40.04	14.33	1.82	2.63	5.72	2.66	59.73
10318	13.01	225.58	34.99	16.01	1.15	2.77	1.61	2.60	60.78
10319	10.08	524.08	29.57	15.02	2.17	15.82	7.78	3.29	73.96
10320	7.72	207.42	39.84	13.13	1.35	10.86	3.06	3.14	70.76
10331	5.18	191.52	38.07	22.66	1.48	7.09	2.05	2.50	76.39
10332	6.73	1010.60	53.24	15.30	1.76	3.75	22.73	2.43	74.62
10333	19.19	286.04	33.20	15.61	2.11	2.38	3.50	1.40	67.70
10334	7.81	257.54	38.39	17.16	1.52	9.02	2.55	2.23	55.31
10335	9.85	231.22	29.11	13.58	1.24	15.79	2.77	2.06	62.58
10346	8.40	127.77	42.72	17.41	2.20	28.61	0.82	1.27	53.44
10347	11.93	354.67	45.48	15.98	1.41*	1.41	5.13	1.29*	54.62
10348	8.35	229.87	41.48	13.68	1.77	1.01	3.87	1.11*	62.11
10349	14.59	194.13	35.27	17.84	1.23	1.26	1.54	2.49	69.79
10350	10.52	556.16	35.64	19.96	2.21	2.24	7.65	1.31	71.68
10361	19.57	205.30	30.33	14.50	1.60	2.63	1.89	3.09	53.38
10362	52.10	764.62	45.48	14.13	1.82	1.73	15.23	3.03	54.31
10363	28.04	613.09	33.44	14.59	1.82	1.86	11.45	4.34	83.72
10364	34.00	393.86	37.36	14.63	1.77	3.68	4.91	4.25	62.98
10365	13.77	320.81	39.33	15.14	1.40	11.36	3.16	4.89	81.01
10376	25.33	212.17	48.59	13.39	1.88	12.27	3.11	3.70	59.67
10377	29.03	787.39*	39.26	13.02	1.34	4.62	10.21	2.63	47.77
10378	34.79	219.04	30.87	14.26	1.40	1.96	2.38	3.72	55.51
10379	18.55	172.01	45.68	12.60	1.36	1.77	1.25	4.87	94.14
10380	25.16	146.38	44.10	11.50	1.28	13.91	1.34	3.61	70.98
10391	41.37	144.54	55.36	13.20	1.39	1.89	1.44	2.20	49.20
10392	23.45	165.85	36.94	12.14	1.00	1.89	1.27	2.15	58.29
10393	30.01	189.19	37.53	11.35	1.62	3.77	1.80	2.28	63.00

Table 1. Summary of Individual Hormone Results (page 2 of 4)

Animal Number	TSH (ng/mL)	DHT (pg/mL)	Estradiol (pg/mL)	FSH (ng/mL)	LH (ng/mL)	Prolactin (ng/mL)	Testosterone (ng/mL)	Thyroxine (µg/dL)	Triiodothyronine (ng/dL)
10394	37.69	172.84	39.80	15.92	1.63	2.27	1.52	2.53	66.07
10395	36.00	77.90	43.01	12.47	1.53	1.28	BDL	4.11	77.61
10306	9.89	256.59	19.12	13.35	1.63	29.54	3.92	6.21	86.87
10307	8.93	218.29	31.35	16.23	2.52	19.20	3.89	4.40	97.13
10308	6.49	140.21	25.65	18.99	2.01	4.09	1.28	3.83	75.76
10309	9.56	1093.40	30.98	12.96	2.14	52.63	28.11	3.87	91.91
10310	9.55	689.34	29.54	16.27	2.82	101.52	21.05	4.90	99.32
10321	10.88	112.90	39.33	11.08	1.24	4.39	0.63	2.15	71.26
10322	8.14	344.55	36.40	13.60	1.41	2.54	4.17	3.07	71.85
10323	10.19	266.34	33.04	16.69	2.01	5.03	3.03	2.63	70.71
10324	33.14	1342.30	40.56	24.04	1.81	1.92	28.37*	3.04	72.18
10325	8.11	471.49	34.43	12.55	1.63	2.19	7.55	2.44	56.43
10336	6.00	109.97	30.39	10.24	1.42	1.45	0.23	1.08	40.94
10337	15.65	143.72	47.70	17.49	1.33	0.98	0.70	1.34	64.67
10338	11.51	854.43	53.15	13.35	2.99	6.17	14.86	1.58	60.40
10339	7.94	419.18	41.80	18.61	1.78	1.64	5.40	1.72	71.92
10340	9.08	124.29	39.96	17.42	1.56	1.50	0.50	1.51	69.37
10351	9.21	200.02	59.79	17.51	2.85	3.25	1.81	BDL	56.31
10352	12.96	146.12	43.09	15.79	1.38	1.63	0.82	1.56	56.42
10353	17.47	290.56	40.29	16.95	2.29	4.26	3.81	1.22	70.28
10354	5.54	670.40*	31.11	14.14	1.76	1.40*	15.16	1.57	59.46
10355	10.52	163.40	35.41	16.56	1.73	1.34	1.98	1.77	67.93
10366	22.08	213.24	32.66	10.33	1.58	36.20	2.25	3.40	65.38
10367	17.47	246.69	33.63	13.25	1.88	5.28	3.85	3.59	62.70
10368	26.34	762.86*	26.06	15.22	2.02	15.86	12.39	3.58	60.37
10369	27.03	201.19	34.97	14.93	1.96	4.58	2.15	4.10	65.16
10370	17.47	260.81	36.24	14.34	1.80	7.20	3.18	4.00	73.70
10381	17.68	122.92	35.43	11.10	1.23	20.11	1.13	3.61	67.40
10382	31.73	314.37	40.08	11.10	1.39	2.95	4.38	3.37	67.88
10383	17.78	166.50	33.45	10.96	1.28	10.97	1.36	3.93	65.64
10384	20.58	258.06	26.35	10.56	1.29	58.20	2.21	3.35	57.07

Table 1. Summary of Individual Hormone Results (page 3 of 4)

Animal Number	TSH (ng/mL)	DHT (pg/mL)	Estradiol (pg/mL)	FSH (ng/mL)	LH (ng/mL)	Prolactin (ng/mL)	Testosterone (ng/mL)	Thyroxine (µg/dL)	Triiodothyronine (ng/dL)
10385	29.85	219.19	30.86	10.59	1.38	11.48	2.47	4.13	79.43
10397	18.14	306.04	27.99	10.98	1.37	1.09*	3.51	2.70	56.99
10398	36.20	126.62	36.60	9.43	1.49	1.36	0.71	2.44	60.22
10399	28.77	273.74	46.87	12.26	1.58	1.59	3.59	2.37	58.35
10400	32.77	285.18	28.68	10.71	1.53	3.08	2.94	3.26	50.56
10311	9.05	515.79	23.83	12.95	2.76	44.37*	8.87	6.36	94.55
10312	8.32	497.67	27.01	10.96	2.31	66.61	9.13	5.28	78.52
10313	11.24	667.62	23.71	16.02	2.73	42.96	15.22	4.63	72.42
10314	24.73	370.52	23.67	15.43	1.83	14.67	6.63	4.18	83.16
10315	23.41	728.19	23.68	17.40	3.04	48.13	11.92	5.11	77.01
10326	10.36	555.45	26.64	12.88	2.23	5.81	14.36	4.48	67.21
10327	6.77	241.09	29.21	16.47	2.04	4.98	3.64	2.86	60.80
10328	14.78	528.05	24.65	15.21	2.35	54.31	7.65	3.08	66.13
10329	7.14	368.41	31.48	14.38	1.91	4.77	3.43	3.21	62.11
10330	9.42	347.10	28.89	12.51	1.60	1.02	3.65	4.51	85.20
10341	21.69	339.21	35.60	13.34	2.18	5.43	4.64	2.25	84.34
10342	14.15	276.79	41.43	16.75	2.72	13.36	4.37	1.74	64.13
10343	16.54	407.41	36.69	11.39	1.13	3.35	4.80	1.64	65.20
10344	23.42	592.08	46.26	17.85	1.92	9.59	9.11	1.53	65.38
10345	8.44	122.54	49.20	13.58	1.59	1.99	0.25	2.28	61.36
10356	11.80	131.37	38.73	12.73	1.29	1.48	BDL	1.22	60.65
10357	14.09	557.25	41.03	11.40	1.06	1.42	7.67	1.80	85.89
10358	6.36	313.86	33.80	17.51	3.67	1.72	4.80	1.18	60.93
10359	8.07	341.44	33.93	12.90	1.64	1.19	3.68	1.68	70.67
10360	7.14	220.97	30.38	18.27	1.48	4.66	2.09	2.59	66.85
10371	13.84	275.23	38.76	16.30	2.70	15.72	5.08	3.48	66.47
10372	13.43	277.14	29.15	10.35	1.12	45.56	3.39	3.51	63.06
10373	11.30	250.37	22.19	13.46	1.59	7.72	3.31	3.92	66.41
10374	16.45	329.03	30.75	11.09	1.69	47.41	4.58	4.04	68.69
10375	37.34	730.14	35.28	15.36	2.41	3.96	14.26	3.04	45.37
10386	16.82	178.94	33.53	14.08	1.74	1.54*	1.69	3.24	64.58
10387	39.10	523.95	35.72	14.26	1.72	2.47	7.36	3.42	47.82

Table 1. Summary of Individual Hormone Results (page 4 of 4)

Animal Number	TSH (ng/mL)	DHT (pg/mL)	Estradiol (pg/mL)	FSH (ng/mL)	LH (ng/mL)	Prolactin (ng/mL)	Testosterone (ng/mL)	Thyroxine (µg/dL)	Triiodothyronine (ng/dL)
10388	39.37	200.60	39.22	12.80	1.48	12.47	2.76	3.59	54.83
10389	19.22	688.08	30.06	12.69	1.60	9.58	8.03	3.37	76.08
10390	21.13	311.18	34.68	13.97	1.15	7.59	2.74	4.09	72.32
10401	24.88	357.88	34.82	10.79	1.54	1.49	5.01	2.94	49.71
10402	18.69	505.90	43.40	13.56	2.10	19.48	10.39	3.35	56.07
10403	17.38	247.72	34.63	11.14	1.56	44.86	1.36	1.86	45.12
10404	27.99	394.40	31.85	11.62	1.47	11.04	1.52	2.52	55.30
10405	42.84	236.08	41.84	16.32	1.96	4.57	3.62	2.01	39.56

*Value is based on a single determination

BDL = below detection limit, for testosterone = <0.2 ng/mL, for thyroxine = <1 µg/dL

APPENDIX 9 - STATISTICAL REPORT

Final Report

Interlaboratory Validation of the 15-Day Adult Intact Male Rat Assay Intra-Laboratory Statistical Analysis for Charles River Laboratories

EPA CONTRACT NUMBER 68-W-01-023
WORK ASSIGNMENT 5-15

March 31, 2006

Prepared for

U.S. ENVIRONMENTAL PROTECTION AGENCY
ENDOCRINE DISRUPTOR SCREENING PROGRAM
WASHINGTON, D.C.

Prepared by

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Interlaboratory Validation of the 15-Day Adult Intact Male Rat Assay
Intra-Laboratory Statistical Analysis for Charles River Laboratories

EPA CONTRACT NUMBER 68-W-01-023
WORK ASSIGNMENT 5-15

Zhenxu J. Ma, Author — Date: 3/31/06

Paul I. Feder, Reviewer — Date: March 31, 2006

Offsite Quality Assurance Statement

Study Number: WA 5-15

This study was inspected by the Quality Assurance Unit and reports were submitted to the Study Director and Management as follows:

Phase Inspected	Inspection Date	Date Reported to Battelle Task Leader/Battelle Management	Date Reported to Offsite Study Director /Management
Audit statistics data	1/18/2006	1/18/2006	1/23/2006
Audit statistics report	1/18/2006	1/18/2006	1/23/2006

Kathleen C. Reed 3-28-06

Quality Assurance Unit **Date**

Interlaboratory Validation of the 15-Day Adult Intact Male Rat Assay
Intra-Laboratory Statistical Analysis for Charles River Laboratories

EPA CONTRACT NUMBER 68-W-01-023
WORK ASSIGNMENT 5-15

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

All aspects of the above-referenced study performed by Battelle were conducted according to Good Laboratory Practice regulations.

No deviations were noted that had any significant impact on the study.

Paul I. Feder

March 31, 2006

Paul I. Feder

Date

INTRODUCTION

Charles River Laboratories, Preclinical Services (Charles River) conducted a 15-day adult intact male rat assay according to the test method provided by the EPA.

Two substances Linuron and Phenobarbital were tested, each at three dose levels. In addition a vehicle control group was tested. The sample size was n=15 adult male rats per group, for a total of seven groups and 105 animals per laboratory. This statistical report specifies the summaries, displays, and statistical analyses that were used to summarize the results within Charles River Laboratories.

STATISTICAL METHODS

Data

The test method specifies four categories of data:

1. Growth - body weights and food consumption – (7 endpoints)
   - Body weight change (TD8 – TD1)
   - Body weight change (TD15 – TD8)
   - Body weight change (TD15 – TD1)
   - Final body weights (TD15)
   - Food consumption (TD8 - TD1)
   - Food consumption (TD15 - TD8)
   - Food consumption (TD15 - TD1)

The TD15 body weight is the live weight before sacrifice. Body weights were reported in grams (g). Body weight changes for a given period were reported in g/day, which were calculated as the differences between the body weights at the start and the end of the given period divided by the length of the period (i.e., the daily average within the weekly or bi-weekly interval). Food consumption for each animal was reported in g/kg/day, which was calculated as follows. The average of two body weights for a period (Day 1 and Day 8 for period TD8-TD1 and Day 8 and Day 15 for period TD15-TD8) was calculated. The average body weight in grams was transformed to kilograms. The food consumption for the weekly period (in grams) was divided by the average body weight in kilograms. This ratio was divided by 7 days in the period to get the food consumption in g/kg/day for that animal. The food consumption for the period TD15-TD1 was determined as the average of the two weekly average values if both were present. If one weekly value was missing the average for the period TD15-TD1 was reported as missing.

2. Hormonal analysis - (9 hormones)
   - Testosterone (ng/ml)
   - LH (ng/ml)
   - TSH (ng/ml)

T_4 (μg/dl)
T_3 (ng/dl)
FSH (ng/ml)
Estradiol (pg/ml)
Prolactin (ng/ml)
DHT (pg/ml)

3. Organ weights – (9 organs)
   Liver
   Right testis
   Left testis
   Testes paired (sum of left and right testis weights)
   Epididymides (paired weight)
   Entire prostate
   Seminal vesicles with fluid and coagulating gland
   Accessory sex gland (ASG) (sum of entire prostate and seminal vesicles with fluid and coagulating gland weights)
   Thyroid

Organ weights were reported in grams (g). Organ weights were reported wet to the nearest 0.0001 g. Organ weights were analyzed in two ways: unadjusted and adjusted. Adjusted organ weights were calculated as organ weight to final body weight ratio (expressed as percent). Note that paired testes weights and ASG weights were derived values, based on the constituent weights of their derived organs.

4. Histology – (5 organs)
   Right testis
   Left testis
   Right Epididymus
   Left Epididymus
   Thyroid

Histology data were not analyzed statistically.

The test method specifies that all rats were to be sacrificed on Test Day (TD) 15. If animals died prior to necropsy their body weights were included in summaries and displays up to the time of death, but were not imputed beyond date of death nor they were included in the final body weight gain summaries (in either the initial or final weight average). One animal died prior to TD15. This was animal 10396 (Phenobarbitol 100) that died on TD9. This animal was not included in the data summaries except for those (change in body weight and food consumption) involving only TD1 to TD8.

All data that entered into the statistical analyses were *a priori* valid data. Appendix C contains a preliminary summary of these data.

Outlier Detection

Outlier screens were carried out prior to analysis. Screens were carried out separately for each endpoint, based on untransformed data. When both unadjusted and body weight adjusted values are called for in the statistical analysis plan (organ weights), the outlier screens were only carried out based on the unadjusted values.

For each endpoint a one way analysis of variance model was fitted to the data. The data include seven groups with n=15 animals per group, less any data omitted due to deaths, missing values, or procedural errors. For purposes of outlier screening separate standard deviations were assumed within each group. Studentized residuals were determined based on the analysis of variance fit and ordered in absolute value. Assuming no data had been omitted, there would have been 105 values. A procedure which generalizes Grubbs (1969) procedure to accommodate heterogeneous variances was used. The absolute studentized residuals were compared to a cutoff value corresponding to a 2.5% significance level (for a two-sided 5% level test) of the maximum of seven component maximum studentized residuals, each component maximum studentized residual based on 15 observations. The cutoff value was based on a simulation study to determine the upper 97.5% point of the distribution of the maximum of seven independent maximum studentized residuals, each with 14 degrees of freedom, from standard normal distributions. This cutoff value is 2.84. Any studentized residual in excess of 2.84 in absolute value was flagged. Just a single iteration of the outlier screening procedure was carried out.

Normal probability plots of the studentized residuals were prepared (Appendix A). If the flagged values appeared to be outliers in the probability plots, in that they departed from the trend in the body of the residuals, they were treated as potential outliers. If the trend observed in the tails of the normal probability plot was continuous but heavily skewed or considerably heavier tailed than normal, a data transformation (e.g. square root, (natural) logarithm) might be attempted to improve agreement with normal distribution assumptions. The outlier screen would be repeated on the transformed data. However, if the tails of the normal probability plot depart just slightly or moderately from straight line behavior, the data would be analyzed without transformation. No transformations were attempted for this analysis due to the consideration of applying a uniform approach across laboratories, so the results could be more easily combined.

The flagged values were sent to the study director who determined whether these values were to be included in all the analyses, were to be treated as outliers (i.e. both included in analyses and excluded from analyses), or were to be excluded from all analyses. Subsequent statistical analyses were carried out both including and excluding the outliers that were specified by the study director to be treated as outliers. The disposition of each flagged value is summarized in Appendix B.

Heterogeneity of Residual Variances among Treatment Groups

Tests for heterogeneity of variance were carried out on the data excluding the flagged potential outliers. For each endpoint extent of heterogeneity of variability was assessed across treatment groups. A one-way analysis of variance model was fitted to the data, including the factor treatment (fixed). Three versions of the model were fitted to test for heterogeneity of residual variance.

1. Separate variances for each treatment group (7 variances)
2. Separate variances for each substance (or control) (3 variances)
3. Common variances across all groups

For each endpoint, these models were compared by likelihood ratio tests and a "best" model was selected for further statistical analyses (Table 1).

Data Summaries

Data summaries include tables and figures. Summary tables were prepared including all the data and excluding the outliers. Summary figures were prepared only including all the data.

Tables

Summary values for the seven body weight and food consumption endpoints are displayed in Tables 2 and 3. There is one table per substance.

For each endpoint and each dose group the following statistics are reported:

- Number of animals on which the statistic is based
- Mean ± standard error
- Coefficient of variation
- Difference of mean from control group mean ± standard error
- Ratio of mean to control group mean ± standard error[1]

In addition, the linear trend slope contrast was estimated for each substance based on the control group and the three graded dose groups, treating the control group and the

[1] If X, Y denote the control group least squares mean and the dose group least squares mean respectively, with variance-covariance matrix (S_X^2, S_Y^2, S_{XY}), where S_{XY} is zero because X and Y are independent, an approximate standard error for $R \equiv (Y/X) \times 100\%$ is

$$Se[R(X, Y)] \approx |1/X| \, [(Y/X)^2 \, S_X^2 + S_Y^2]^{1/2} \times 100\%$$

three dose groups as equally spaced[2]. The estimated slope and its standard error are reported.

For ease of presentation each table is broken into three pages, one page per dose level. The summary results for the vehicle control and the linear dose trend test results are presented on each page. For the same test substance they are same on all three pages of the table.

Tables 4 and 5 display summary values for the nine organ weight endpoints specified in the test method. These results include both unadjusted and body-weight adjusted organ weights. The tables include the same summary statistics as those discussed for Tables 2 and 3.

Tables 6 and 7 display summary values for the nine hormonal analysis endpoints specified in the test method. There is one table per substance. The tables include the same summary statistics as discussed for Tables 2 and 3.

Figures

The figures include mean daily body weights figures and figures to compare the various endpoints across substances and dose groups. The figures include all the data. For organ weights, figures were prepared based on both the unadjusted weights and the adjusted organ weights (i.e., organ to body weight ratios).

Figures 1-2 display mean body weight ± 2 standard errors for each day from TD1 to TD15 for the control group and for each dose group. Figure 1 corresponds to Linuron and Figure 2 corresponds to Phenobarbitol.

For the 7 body weight and food consumption measures, the 9 unadjusted organ weights, the 9 organ weight to body weight ratios, and the 9 hormone concentrations (34 endpoints) summarized in Tables 2-7, Figures 3 through 36 were prepared to display the least squares means ± 2 standard errors for each of the seven dose groups (control group + three dose groups × 2 substances). Each figure contains seven bars, corresponding to a control group or substance and dose group. Each bar is centered at the least squares mean and extends two standard errors above and below the least squares mean.

Analysis of Variance

For each of the 34 endpoints summarized in Tables 2-7 analysis of variance models were fitted to the data to estimate treatment effects. For the nine organ weight

[2] If X_0, X_1, X_2, X_3 denote the least squares means for the control group "0" and (equally spaced) dose groups "1", "2", "3" then the linear contrast among these is defined to be

$$\text{Linear Contrast} \equiv [-3X_0 - X_1 + X_2 + 3X_3]/[20]^{1/2}$$

responses the unadjusted responses were analyzed as well as the organ to final body weight ratio (percent) responses.

Analyses were carried out based on all the data and after omitting outliers (enumerated in Appendix B). The (possibly heterogeneous) residual variance structure assumed in these analyses is as discussed in the section - "Heterogeneity of Variance across Treatment Groups." Analyses were carried out on the untransformed data, using the simplest variance structure compatible with the data.

For each response the following one-way analysis of variance model was fitted to the data. The treatment group in the analysis of variance model is the fixed effect. The factors in the analysis of variance model are as shown below.

Source	df
Treatment	6
Residual = Replicate (Treatment)	14×7=98
	104

Least squares (LS) means for individual treatment groups and for differences between dose groups and control group and associated standard errors and ± 2 standard error intervals were calculated based on the above model. (For these data the least squares mean coincides with the simple arithmetic mean.) In addition linear trend contrasts among the control group and the three dose groups within a substance were calculated, treating the control group and the three dose groups as equally spaced (using the linear contrast shown in footnote 2). For each substance separately, least squares means were compared between the treatment groups and the control group by means of two-sample t-tests. Linear trend statistics were compared to 0 trend by means of one-sample t-tests.

Two-tailed unadjusted significance levels were reported. If the unadjusted significance levels were less than 0.05, they were indicated with a single asterisk, '*'. If they were less than 0.00625 for the comparisons of test substance dose to vehicle or for the linear trend test, they were indicated with two asterisks, '**'. The significance level 0.00625 provides overall family wise protection at the 0.05 significance level against making any false positive or Type 1 errors within a family of eight inferences, consisting of comparison of each of three dose levels to the control and comparison of a linear trend to 0, for both Linuron and Phenobarbitol (i.e. two substances × three doses per substance plus two linear trend comparisons, for a total of eight inferences). This is referred to as Bonferroni's simultaneity adjusted significance level of 0.05/8 (0.00625).

Round Off

Derived numbers in the tables may differ from computer listings or hand calculations by one or several digits in the least significant figure due to round off in intermediate calculations.

Archive

Upon completion of the project the report and supporting data were archived at Battelle.

Table 1. Likelihoods for Various Heterogeneous Covariance Structures, Likelihood Ratio Goodness of Fit Statistics, and Selections of Covariance Structure[1, 2].

Parameter	Selected Covariance Structure	TestChem*DoseLevel (T*D)	TestChem (T)	All (A)	(T*D)-(T)		(T)-(A)	
					Estimate	p-value (Chisq;df=4)	Estimate	p-value (Chisq;df=2)
Body Weight Change (TD8-TD1)	All	434.0	438.5	438.8	4.4828	0.34459	0.3545	0.83757
Body Weight Change (TD15-TD8)	All	373.1	381.0	382.5	7.8611	0.09680	1.5293	0.46549
Body Weight Change (TD15-TD1)	All	362.1	363.8	366.0	1.7143	0.78811	2.2458	0.32533
Final Body Weight	All	930.1	932.3	936.3	2.1963	0.69971	4.0024	0.13518
Food Consumption (TD8-TD1)	All	655.2	660.2	666.1	4.9603	0.29139	5.9213	0.05178
Food Consumption (TD15-TD8)	T	623.3	632.4	645.0	9.0859	0.05899	12.5767	0.00186
Food Consumption (TD15-TD1)	T	579.9	581.5	596.9	1.6583	0.79828	15.3252	0.00047
Liver	All	40.8	47.7	50.6	6.9151	0.14044	2.8996	0.23461
Left Testis	All	-337.2	-335.1	-331.4	2.1597	0.70641	3.6369	0.16227
Right Testis	All	-346.1	-343.8	-340.6	2.3389	0.67370	3.2279	0.19910
Paired Testes	All	-210.7	-208.9	-205.2	1.8491	0.76349	3.6251	0.16324
Paired Epididymides	All	-354.1	-353.0	-352.3	1.1600	0.88463	0.6635	0.71766
Entire Prostate	All	-245.6	-244.3	-241.1	1.3605	0.85102	3.1465	0.20737
SeminalVesicleCoagGlandFluid	All	-205.2	-202.6	-200.4	2.5909	0.62844	2.1975	0.33329
ASG	All	-128.2	-127.0	-127.0	1.1800	0.88138	0.0107	0.99466
Thyroid Glands	T	-887.1	-878.7	-865.4	8.3862	0.07841	13.3052	0.00129
Testosterone	T*D	510.2	529.4	548.7	19.2075	0.00072	19.3391	0.00006
LH	T*D	112.1	122.6	138.2	10.4888	0.03295	15.5560	0.00042
TSH	T*D	629.0	643.2	667.5	14.1926	0.00670	24.3109	0.00001
T4	All	182.3	185.6	190.0	3.2831	0.51162	4.4116	0.11016
T3	All	736.6	739.1	741.7	2.4347	0.65637	2.6235	0.26935
FSH	All	427.5	434.0	439.5	6.5492	0.16172	5.5139	0.06349
Estradiol	All	622.6	627.2	632.9	4.5934	0.33162	5.6495	0.05932
Prolactin	T*D	621.0	661.3	755.8	40.2788	0.00000	94.5140	0.00000
DHT	T*D	1297.2	1308.5	1310.4	11.2509	0.02388	1.8693	0.39273

1. A one-way ANOVA model was fitted to the data separately for each parameter, in which test chemical and dosage level interaction (i.e., dose group) were fixed effects. For organ weight parameters, organ weight to final body weight ratios (%) were used. Outliers were excluded from the model fits.
2. Two heterogeneous covariance models and a homogenous covariance were compared. The steps for selecting a covariance structure were: starting from the most complex structure in (T*D), if (T*D) was significantly better than the next less complex one in (T), then (T*D) was picked. Otherwise comparing (T) with the homogenous model (All). If (T) was significantly better, then pick (T). If not, (All) was picked.

Table 2. Summary Statistics between Vehicle and Linuron in Adult Intact Male Assay for Body Weight Changes (g/day), Final Body Weight (g), and Food Consumptions (g/kg/day) for Charles River Laboratories[1,2]

Parameter	Vehicle			Linuron (50 mg/kg/day)					
	N	LS Mean (SE)	CV (%)	N	LS Mean(SE)	CV (%)	Diff from Vehicle[3]	Ratio to Vehicle (%)[4]	Linear Trend[5]
Body Weight Change (TD8-TD1)	15	5.762 (0.532)	35.8	15	0.124 (0.532)	1665	-5.638 (0.753)**	2.149 (9.239)	-7.260 (0.532)**
Body Weight Change (TD15-TD8)	15	4.371 (0.407)	36.1	15	2.562 (0.407)	61.6	-1.810 (0.576)**	58.606 (10.796)	-1.352 (0.407)**
Body Weight Change (TD15-TD1)	15	5.067 (0.374)	28.6	15	1.343 (0.374)	107.9	-3.724 (0.529)**	26.504 (7.636)	-4.306 (0.374)**
Final Body Weight (g)	15	403.400 (7.072)	6.8	15	353.067 (7.072)	7.8	-50.333 (10.001)**	87.523 (2.330)	-57.050 (7.072)**
Food Consumption (TD8-TD1)	15	71.229 (1.885)	10.3	13	54.128 (2.025)	13.5	-17.101 (2.767)**	75.992 (3.483)	-23.876 (1.923)**
Food Consumption (TD15-TD8)	15	61.851 (1.270)	8.0	15	55.057 (2.120)	14.9	-6.794 (2.471)*	89.016 (3.885)	-9.834 (1.828)**
Food Consumption (TD15-TD1)	15	66.540 (1.206)	7.0	13	54.386 (2.005)	13.3	-12.154 (2.340)**	81.735 (3.358)	-17.341 (1.685)**

1. Least squares means and standard errors were estimated based on a one-way ANOVA model applied to all data for each parameter.
2. CV was calculated as residual standard deviation/LS Mean.
3. Significant differences from the vehicle were indicated by "*" for the 0.05 level and "**" for the 0.05/8 level (a Bonferroni adjusted p-level).
4. Ratio to the vehicle was calculated as percent of vehicle group mean, The standard error for the ratio was $Se[R(X, Y)] \approx |1/X| [(Y/X)^2 S_X^2 + S_Y^2]^{1/2} \times 100\%$
5. Linear Contrast $\equiv[-3X_0 - X_1 + X_2 + 3X_3]/[20]^{1/2}$, where X_0 is vehicle, X_1, X_2, and X_3 are the low, mid, and high dosage levels of Linuron respectively. Significant dose trend was indicated by "*" for the 0.05 level and "**" for the 0.05/8 level.

Table 2(cont.). Summary Statistics between Vehicle and Linuron in Adult Intact Male Assay for Body Weight Changes (g/day), Final Body Weight (g), and Food Consumptions (g/kg/day) for Charles River Laboratories[1,2]

Parameter	Vehicle			Linuron (100 mg/kg/day)					
	N	LS Mean (SE)	CV (%)	N	LS Mean(SE)	CV (%)	Diff from Vehicle[3]	Ratio to Vehicle (%)[4]	Linear Trend[5]
Body Weight Change (TD8-TD1)	15	5.762 (0.532)	35.8	15	-1.086 (0.532)	-190	-6.848 (0.753)**	-18.843 (9.399)	-7.260 (0.532)**
Body Weight Change (TD15-TD8)	15	4.371 (0.407)	36.1	15	2.314 (0.407)	68.1	-2.057 (0.576)**	52.941 (10.539)	-1.352 (0.407)**
Body Weight Change (TD15-TD1)	15	5.067 (0.374)	28.6	15	0.614 (0.374)	235.8	-4.452 (0.529)**	12.124 (7.435)	-4.306 (0.374)**
Final Body Weight (g)	15	403.400 (7.072)	6.8	15	344.533 (7.072)	7.9	-58.867 (10.001)**	85.407 (2.305)	-57.050 (7.072)**
Food Consumption (TD8-TD1)	15	71.229 (1.885)	10.3	15	48.448 (1.885)	15.1	-22.781 (2.666)**	68.017 (3.201)	-23.876 (1.923)**
Food Consumption (TD15-TD8)	15	61.851 (1.270)	8.0	15	50.378 (2.120)	16.3	-11.473 (2.471)**	81.451 (3.814)	-9.834 (1.828)**
Food Consumption (TD15-TD1)	15	66.540 (1.206)	7.0	15	49.413 (1.867)	14.6	-17.127 (2.223)**	74.261 (3.112)	-17.341 (1.685)**

1. Least squares means and standard errors were estimated based on a one-way ANOVA model applied to all data for each parameter.
2. CV was calculated as residual standard deviation/LS Mean.
3. Significant differences from the vehicle were indicated by "*" for the 0.05 level and "**" for the 0.05/8 level (a Bonferroni adjusted p-level).
4. Ratio to the vehicle was calculated as percent of vehicle group mean. The standard error for the ratio was $Se[R(X, Y)] \approx |1/X| [(Y/X)^2 S_X^2 + S_Y^2]^{1/2} \times 100\%$
5. Linear Contrast $\equiv[-3X_0 - X_1 + X_2 + 3X_3]/[20]^{1/2}$, where X_0 is vehicle, X_1, X_2, and X_3 are the low, mid, and high dosage levels of Linuron respectively. Significant dose trend was indicated by "*" for the 0.05 level and "**" for the 0.05/8 level.

Table 2(cont.). Summary Statistics between Vehicle and Linuron in Adult Intact Male Assay for Body Weight Changes (g/day), Final Body Weight (g), and Food Consumptions (g/kg/day) for Charles River Laboratories[1,2]

Parameter	Vehicle			Linuron (150 mg/kg/day)					
	N	LS Mean (SE)	CV (%)	N	LS Mean(SE)	CV (%)	Diff from Vehicle[3]	Ratio to Vehicle (%)[4]	Linear Trend[5]
Body Weight Change (TD8-TD1)	15	5.762 (0.532)	35.8	15	-4.657 (0.532)	-44.3	-10.419 (0.753)**	-80.826 (11.877)	-7.260 (0.532)**
Body Weight Change (TD15-TD8)	15	4.371 (0.407)	36.1	15	2.438 (0.407)	64.7	-1.933 (0.576)**	55.773 (10.665)	-1.352 (0.407)**
Body Weight Change (TD15-TD1)	15	5.067 (0.374)	28.6	15	-1.110 (0.374)	-131	-6.176 (0.529)**	-21.898 (7.556)	-4.306 (0.374)**
Final Body Weight (g)	15	403.400 (7.072)	6.8	15	321.200 (7.072)	8.5	-82.200 (10.001)**	79.623 (2.241)	-57.050 (7.072)**
Food Consumption (TD8-TD1)	15	71.229 (1.885)	10.3	14	37.530 (1.952)	19.5	-33.699 (2.714)**	52.689 (3.074)	-23.876 (1.923)**
Food Consumption (TD15-TD8)	15	61.851 (1.270)	8.0	14	48.751 (2.195)	16.8	-13.100 (2.536)**	78.821 (3.900)	-9.834 (1.828)**
Food Consumption (TD15-TD1)	15	66.540 (1.206)	7.0	13	42.347 (2.005)	17.1	-24.193 (2.340)**	63.641 (3.227)	-17.341 (1.685)**

1. Least squares means and standard errors were estimated based on a one-way ANOVA model applied to all data for each parameter.
2. CV was calculated as residual standard deviation/LS Mean.
3. Significant differences from the vehicle were indicated by "*" for the 0.05 level and "**" for the 0.05/8 level (a Bonferroni adjusted p-level).
4. Ratio to the vehicle was calculated as percent of vehicle group mean. The standard error for the ratio was $Se[R(X, Y)] \approx |1/X| [(Y/X)^2 S_X^2 + S_Y^2]^{1/2} \times 100\%$
5. Linear Contrast $\equiv [-3X_0 - X_1 + X_2 + 3X_3]/[20]^{1/2}$, where X_0 is vehicle, X_1, X_2, and X_3 are the low, mid, and high dosage levels of Linuron respectively. Significant dose trend was indicated by "*" for the 0.05 level and "**" for the 0.05/8 level.

Table 3. Summary Statistics between Vehicle and Phenobarbital in Adult Intact Male Assay for Body Weight Changes (g/day), Final Body Weight (g), and Food Consumptions (g/kg/day) for Charles River Laboratories[1,2]

Parameter	Vehicle			Phenobarbital (25 mg/kg/day)					
	N	LS Mean (SE)	CV (%)	N	LS Mean (SE)	CV (%)	Diff from Vehicle[3]	Ratio to Vehicle (%)[4]	Linear Trend[5]
Body Weight Change (TD8-TD1)	15	5.762 (0.532)	35.8	15	5.971 (0.532)	34.5	0.210 (0.753)	103.636 (13.302)	-2.594 (0.532)**
Body Weight Change (TD15-TD8)	15	4.371 (0.407)	36.1	15	4.800 (0.407)	32.9	0.429 (0.576)	109.804 (13.834)	0.077 (0.414)
Body Weight Change (TD15-TD1)	15	5.067 (0.374)	28.6	15	5.386 (0.374)	26.9	0.319 (0.529)	106.297 (10.772)	-1.238 (0.380)**
Final Body Weight (g)	15	403.400 (7.072)	6.8	15	412.000 (7.072)	6.6	8.600 (10.001)	102.132 (2.506)	-15.800 (7.185)*
Food Consumption (TD8-TD1)	15	71.229 (1.885)	10.3	15	71.532 (1.885)	10.2	0.303 (2.666)	100.425 (3.751)	-6.819 (1.885)**
Food Consumption (TD15-TD8)	15	61.851 (1.270)	8.0	14	60.411 (1.304)	8.1	-1.440 (1.820)	97.672 (2.909)	1.164 (1.286)
Food Consumption (TD15-TD1)	15	66.540 (1.206)	7.0	14	65.640 (1.036)	5.9	-0.900 (1.590)	98.647 (2.372)	-2.813 (1.114)*

1. Least squares means and standard errors were estimated based on a one-way ANOVA model applied to all data for each parameter.
2. CV was calculated as residual standard deviation/LS Mean.
3. Significant differences from the vehicle were indicated by "*" for the 0.05 level and "**" for the 0.05/8 level (a Bonferroni adjusted p-level).
4. Ratio to the vehicle was calculated as percent of vehicle group mean. The standard error for the ratio was $Se[R(X, Y)] \approx |1/X| [(Y/X)^2 S_X^2 + S_Y^2]^{1/2} \times 100\%$
5. Linear Contrast $= [-3X_0 - X_1 + X_2 + 3X_3]/[20]^{1/2}$, where X_0 is vehicle, X_1, X_2, and X_3 are the low, mid, and high dosage levels of Phenobarbital respectively. Significant dose trend was indicated by "*" for the 0.05 level and "**" for the 0.05/8 level.

Table 3(cont.). **Summary Statistics between Vehicle and Phenobarbital in Adult Intact Male Assay for Body Weight Changes (g/day), Final Body Weight (g), and Food Consumptions (g/kg/day) for Charles River Laboratories[1,2]**

Parameter	Vehicle			Phenobarbital (50 mg/kg/day)					
	N	LS Mean (SE)	CV (%)	N	LS Mean (SE)	CV (%)	Diff from Vehicle[3]	Ratio to Vehicle (%)[4]	Linear Trend[5]
Body Weight Change (TD8-TD1)	15	5.762 (0.532)	35.8	15	5.286 (0.532)	39.0	-0.476 (0.753)	91.736 (12.535)	-2.594 (0.532)**
Body Weight Change (TD15-TD8)	15	4.371 (0.407)	36.1	15	4.790 (0.407)	32.9	0.419 (0.576)	109.586 (13.819)	0.077 (0.414)
Body Weight Change (TD15-TD1)	15	5.067 (0.374)	28.6	15	5.038 (0.374)	28.7	-0.029 (0.529)	99.436 (10.409)	-1.238 (0.380)**
Final Body Weight (g)	15	403.400 (7.072)	6.8	15	407.467 (7.072)	6.7	4.067 (10.001)	101.008 (2.492)	-15.800 (7.185)*
Food Consumption (TD8-TD1)	15	71.229 (1.885)	10.3	15	71.374 (1.885)	10.2	0.145 (2.666)	100.204 (3.747)	-6.819 (1.885)**
Food Consumption (TD15-TD8)	15	61.851 (1.270)	8.0	15	62.464 (1.260)	7.8	0.612 (1.789)	100.990 (2.906)	1.164 (1.286)
Food Consumption (TD15-TD1)	15	66.540 (1.206)	7.0	15	66.919 (1.001)	5.8	0.379 (1.568)	100.569 (2.364)	-2.813 (1.114)*

1. Least squares means and standard errors were estimated based on a one-way ANOVA model applied to all data for each parameter.
2. CV was calculated as residual standard deviation/LS Mean.
3. Significant differences from the vehicle were indicated by "*" for the 0.05 level and "**" for the 0.05/8 level (a Bonferroni adjusted p-level).
4. Ratio to the vehicle was calculated as percent of vehicle group mean. The standard error for the ratio was $Se[R(X, Y)] \approx |1/X| [(Y/X)^2 S_X^2 + S_Y^2]^{1/2} \times 100\%$
5. Linear Contrast $=[-3X_0 - X_1 + X_2 + 3X_3]/[20]^{1/2}$, where X_0 is vehicle, X_1, X_2, and X_3 are the low, mid, and high dosage levels of Phenobarbital respectively. Significant dose trend was indicated by "*" for the 0.05 level and "**" for the 0.05/8 level.

Table 3(cont.). Summary Statistics between Vehicle and Phenobarbital in Adult Intact Male Assay for Body Weight Changes (g/day), Final Body Weight (g), and Food Consumptions (g/kg/day) for Charles River Laboratories[1,2]

Parameter	Vehicle			Phenobarbital (100 mg/kg/day)					
	N	LS Mean (SE)	CV (%)	N	LS Mean(SE)	CV (%)	Diff from Vehicle[3]	Ratio to Vehicle (%)[4]	Linear Trend[5]
Body Weight Change (TD8-TD1)	15	5.762 (0.532)	35.8	15	2.124 (0.532)	97.1	-3.638 (0.753)**	36.860 (9.844)	-2.594 (0.532)**
Body Weight Change (TD15-TD8)	15	4.371 (0.407)	36.1	14	4.490 (0.421)	35.1	0.118 (0.586)	102.708 (13.583)	0.077 (0.414)
Body Weight Change (TD15-TD1)	15	5.067 (0.374)	28.6	14	3.337 (0.387)	43.4	-1.730 (0.538)**	65.857 (9.055)	-1.238 (0.380)**
Final Body Weight (g)	15	403.400 (7.072)	6.8	14	381.357 (7.320)	7.2	-22.043 (10.178)*	94.536 (2.458)	-15.800 (7.185)*
Food Consumption (TD8-TD1)	15	71.229 (1.885)	10.3	15	61.116 (1.885)	11.9	-10.113 (2.666)**	85.803 (3.488)	-6.819 (1.885)**
Food Consumption (TD15-TD8)	15	61.851 (1.270)	8.0	14	62.902 (1.304)	7.8	1.051 (1.820)	101.699 (2.967)	1.164 (1.286)
Food Consumption (TD15-TD1)	15	66.540 (1.206)	7.0	14	61.920 (1.036)	6.3	-4.620 (1.590)*	93.057 (2.296)	-2.813 (1.114)*

1. Least squares means and standard errors were estimated based on a one-way ANOVA model applied to all data for each parameter.
2. CV was calculated as residual standard deviation/LS Mean.
3. Significant differences from the vehicle were indicated by "*" for the 0.05 level and "**" for the 0.05/8 level (a Bonferroni adjusted p-level).
4. Ratio to the vehicle was calculated as percent of vehicle group mean. The standard error for the ratio was $Se[R(X, Y)] \approx |1/X| [(Y/X)^2 S_X^2 + S_Y^2]^{1/2} \times 100\%$
5. Linear Contrast $\equiv [-3X_0 - X_1 + X_2 + 3X_3]/[20]^{1/2}$ where X_0 is vehicle, X_1, X_2, and X_3 are the low, mid, and high dosage levels of Phenobarbital respectively. Significant dose trend was indicated by "*" for the 0.05 level and "**" for the 0.05/8 level.

Table 4. Summary Statistics between Vehicle and Linuron in Adult Intact Male Assay for Unadjusted Organ Weights (g) and Adjusted Organ Weights for Charles River Laboratories[1,2,6]

Parameter	Vehicle			Linuron (50 mg/kg/day)					
	N	LS Mean (SE)	CV (%)	N	LS Mean(SE)	CV (%)	Diff from Vehicle[3]	Ratio to Vehicle (%)[4]	Linear Trend[5]
Liver	15	14.051 (0.458)	12.6	15	11.945 (0.458)	14.9	-2.106 (0.648)**	85.014 (4.279)	-1.129 (0.458)*
Right Testis	15	1.657 (0.033)	7.8	15	1.726 (0.033)	7.4	0.069 (0.047)	104.172 (2.892)	-0.021 (0.033)
Left Testis	15	1.680 (0.032)	7.3	15	1.739 (0.032)	7.1	0.059 (0.045)	103.484 (2.730)	-0.022 (0.032)
Paired Testes	15	3.337 (0.063)	7.4	15	3.465 (0.063)	7.1	0.128 (0.090)	103.825 (2.743)	-0.043 (0.063)
Paired Epididymides	15	1.223 (0.029)	9.2	15	1.156 (0.029)	9.7	-0.067 (0.041)	94.545 (3.269)	-0.115 (0.029)**
Entire Prostate	15	1.106 (0.057)	19.9	15	1.002 (0.057)	22.0	-0.104 (0.080)	90.559 (6.933)	-0.209 (0.057)**
Seminal Vesicles with fluid and Coagulating Gland	15	1.336 (0.073)	21.2	15	1.157 (0.073)	24.4	-0.179 (0.103)	86.625 (7.230)	-0.263 (0.073)**
Accessory Sex Gland	15	2.442 (0.101)	16.0	15	2.159 (0.101)	18.1	-0.283 (0.143)*	88.407 (5.511)	-0.473 (0.101)**
Thyroid Glands	15	0.029 (0.002)	21.4	15	0.034 (0.003)	32.5	0.004 (0.003)	114.156 (11.471)	-0.003 (0.002)
Adj Liver	15	3.480 (0.074)	8.2	15	3.386 (0.074)	8.4	-0.094 (0.104)	97.305 (2.950)	0.260 (0.074)**
Adj Right Testis	15	0.412 (0.010)	9.2	15	0.491 (0.010)	7.7	0.079 (0.014)**	119.060 (3.693)	0.066 (0.010)**
Adj Left Testis	15	0.418 (0.010)	9.5	15	0.495 (0.010)	8.0	0.077 (0.015)**	118.353 (3.803)	0.067 (0.010)**
Adj Paired Testes	15	0.831 (0.020)	9.2	15	0.986 (0.020)	7.7	0.155 (0.028)**	118.704 (3.676)	0.133 (0.020)**
Adj Paired Epididymides	15	0.305 (0.009)	11.7	15	0.329 (0.009)	10.8	0.024 (0.013)	107.947 (4.449)	0.017 (0.009)
Adj Entire Prostate	15	0.278 (0.016)	22.8	15	0.285 (0.016)	22.2	0.008 (0.023)	102.704 (8.436)	-0.020 (0.016)
Adj Seminal Vesicles with fluid and Coagulating Gland	15	0.333 (0.020)	23.4	15	0.327 (0.020)	23.9	-0.006 (0.029)	98.105 (8.474)	-0.025 (0.020)
Adj Accessory Sex Gland	15	0.611 (0.029)	18.7	15	0.613 (0.029)	18.6	0.001 (0.042)	100.195 (6.820)	-0.046 (0.029)
Adj Thyroid Glands	15	0.007 (0.000)	23.1	15	0.010 (0.001)	33.5	0.002 (0.001)*	129.691 (13.612)	0.001 (0.001)

1. Least squares means and standard errors were estimated based on a one-way ANOVA model applied to all data for each parameter.
2. CV was calculated as residual standard deviation/LS Mean.
3. Significant differences from the vehicle were indicated by "*" for the 0.05 level and "**" for the 0.05/8 level (a Bonferroni adjusted p-level).
4. Ratio to the vehicle was calculated as percent of vehicle group mean. The standard error for the ratio was $Se[R(X, Y)] \approx |1/X| [(Y/X)^2 S_X^2 + S_Y^2]^{1/2} \times 100\%$
5. Linear Contrast $=[-3X_0 - X_1 + X_2 + 3X_3]/[20]^{1/2}$ where X_0 is vehicle, X_1, X_2, and X_3 are the low, mid, and high dosage levels. Significant dose trend was indicated by "*" for the 0.05 level and "**" for the 0.05/8 level.
6. Adjusted organ weights are defined as organ weight to final body weight ratios (expressed as %).

Table 4(cont.). Summary Statistics between Vehicle and Linuron in Adult Intact Male Assay for Unadjusted Organ Weights (g) and Adjusted Organ Weights for Charles River Laboratories[1,2,6]

Parameter	Vehicle			Linuron (100 mg/kg/day)					
	N	LS Mean (SE)	CV (%)	N	LS Mean (SE)	CV (%)	Diff from Vehicle[3]	Ratio to Vehicle (%)[4]	Linear Trend[5]
Liver	15	14.051 (0.458)	12.6	15	12.335 (0.458)	14.4	-1.717 (0.648)*	87.784 (4.338)	-1.129 (0.458)*
Right Testis	15	1.657 (0.033)	7.8	15	1.666 (0.033)	7.7	0.010 (0.047)	100.590 (2.840)	-0.021 (0.033)
Left Testis	15	1.680 (0.032)	7.3	15	1.680 (0.032)	7.3	-0.000 (0.045)	99.995 (2.683)	-0.022 (0.032)
Paired Testes	15	3.337 (0.063)	7.4	15	3.347 (0.063)	7.3	0.010 (0.090)	100.290 (2.695)	-0.043 (0.063)
Paired Epididymides	15	1.223 (0.029)	9.2	15	1.136 (0.029)	9.9	-0.087 (0.041)*	92.895 (3.242)	-0.115 (0.029)**
Entire Prostate	15	1.106 (0.057)	19.9	15	0.928 (0.057)	23.7	-0.178 (0.080)*	83.941 (6.709)	-0.209 (0.057)**
Seminal Vesicles with fluid and Coagulating Gland	15	1.336 (0.073)	21.2	15	1.155 (0.073)	24.5	-0.181 (0.103)	86.441 (7.224)	-0.263 (0.073)**
Accessory Sex Gland	15	2.442 (0.101)	16.0	15	2.083 (0.101)	18.7	-0.359 (0.143)*	85.309 (5.428)	-0.473 (0.101)**
Thyroid Glands	15	0.029 (0.002)	21.4	15	0.031 (0.003)	35.2	0.002 (0.003)	105.459 (11.212)	-0.003 (0.002)
Adj Liver	15	3.480 (0.074)	8.2	15	3.578 (0.074)	8.0	0.099 (0.104)	102.832 (3.033)	0.260 (0.074)**
Adj Right Testis	15	0.412 (0.010)	9.2	15	0.484 (0.010)	7.8	0.072 (0.014)**	117.446 (3.663)	0.066 (0.010)**
Adj Left Testis	15	0.418 (0.010)	9.5	15	0.489 (0.010)	8.1	0.071 (0.015)**	116.859 (3.775)	0.067 (0.010)**
Adj Paired Testes	15	0.831 (0.020)	9.2	15	0.973 (0.020)	7.8	0.142 (0.028)**	117.151 (3.648)	0.133 (0.020)**
Adj Paired Epididymides	15	0.305 (0.009)	11.7	15	0.330 (0.009)	10.8	0.026 (0.013)	108.374 (4.458)	0.017 (0.009)
Adj Entire Prostate	15	0.278 (0.016)	22.8	15	0.269 (0.016)	23.5	-0.009 (0.023)	96.795 (8.191)	-0.020 (0.016)
Adj Seminal Vesicles with fluid and Coagulating Gland	15	0.333 (0.020)	23.4	15	0.333 (0.020)	23.4	-0.000 (0.029)	99.975 (8.553)	-0.025 (0.020)
Adj Accessory Sex Gland	15	0.611 (0.029)	18.7	15	0.602 (0.029)	18.9	-0.009 (0.042)	98.530 (6.764)	-0.046 (0.029)
Adj Thyroid Glands	15	0.007 (0.000)	23.1	15	0.009 (0.001)	35.5	0.002 (0.001)	122.470 (13.373)	0.001 (0.001)

1. Least squares means and standard errors were estimated based on a one-way ANOVA model applied to all data for each parameter.
2. CV was calculated as residual standard deviation/LS Mean.
3. Significant differences from the vehicle were indicated by "*" for the 0.05 level and "**" for the 0.05/8 level (a Bonferroni adjusted p-level).
4. Ratio to the vehicle was calculated as percent of vehicle group mean. The standard error for the ratio was $Se[R(X, Y)] \approx |1/X| [(Y/X)^2 S_X^2 + S_Y^2]^{1/2} \times 100\%$
5. Linear Contrast $= [-3X_0 - X_1 + X_2 + 3X_3]/[20]^{1/2}$, where X_0 is vehicle, X_1, X_2, and X_3 are the low, mid, and high dosage levels. Significant dose trend was indicated by "*" for the 0.05 level and "**" for the 0.05/8 level.
6. Adjusted organ weights are defined as organ weight to final body weight ratios (expressed as %).

Table 4(cont.). Summary Statistics between Vehicle and Linuron in Adult Intact Male Assay for Unadjusted Organ Weights (g) and Adjusted Organ Weights for Charles River Laboratories[1,2,6]

Parameter	Vehicle			Linuron (150 mg/kg/day)					
	N	LS Mean (SE)	CV (%)	N	LS Mean (SE)	CV (%)	Diff from Vehicle[3]	Ratio to Vehicle (%)[4]	Linear Trend[5]
Liver	15	14.051 (0.458)	12.6	15	12.239 (0.458)	14.5	-1.812 (0.648)**	87.101 (4.323)	-1.129 (0.458)*
Right Testis	15	1.657 (0.033)	7.8	15	1.645 (0.033)	7.8	-0.011 (0.047)	99.310 (2.822)	-0.021 (0.033)
Left Testis	15	1.680 (0.032)	7.3	15	1.667 (0.032)	7.4	-0.014 (0.045)	99.183 (2.672)	-0.022 (0.032)
Paired Testes	15	3.337 (0.063)	7.4	15	3.312 (0.063)	7.4	-0.025 (0.090)	99.246 (2.681)	-0.043 (0.063)
Paired Epididymides	15	1.223 (0.029)	9.2	15	1.058 (0.029)	10.6	-0.165 (0.041)**	86.479 (3.140)	-0.115 (0.029)**
Entire Prostate	15	1.106 (0.057)	19.9	15	0.818 (0.057)	26.9	-0.288 (0.080)**	73.991 (6.393)	-0.209 (0.057)**
Seminal Vesicles with fluid and Coagulating Gland	15	1.336 (0.073)	21.2	15	0.944 (0.073)	29.9	-0.392 (0.103)**	70.685 (6.692)	-0.263 (0.073)**
Accessory Sex Gland	15	2.442 (0.101)	16.0	15	1.762 (0.101)	22.2	-0.679 (0.143)**	72.183 (5.093)	-0.473 (0.101)**
Thyroid Glands	15	0.029 (0.002)	21.4	15	0.027 (0.003)	41.1	-0.003 (0.003)	90.170 (10.796)	-0.003 (0.002)
Adj Liver	15	3.480 (0.074)	8.2	15	3.802 (0.074)	7.5	0.323 (0.104)**	109.276 (3.132)	0.260 (0.074)**
Adj Right Testis	15	0.412 (0.010)	9.2	15	0.513 (0.010)	7.4	0.101 (0.014)**	124.393 (3.790)	0.066 (0.010)**
Adj Left Testis	15	0.418 (0.010)	9.5	15	0.520 (0.010)	7.6	0.102 (0.015)**	124.278 (3.915)	0.067 (0.010)**
Adj Paired Testes	15	0.831 (0.020)	9.2	15	1.033 (0.020)	7.4	0.202 (0.028)**	124.335 (3.779)	0.133 (0.020)**
Adj Paired Epididymides	15	0.305 (0.009)	11.7	15	0.330 (0.009)	10.8	0.025 (0.013)	108.077 (4.452)	0.017 (0.009)
Adj Entire Prostate	15	0.278 (0.016)	22.8	15	0.253 (0.016)	25.0	-0.025 (0.023)	91.078 (7.960)	-0.020 (0.016)
Adj Seminal Vesicles with fluid and Coagulating Gland	15	0.333 (0.020)	23.4	15	0.294 (0.020)	26.6	-0.040 (0.029)	88.013 (8.058)	-0.025 (0.020)
Adj Accessory Sex Gland	15	0.611 (0.029)	18.7	15	0.547 (0.029)	20.9	-0.065 (0.042)	89.406 (6.463)	-0.046 (0.029)
Adj Thyroid Glands	15	0.007 (0.000)	23.1	15	0.008 (0.001)	38.3	0.001 (0.001)	113.372 (13.085)	0.001 (0.001)

1. Least squares means and standard errors were estimated based on a one-way ANOVA model applied to all data for each parameter.
2. CV was calculated as residual standard deviation/LS Mean.
3. Significant differences from the vehicle were indicated by "*" for the 0.05 level and "**" for the 0.05/8 level (a Bonferroni adjusted p-level).
4. Ratio to the vehicle was calculated as percent of vehicle group mean. The standard error for the ratio was $Se[R(X, Y)] \approx |1/X| [(Y/X)^2 S_X^2 + S_Y^2]^{1/2} \times 100\%$
5. Linear Contrast $=[-3X_0 - X_1 + X_2 + 3X_3]/[20]^{1/2}$ where X_0 is vehicle, X_1, X_2, and X_3 are the low, mid, and high dosage levels. Significant dose trend was indicated by "*" for the 0.05 level and "**" for the 0.05/8 level.
6. Adjusted organ weights are defined as organ weight to final body weight ratios (expressed as %).

Table 5. Summary Statistics between Vehicle and Phenobarbital in Adult Intact Male Assay for Unadjusted Organ Weights (g) and Adjusted Organ Weights for Charles River Laboratories[1,2,6]

Parameter	Vehicle			Phenobarbital (25 mg/kg/day)					
	N	LS Mean (SE)	CV (%)	N	LS Mean (SE)	CV (%)	Diff from Vehicle[3]	Ratio to Vehicle (%)[4]	Linear Trend[5]
Liver	15	14.051 (0.458)	12.6	15	18.182 (0.458)	9.8	4.130 (0.648)**	129.396 (5.331)	4.441 (0.465)**
Right Testis	15	1.657 (0.033)	7.8	15	1.677 (0.033)	7.7	0.020 (0.047)	101.227 (2.849)	0.045 (0.034)
Left Testis	15	1.680 (0.032)	7.3	15	1.708 (0.032)	7.2	0.028 (0.045)	101.637 (2.705)	0.032 (0.032)
Paired Testes	15	3.337 (0.063)	7.4	15	3.385 (0.063)	7.3	0.048 (0.090)	101.434 (2.710)	0.077 (0.065)
Paired Epididymides	15	1.223 (0.029)	9.2	15	1.190 (0.029)	9.5	-0.033 (0.041)	97.303 (3.314)	0.031 (0.030)
Entire Prostate	15	1.106 (0.057)	19.9	15	1.139 (0.057)	19.3	0.033 (0.080)	102.961 (7.376)	-0.028 (0.058)
Seminal Vesicles with fluid and Coagulating Gland	15	1.336 (0.073)	21.2	15	1.366 (0.073)	20.7	0.030 (0.103)	102.275 (7.817)	0.009 (0.074)
Accessory Sex Gland	15	2.442 (0.101)	16.0	15	2.505 (0.101)	15.6	0.063 (0.143)	102.586 (5.916)	-0.018 (0.102)
Thyroid Glands	15	0.029 (0.002)	21.4	15	0.039 (0.002)	20.8	0.009 (0.003)**	131.212 (10.124)	0.004 (0.002)*
Adj Liver	15	3.480 (0.074)	8.2	15	4.408 (0.074)	6.5	0.929 (0.104)**	126.684 (3.413)	1.304 (0.075)**
Adj Right Testis	15	0.412 (0.010)	9.2	15	0.409 (0.010)	9.3	-0.004 (0.014)	99.131 (3.344)	0.028 (0.010)*
Adj Left Testis	15	0.418 (0.010)	9.5	15	0.417 (0.010)	9.5	-0.002 (0.015)	99.552 (3.463)	0.025 (0.010)*
Adj Paired Testes	15	0.831 (0.020)	9.2	15	0.825 (0.020)	9.2	-0.005 (0.028)	99.343 (3.339)	0.053 (0.020)*
Adj Paired Epididymides	15	0.305 (0.009)	11.7	15	0.291 (0.009)	12.3	-0.014 (0.013)	95.362 (4.178)	0.020 (0.009)*
Adj Entire Prostate	15	0.278 (0.016)	22.8	15	0.279 (0.016)	22.7	0.001 (0.023)	100.376 (8.338)	0.002 (0.017)
Adj Seminal Vesicles with fluid and Coagulating Gland	15	0.333 (0.020)	23.4	15	0.336 (0.020)	23.3	0.002 (0.029)	100.722 (8.585)	0.013 (0.020)
Adj Accessory Sex Gland	15	0.611 (0.029)	18.7	15	0.615 (0.029)	18.6	0.003 (0.042)	100.565 (6.833)	0.016 (0.030)
Adj Thyroid Glands	15	0.007 (0.000)	23.1	15	0.009 (0.001)	20.8	0.002 (0.001)**	128.195 (10.286)	0.001 (0.000)**

1. Least squares means and standard errors were estimated based on a one-way ANOVA model applied to all data for each parameter.
2. CV was calculated as residual standard deviation/LS Mean.
3. Significant differences from the vehicle were indicated by "*" for the 0.05 level and "**" for the 0.05/8 level (a Bonferroni adjusted p-level).
4. Ratio to the vehicle was calculated as percent of vehicle group mean. The standard error for the ratio was $Se[R(X, Y)] \approx |1/X| [(Y/X)^2 S_X^2 + S_Y^2]^{1/2} \times 100\%$
5. Linear Contrast $\equiv [-3X_0 - X_1 + X_2 + 3X_3]/[20]^{1/2}$, where X_0 is vehicle, X_1, X_2, and X_3 are the low, mid, and high dosage levels. Significant dose trend was indicated by "*" for the 0.05 level and "**" for the 0.05/8 level.
6. Adjusted organ weights are defined as organ weight to final body weight ratios (expressed as %).

Table 5(cont.). Summary Statistics between Vehicle and Phenobarbital in Adult Intact Male Assay for Unadjusted Organ Weights (g) and Adjusted Organ Weights for Charles River Laboratories[1,2,6]

Parameter	Vehicle			Phenobarbital (50 mg/kg/day)					
	N	LS Mean (SE)	CV (%)	N	LS Mean (SE)	CV (%)	Diff from Vehicle[3]	Ratio to Vehicle (%)[4]	Linear Trend[5]
Liver	15	14.051 (0.458)	12.6	15	18.906 (0.458)	9.4	4.854 (0.648)**	134.548 (5.465)	4.441 (0.465)**
Right Testis	15	1.657 (0.033)	7.8	15	1.711 (0.033)	7.5	0.054 (0.047)	103.277 (2.879)	0.045 (0.034)
Left Testis	15	1.680 (0.032)	7.3	15	1.690 (0.032)	7.3	0.010 (0.045)	100.582 (2.691)	0.032 (0.032)
Paired Testes	15	3.337 (0.063)	7.4	15	3.401 (0.063)	7.2	0.064 (0.090)	101.920 (2.717)	0.077 (0.065)
Paired Epididymides	15	1.223 (0.029)	9.2	15	1.235 (0.029)	9.1	0.012 (0.041)	101.001 (3.376)	0.031 (0.030)
Entire Prostate	15	1.106 (0.057)	19.9	15	1.206 (0.057)	18.3	0.100 (0.080)	109.046 (7.603)	-0.028 (0.058)
Seminal Vesicles with fluid and Coagulating Gland	15	1.336 (0.073)	21.2	15	1.409 (0.073)	20.1	0.073 (0.103)	105.491 (7.944)	0.009 (0.074)
Accessory Sex Gland	15	2.442 (0.101)	16.0	15	2.615 (0.101)	14.9	0.173 (0.143)	107.101 (6.050)	-0.018 (0.102)
Thyroid Glands	15	0.029 (0.002)	21.4	15	0.036 (0.002)	22.2	0.007 (0.003)*	123.194 (9.810)	0.004 (0.002)*
Adj Liver	15	3.480 (0.074)	8.2	15	4.634 (0.074)	6.1	1.154 (0.104)**	133.164 (3.521)	1.304 (0.075)**
Adj Right Testis	15	0.412 (0.010)	9.2	15	0.421 (0.010)	9.0	0.009 (0.014)	102.064 (3.393)	0.028 (0.010)*
Adj Left Testis	15	0.418 (0.010)	9.5	15	0.416 (0.010)	9.6	-0.002 (0.015)	99.417 (3.461)	0.025 (0.010)*
Adj Paired Testes	15	0.831 (0.020)	9.2	15	0.837 (0.020)	9.1	0.006 (0.028)	100.731 (3.362)	0.053 (0.020)*
Adj Paired Epididymides	15	0.305 (0.009)	11.7	15	0.305 (0.009)	11.7	-0.000 (0.013)	99.892 (4.273)	0.020 (0.009)*
Adj Entire Prostate	15	0.278 (0.016)	22.8	15	0.298 (0.016)	21.2	0.020 (0.023)	107.269 (8.631)	0.002 (0.017)
Adj Seminal Vesicles with fluid and Coagulating Gland	15	0.333 (0.020)	23.4	15	0.349 (0.020)	22.4	0.016 (0.029)	104.673 (8.756)	0.013 (0.020)
Adj Accessory Sex Gland	15	0.611 (0.029)	18.7	15	0.647 (0.029)	17.6	0.036 (0.042)	105.853 (7.016)	0.016 (0.030)
Adj Thyroid Glands	15	0.007 (0.000)	23.1	15	0.009 (0.001)	22.0	0.002 (0.001)*	121.560 (9.996)	0.001 (0.000)**

1. Least squares means and standard errors were estimated based on a one-way ANOVA model applied to all data for each parameter.
2. CV was calculated as residual standard deviation/LS Mean.
3. Significant differences from the vehicle were indicated by "*" for the 0.05 level and "**" for the 0.05/8 level (a Bonferroni adjusted p-level).
4. Ratio to the vehicle was calculated as percent of vehicle group mean. The standard error for the ratio was $Se[R(X, Y)] \approx |1/X| [(Y/X)^2 S_X^2 + S_Y^2]^{1/2} \times 100\%$
5. Linear Contrast $=[-3X_0 - X_1 + X_2 + 3X_3]/[20]^{1/2}$, where X_0 is vehicle, X_1, X_2, and X_3 are the low, mid, and high dosage levels. Significant dose trend was indicated by "*" for the 0.05 level and "**" for the 0.05/8 level.
6. Adjusted organ weights are defined as organ weight to final body weight ratios (expressed as %).

Table 5(cont.). Summary Statistics between Vehicle and Phenobarbital in Adult Intact Male Assay for Unadjusted Organ Weights (g) and Adjusted Organ Weights for Charles River Laboratories[1,2,6]

Parameter	Vehicle			Phenobarbital (100 mg/kg/day)					
	N	LS Mean (SE)	CV (%)	N	LS Mean (SE)	CV (%)	Diff from Vehicle[3]	Ratio to Vehicle (%)[4]	Linear Trend[5]
Liver	15	14.051 (0.458)	12.6	14	20.430 (0.474)	8.7	6.379 (0.659)**	145.395 (5.818)	4.441 (0.465)**
Right Testis	15	1.657 (0.033)	7.8	14	1.713 (0.034)	7.5	0.056 (0.048)	103.396 (2.930)	0.045 (0.034)
Left Testis	15	1.680 (0.032)	7.3	14	1.734 (0.033)	7.1	0.054 (0.046)	103.204 (2.773)	0.032 (0.032)
Paired Testes	15	3.337 (0.063)	7.4	14	3.447 (0.066)	7.1	0.110 (0.091)	103.299 (2.782)	0.077 (0.065)
Paired Epididymides	15	1.223 (0.029)	9.2	14	1.255 (0.030)	9.0	0.032 (0.042)	102.591 (3.462)	0.031 (0.030)
Entire Prostate	15	1.106 (0.057)	19.9	14	1.042 (0.059)	21.1	-0.064 (0.082)	94.225 (7.193)	-0.028 (0.058)
Seminal Vesicles with fluid and Coagulating Gland	15	1.336 (0.073)	21.2	14	1.335 (0.076)	21.2	-0.000 (0.105)	99.966 (7.864)	0.009 (0.074)
Accessory Sex Gland	15	2.442 (0.101)	16.0	14	2.377 (0.104)	16.4	-0.064 (0.145)	97.365 (5.868)	-0.018 (0.102)
Thyroid Glands	15	0.029 (0.002)	21.4	14	0.037 (0.002)	21.9	0.007 (0.003)*	124.519 (10.039)	0.004 (0.002)*
Adj Liver	15	3.480 (0.074)	8.2	14	5.349 (0.076)	5.3	1.869 (0.106)**	153.712 (3.919)	1.304 (0.075)**
Adj Right Testis	15	0.412 (0.010)	9.2	14	0.450 (0.010)	8.4	0.037 (0.014)*	109.061 (3.571)	0.028 (0.010)*
Adj Left Testis	15	0.418 (0.010)	9.5	14	0.456 (0.011)	8.7	0.038 (0.015)*	109.040 (3.690)	0.025 (0.010)*
Adj Paired Testes	15	0.831 (0.020)	9.2	14	0.906 (0.020)	8.4	0.075 (0.028)*	109.050 (3.561)	0.053 (0.020)*
Adj Paired Epididymides	15	0.305 (0.009)	11.7	14	0.330 (0.010)	10.8	0.025 (0.013)	108.355 (4.530)	0.020 (0.009)*
Adj Entire Prostate	15	0.278 (0.016)	22.8	14	0.275 (0.017)	23.0	-0.003 (0.024)	98.998 (8.429)	0.002 (0.017)
Adj Seminal Vesicles with fluid and Coagulating Gland	15	0.333 (0.020)	23.4	14	0.349 (0.021)	22.4	0.015 (0.029)	104.635 (8.903)	0.013 (0.020)
Adj Accessory Sex Gland	15	0.611 (0.029)	18.7	14	0.624 (0.030)	18.3	0.013 (0.042)	102.073 (7.004)	0.016 (0.030)
Adj Thyroid Glands	15	0.007 (0.000)	23.1	14	0.010 (0.001)	20.4	0.002 (0.001)**	130.770 (10.562)	0.001 (0.000)**

1. Least squares means and standard errors were estimated based on a one-way ANOVA model applied to all data for each parameter.
2. CV was calculated as residual standard deviation/LS Mean.
3. Significant differences from the vehicle were indicated by "*" for the 0.05 level and "**" for the 0.05/8 level (a Bonferroni adjusted p-level).
4. Ratio to the vehicle was calculated as percent of vehicle group mean. The standard error for the ratio was $Se[R(X, Y)] \approx |1/X| [(Y/X)^2 S_X^2 + S_Y^2]^{1/2} \times 100\%$
5. Linear Contrast $\equiv [-3X_0 - X_1 + X_2 + 3X_3]/[20]^{1/2}$ where X_0 is vehicle, X_1, X_2, and X_3 are the low, mid, and high dosage levels. Significant dose trend was indicated by "*" for the 0.05 level and "**" for the 0.05/8 level.
6. Adjusted organ weights are defined as organ weight to final body weight ratios (expressed as %).

Table 6. Summary Statistics between Vehicle and Linuron in Adult Intact Male Assay for Hormonal Parameters for Charles River Laboratories[1,2]

Parameter	Vehicle			Linuron (50 mg/kg/day)					
	N	LS Mean (SE)	CV (%)	N	LS Mean(SE)	CV (%)	Diff from Vehicle[3]	Ratio to Vehicle (%)[4]	Linear Trend[5]
Testosterone (ng/ml)	15	9.927 (1.873)	73.1	14	4.826 (0.960)	74.4	-5.101 (2.105)*	48.618 (13.330)	-4.651 (1.366)**
LH (ng/ml)	15	2.178 (0.127)	22.6	15	1.753 (0.095)	21.1	-0.425 (0.159)*	80.502 (6.420)	-0.204 (0.152)
TSH (ng/ml)	15	13.095 (1.681)	49.7	14	9.844 (0.601)	22.8	-3.251 (1.785)	75.172 (10.686)	-1.236 (1.320)
T4 (µg/dl)	15	4.729 (0.151)	12.4	15	3.099 (0.151)	18.9	-1.631 (0.213)**	65.520 (3.816)	-2.427 (0.151)**
T3 (ng/dl)	15	81.653 (2.593)	12.3	15	68.196 (2.593)	14.7	-13.457 (3.667)**	83.520 (4.138)	-12.103 (2.593)**
FSH (ng/ml)	15	14.807 (0.559)	14.6	14	14.269 (0.579)	15.2	-0.538 (0.804)	96.366 (5.338)	1.042 (0.560)
Estradiol (pg/ml)	15	25.403 (1.530)	23.3	15	33.199 (1.530)	17.8	7.795 (2.164)**	130.686 (9.911)	10.008 (1.554)**
Prolactin (ng/ml)	15	36.476 (6.999)	74.3	14	4.735 (1.082)	85.5	-31.741 (7.083)**	12.981 (3.874)	-22.928 (4.714)**
DHT (pg/ml)	15	487.734 (63.273)	50.2	14	345.972 (37.905)	41.0	-141.762 (73.758)	70.935 (12.045)	-123.388 (54.655)*

1. Least squares means and standard errors were estimated based on a one-way ANOVA model applied to the data for each parameter, after excluding some extreme observations.
2. CV was calculated as residual standard deviation/LS Mean.
3. Significant differences from the vehicle were indicated by "*" for the 0.05 level and "**" for the 0.05/8 level (a Bonferroni adjusted p-level).
4. Ratio to the vehicle was calculated as percent of vehicle group mean. The standard error for the ratio was $Se[R(X, Y)] \approx |1/X| [(Y/X)^2 S_X^2 + S_Y^2]^{1/2} \times 100\%$
5. Linear Contrast $\equiv[-3X_0 - X_1 + X_2 + 3X_3]/[20]^{1/2}$, where X_0 is vehicle, X_1, X_2, and X_3 are the low, mid, and high dosage levels of Linuron respectively. Significant dose trend was indicated by "*" for the 0.05 level and "**" for the 0.05/8 level.

Table 6 (cont.). Summary Statistics between Vehicle and Linuron in Adult Intact Male Assay for Hormonal Parameters for Charles River Laboratories[1,2]

Parameter	Vehicle			Linuron (100 mg/kg/day)					
	N	LS Mean (SE)	CV (%)	N	LS Mean(SE)	CV (%)	Diff from Vehicle[3]	Ratio to Vehicle (%)[4]	Linear Trend[5]
Testosterone (ng/ml)	15	9.927 (1.873)	73.1	14	3.981 (1.065)	100.1	-5.947 (2.154)*	40.099 (13.124)	-4.651 (1.366)**
LH (ng/ml)	15	2.178 (0.127)	22.6	15	1.782 (0.137)	29.7	-0.396 (0.187)*	81.818 (7.882)	-0.204 (0.152)
TSH (ng/ml)	15	13.095 (1.681)	49.7	15	12.212 (1.519)	48.2	-0.883 (2.266)	93.259 (16.670)	-1.236 (1.320)
T4 (µg/dl)	15	4.729 (0.151)	12.4	15	1.819 (0.151)	32.1	-2.910 (0.213)**	38.469 (3.420)	-2.427 (0.151)**
T3 (ng/dl)	15	81.653 (2.593)	12.3	15	65.621 (2.593)	15.3	-16.032 (3.667)**	80.366 (4.074)	-12.103 (2.593)**
FSH (ng/ml)	15	14.807 (0.559)	14.6	15	15.622 (0.559)	13.9	0.815 (0.790)	105.507 (5.487)	1.042 (0.560)
Estradiol (pg/ml)	15	25.403 (1.530)	23.3	15	40.946 (1.530)	14.5	15.543 (2.164)**	161.184 (11.424)	10.008 (1.554)**
Prolactin (ng/ml)	15	36.476 (6.999)	74.3	15	5.566 (1.190)	82.8	-30.910 (7.100)**	15.259 (4.383)	-22.928 (4.714)**
DHT (pg/ml)	15	487.734 (63.273)	50.2	15	357.769 (69.584)	75.3	-129.965 (94.050)	73.353 (17.149)	-123.388 (54.655)*

1. Least squares means and standard errors were estimated based on a one-way ANOVA model applied to the data for each parameter, after excluding some extreme observations.
2. CV was calculated as residual standard deviation/LS Mean.
3. Significant differences from the vehicle were indicated by "*" for the 0.05 level and "**" for the 0.05/8 level (a Bonferroni adjusted p-level).
4. Ratio to the vehicle was calculated as percent of vehicle group mean. The standard error for the ratio was $Se[R(X, Y)] \approx |1/X| [(Y/X)^2 S_X^2 + S_Y^2]^{1/2} \times 100\%$
5. Linear Contrast $\equiv [-3X_0 - X_1 + X_2 + 3X_3]/[20]^{1/2}$, where X_0 is vehicle, X_1, X_2, and X_3 are the low, mid, and high dosage levels of Linuron respectively. Significant dose trend was indicated by "*" for the 0.05 level and "**" for the 0.05/8 level.

Table 6 (cont.). Summary Statistics between Vehicle and Linuron in Adult Intact Male Assay for Hormonal Parameters for Charles River Laboratories[1,2]

Parameter	Vehicle			Linuron (150 mg/kg/day)					
	N	LS Mean (SE)	CV (%)	N	LS Mean(SE)	CV (%)	Diff from Vehicle[3]	Ratio to Vehicle (%)[4]	Linear Trend[5]
Testosterone (ng/ml)	15	9.927 (1.873)	73.1	14	3.276 (0.641)	73.2	-6.651 (1.980)**	33.004 (8.970)	-4.651 (1.366)**
LH (ng/ml)	15	2.178 (0.127)	22.6	15	1.865 (0.179)	37.1	-0.313 (0.219)	85.614 (9.600)	-0.204 (0.152)
TSH (ng/ml)	15	13.095 (1.681)	49.7	15	10.463 (0.866)	32.1	-2.631 (1.891)	79.905 (12.208)	-1.236 (1.320)
T4 (μg/dl)	15	4.729 (0.151)	12.4	15	1.537 (0.151)	38.0	-3.192 (0.213)**	32.506 (3.356)	-2.427 (0.151)**
T3 (ng/dl)	15	81.653 (2.593)	12.3	15	64.469 (2.593)	15.6	-17.184 (3.667)**	78.955 (4.046)	-12.103 (2.593)**
FSH (ng/ml)	15	14.807 (0.559)	14.6	15	15.909 (0.559)	13.6	1.102 (0.790)	107.443 (5.540)	1.042 (0.560)
Estradiol (pg/ml)	15	25.403 (1.530)	23.3	14	37.740 (1.584)	15.7	12.337 (2.202)**	148.563 (10.905)	10.008 (1.554)**
Prolactin (ng/ml)	15	36.476 (6.999)	74.3	14	2.019 (0.314)	58.2	-34.457 (7.006)**	5.536 (1.367)	-22.928 (4.714)**
DHT (pg/ml)	15	487.734 (63.273)	50.2	15	299.866 (44.012)	56.8	-187.868 (77.075)*	61.481 (12.043)	-123.388 (54.655)*

1. Least squares means and standard errors were estimated based on a one-way ANOVA model applied to the data for each parameter, after excluding some extreme observations.
2. CV was calculated as residual standard deviation/LS Mean.
3. Significant differences from the vehicle were indicated by "*" for the 0.05 level and "**" for the 0.05/8 level (a Bonferroni adjusted p-level).
4. Ratio to the vehicle was calculated as percent of vehicle group mean. The standard error for the ratio was $Se[R(X, Y)] \approx |1/X| [(Y/X)^2 S_X^2 + S_Y^2]^{1/2} \times 100\%$
5. Linear Contrast $\equiv [-3X_0 - X_1 + X_2 + 3X_3]/[20]^{1/2}$, where X_0 is vehicle, X_1, X_2, and X_3 are the low, mid, and high dosage levels of Linuron respectively. Significant dose trend was indicated by "*" for the 0.05 level and "**" for the 0.05/8 level.

Table 7. Summary Statistics between Vehicle and Phenobarbital in Adult Intact Male Assay for Hormonal Parameters for Charles River Laboratories[1,2]

Parameter	Vehicle			Phenobarbital (25 mg/kg/day)					
	N	LS Mean (SE)	CV (%)	N	LS Mean(SE)	CV (%)	Diff from Vehicle[3]	Ratio to Vehicle (%)[4]	Linear Trend[5]
Testosterone (ng/ml)	15	9.927 (1.873)	73.1	15	6.072 (1.212)	77.3	-3.855 (2.231)	61.164 (16.798)	-5.766 (1.321)**
LH (ng/ml)	15	2.178 (0.127)	22.6	15	1.811 (0.098)	21.0	-0.367 (0.161)*	83.134 (6.621)	-0.502 (0.100)**
TSH (ng/ml)	15	13.095 (1.681)	49.7	15	23.349 (2.877)	47.7	10.254 (3.333)**	178.307 (31.733)	11.692 (2.051)**
T4 (µg/dl)	15	4.729 (0.151)	12.4	15	3.751 (0.151)	15.6	-0.979 (0.213)**	79.306 (4.073)	-1.437 (0.153)**
T3 (ng/dl)	15	81.653 (2.593)	12.3	15	64.847 (2.593)	15.5	-16.805 (3.667)**	79.419 (4.055)	-16.985 (2.634)**
FSH (ng/ml)	15	14.807 (0.559)	14.6	15	13.841 (0.559)	15.6	-0.965 (0.790)	93.480 (5.167)	-2.006 (0.568)**
Estradiol (pg/ml)	15	25.403 (1.530)	23.3	15	33.709 (1.530)	17.6	8.305 (2.164)**	132.694 (10.007)	9.431 (1.554)**
Prolactin (ng/ml)	15	36.476 (6.999)	74.3	15	14.050 (4.084)	112.6	-22.426 (8.104)*	38.518 (13.417)	-22.967 (4.896)**
DHT (pg/ml)	15	487.734 (63.273)	50.2	15	389.625 (55.075)	54.7	-98.109 (83.886)	79.885 (15.327)	-179.980 (50.247)**

1. Least squares means and standard errors were estimated based on a one-way ANOVA model applied to the data for each parameter, after excluding some extreme observations.
2. CV was calculated as residual standard deviation/LS Mean.
3. Significant differences from the vehicle were indicated by "*" for the 0.05 level and "**" for the 0.05/8 level (a Bonferroni adjusted p-level).
4. Ratio to the vehicle was calculated as percent of vehicle group mean. The standard error for the ratio was $Se[R(X, Y)] \approx |1/X| [(Y/X)^2 S_X^2 + S_Y^2]^{1/2} \times 100\%$
5. Linear Contrast $\equiv[-3X_0 - X_1 + X_2 + 3X_3]/[20]^{1/2}$, where X_0 is vehicle, X_1, X_2, and X_3 are the low, mid, and high dosage levels of phenobarbital respectively. Significant dose trend was indicated by "*" for the 0.05 level and "**" for the 0.05/8 level.

Table 7 (cont.). Summary Statistics between Vehicle and Phenobarbital in Adult Intact Male Assay for Hormonal Parameters for Charles River Laboratories[1,2]

Parameter	Vehicle			Phenobarbital (50 mg/kg/day)					
	N	LS Mean (SE)	CV (%)	N	LS Mean(SE)	CV (%)	Diff from Vehicle[3]	Ratio to Vehicle (%)[4]	Linear Trend[5]
Testosterone (ng/ml)	15	9.927 (1.873)	73.1	15	3.495 (0.723)	80.1	-6.433 (2.008)**	35.202 (9.854)	-5.766 (1.321)**
LH (ng/ml)	15	2.178 (0.127)	22.6	15	1.435 (0.054)	14.6	-0.743 (0.138)**	65.871 (4.572)	-0.502 (0.100)**
TSH (ng/ml)	15	13.095 (1.681)	49.7	15	25.741 (2.024)	30.5	12.647 (2.631)**	196.579 (29.597)	11.692 (2.051)**
T4 (µg/dl)	15	4.729 (0.151)	12.4	15	3.642 (0.151)	16.1	-1.087 (0.213)**	77.009 (4.028)	-1.437 (0.153)**
T3 (ng/dl)	15	81.653 (2.593)	12.3	15	65.408 (2.593)	15.4	-16.245 (3.667)**	80.105 (4.069)	-16.985 (2.634)**
FSH (ng/ml)	15	14.807 (0.559)	14.6	15	12.459 (0.559)	17.4	-2.348 (0.790)**	84.142 (4.933)	-2.006 (0.568)**
Estradiol (pg/ml)	15	25.403 (1.530)	23.3	15	36.525 (1.530)	16.2	11.122 (2.164)**	143.782 (10.548)	9.431 (1.554)**
Prolactin (ng/ml)	15	36.476 (6.999)	74.3	14	8.121 (1.533)	70.6	-28.355 (7.165)**	22.263 (5.993)	-22.967 (4.896)**
DHT (pg/ml)	15	487.734 (63.273)	50.2	15	301.385 (52.321)	67.2	-186.349 (82.103)*	61.793 (13.392)	-179.980 (50.247)**

1. Least squares means and standard errors were estimated based on a one-way ANOVA model applied to the data for each parameter, after excluding some extreme observations.
2. CV was calculated as residual standard deviation/LS Mean.
3. Significant differences from the vehicle were indicated by "*" for the 0.05 level and "**" for the 0.05/8 level (a Bonferroni adjusted p-level).
4. Ratio to the vehicle was calculated as percent of vehicle group mean. The standard error for the ratio was $Se[R(X, Y)] \approx |1/X| [(Y/X)^2 S_X^2 + S_Y^2]^{1/2} \times 100\%$
5. Linear Contrast $\equiv [-3X_0 - X_1 + X_2 + 3X_3]/[20]^{1/2}$, where X_0 is vehicle, X_1, X_2, and X_3 are the low, mid, and high dosage levels of phenobarbital respectively. Significant dose trend was indicated by "*" for the 0.05 level and "**" for the 0.05/8 level.

Table 7 (cont.). Summary Statistics between Vehicle and Phenobarbital in Adult Intact Male Assay for Hormonal Parameters for Charles River Laboratories[1,2]

Parameter	Vehicle			Phenobarbital (100 mg/kg/day)					
	N	LS Mean (SE)	CV (%)	N	LS Mean(SE)	CV (%)	Diff from Vehicle[3]	Ratio to Vehicle (%)[4]	Linear Trend[5]
Testosterone (ng/ml)	15	9.927 (1.873)	73.1	13	2.192 (0.389)	63.9	-7.736 (1.913)**	22.076 (5.716)	-5.766 (1.321)**
LH (ng/ml)	15	2.178 (0.127)	22.6	14	1.555 (0.068)	16.5	-0.623 (0.144)**	71.396 (5.215)	-0.502 (0.100)**
TSH (ng/ml)	15	13.095 (1.681)	49.7	14	29.727 (2.269)	28.6	16.632 (2.824)**	227.017 (33.909)	11.692 (2.051)**
T4 (µg/dl)	15	4.729 (0.151)	12.4	14	2.623 (0.156)	22.3	-2.106 (0.217)**	55.459 (3.748)	-1.437 (0.153)**
T3 (ng/dl)	15	81.653 (2.593)	12.3	14	56.146 (2.684)	17.9	-25.506 (3.732)**	68.763 (3.946)	-16.985 (2.634)**
FSH (ng/ml)	15	14.807 (0.559)	14.6	14	12.278 (0.579)	17.6	-2.529 (0.804)**	82.921 (5.006)	-2.006 (0.568)**
Estradiol (pg/ml)	15	25.403 (1.530)	23.3	14	38.523 (1.584)	15.4	13.120 (2.202)**	151.645 (11.058)	9.431 (1.554)**
Prolactin (ng/ml)	15	36.476 (6.999)	74.3	13	4.215 (1.469)	125.7	-32.261 (7.152)**	11.557 (4.598)	-22.967 (4.896)**
DHT (pg/ml)	15	487.734 (63.273)	50.2	14	248.849 (31.079)	46.7	-238.885 (70.494)**	51.021 (9.188)	-179.980 (50.247)**

1. Least squares means and standard errors were estimated based on a one-way ANOVA model applied to the data for each parameter, after excluding some extreme observations.
2. CV was calculated as residual standard deviation/LS Mean.
3. Significant differences from the vehicle were indicated by "*" for the 0.05 level and "**" for the 0.05/8 level (a Bonferroni adjusted p-level).
4. Ratio to the vehicle was calculated as percent of vehicle group mean. The standard error for the ratio was $Se[R(X, Y)] \approx |1/X| [(Y/X)^2 S_X^2 + S_Y^2]^{1/2} \times 100\%$
5. Linear Contrast $\equiv [-3X_0 - X_1 + X_2 + 3X_3]/[20]^{1/2}$ where X_0 is vehicle, X_1, X_2, and X_3 are the low, mid, and high dosage levels of phenobarbital respectively. Significant dose trend was indicated by "*" for the 0.05 level and "**" for the 0.05/8 level.

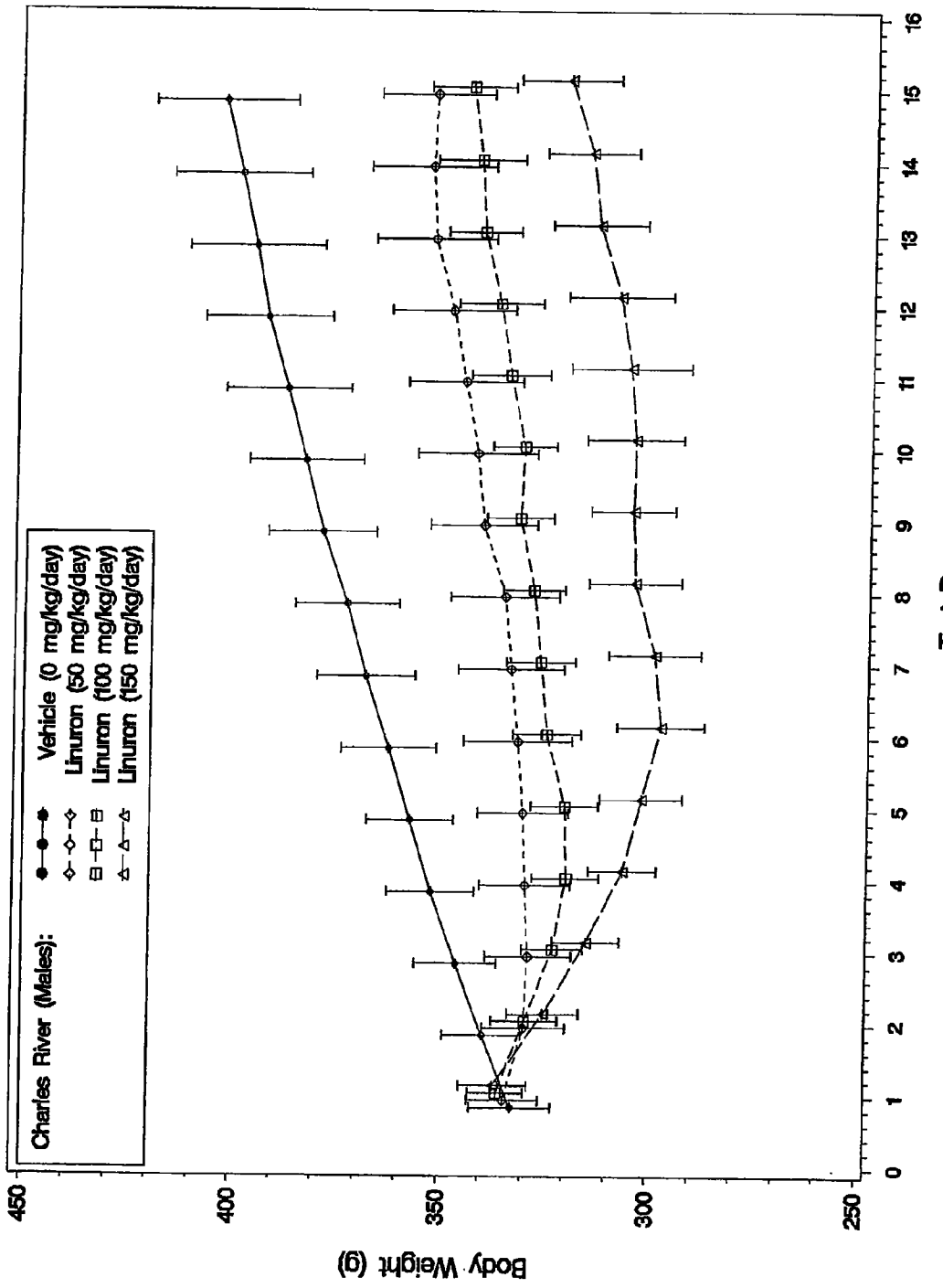


Figure 1. **Charles River Adult Males Means (with ± 2 Standard Error Bars) of Body Weights (g) on Each Day from Test Day 1 to Test Day 15 for the Vehicle (0.25% methylcellulose) Group and the Three Linuron Dose Groups.**

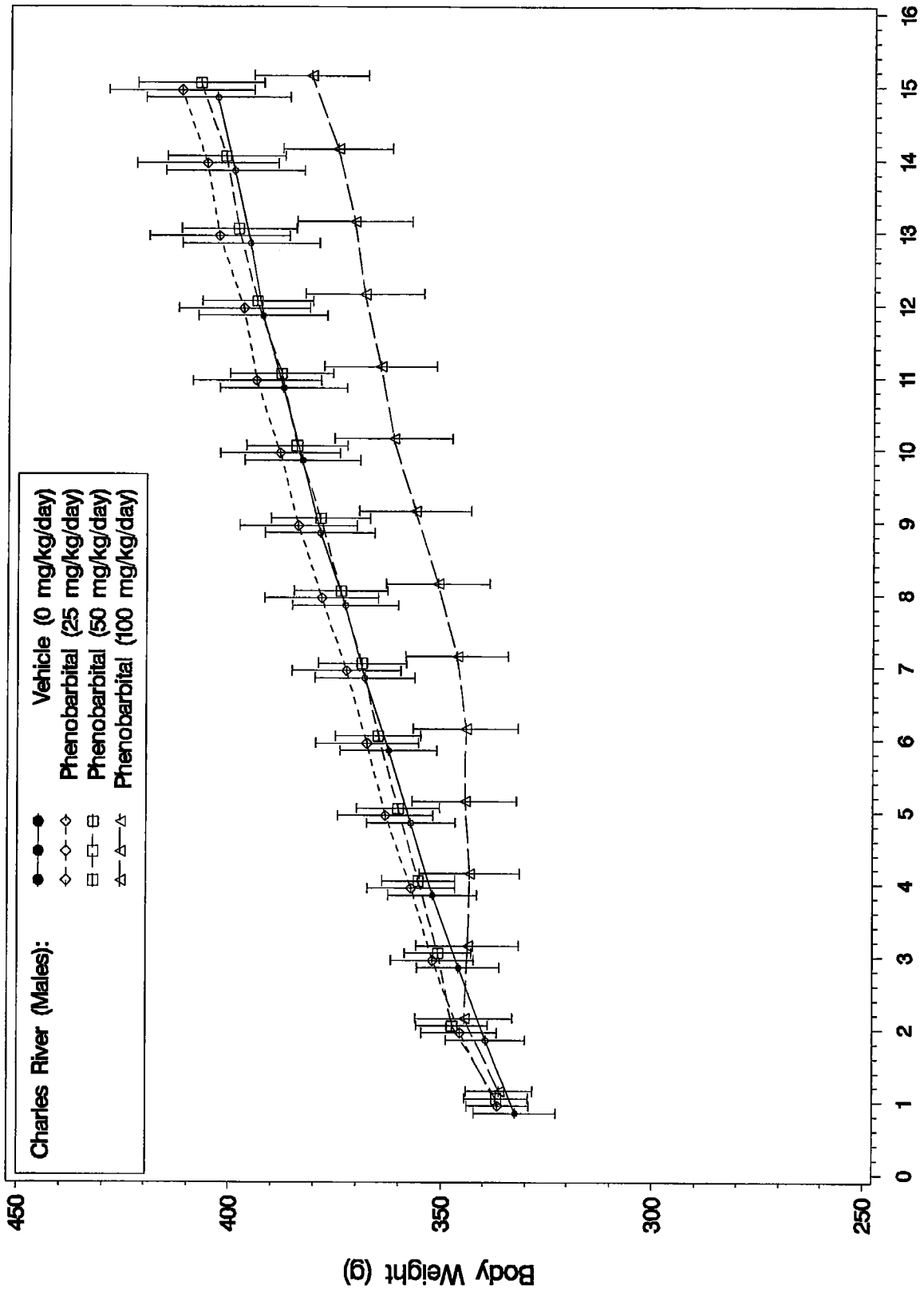


Figure 2. Charles River Adult Males Means (with ± 2 Standard Error Bars) of Body Weights (g) on Each Day from Test Day 1 to Test Day 15 for the Vehicle (0.25% methylcellulose) Group and the Three Phenobarbital Dose Groups.

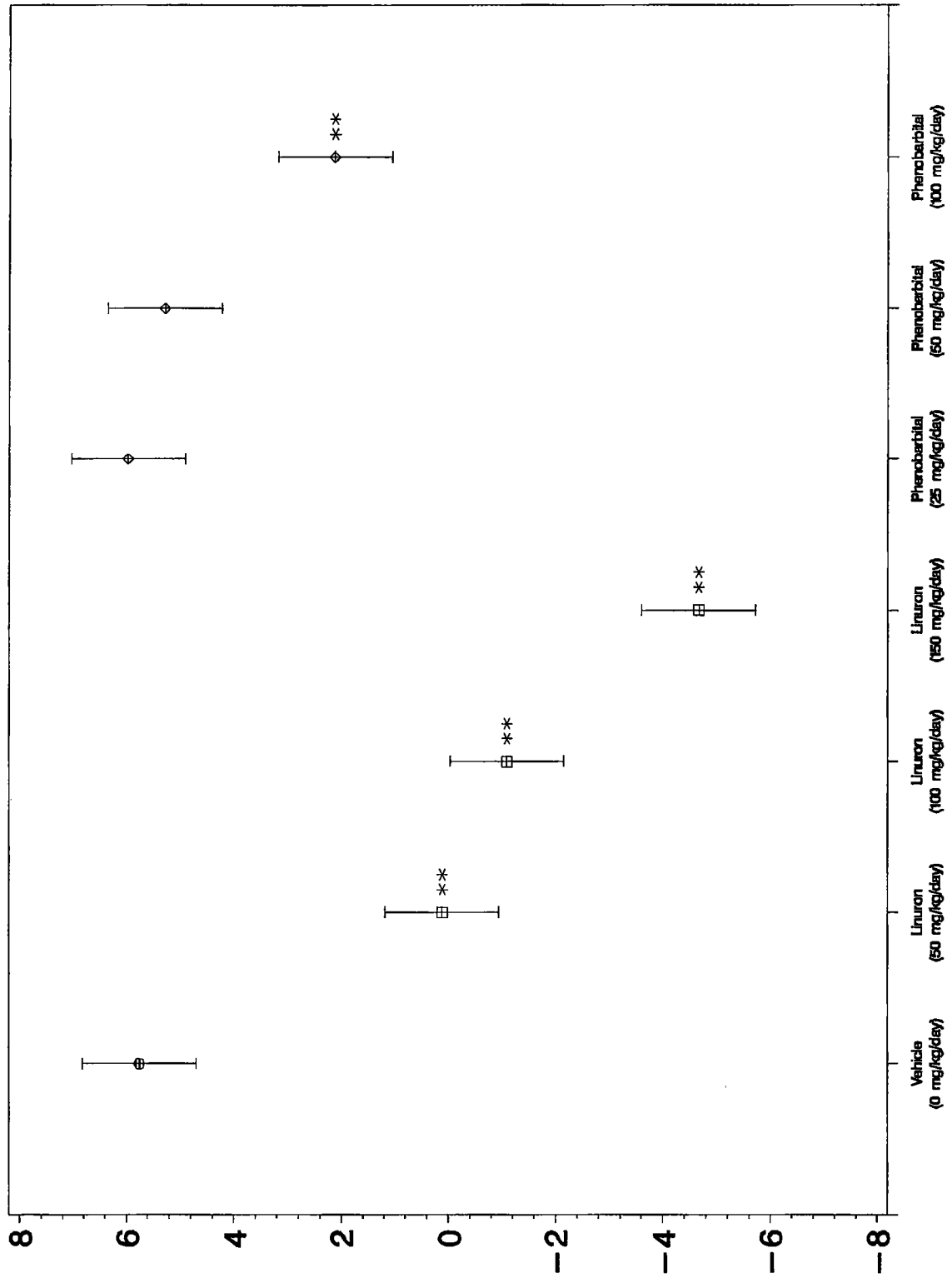


Figure 3. Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for Average Daily Body Weight Changes (g/day) From Day 1 to Day 8 for Each Dose Group (Significant Differences from Vehicle Control are Indicated by "**" for the 0.05 Level, and by "**" for the 0.05/8 Level).

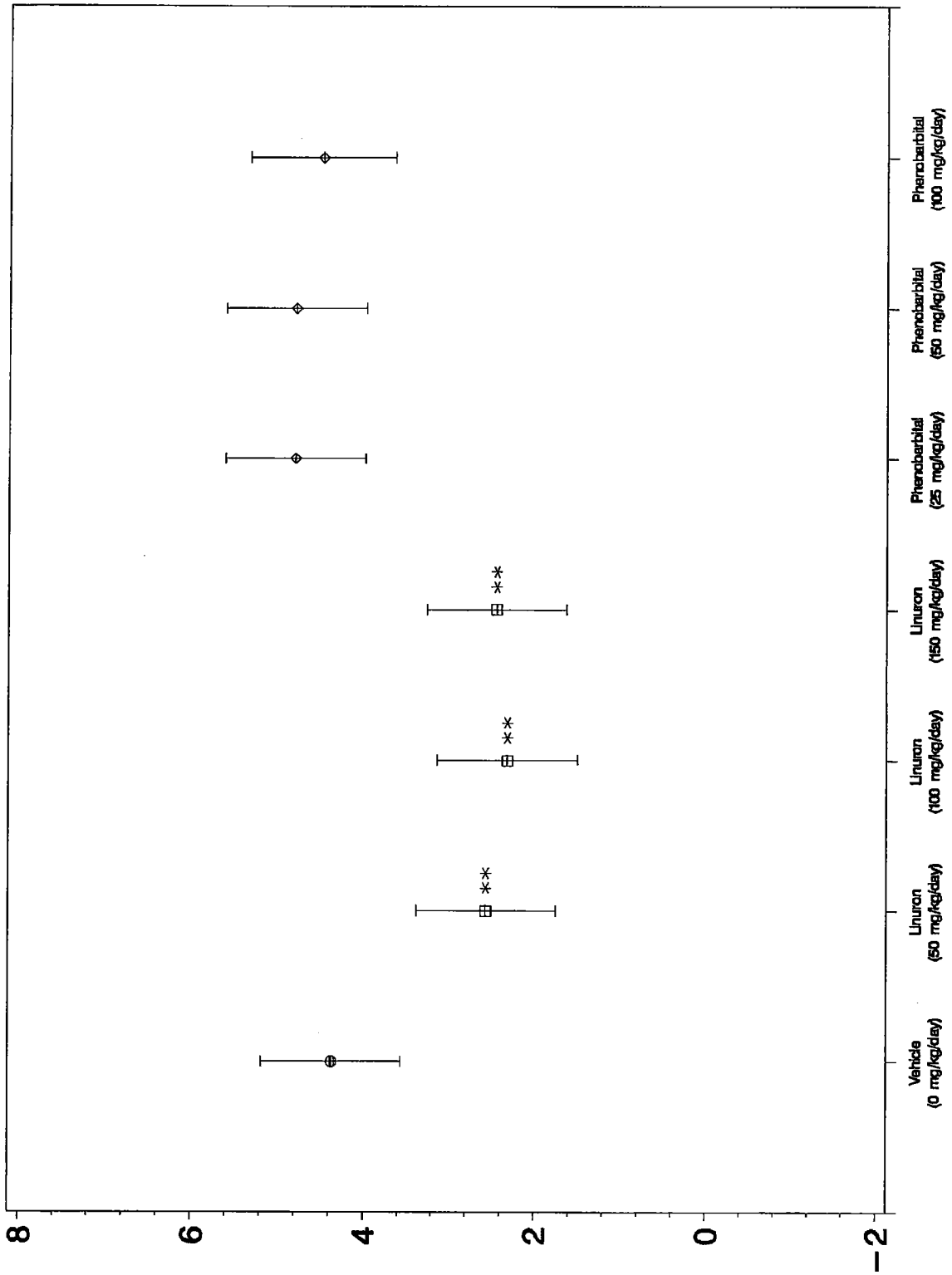


Figure 4. **Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for Average Daily Body Weight Changes (g/day) From Day 8 to Day 15 for Each Dose Group (Significant Differences from Vehicle Control are Indicated by "*" for the 0.05 Level, and by "**" for the 0.05/8 Level).

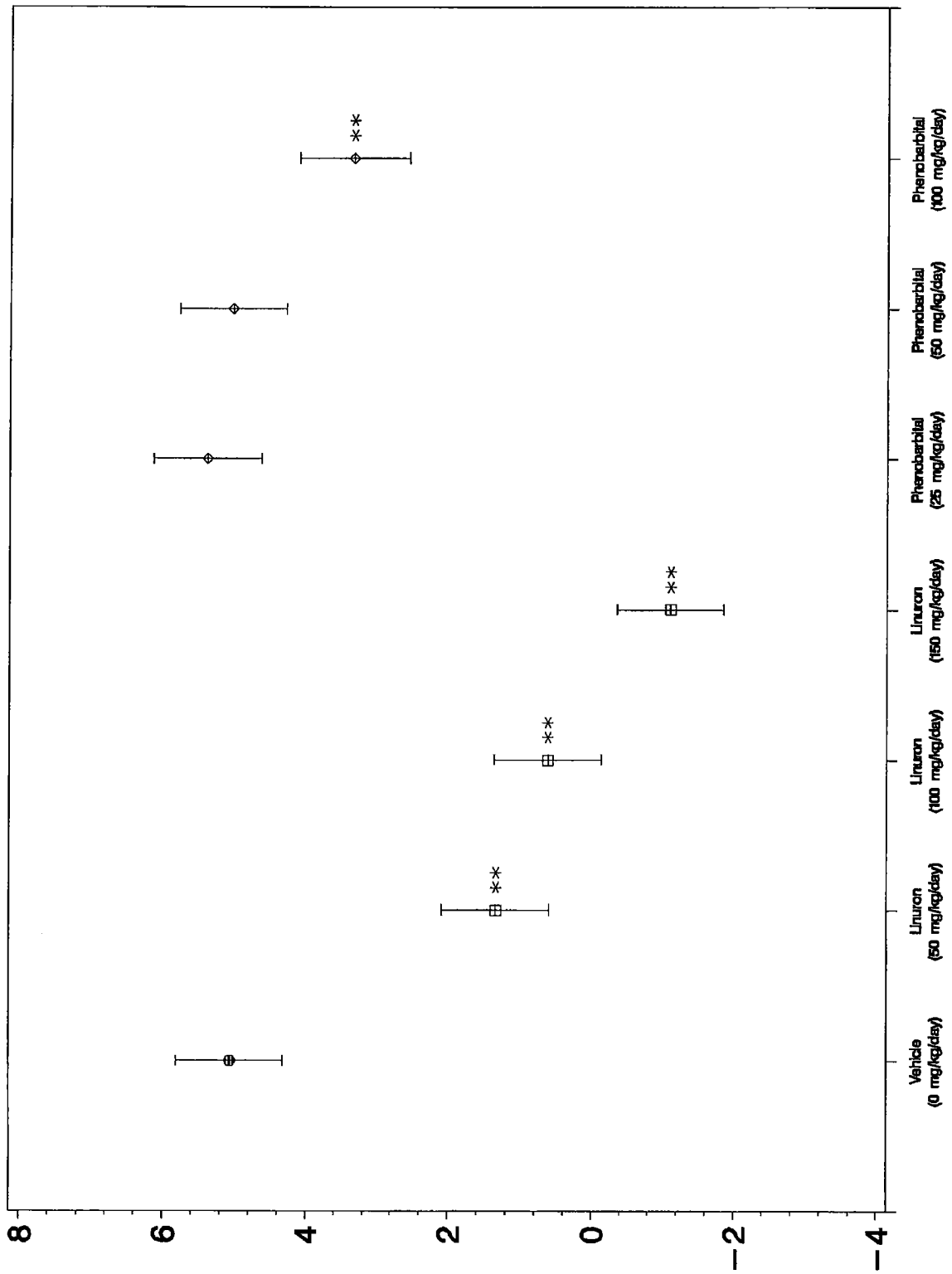


Figure 5. **Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for Average Daily Body Weight Changes (g/day) From Day 1 to Day 15 for Each Dose Group (Significant Differences from Vehicle Control are Indicated by "*" for the 0.05 Level, and by "**" for the 0.05/8 Level).**

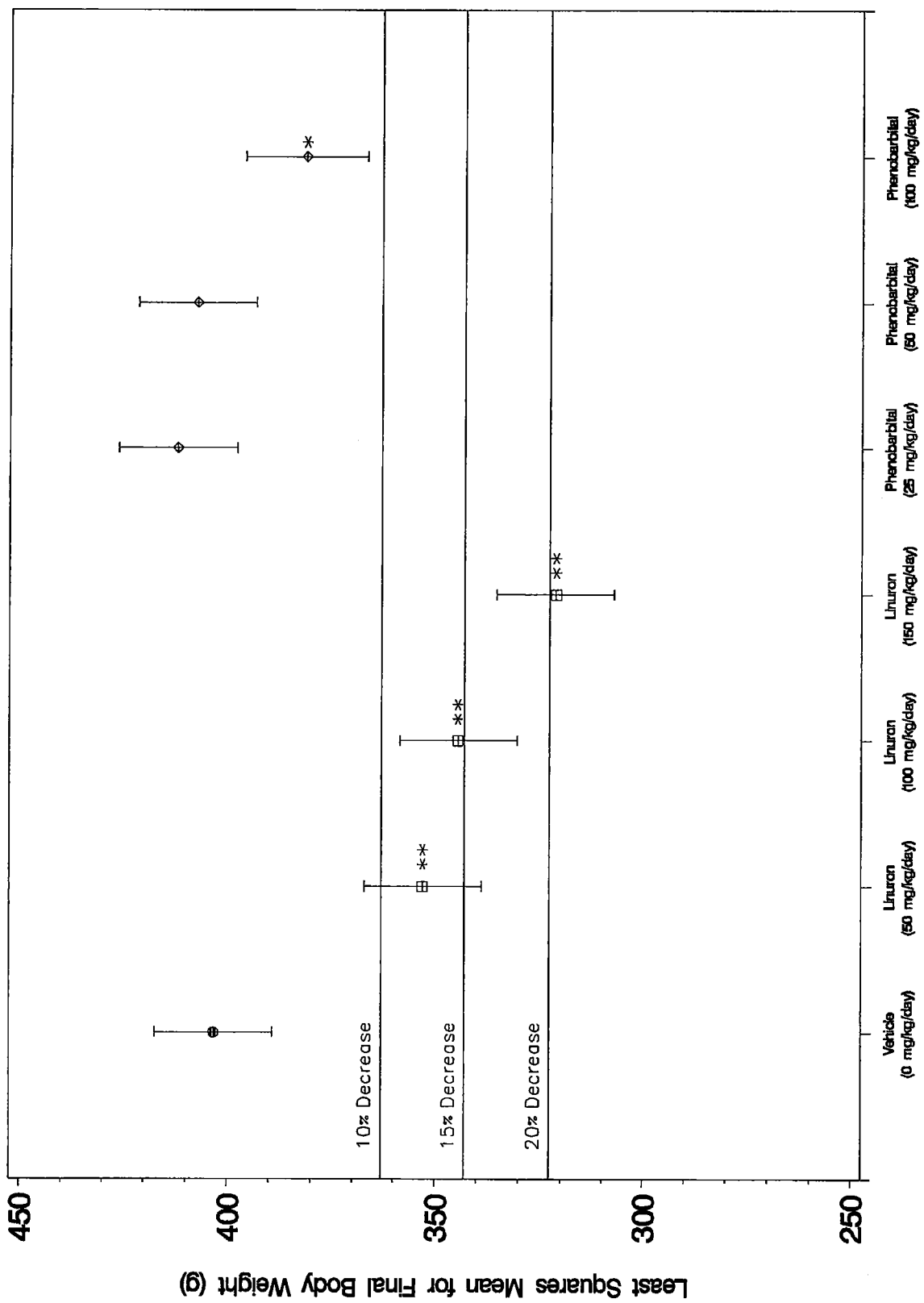


Figure 6. Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for Final Body Weight (g) for Each Dose Group (Significant Differences from Vehicle Control are Indicated by "*" for the 0.05 Level, and by "" for the 0.05/8 Level). The Horizontal Reference Lines Represent 10%, 15% and 20% Decrease in Final Body Weight Relative to Vehicle.**

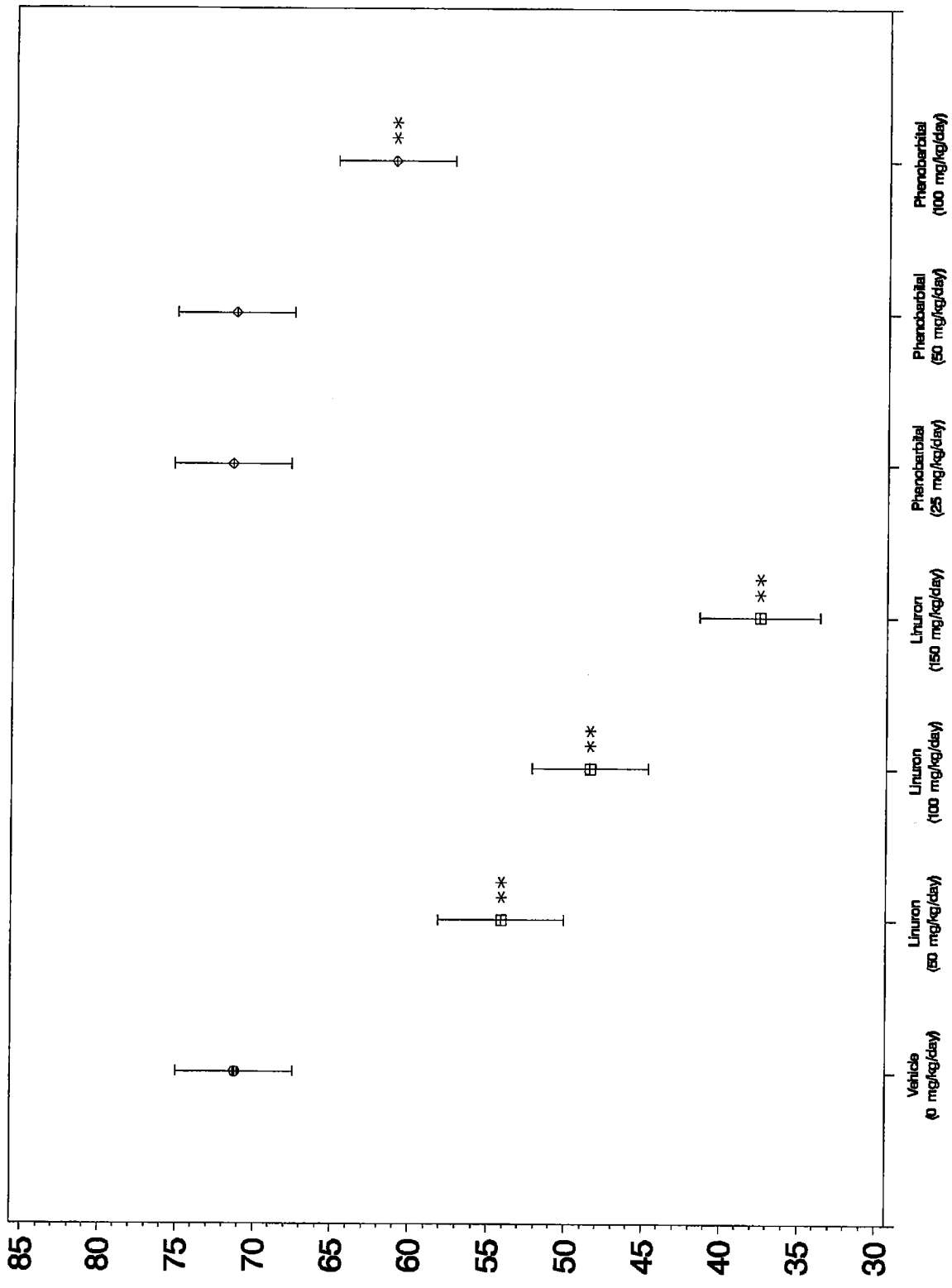


Figure 7. Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for Average Daily Food Consumptions (g/kg/day) From Day 1 to Day 8 for Each Dose Group (Significant Differences from Vehicle Control are Indicated by "**" for the 0.05 Level, and by "***" for the 0.05/8 Level).

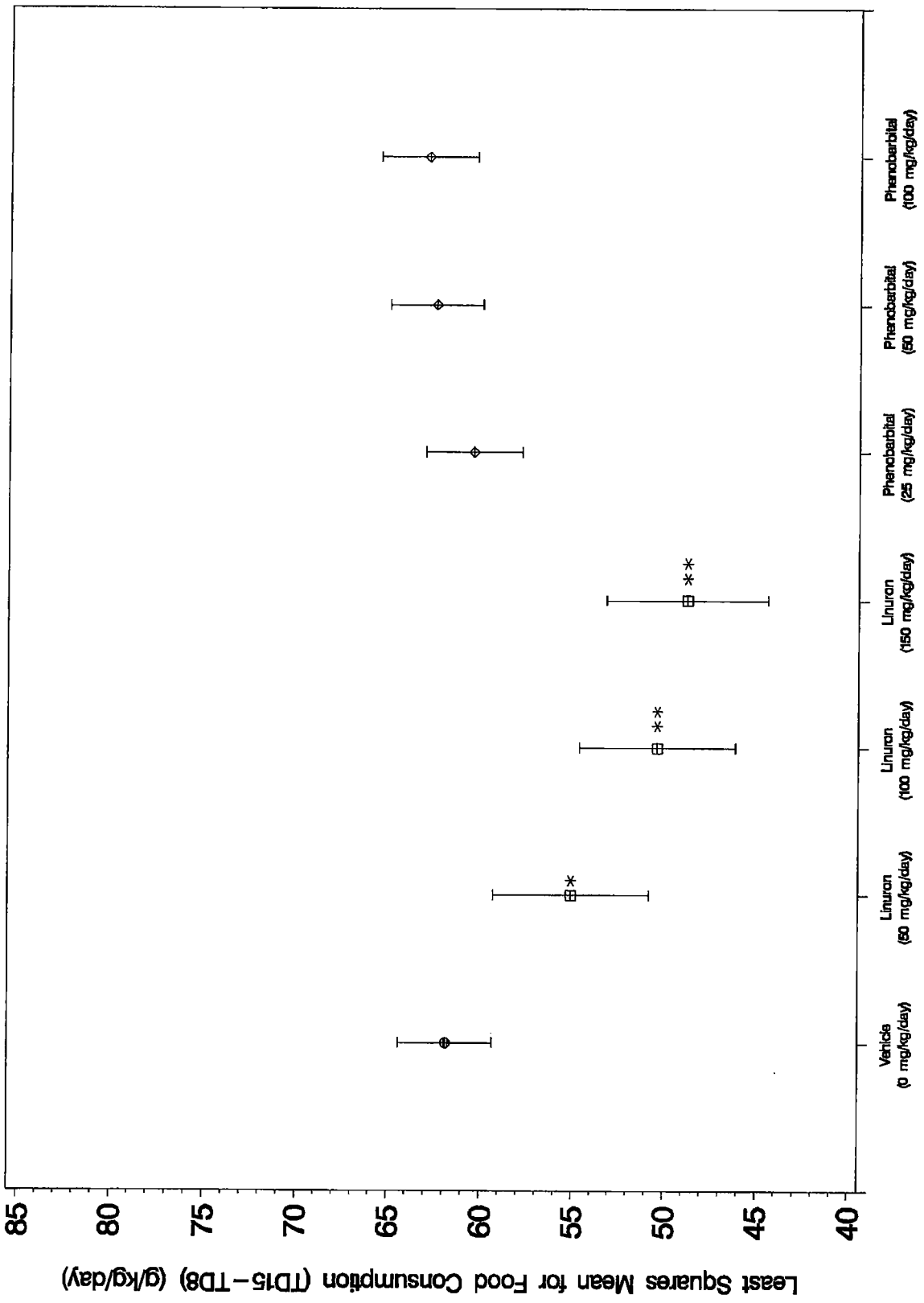


Figure 8. Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for Average Daily Food Consumptions (g/kg/day) From Day 8 to Day 15 for Each Dose Group (Significant Differences from Vehicle Control are Indicated by "*" for the 0.05 Level, and by "" for the 0.05/8 Level).**

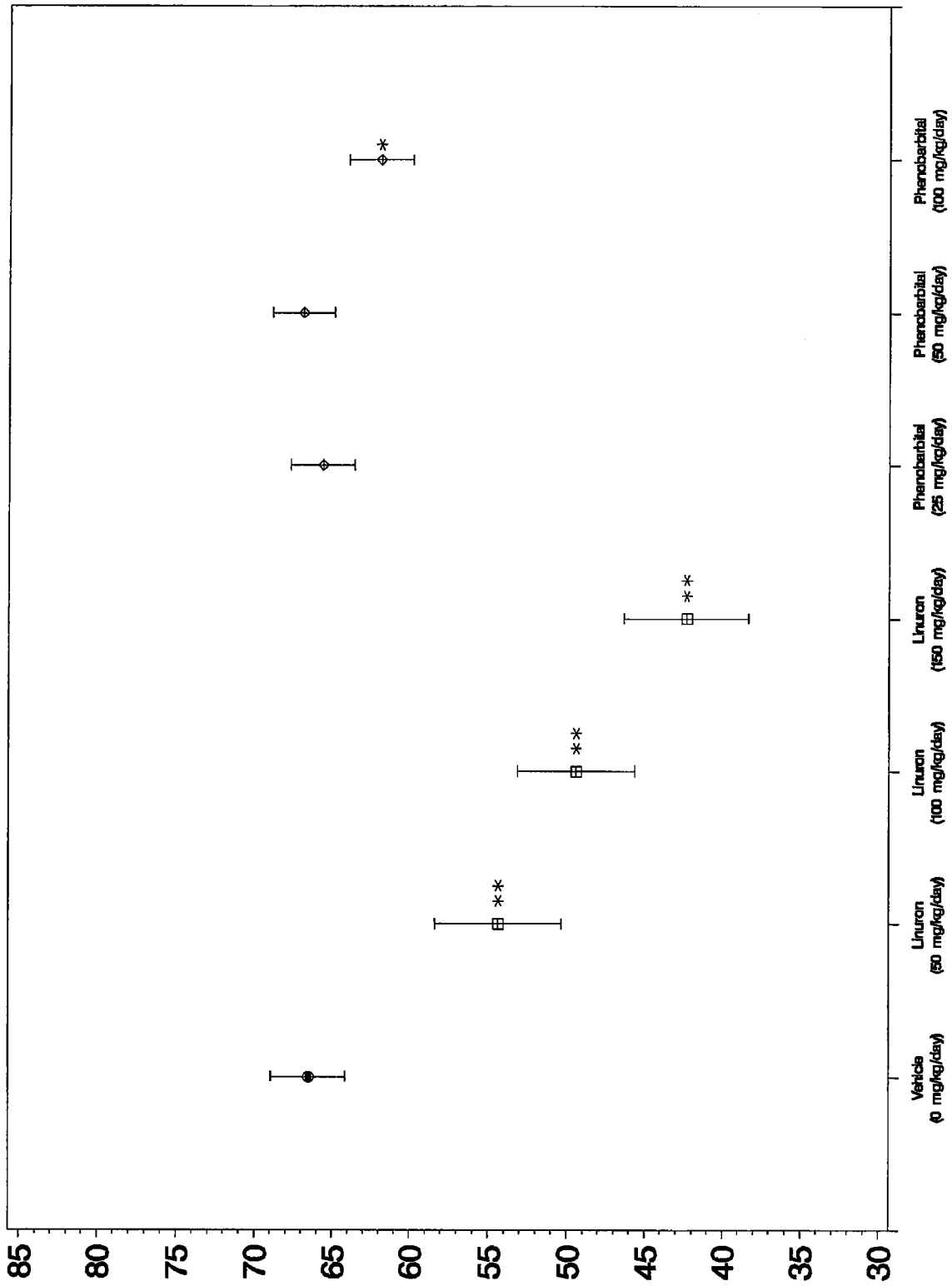


Figure 9. Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for Average Daily Food Consumptions (g/kg/day) From Day 1 to Day 15 for Each Dose Group (Significant Differences from Vehicle Control are Indicated by "*" for the 0.05 Level, and by "**" for the 0.05/8 Level).

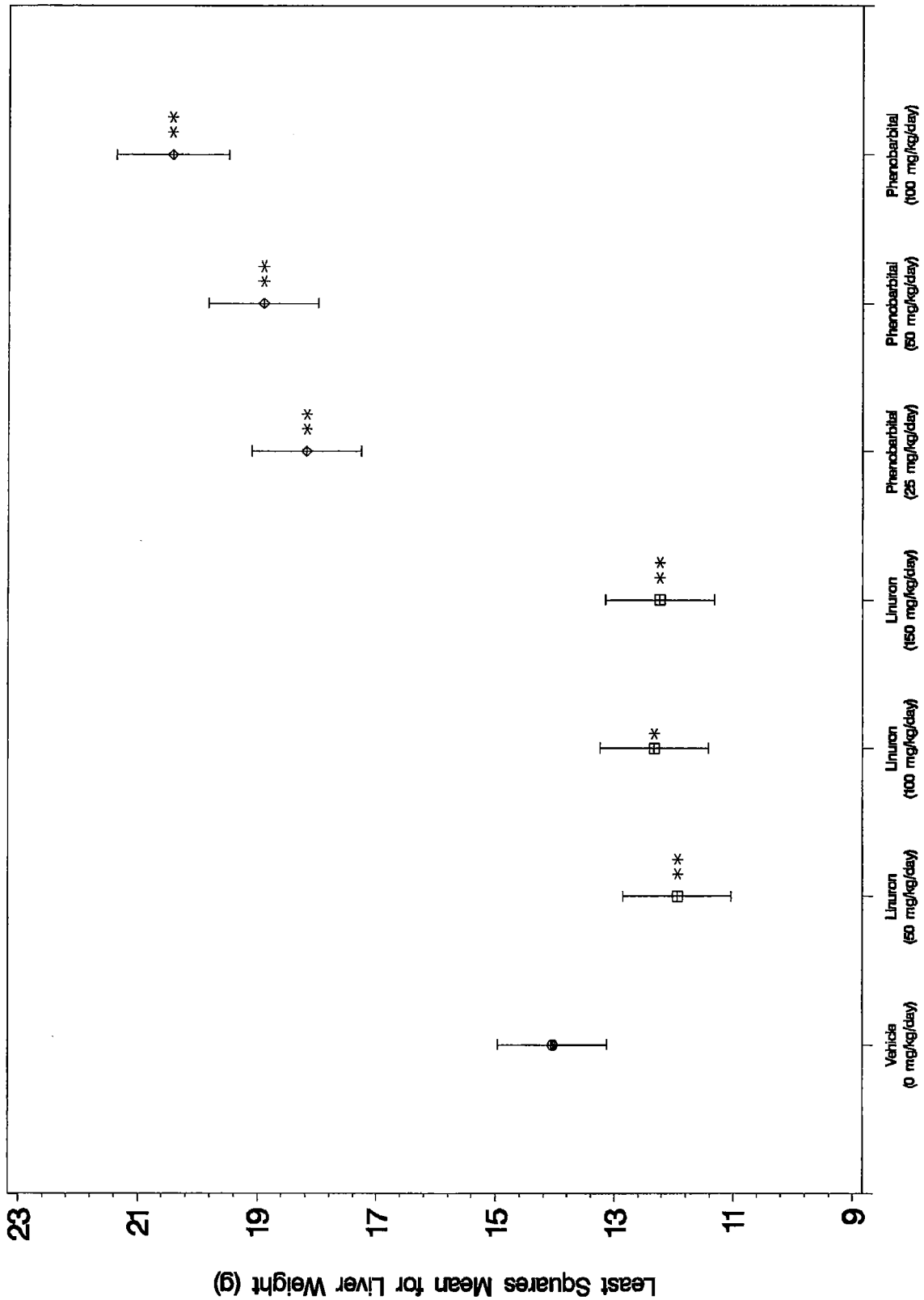


Figure 10. Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for Liver Weight (g) for Each Dose Group (Significant Differences from Vehicle Control are Indicated by "*" for the 0.05 Level, and by "**" for the 0.05/8 Level).

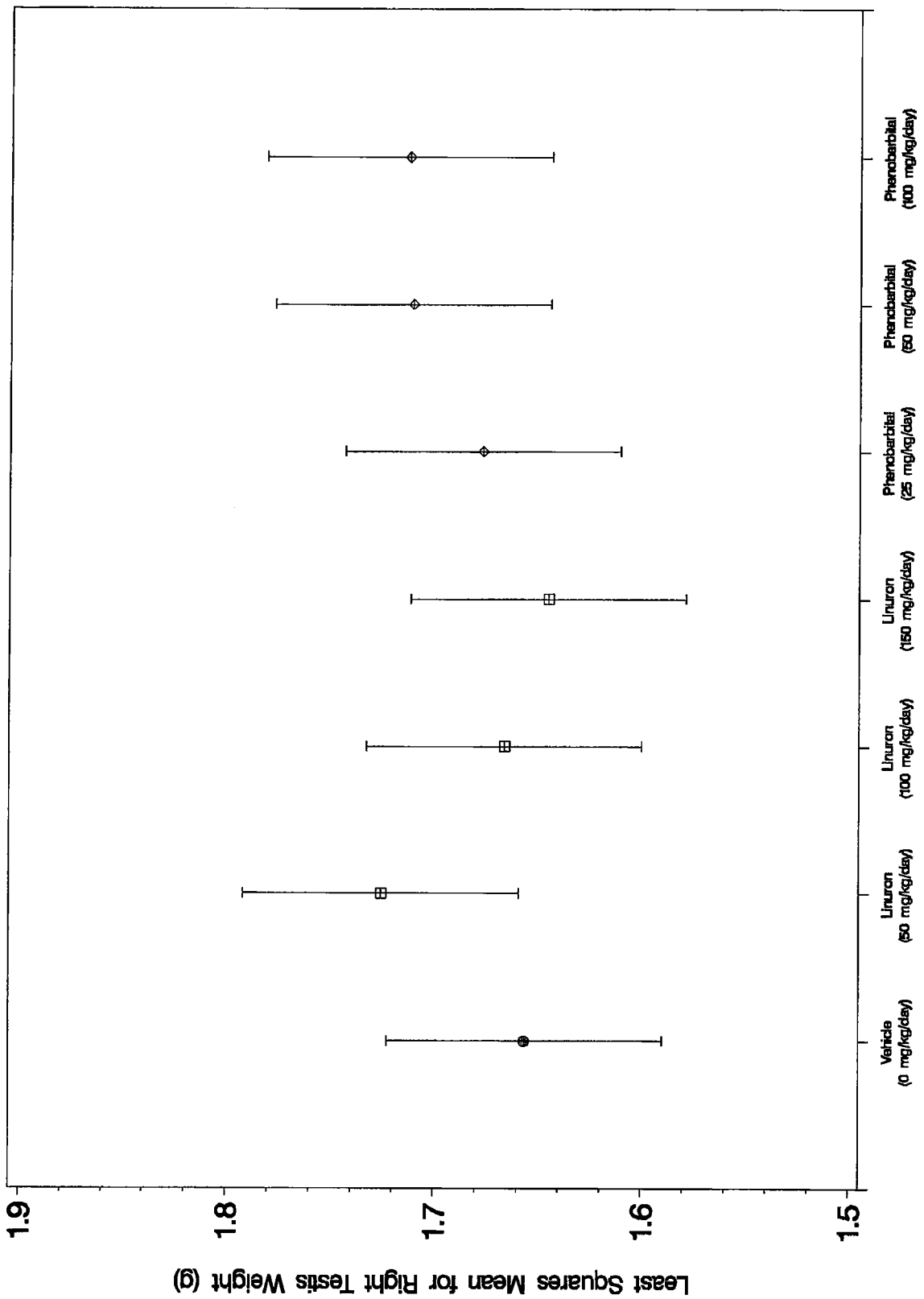


Figure 11. Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for Right Testis Weight (g) for Each Dose Group (Significant Differences from Vehicle Control are Indicated by "*" for the 0.05 Level, and by "" for the 0.05/8 Level).**

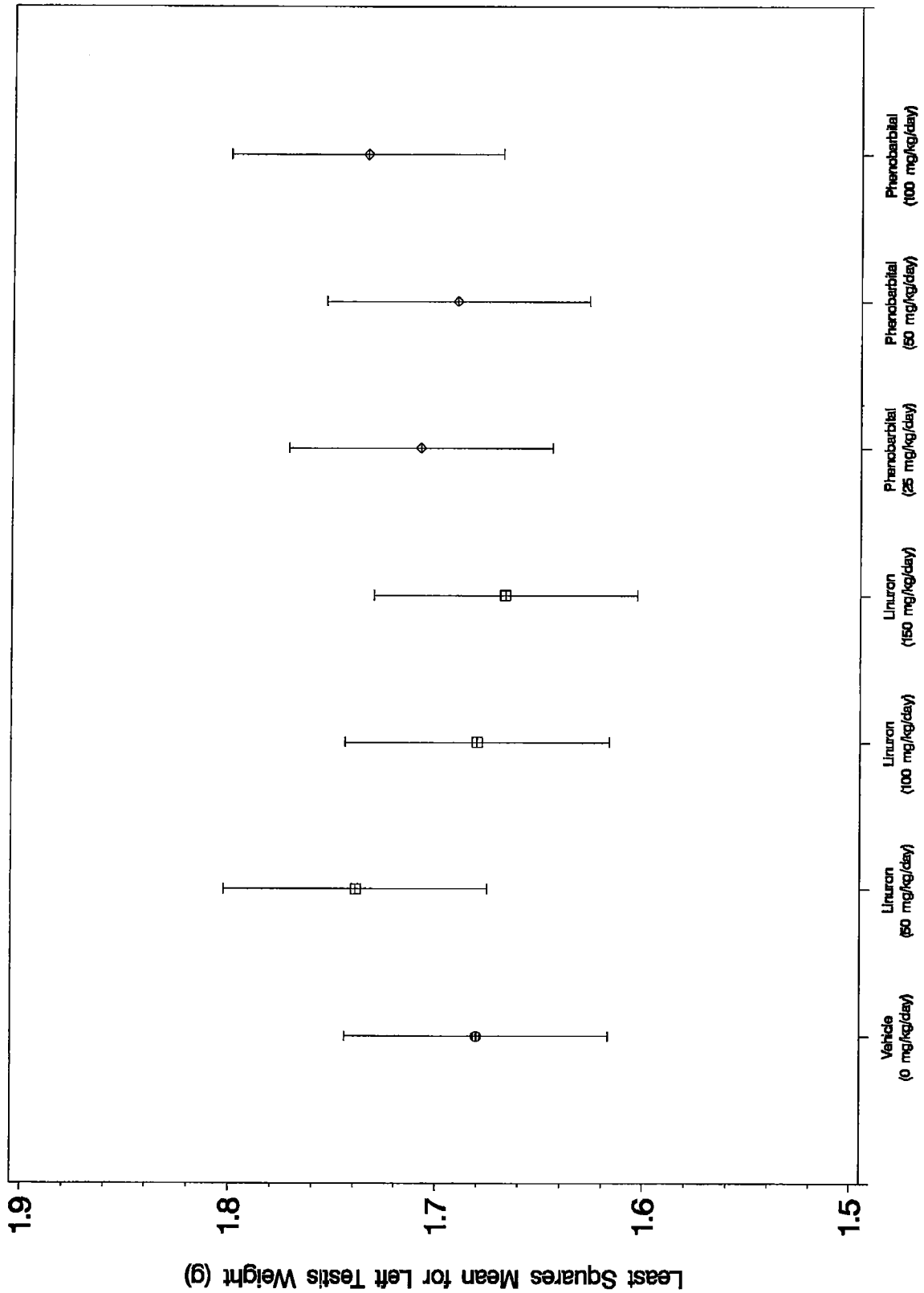


Figure 12. Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for Left Testis Weight (g) for Each Dose Group (Significant Differences from Vehicle Control are Indicated by "*" for the 0.05 Level, and by "**" for the 0.05/8 Level).

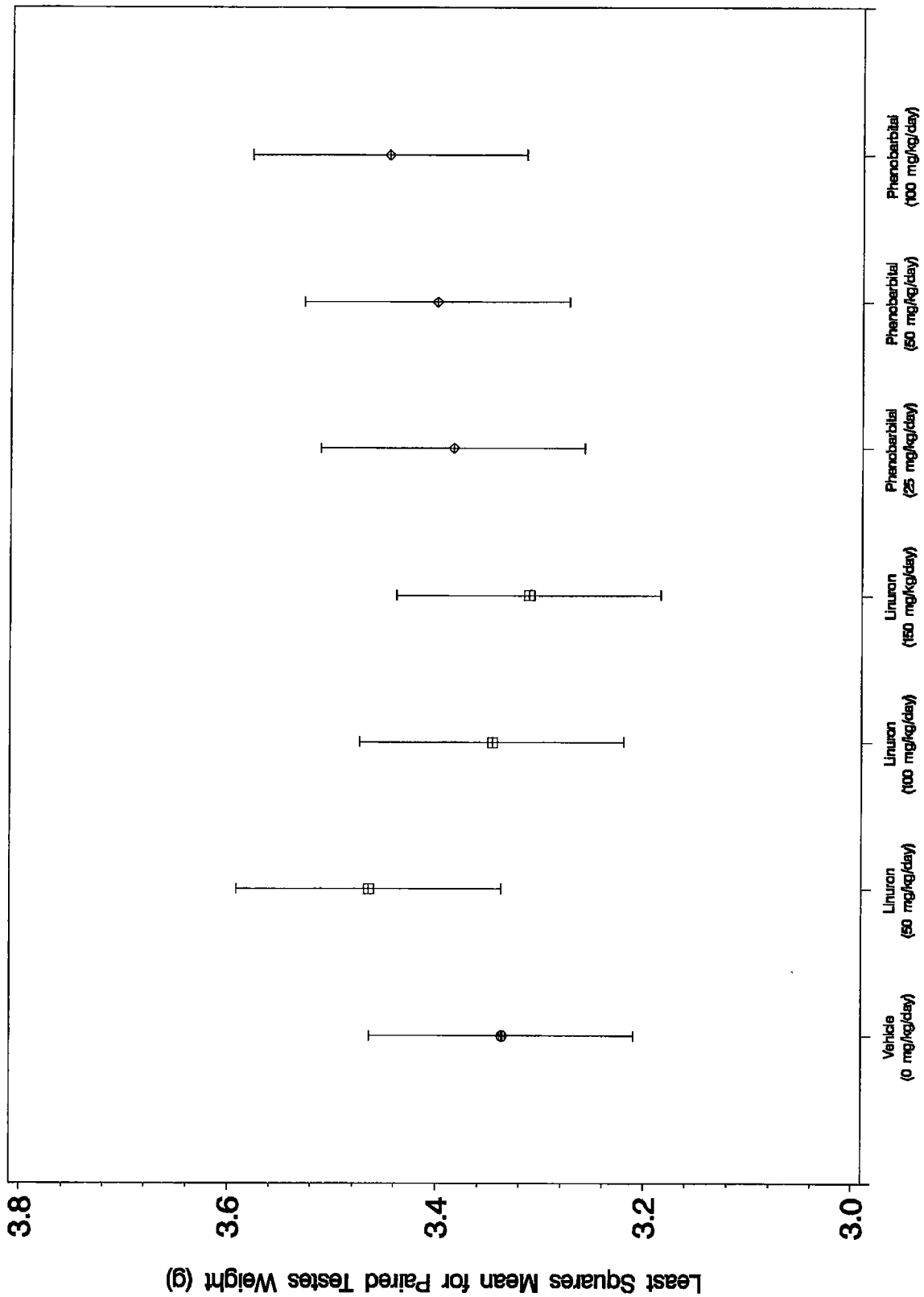


Figure 13. Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for Paired Testes Weight (g) for Each Dose Group (Significant Differences from Vehicle Control are Indicated by "*" for the 0.05 Level, and by "" for the 0.05/8 Level).**

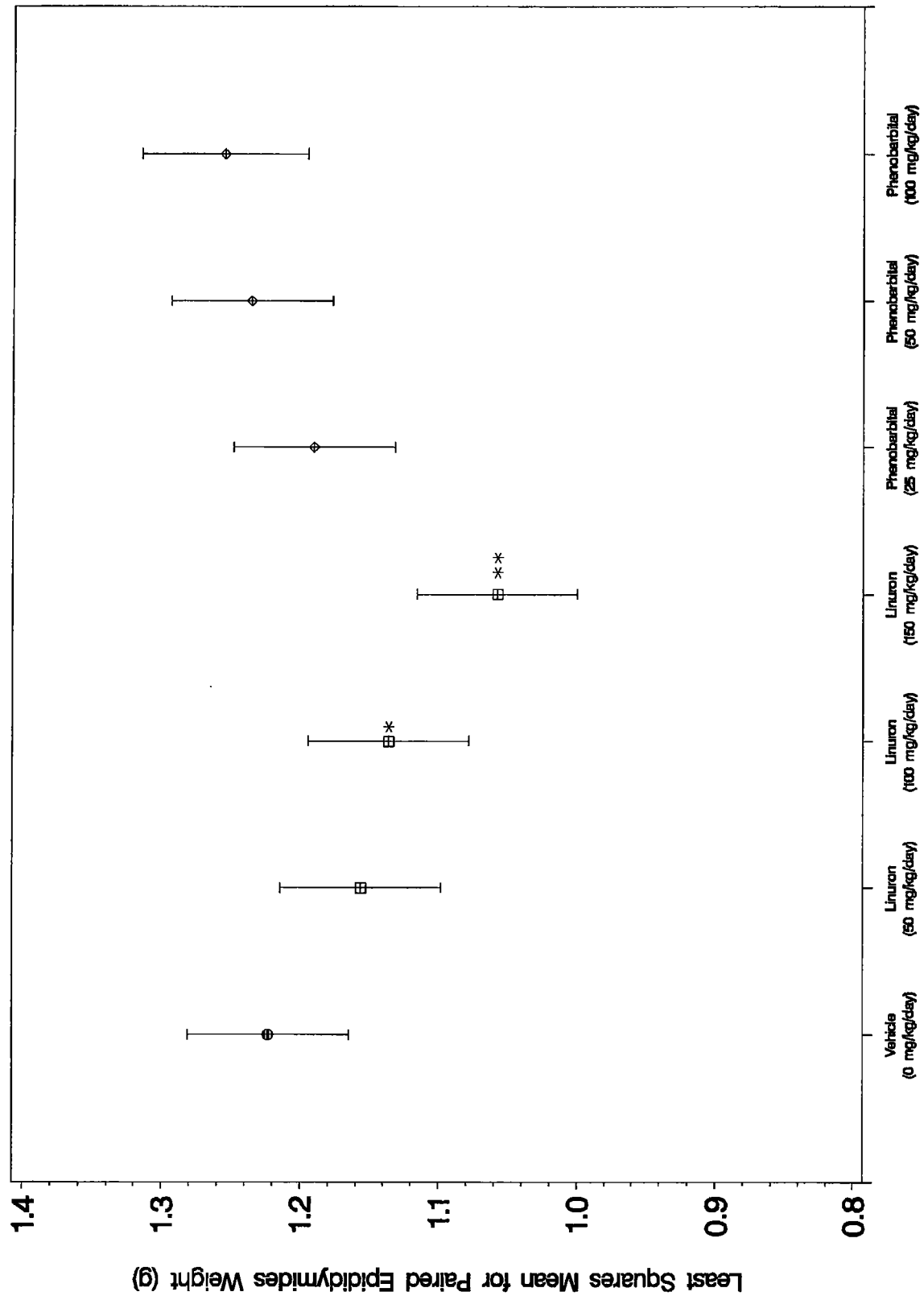


Figure 14. Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for Paired Epididymides Weight (g) For Each Dose Group (Significant Differences from Vehicle Control are Indicated by "*" for the 0.05 Level, and by "" for the 0.05/8 Level).**

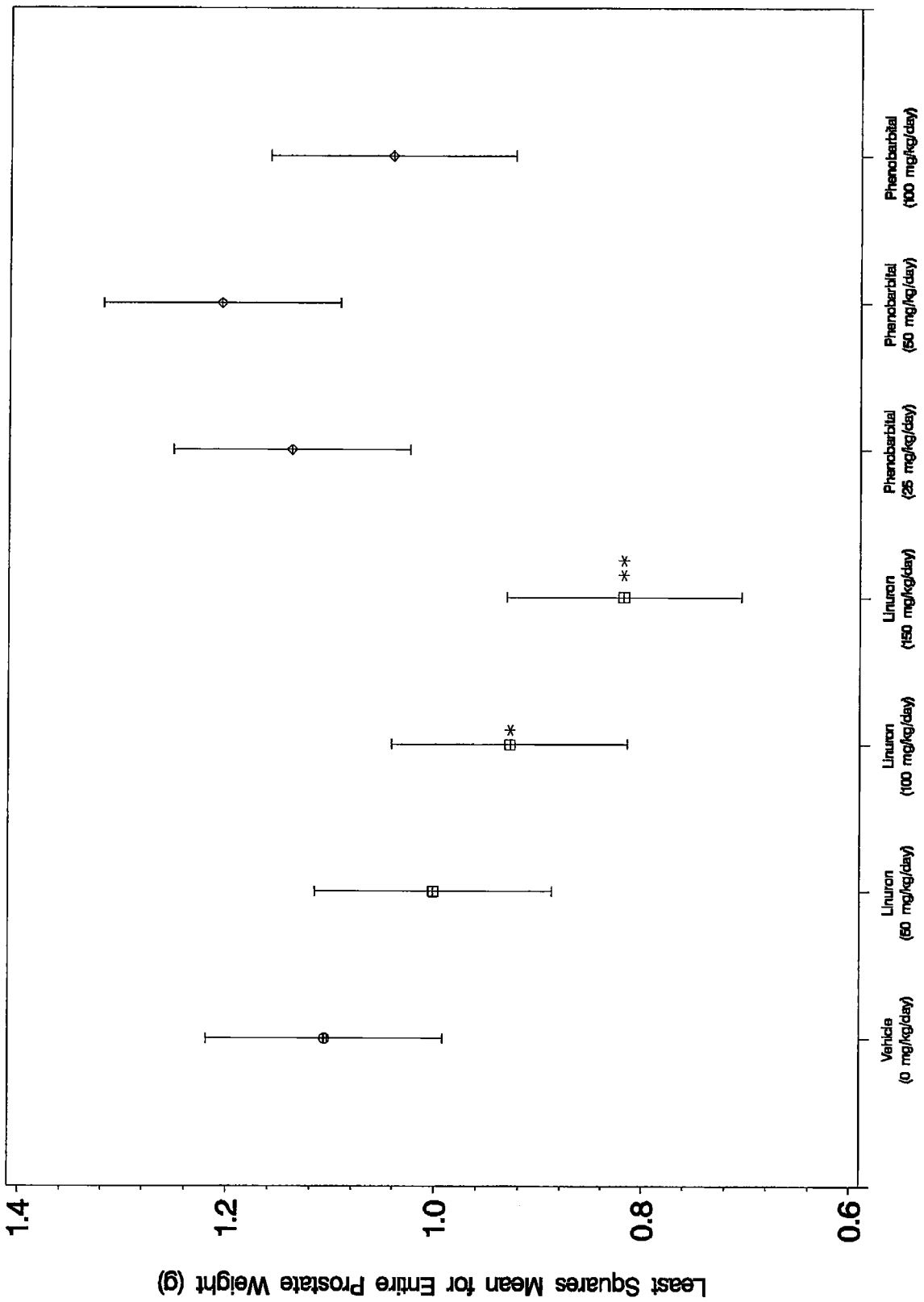


Figure 15. Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for Entire Prostate Weight (g) For Each Dose Group (Significant Differences from Vehicle Control are Indicated by "*" for the 0.05 Level, and by "" for the 0.05/8 Level).**

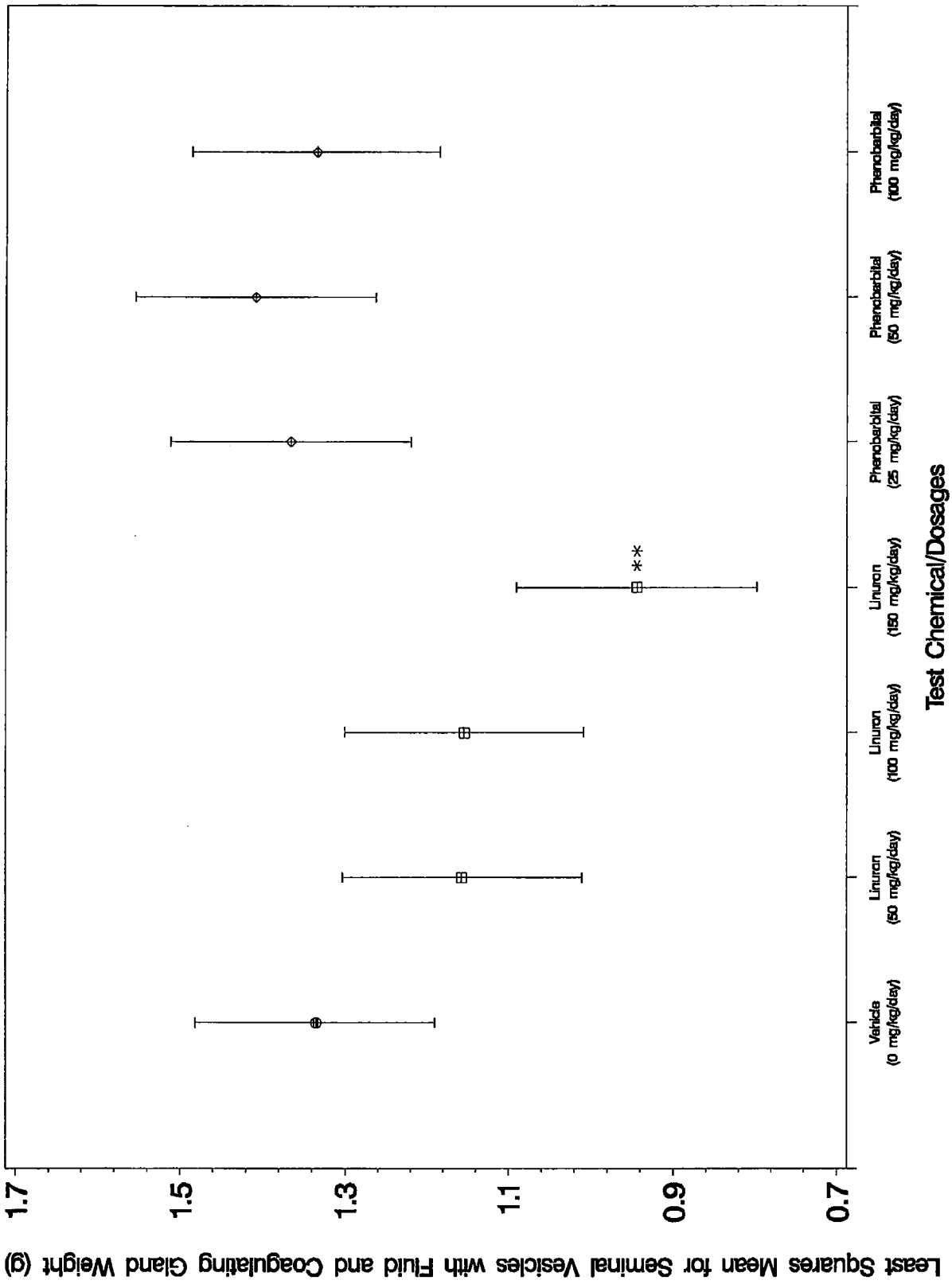


Figure 16. Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for Seminal Vesicles with Fluid And Coagulating Gland Weight (g) for Each Dose Group (Significant Differences from Vehicle Control are Indicated by "**" for the 0.05 Level, and by "***" for the 0.05/8 Level).

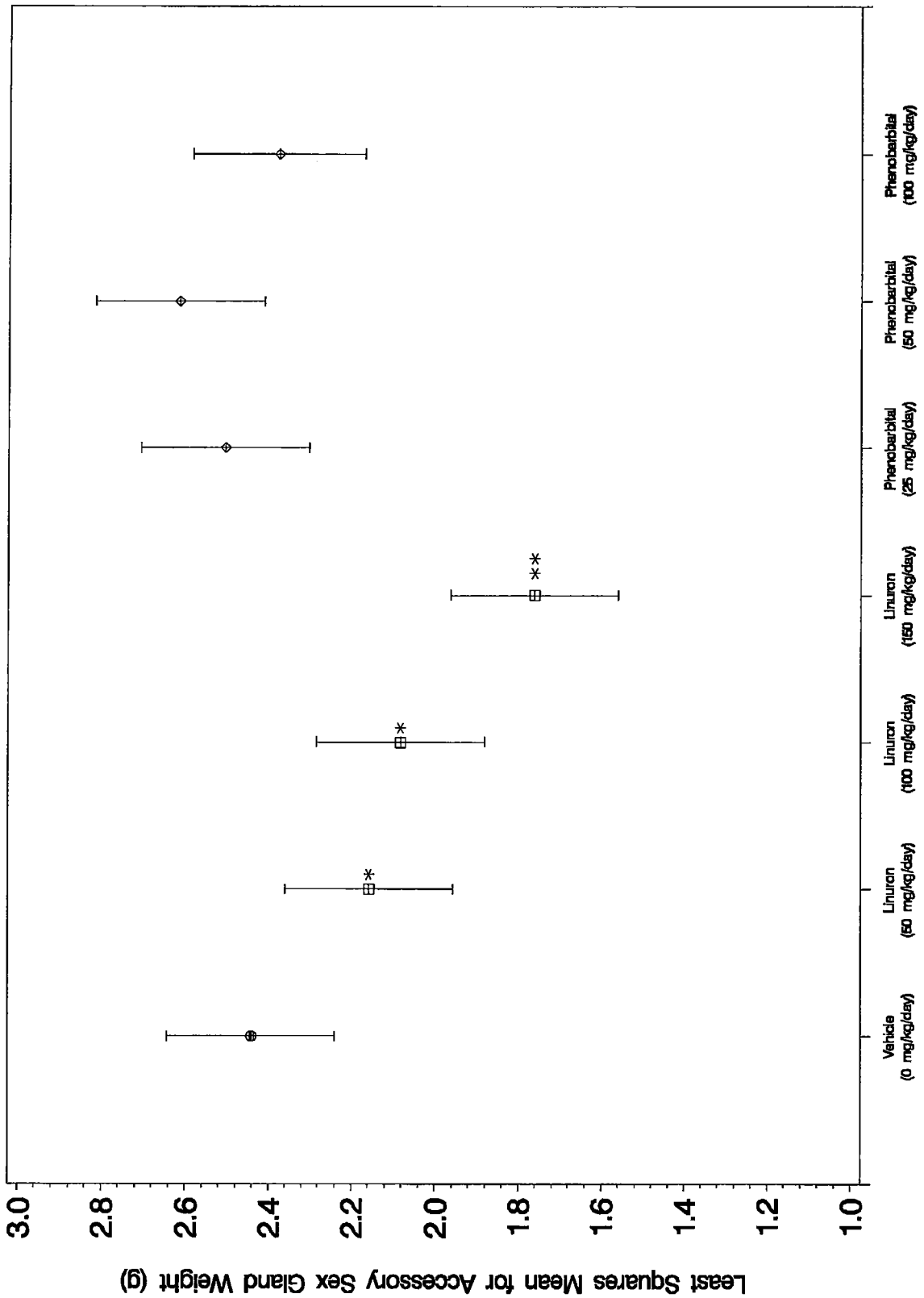


Figure 17. Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for Accessory Sex Gland Weight (g) for Each Dose Group (Significant Differences from Vehicle Control are Indicated by "*" for the 0.05 Level, and by "" for the 0.05/8 Level).**

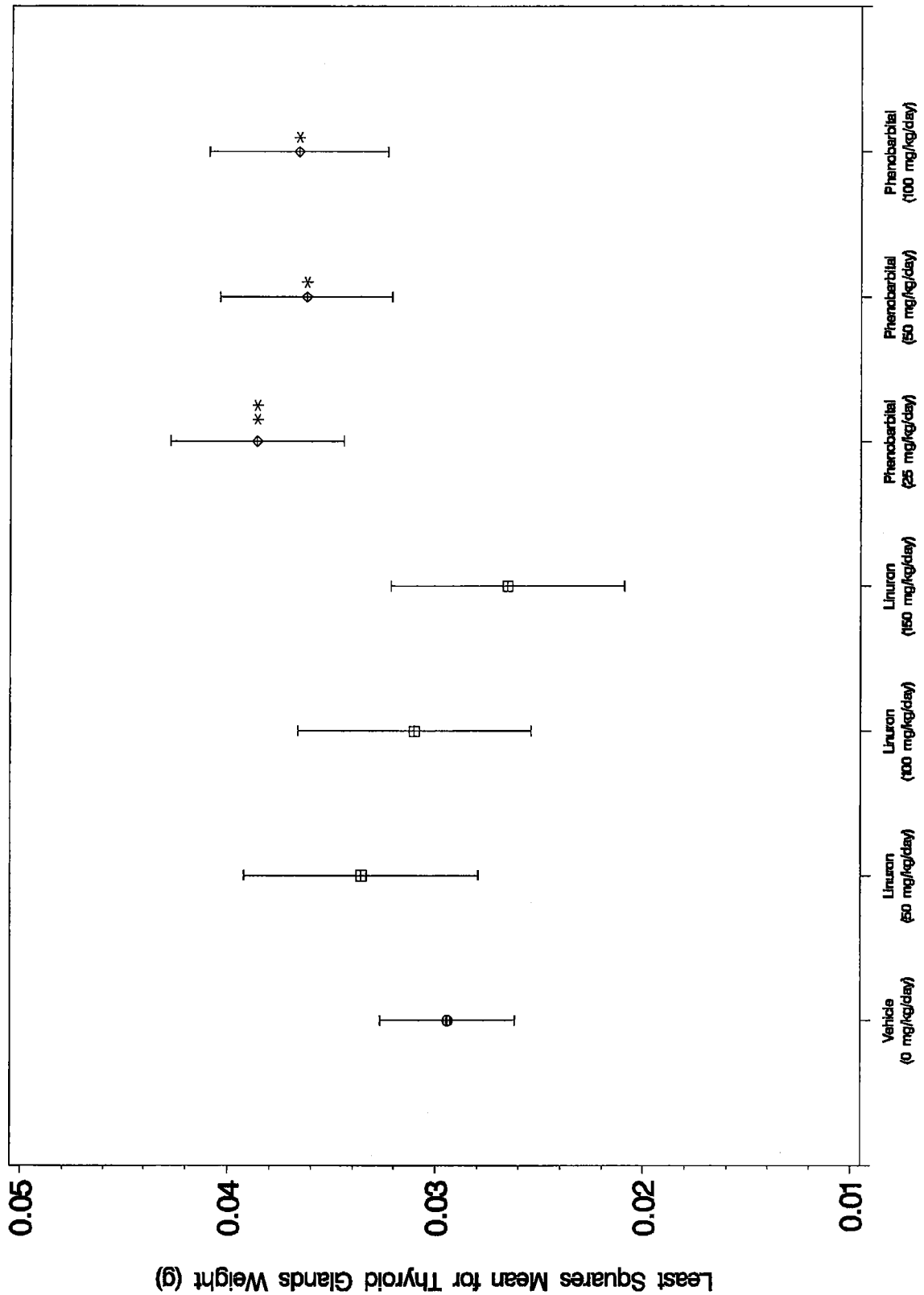


Figure 18. Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for Thyroid Glands Weight (g) For Each Dose Group (Significant Differences from Vehicle Control are Indicated by "*" for the 0.05 Level, and by "**" for the 0.05/8 Level).

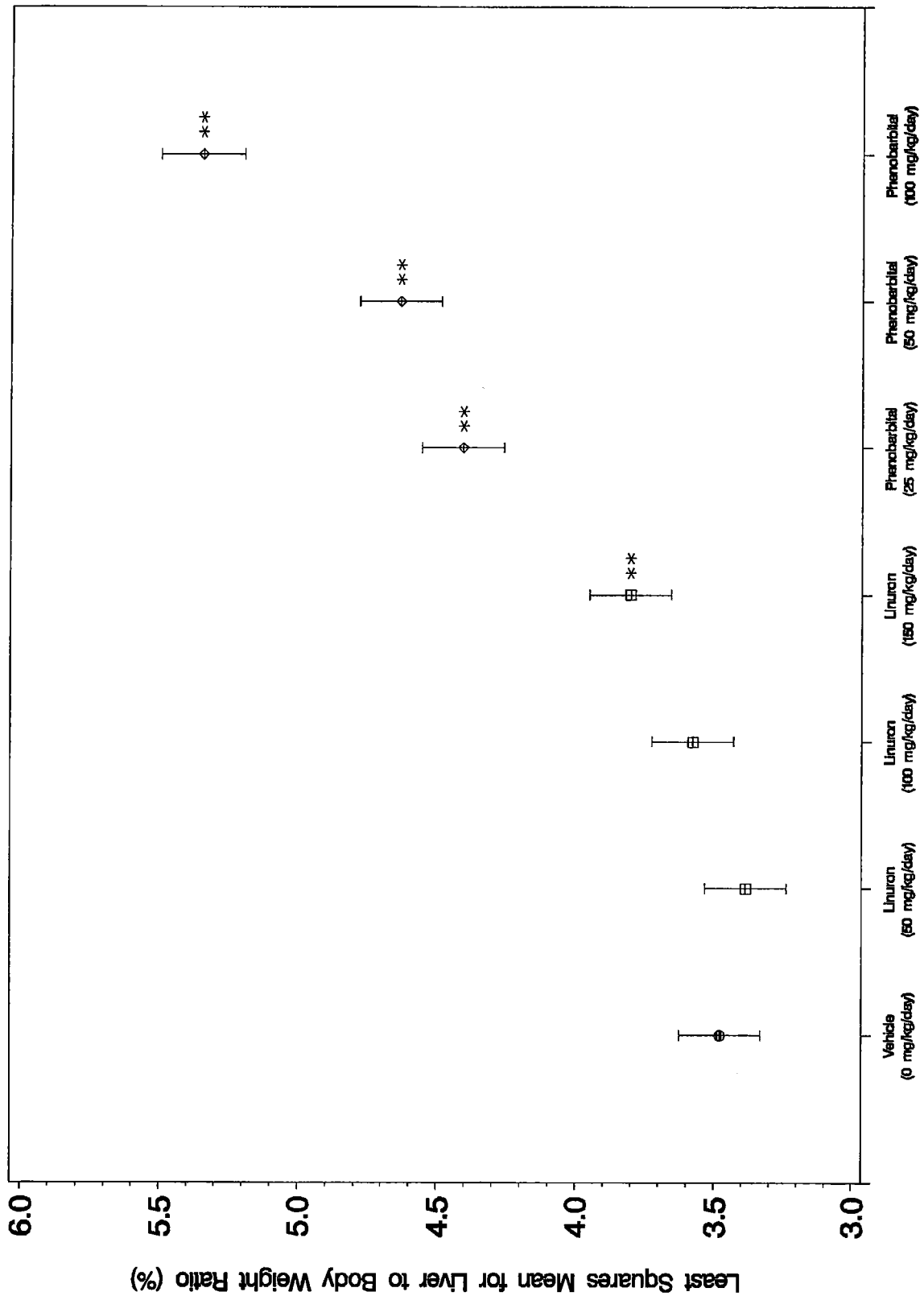


Figure 19. Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for Liver to Body Weight Ratio (%) For Each Dose Group (Significant Differences from Vehicle Control are Indicated by "" for the 0.05 Level, and by "**" for the 0.05/8 Level).**

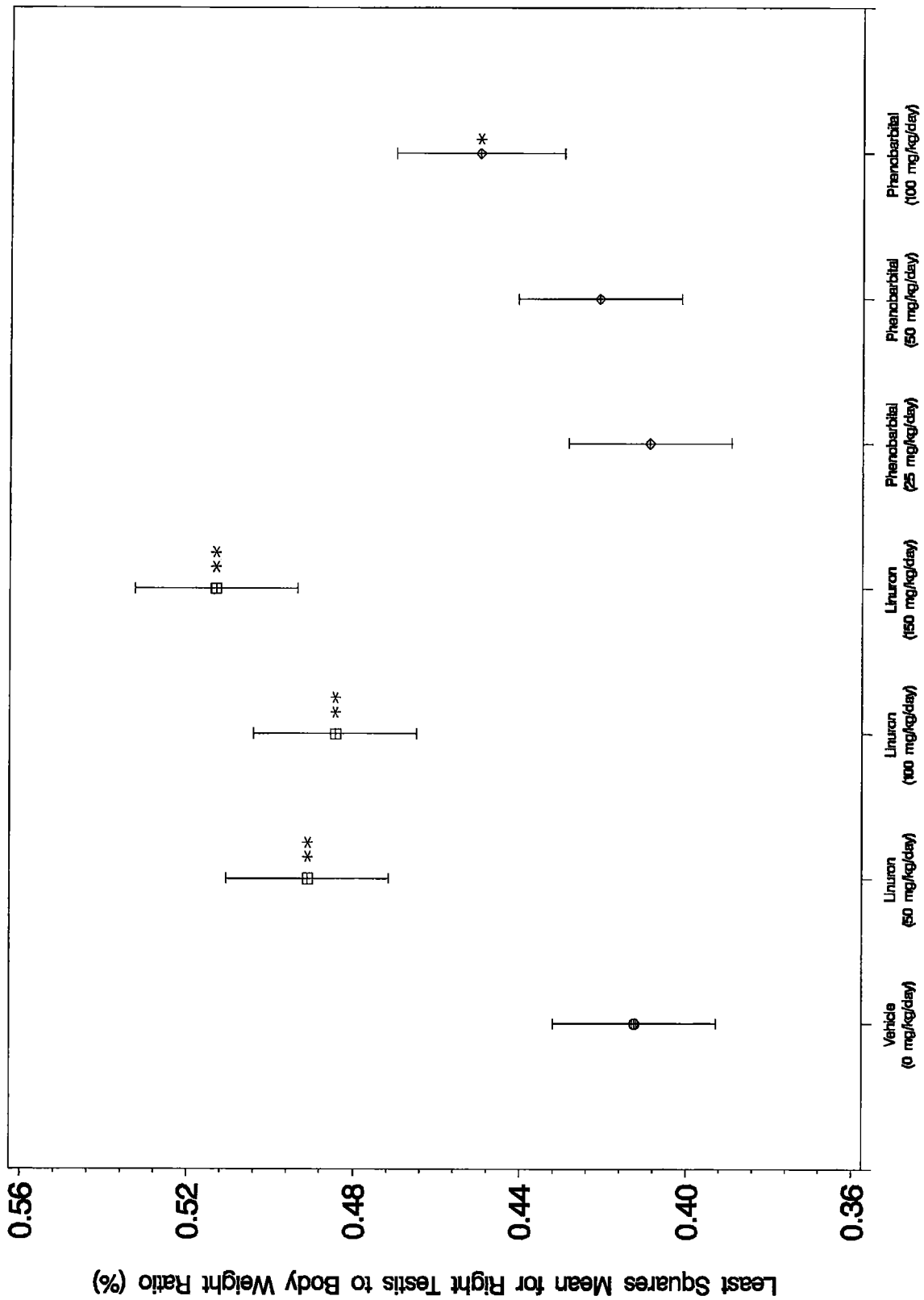


Figure 20. Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for Right Testis to Body Weight Ratio (%) for Each Dose Group (Significant Differences from Vehicle Control are Indicated by "*" for the 0.05 Level, and by "**" for the 0.05/8 Level).

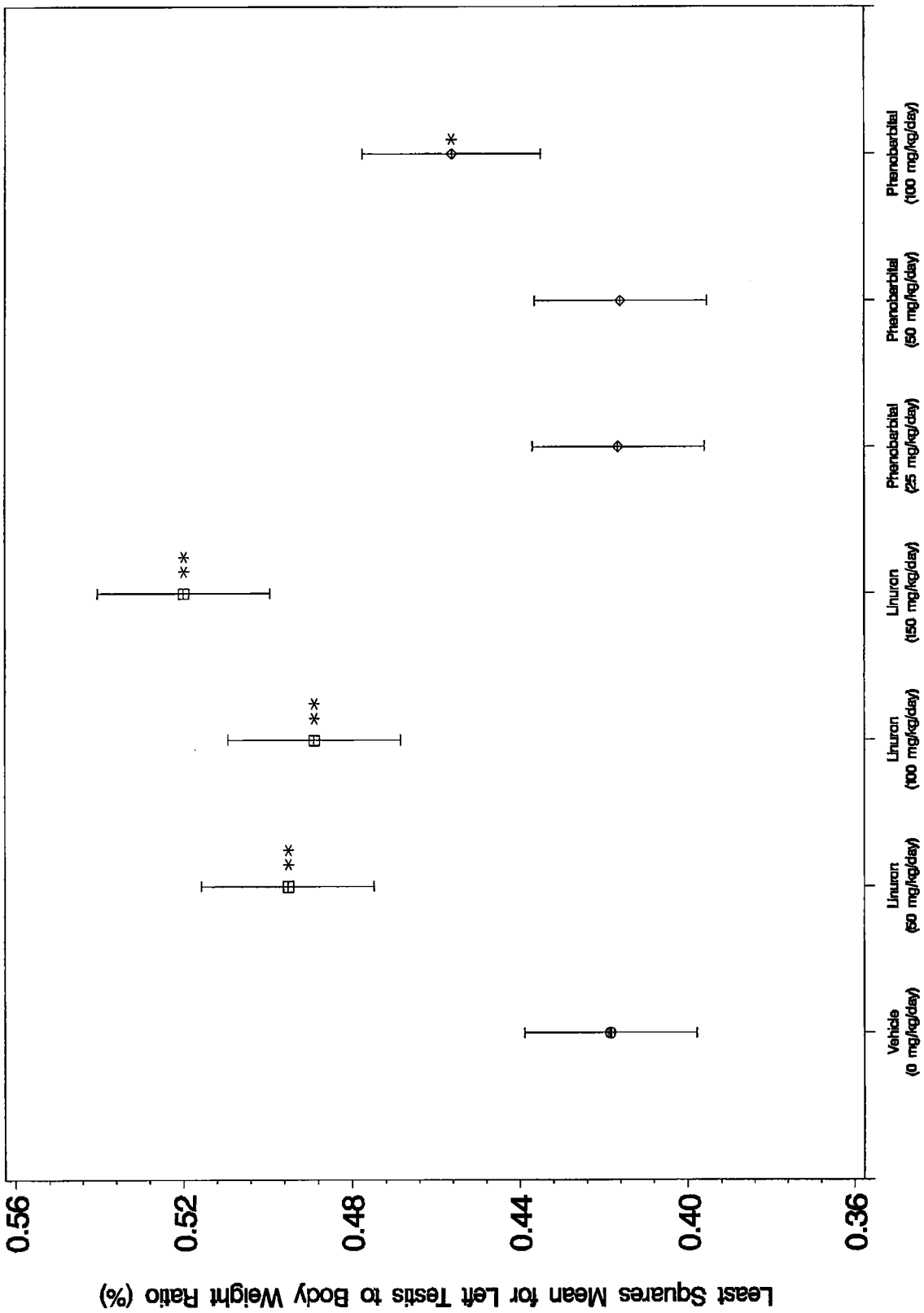


Figure 21. Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for Left Testis to Body Weight Ratio (%) for Each Dose Group (Significant Differences from Vehicle Control are Indicated by "*" for the 0.05 Level, and by "" for the 0.05/8 Level).**

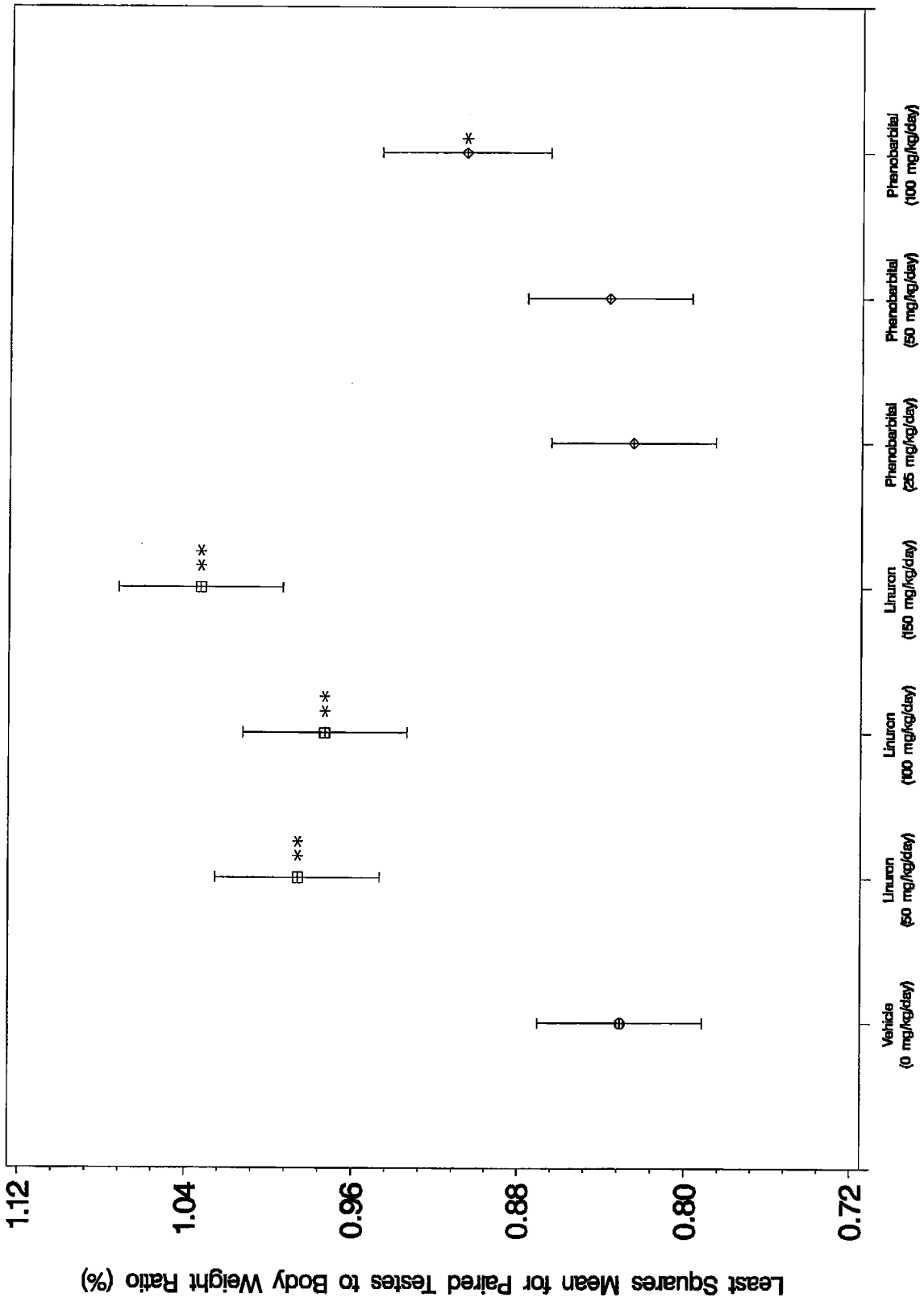


Figure 22. **<u>Charles River Adult Males</u> Least Squares Means (with ± 2 Standard Error Bars) for Paired Testes to Body Weight Ratio (%) for Each Dose Group (Significant Differences from Vehicle Control are Indicated by "**" for the 0.05 Level, and by "***" for the 0.05/8 Level).**

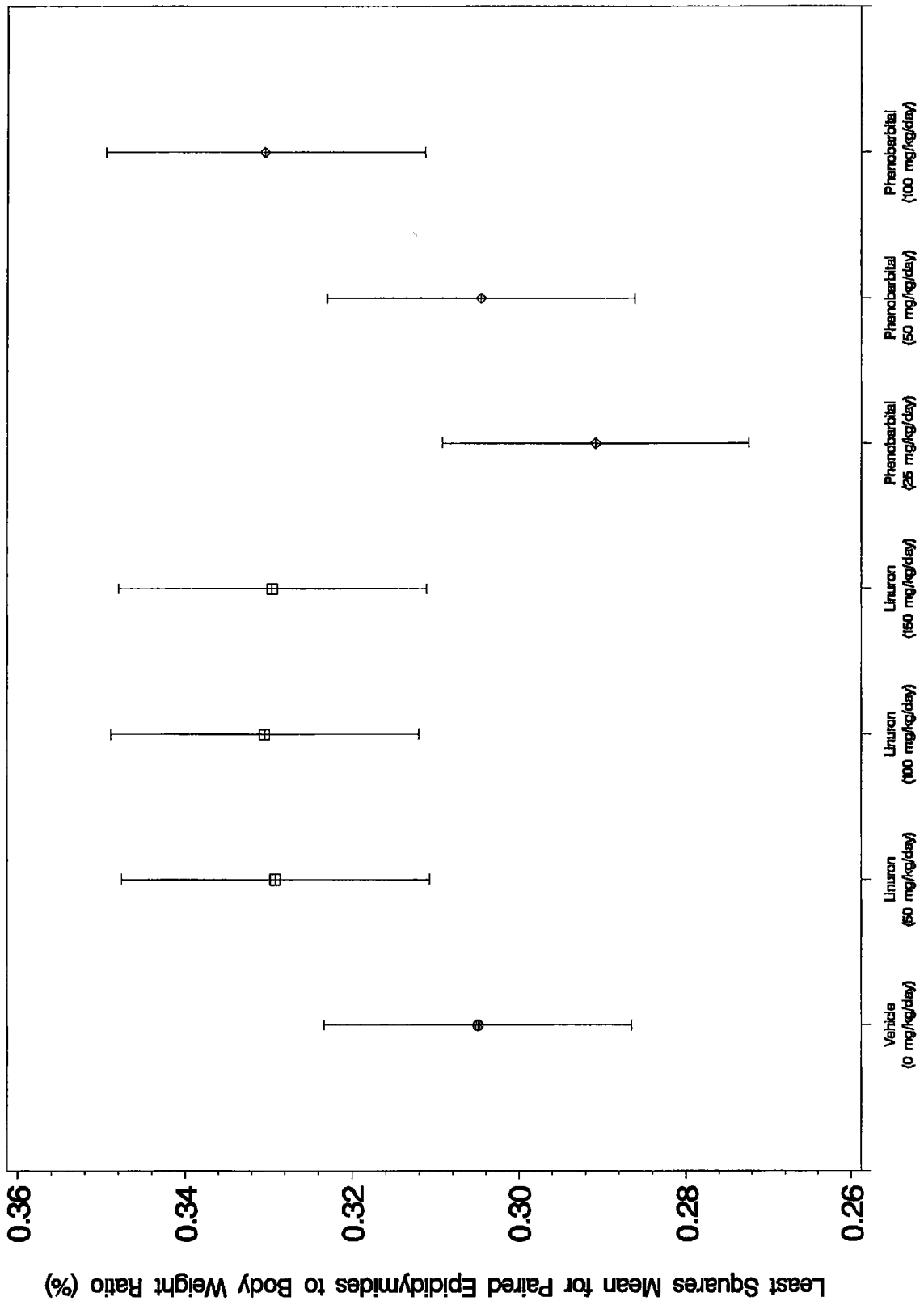


Figure 23. Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for Paired Epididymides to Body Weight Ratio (%) for Each Dose Group (Significant Differences from Vehicle Control are Indicated by "*" for the 0.05 Level, and by "" for the 0.05/8 Level).**

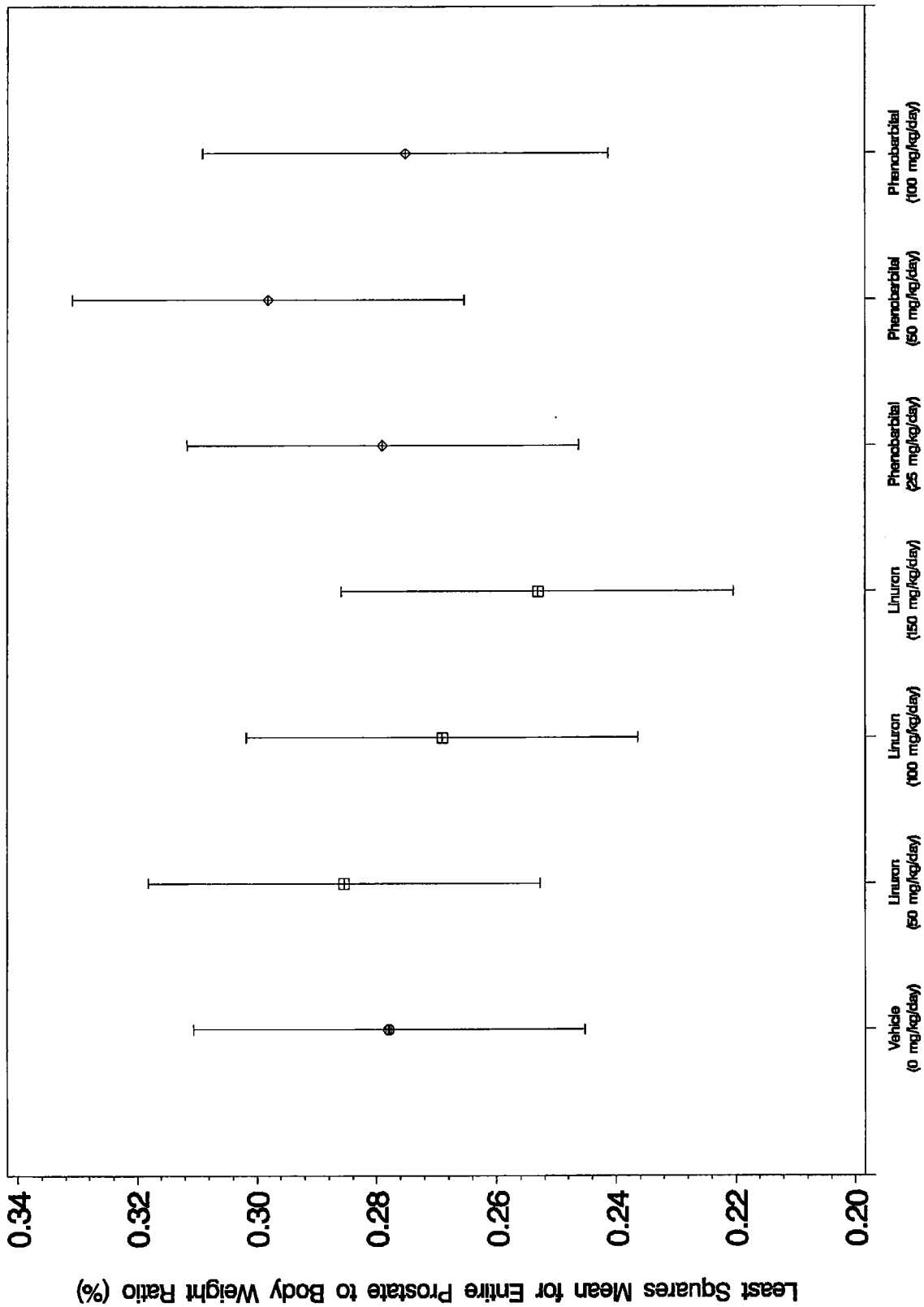


Figure 24. Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for Entire Prostate to Body Weight Ratio (%) for Each Dose Group (Significant Differences from Vehicle Control are Indicated by "*" for the 0.05 Level, and by "*" for the 0.05/8 Level).**

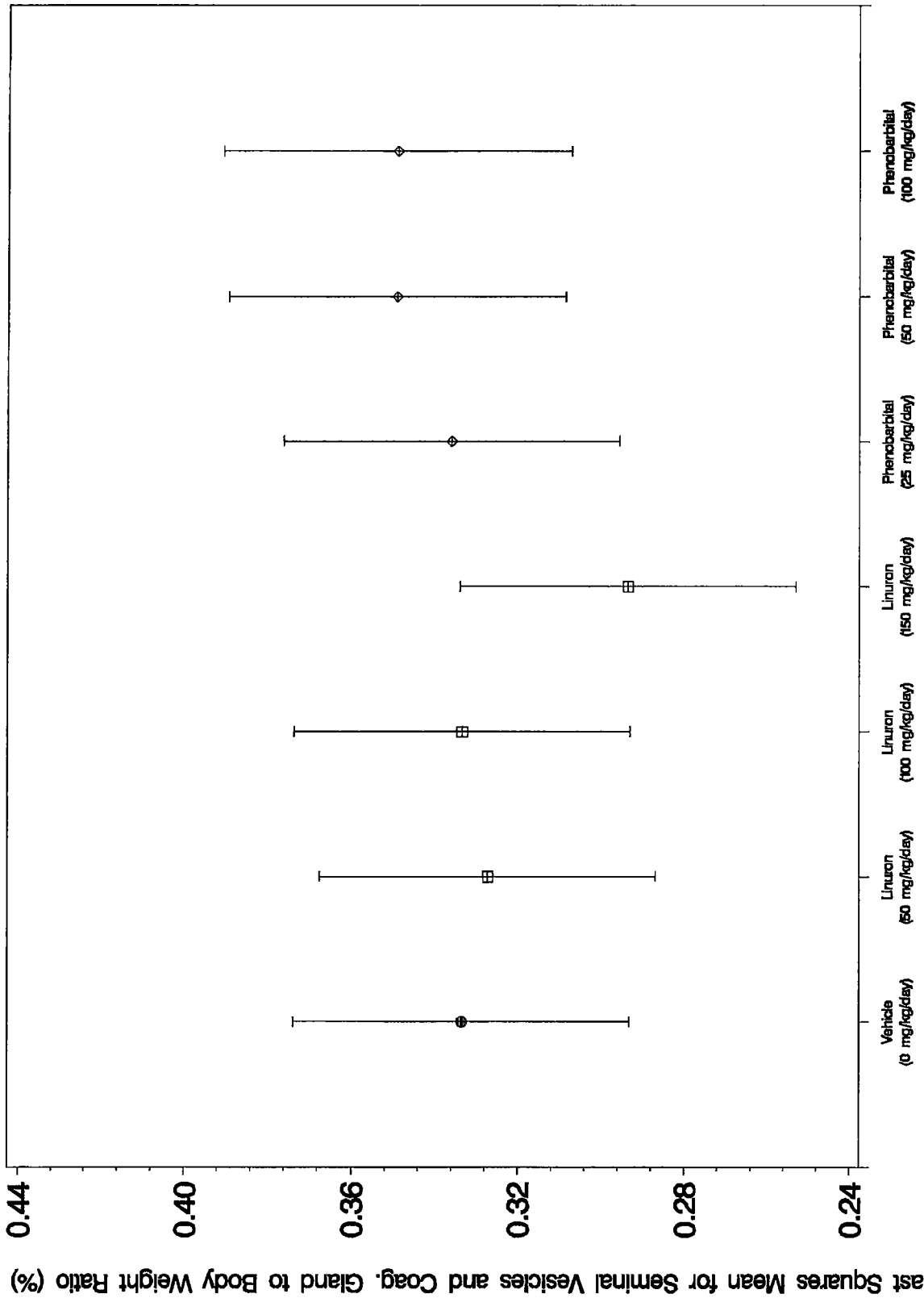


Figure 25. Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for Seminal Vesicles with Fluid and Coagulating Gland to Body Weight Ratio (%) for Each Dose Group (Significant Differences from Vehicle Control are Indicated by "*" for the 0.05 Level, and by "" for the 0.05/8 Level).**

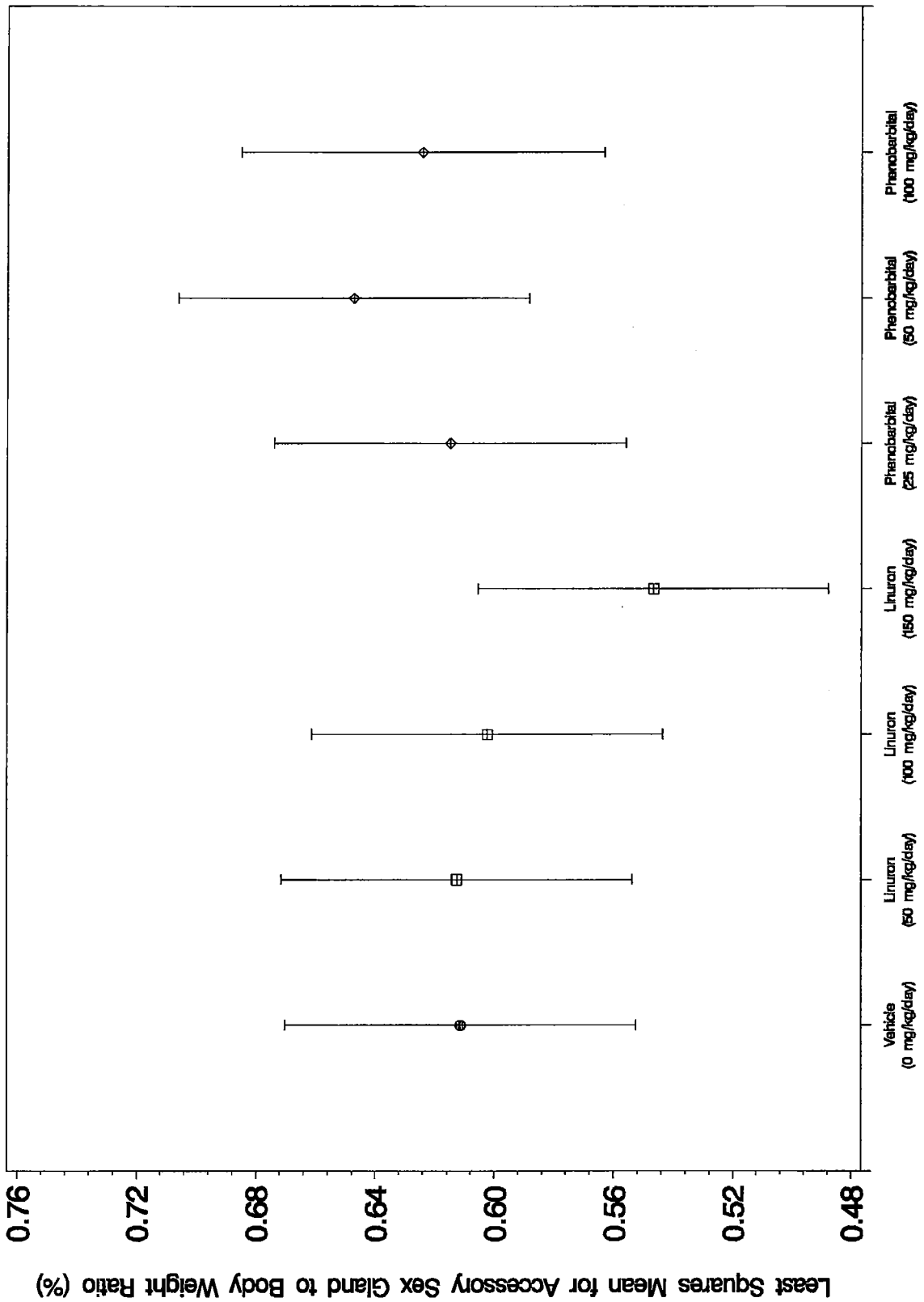


Figure 26. Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for Accessory Sex Gland to Body Weight Ratio (%) for Each Dose Group (Significant Differences from Vehicle Control are Indicated by "*" for the 0.05 Level, and by "" for the 0.05/8 Level).**

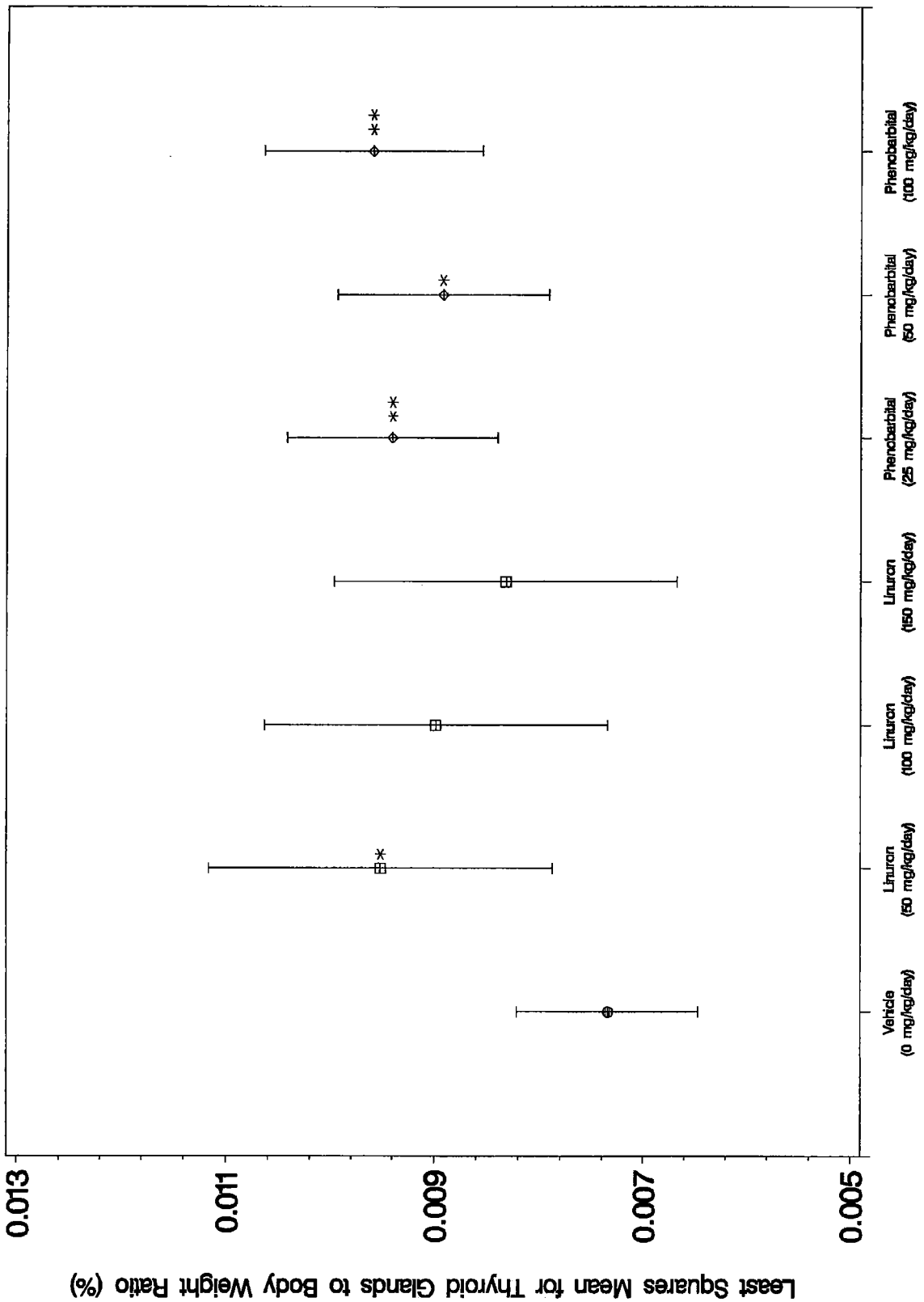


Figure 27. Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for Thyroid Glands to Body Weight Ratio (%) for Each Dose Group (Significant Differences from Vehicle Control are Indicated by "*" for the 0.05 Level, and by "**" for the 0.05/8 Level).

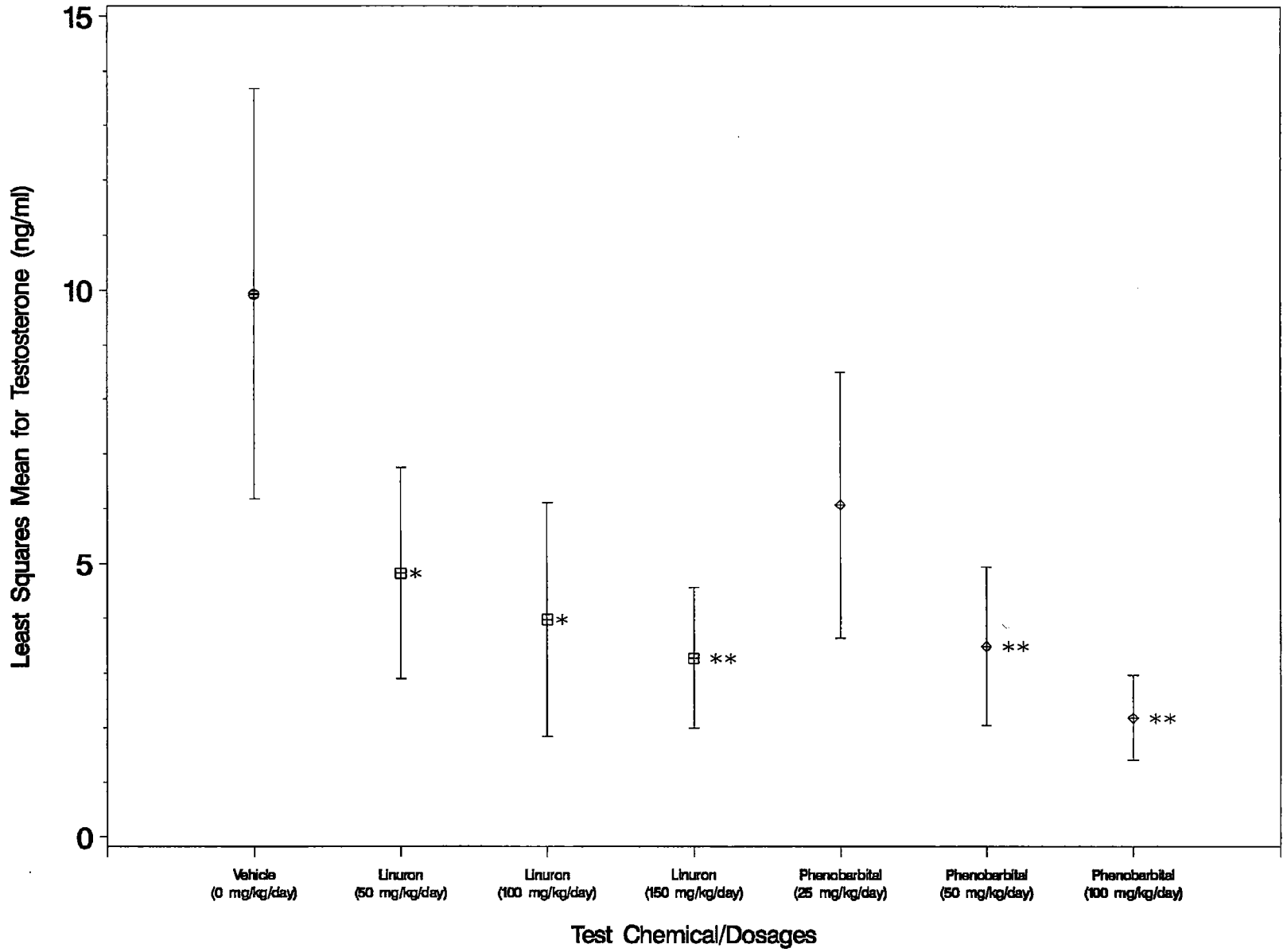


Figure 28. **<u>Charles River Adult Males</u> Least Squares Means (with ± 2 Standard Error Bars) for Testosterone (ng/ml) for Each Dose Group (Significant Differences from Vehicle Control are Indicated by "*" for the 0.05 Level, and by "**" for the 0.05/8 Level).**

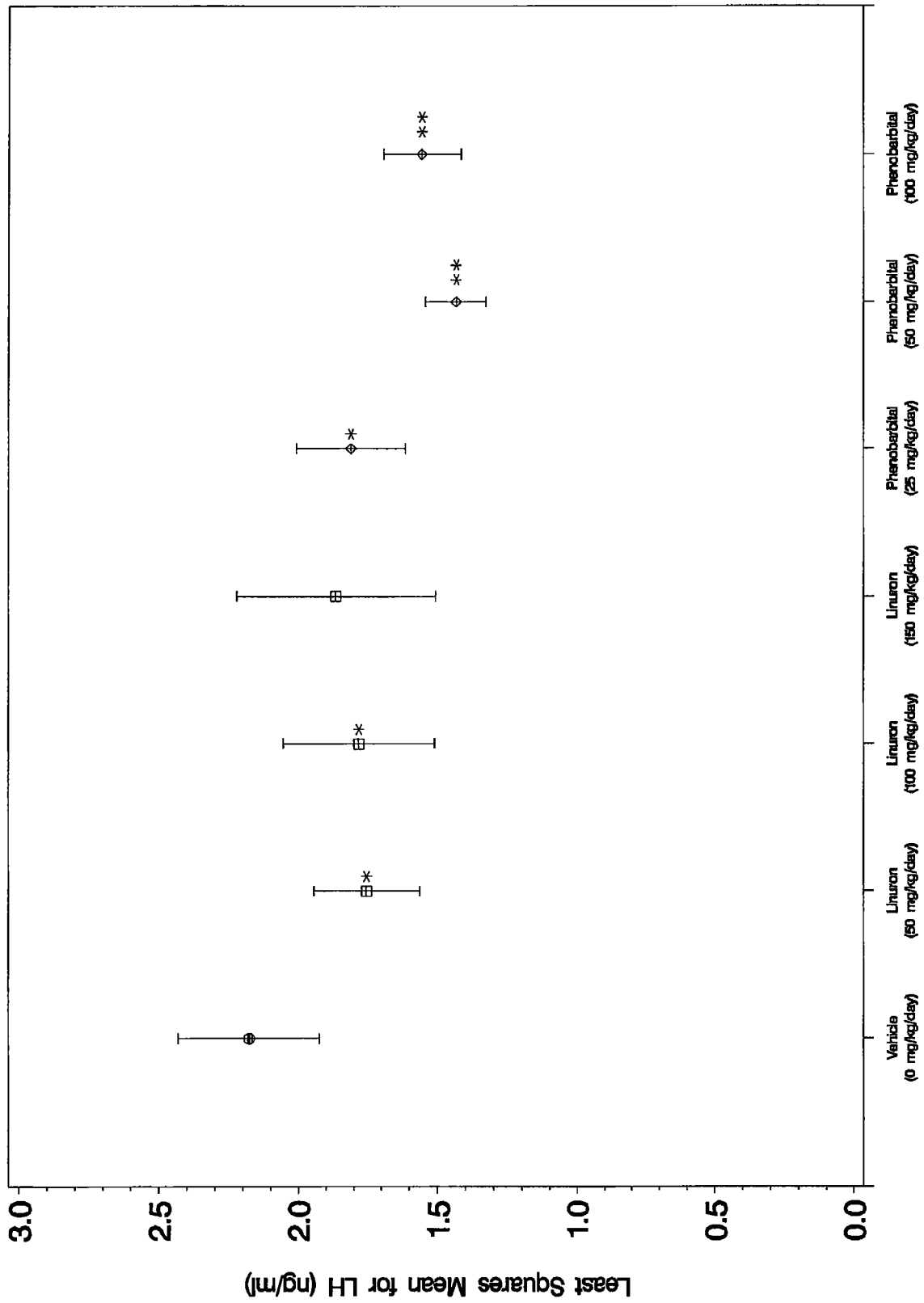


Figure 29. **Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for LH (ng/ml) for Each Dose Group (Significant Differences from Vehicle Control are Indicated by "*" for the 0.05 Level, and by "**" for the 0.05/8 Level).**

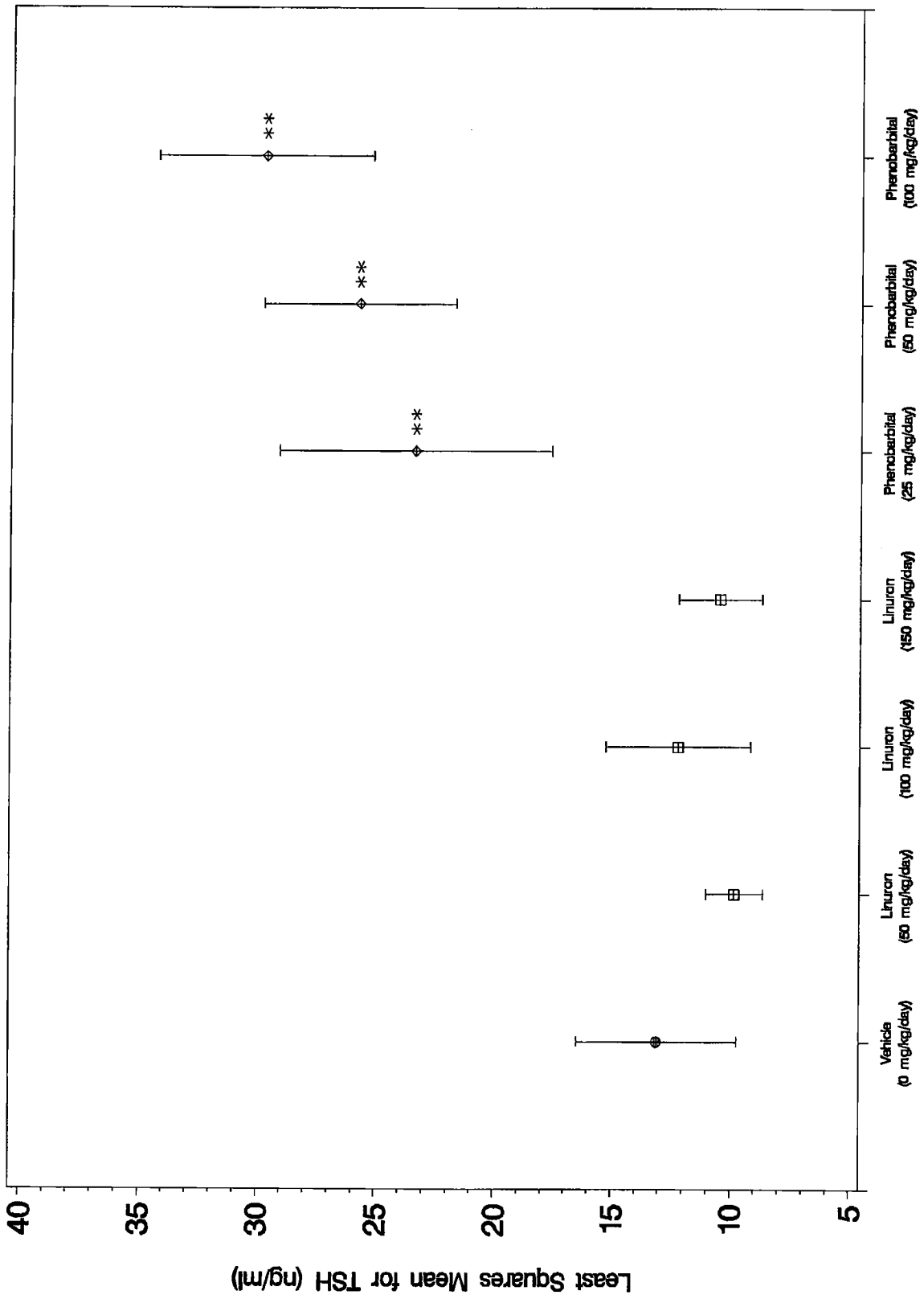


Figure 30. Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for TSH (ng/ml) for Each Dose Group (Significant Differences from Vehicle Control are Indicated by "*" for the 0.05 Level, and by "" for the 0.05/8 Level).**

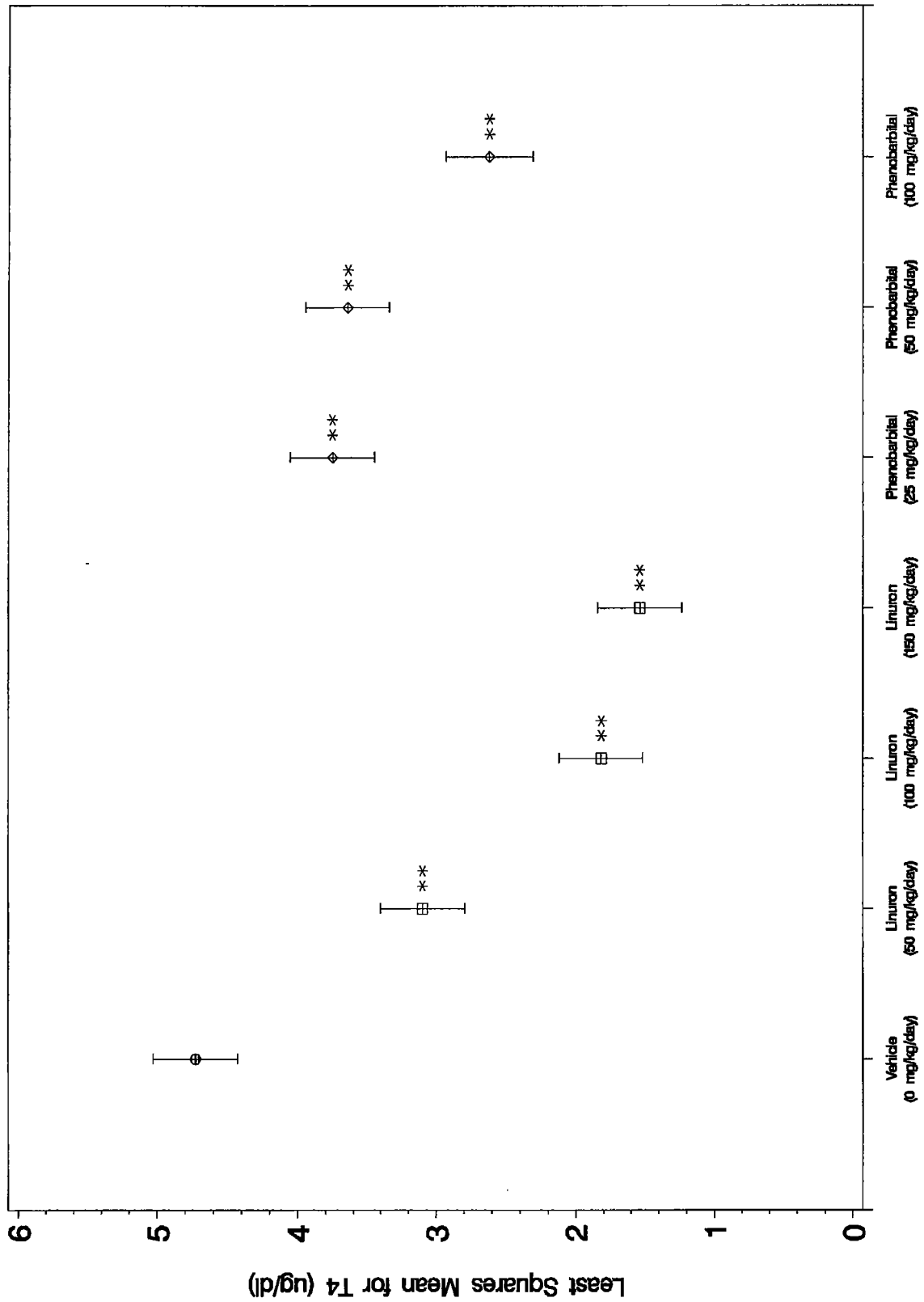


Figure 31. Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for T4 (µg/dl) for Each Dose Group (Significant Differences from Vehicle Control are Indicated by "*" for the 0.05 Level, and by "" for the 0.05/8 Level).**

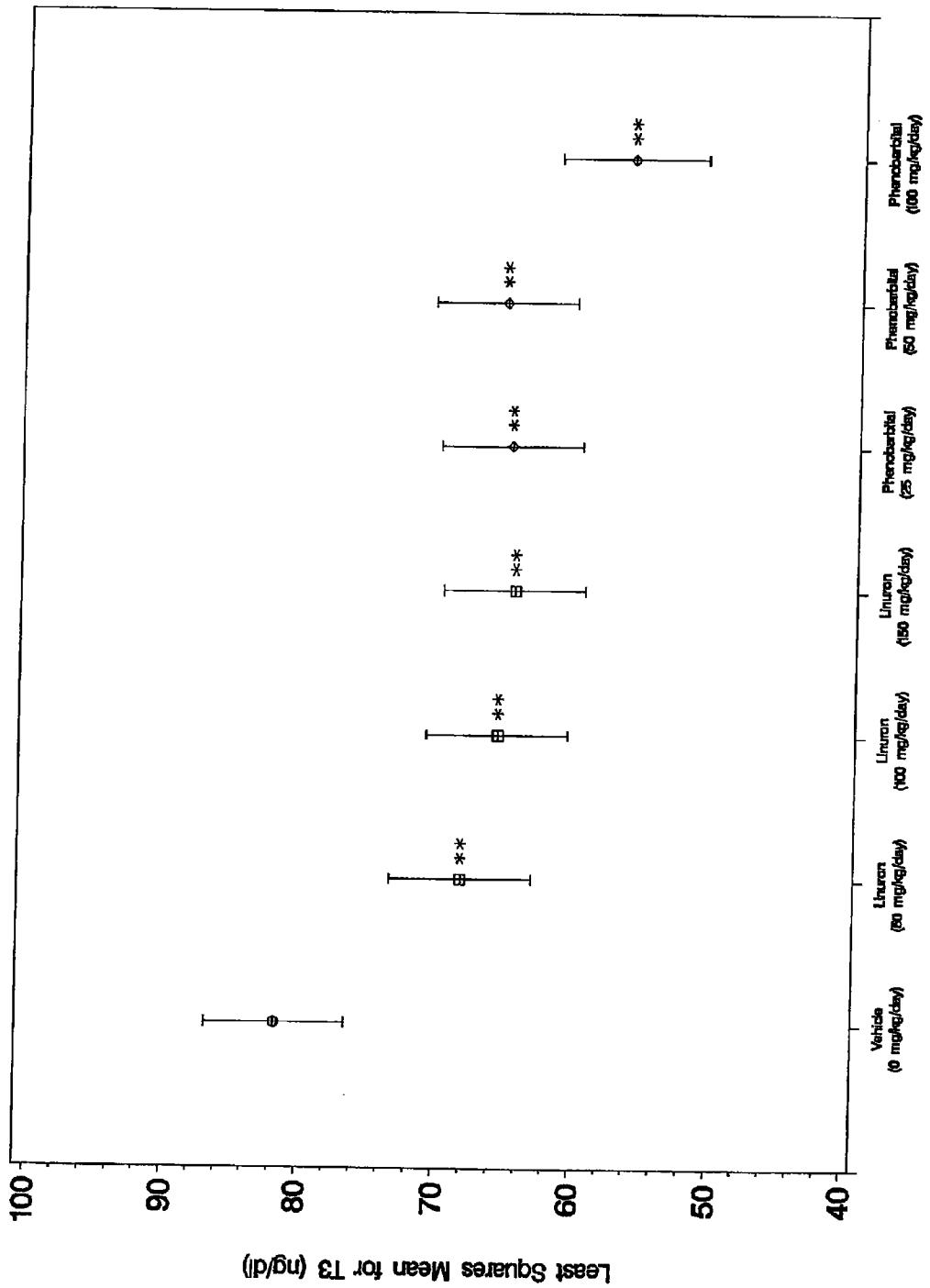


Figure 32. Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for T3 (ng/dl) for Each Dose Group (Significant Differences from Vehicle Control are Indicated by "**" for the 0.05 Level, and by "***" for the 0.05/8 Level).

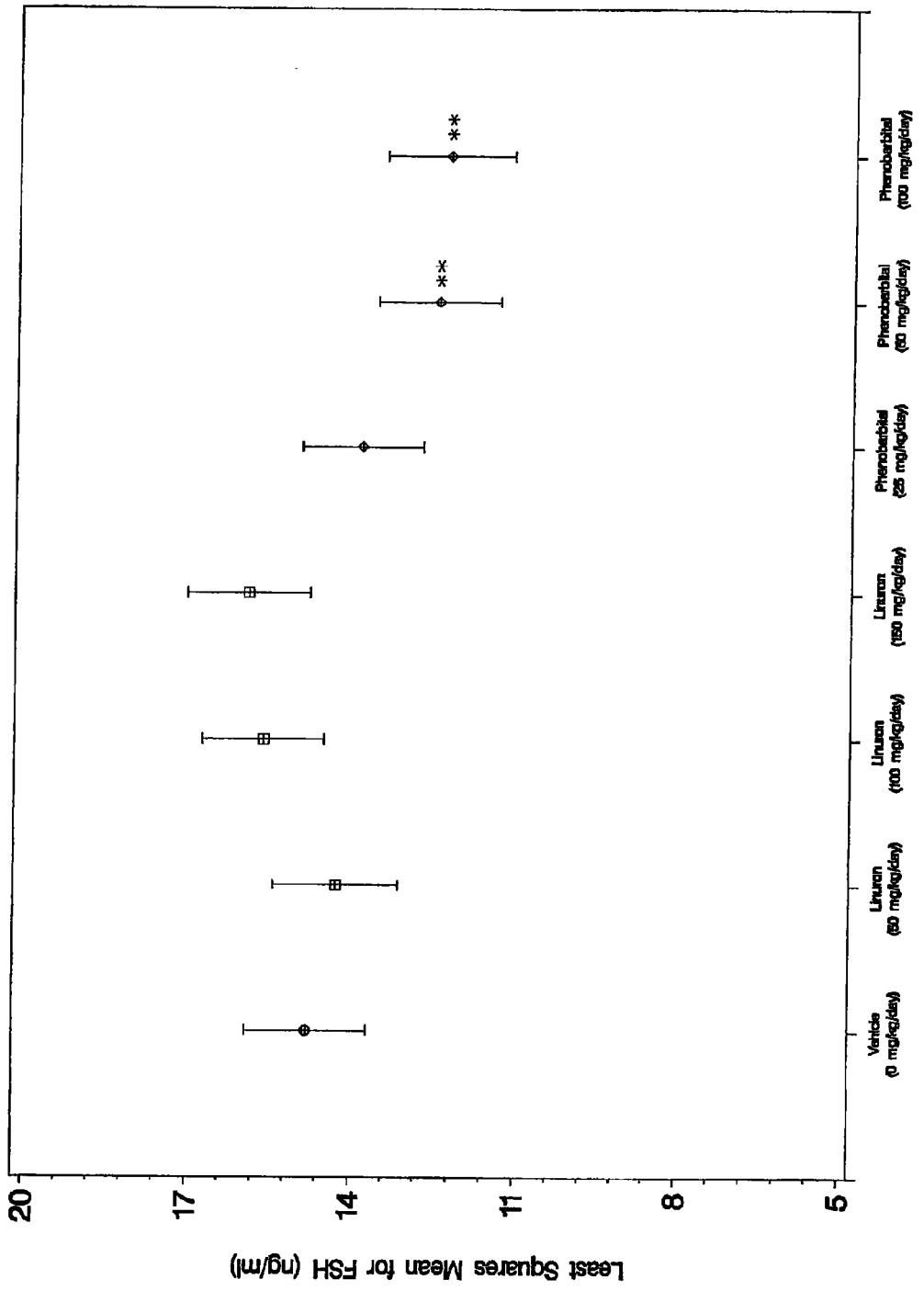


Figure 33. Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for FSH (ng/ml) for Each Dose Group (Significant Differences from Vehicle Control are Indicated by "*" for the 0.05 Level, and by "**" for the 0.05/8 Level).

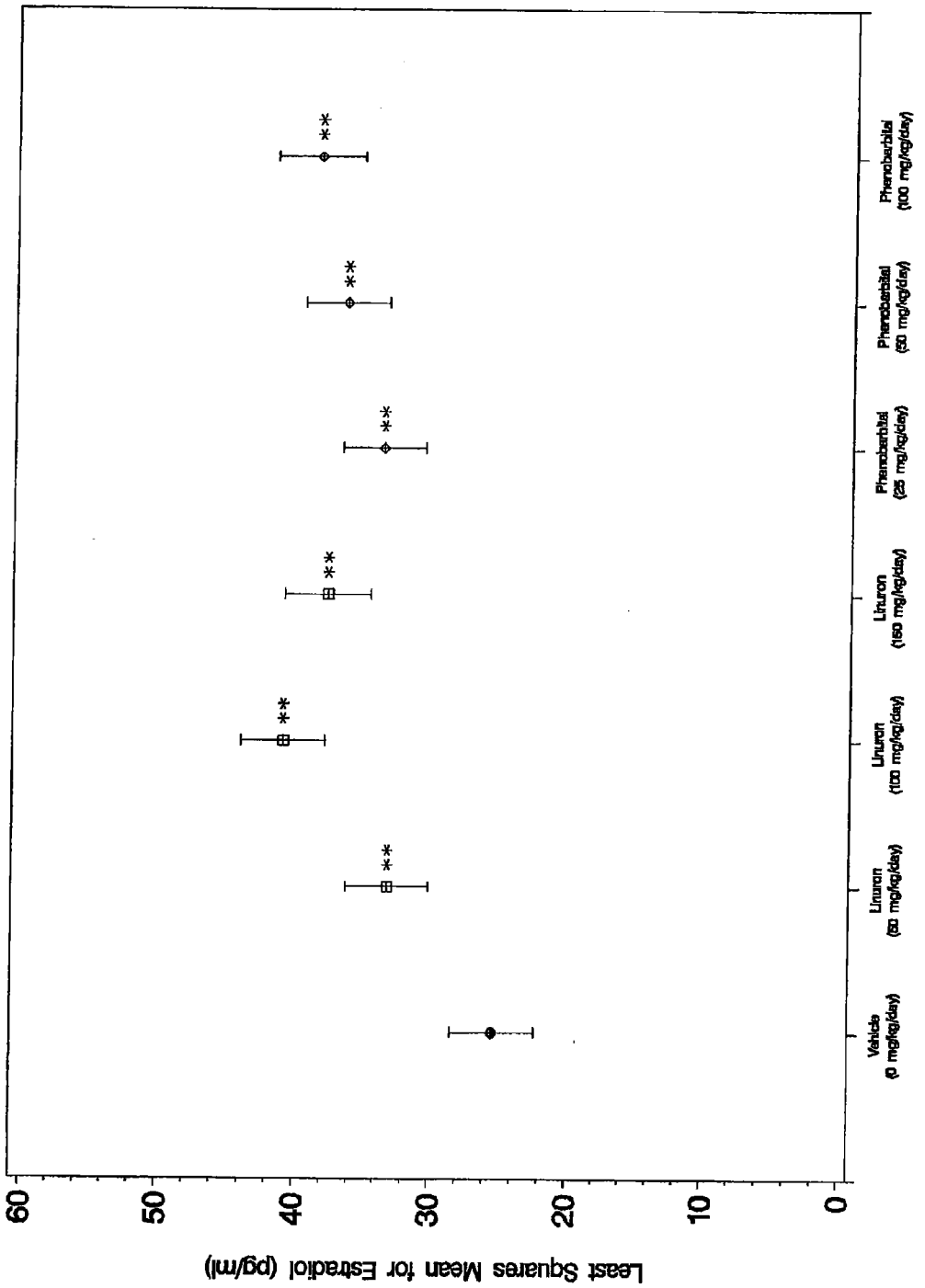


Figure 34. Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for Estradiol (pg/ml) for Each Dose Group (Significant Differences from Vehicle Control are Indicated by "" for the 0.05 Level, and by "**" for the 0.05/8 Level).**

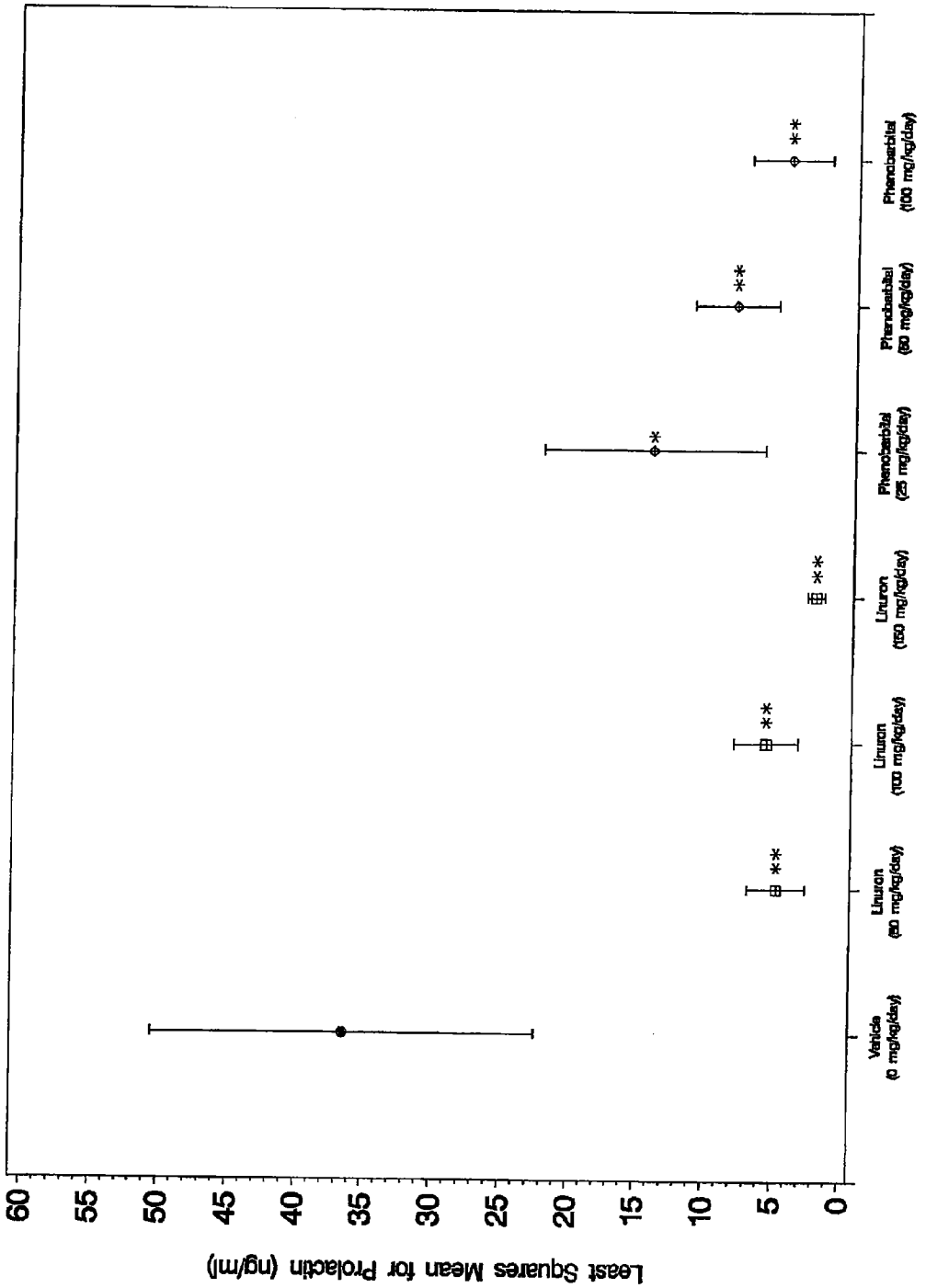


Figure 35. **Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for Prolactin (ng/ml) for Each Dose Group (Significant Differences from Vehicle Control are Indicated by "*" for the 0.05 Level, and by "**" for the 0.05/8 Level).**

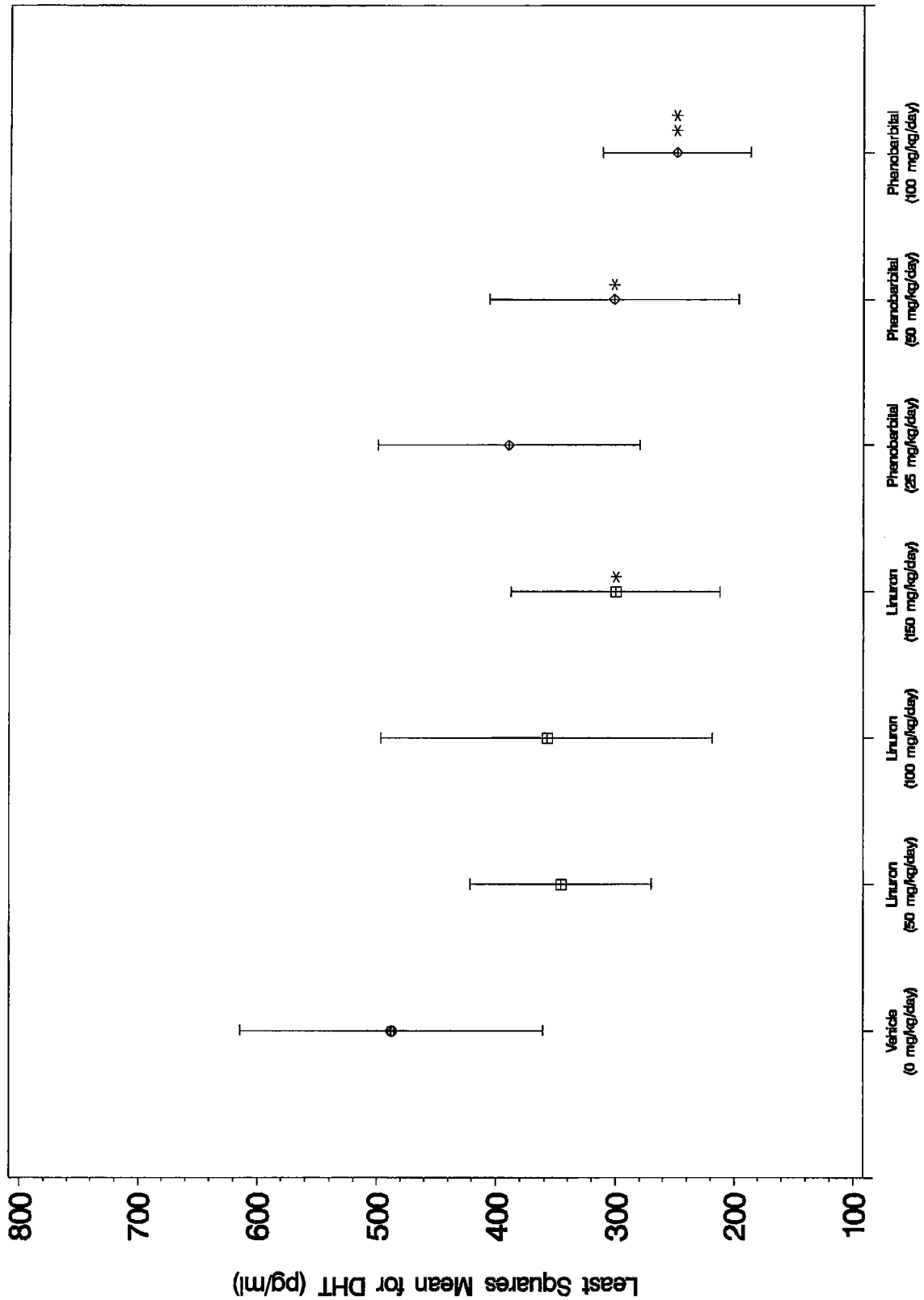


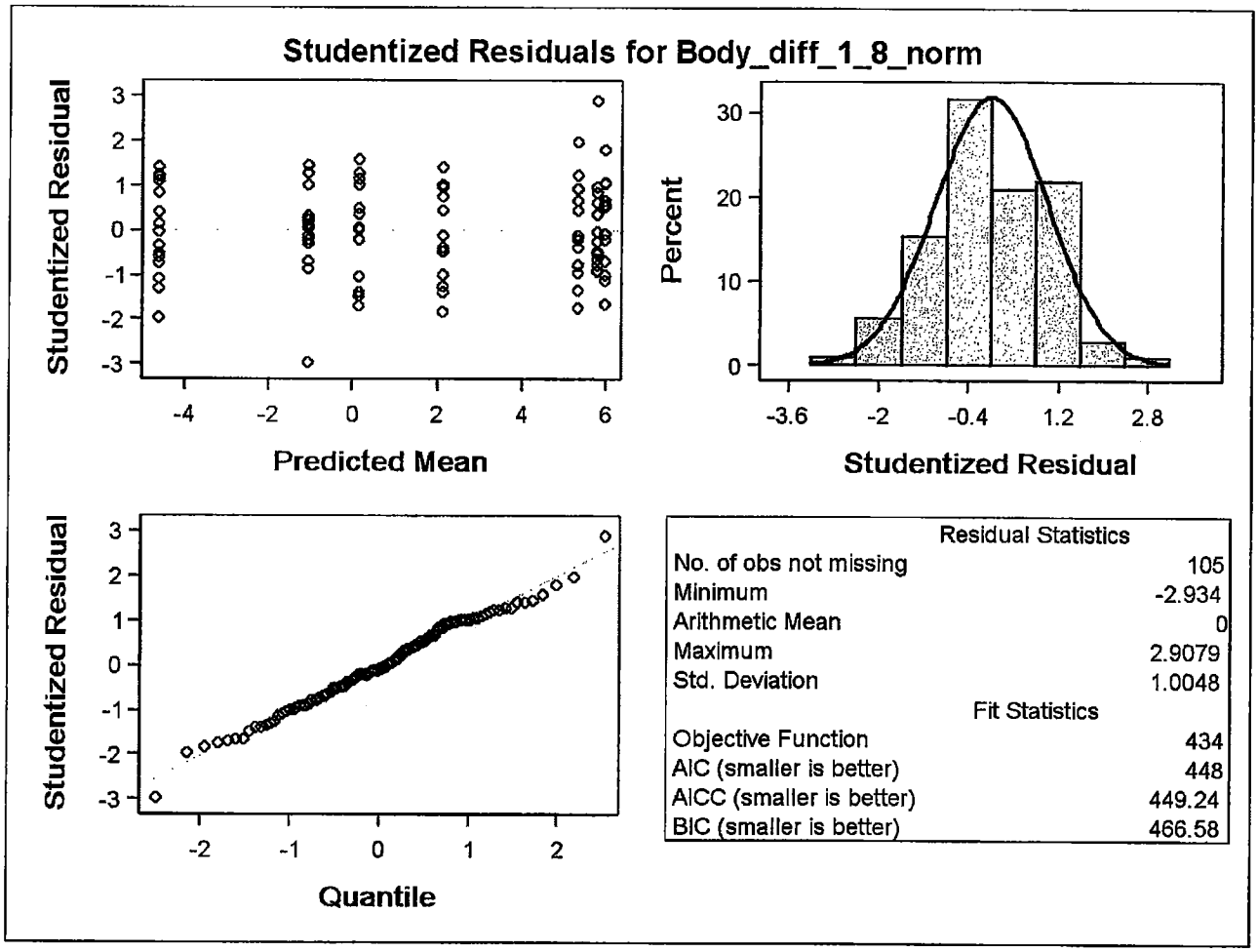
Figure 36. Charles River Adult Males Least Squares Means (with ± 2 Standard Error Bars) for DHT (pg/ml) for Each Dose Group (Significant Differences from Vehicle Control are Indicated by "*" for the 0.05 Level, and by "" for the 0.05/8 Level).**

Appendix A

Normal Probability Plots for Growth, Food Consumptions, Organ Weights, Organ Weight to Body Ratios, and Hormonal Analysis Endpoints.

Charles River: Adult Males
Outlier Screens
Body Weight Change TD8-TD1 (g/day)

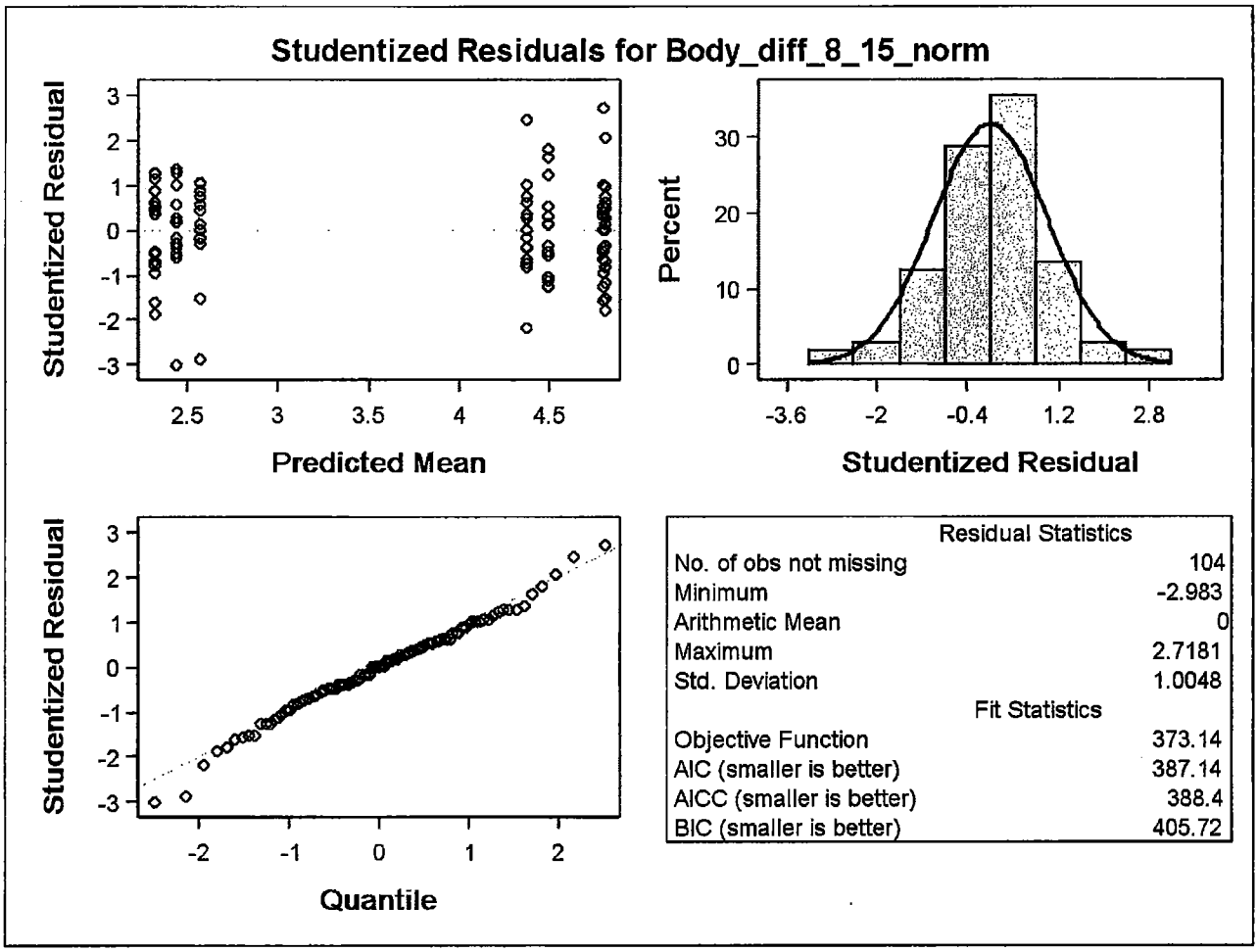
The Mixed Procedure



Residual Statistics	
No. of obs not missing	105
Minimum	-2.934
Arithmetic Mean	0
Maximum	2.9079
Std. Deviation	1.0048
Fit Statistics	
Objective Function	434
AIC (smaller is better)	448
AICC (smaller is better)	449.24
BIC (smaller is better)	466.58

Charles River: Adult Males
Outlier Screens
Body Weight Change TD15-TD8 (g/day)

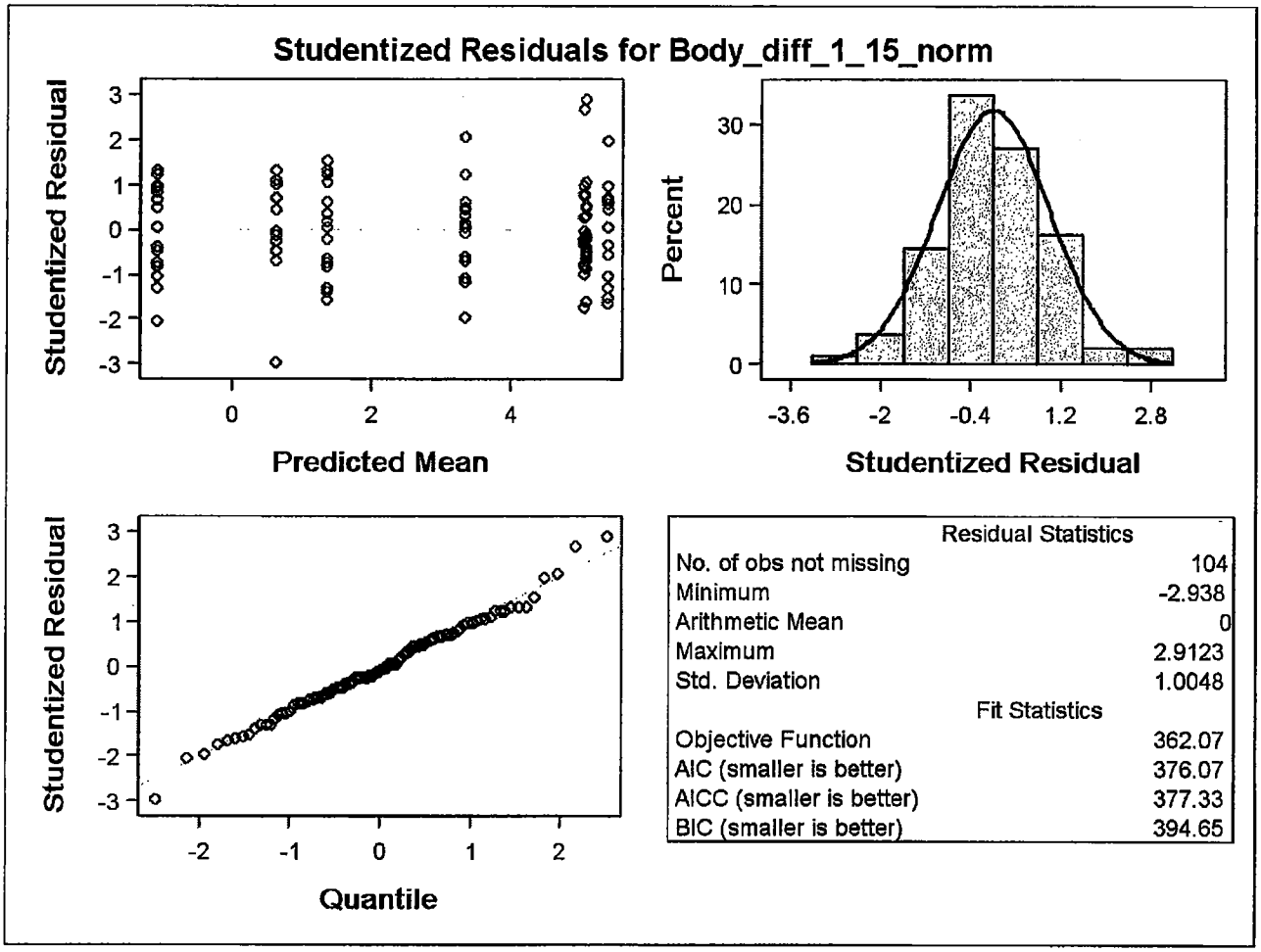
The Mixed Procedure



Residual Statistics	
No. of obs not missing	104
Minimum	-2.983
Arithmetic Mean	0
Maximum	2.7181
Std. Deviation	1.0048
Fit Statistics	
Objective Function	373.14
AIC (smaller is better)	387.14
AICC (smaller is better)	388.4
BIC (smaller is better)	405.72

Charles River: Adult Males
Outlier Screens
Body Weight Change TD15-TD1 (g/day)

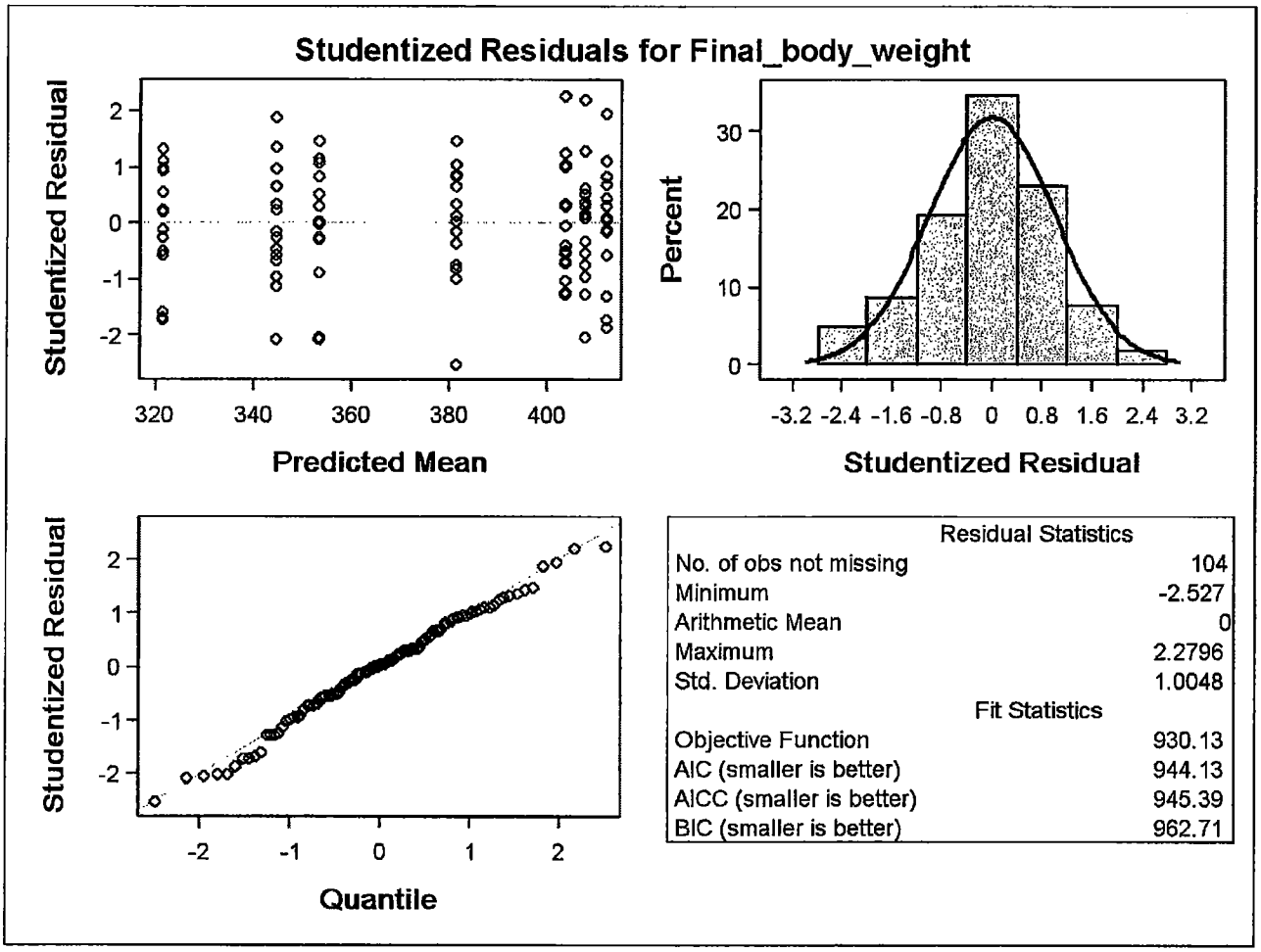
The Mixed Procedure



Residual Statistics	
No. of obs not missing	104
Minimum	-2.938
Arithmetic Mean	0
Maximum	2.9123
Std. Deviation	1.0048
Fit Statistics	
Objective Function	362.07
AIC (smaller is better)	376.07
AICC (smaller is better)	377.33
BIC (smaller is better)	394.65

Charles River: Adult Males
Outlier Screens
Final Body Weight (g)

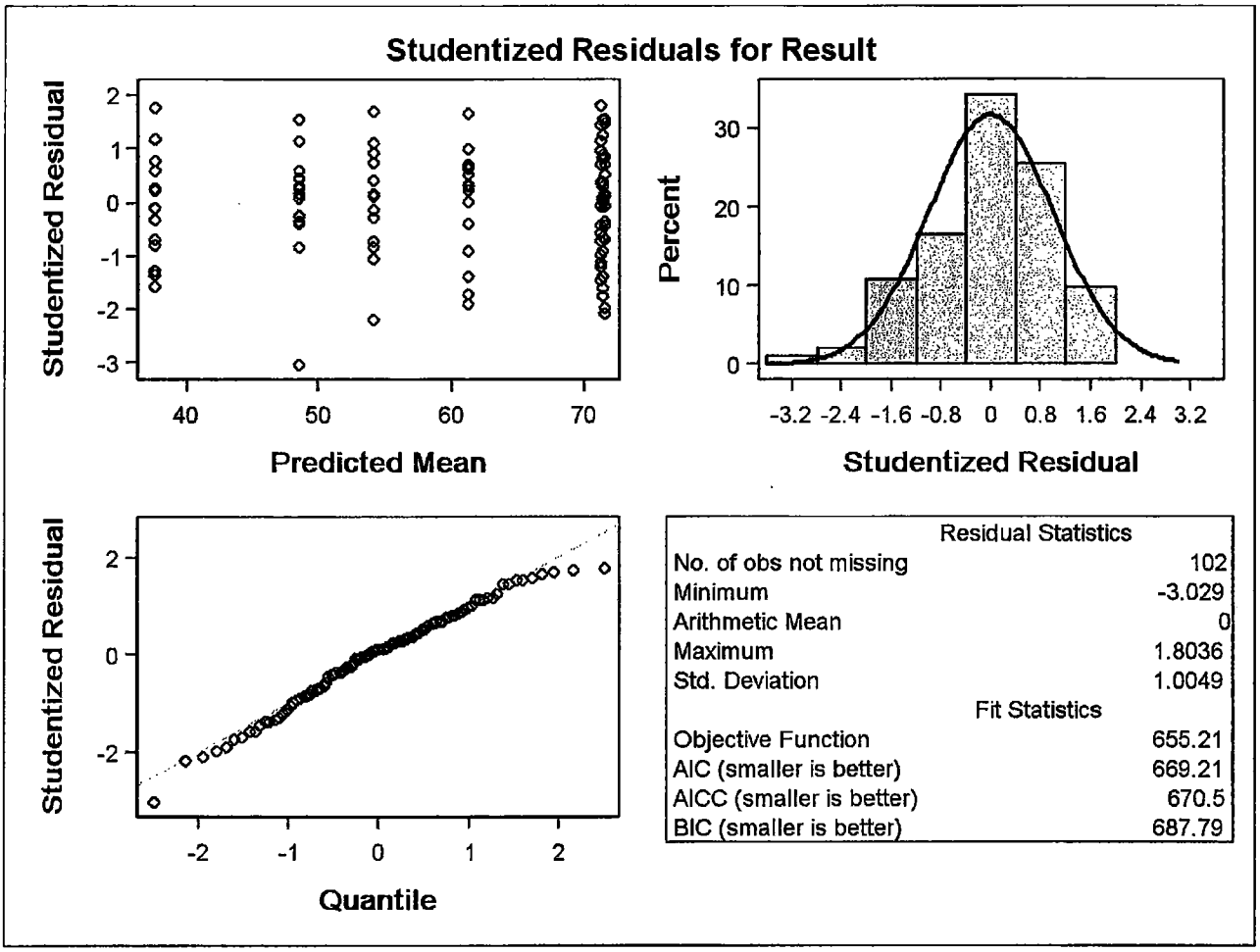
The Mixed Procedure



Residual Statistics	
No. of obs not missing	104
Minimum	-2.527
Arithmetic Mean	0
Maximum	2.2796
Std. Deviation	1.0048
Fit Statistics	
Objective Function	930.13
AIC (smaller is better)	944.13
AICC (smaller is better)	945.39
BIC (smaller is better)	962.71

Charles River: Adult Males
Outlier Screens
Food Consumption TD8-TD1 (g/kg/day)

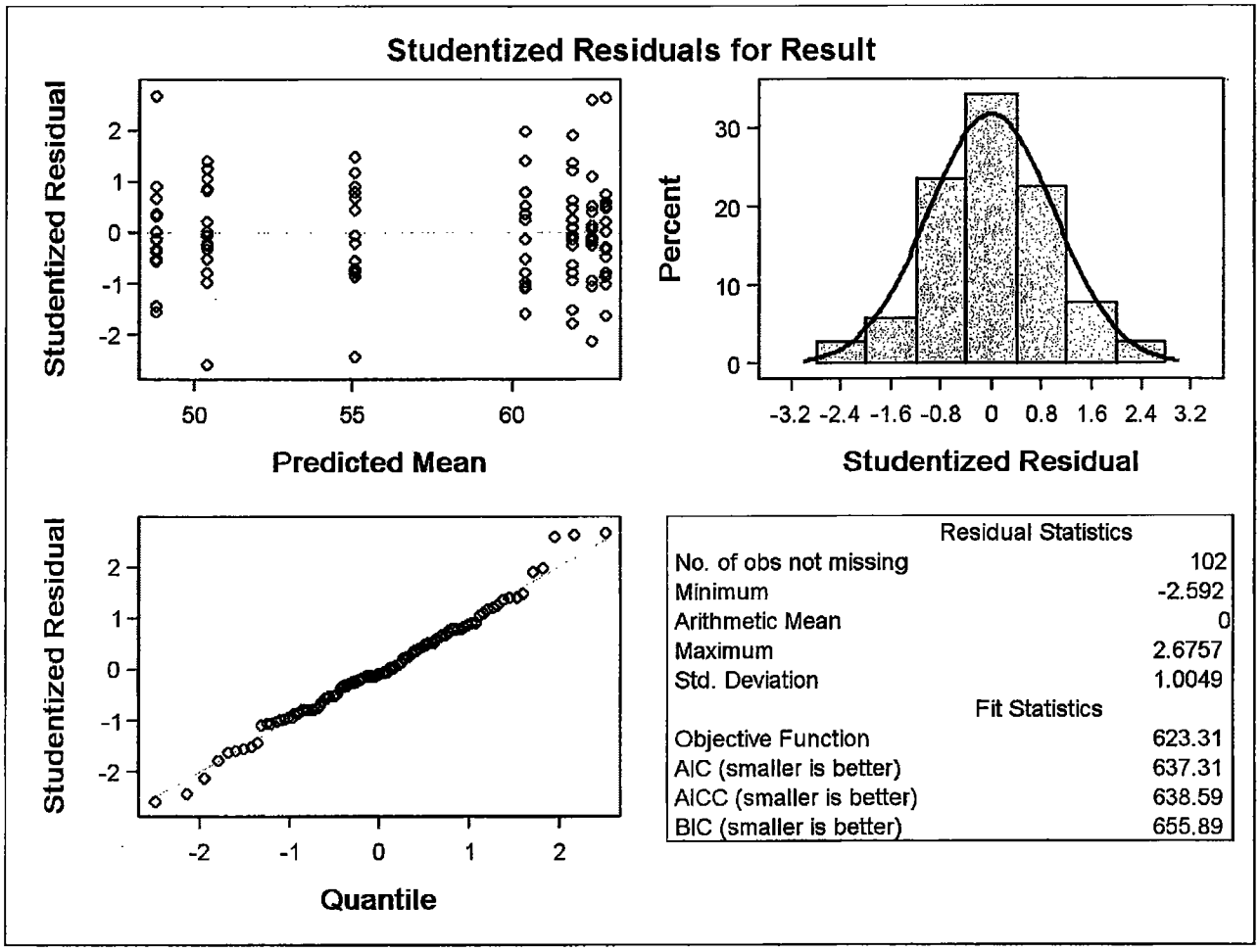
The Mixed Procedure



Residual Statistics	
No. of obs not missing	102
Minimum	-3.029
Arithmetic Mean	0
Maximum	1.8036
Std. Deviation	1.0049
Fit Statistics	
Objective Function	655.21
AIC (smaller is better)	669.21
AICC (smaller is better)	670.5
BIC (smaller is better)	687.79

Charles River: Adult Males
Outlier Screens
Food Consumption TD15-TD8 (g/kg/day)

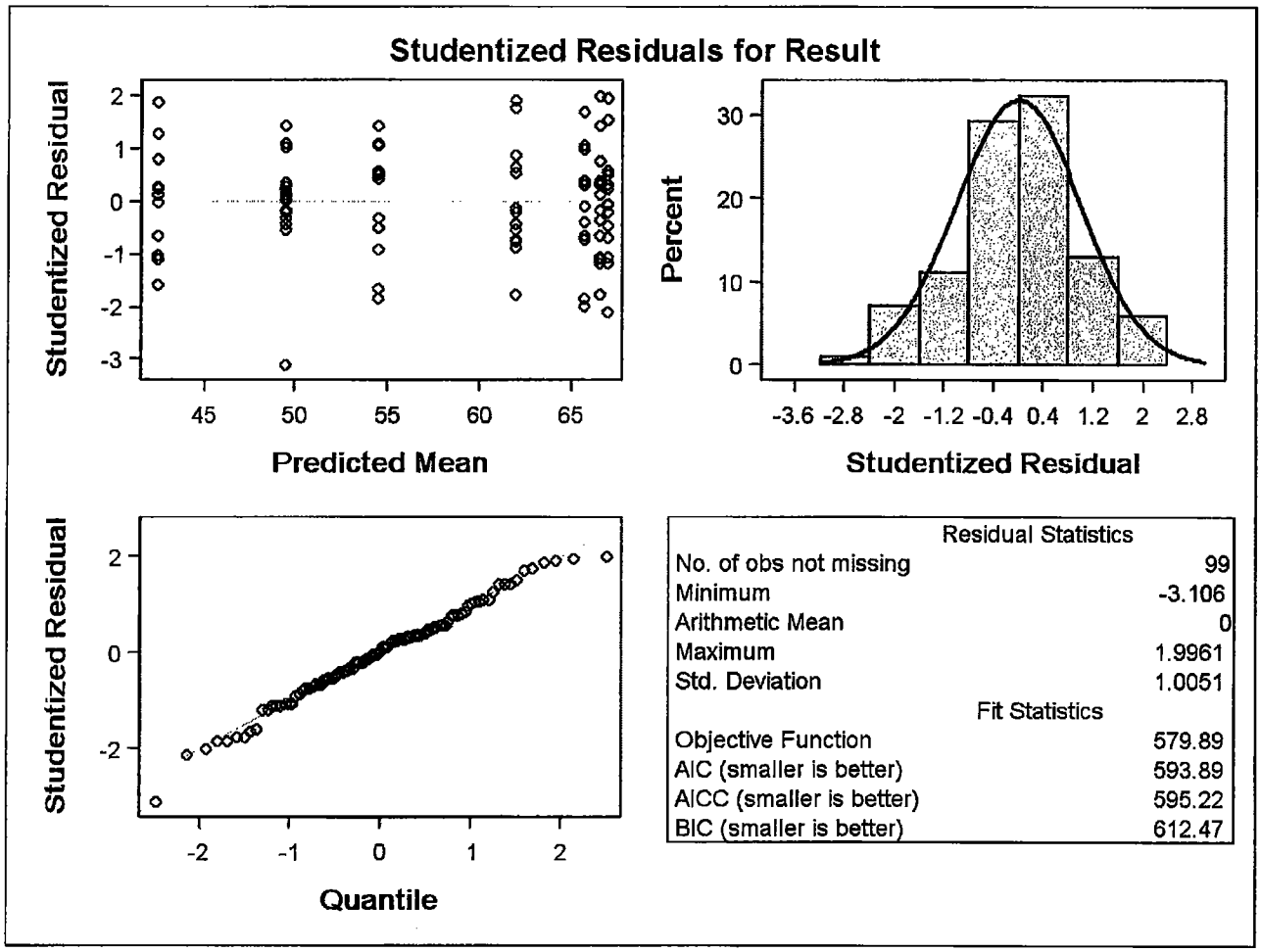
The Mixed Procedure



Residual Statistics	
No. of obs not missing	102
Minimum	-2.592
Arithmetic Mean	0
Maximum	2.6757
Std. Deviation	1.0049
Fit Statistics	
Objective Function	623.31
AIC (smaller is better)	637.31
AICC (smaller is better)	638.59
BIC (smaller is better)	655.89

Charles River: Adult Males
Outlier Screens
Food Consumption TD15-TD1 (g/kg/day)

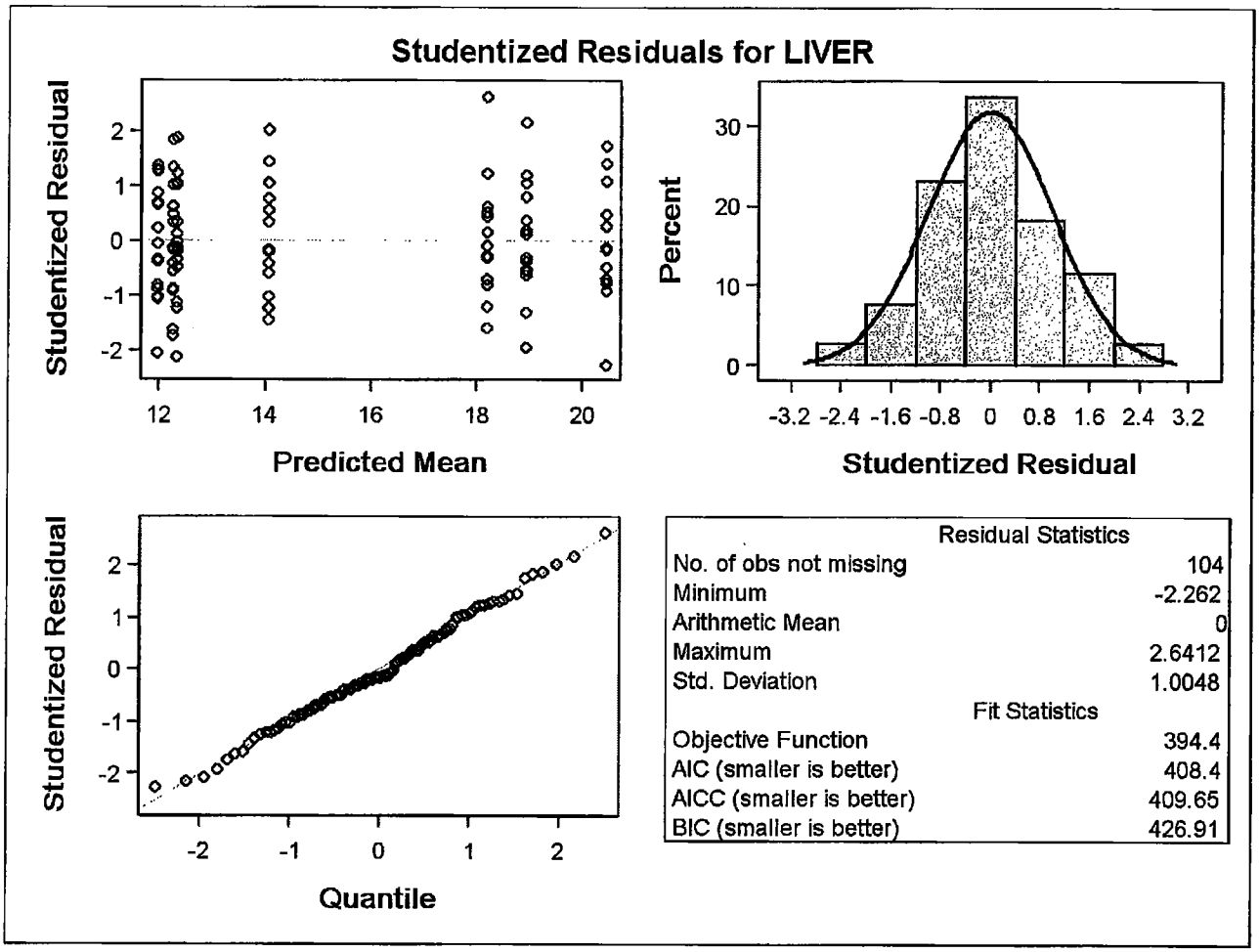
The Mixed Procedure



Residual Statistics	
No. of obs not missing	99
Minimum	-3.106
Arithmetic Mean	0
Maximum	1.9961
Std. Deviation	1.0051
Fit Statistics	
Objective Function	579.89
AIC (smaller is better)	593.89
AICC (smaller is better)	595.22
BIC (smaller is better)	612.47

Charles River: Adult Males
Outlier Screens
Liver Weight (g)

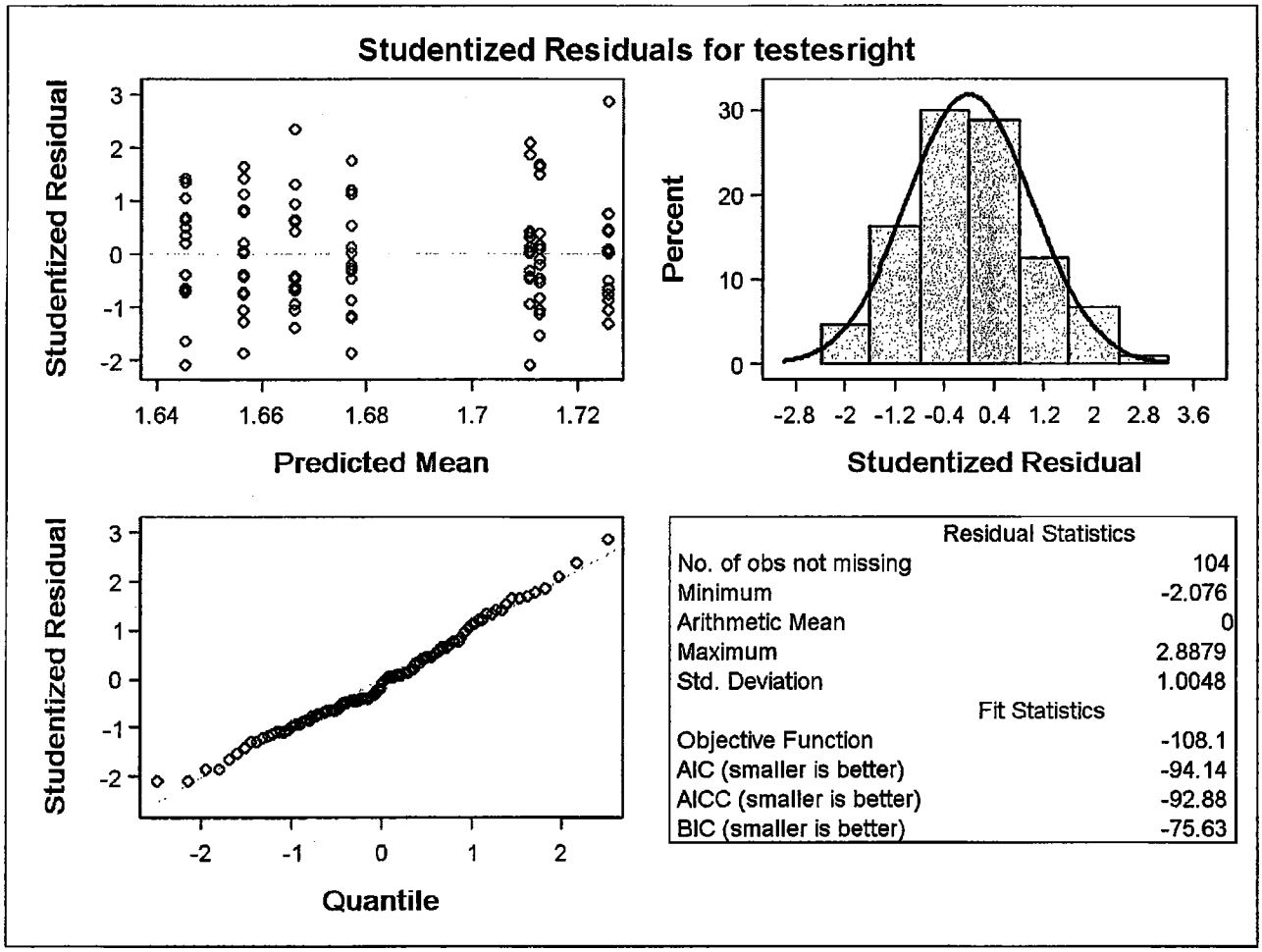
The Mixed Procedure



Residual Statistics	
No. of obs not missing	104
Minimum	-2.262
Arithmetic Mean	0
Maximum	2.6412
Std. Deviation	1.0048
Fit Statistics	
Objective Function	394.4
AIC (smaller is better)	408.4
AICC (smaller is better)	409.65
BIC (smaller is better)	426.91

Charles River: Adult Males
Outlier Screens
Right Testis Weight (g)

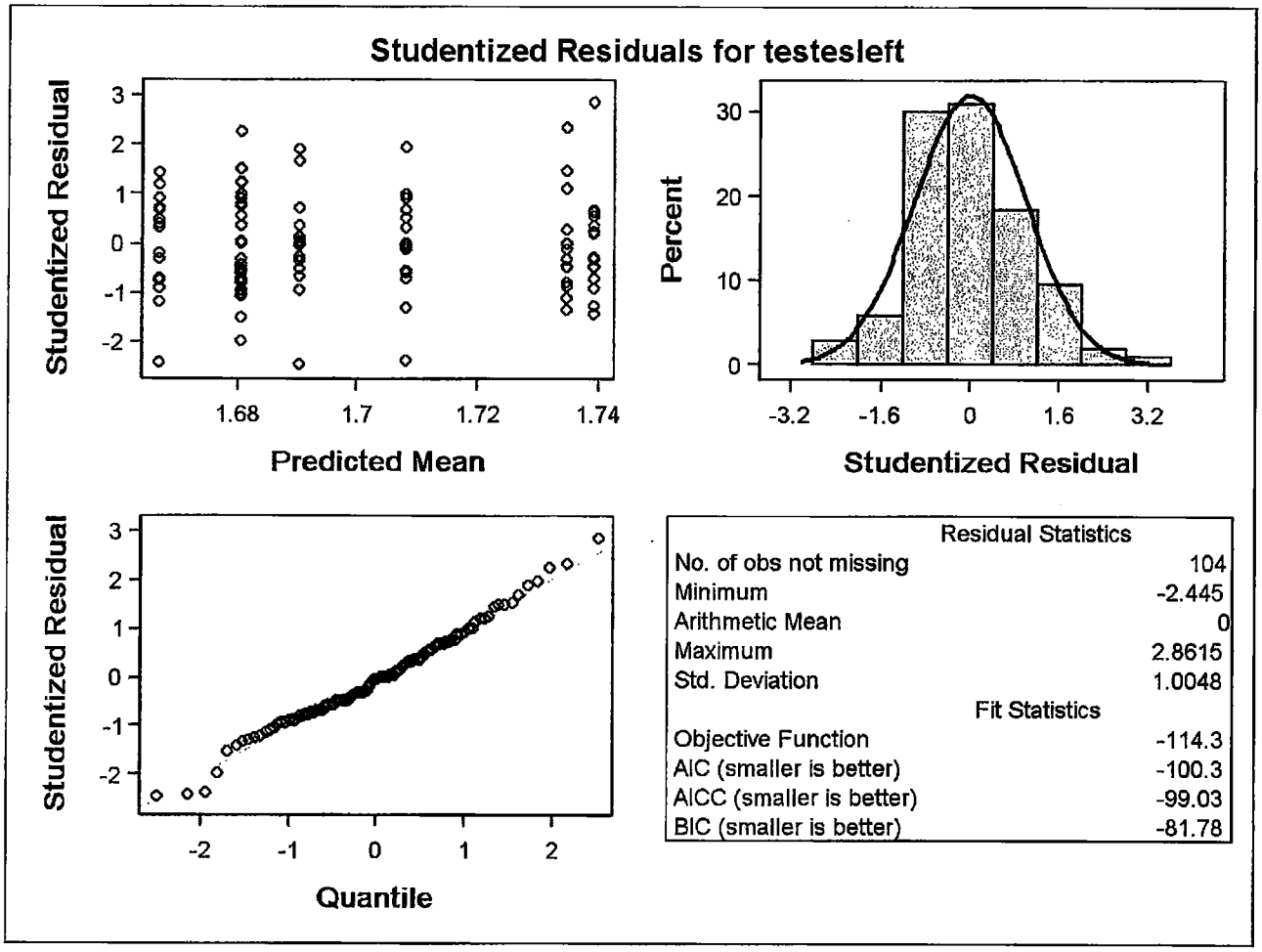
The Mixed Procedure



Residual Statistics	
No. of obs not missing	104
Minimum	-2.076
Arithmetic Mean	0
Maximum	2.8879
Std. Deviation	1.0048
Fit Statistics	
Objective Function	-108.1
AIC (smaller is better)	-94.14
AICC (smaller is better)	-92.88
BIC (smaller is better)	-75.63

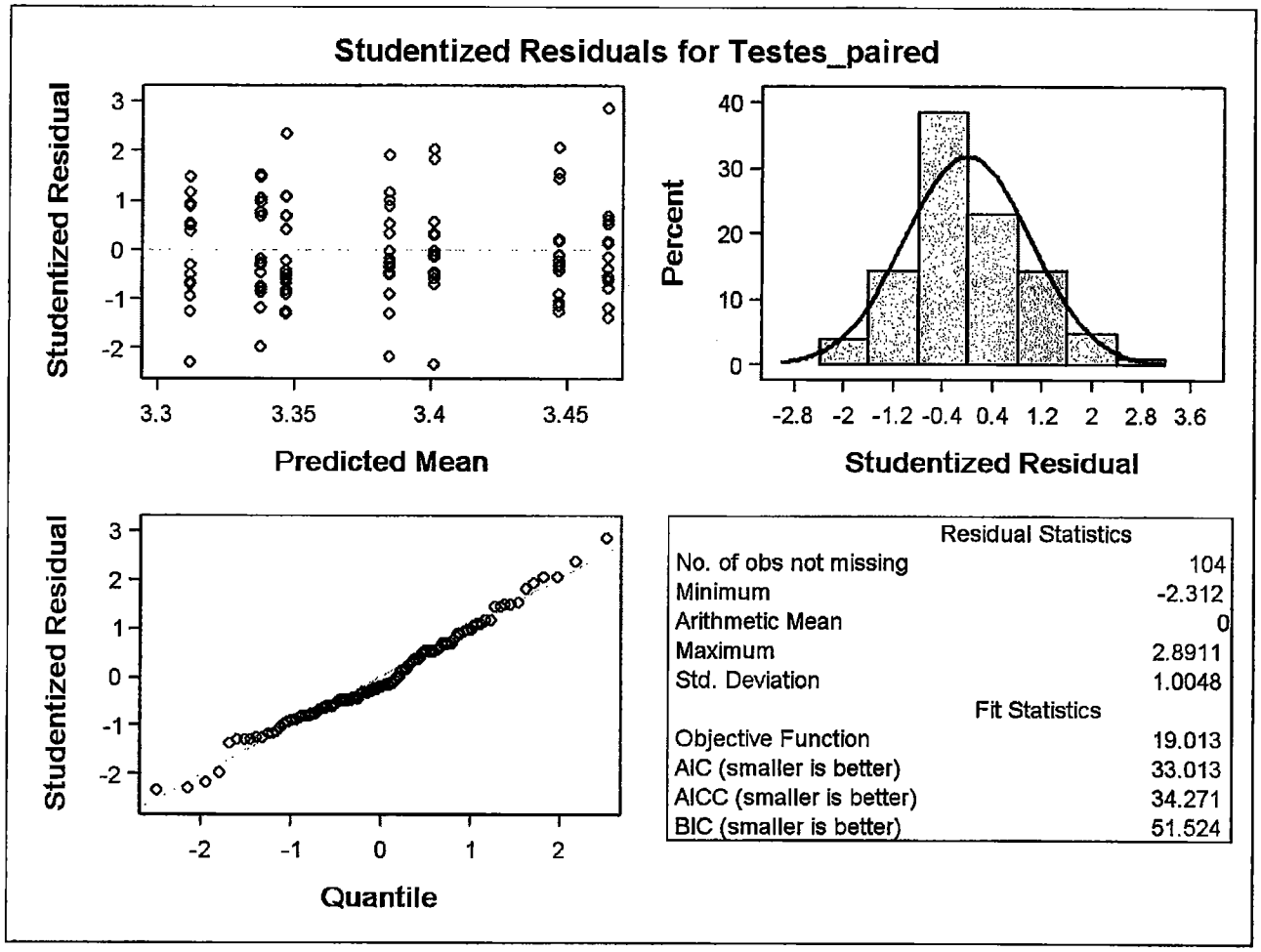
Charles River: Adult Males
Outlier Screens
Left Testis Weight (g)

The Mixed Procedure



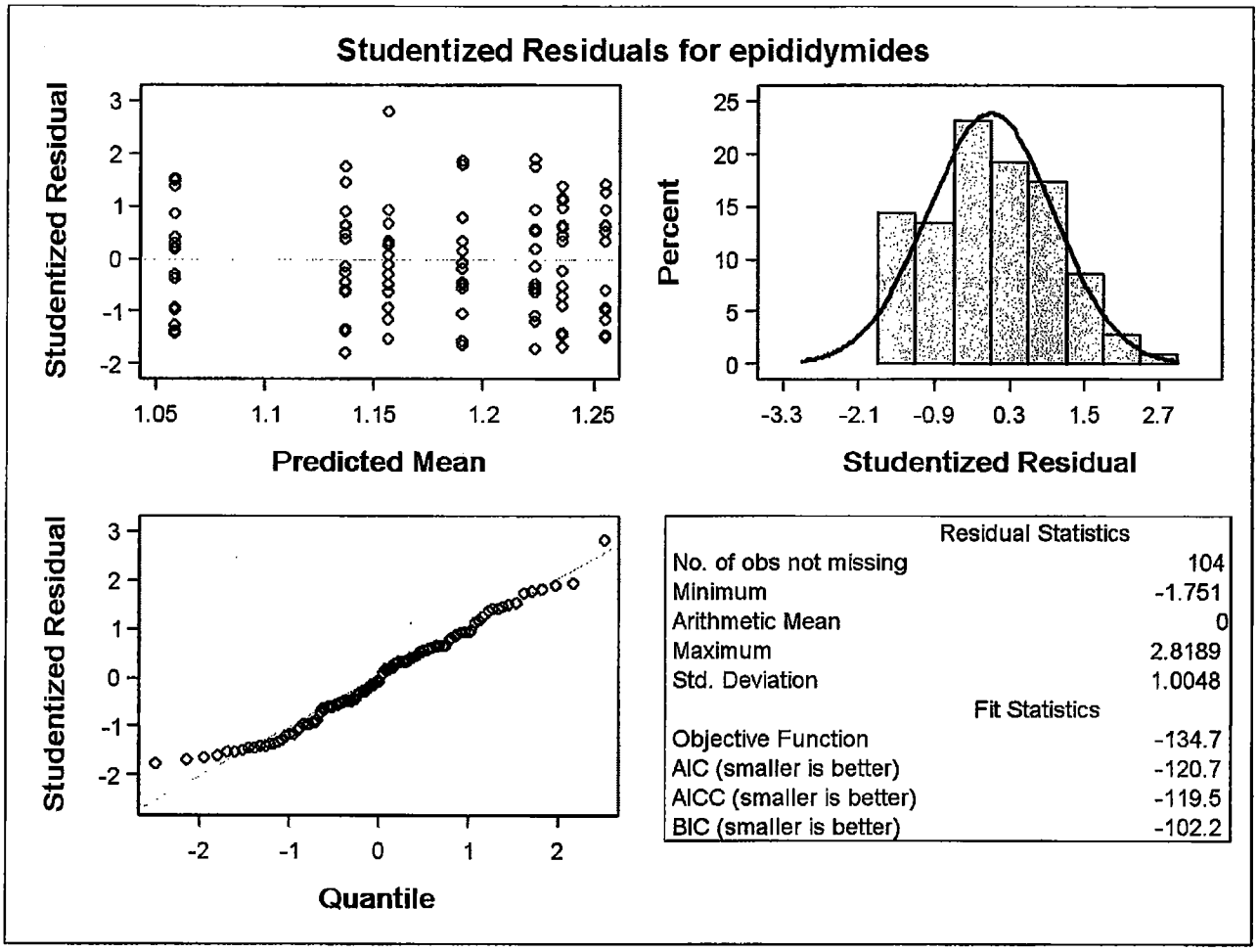
Residual Statistics	
No. of obs not missing	104
Minimum	-2.445
Arithmetic Mean	0
Maximum	2.8615
Std. Deviation	1.0048
Fit Statistics	
Objective Function	-114.3
AIC (smaller is better)	-100.3
AICC (smaller is better)	-99.03
BIC (smaller is better)	-81.78

The Mixed Procedure



Residual Statistics	
No. of obs not missing	104
Minimum	-2.312
Arithmetic Mean	0
Maximum	2.8911
Std. Deviation	1.0048
Fit Statistics	
Objective Function	19.013
AIC (smaller is better)	33.013
AICC (smaller is better)	34.271
BIC (smaller is better)	51.524

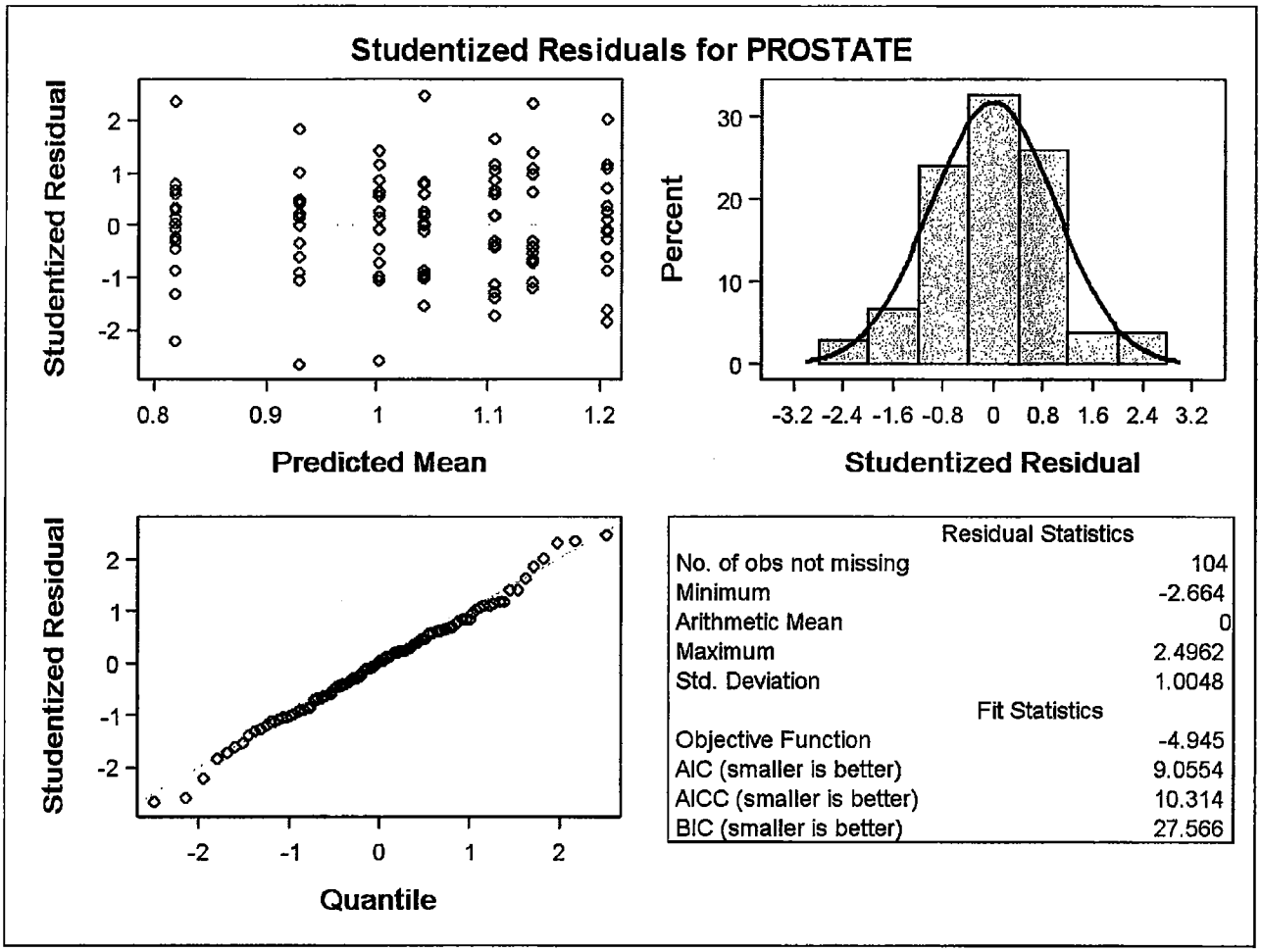
The Mixed Procedure



Residual Statistics	
No. of obs not missing	104
Minimum	-1.751
Arithmetic Mean	0
Maximum	2.8189
Std. Deviation	1.0048
Fit Statistics	
Objective Function	-134.7
AIC (smaller is better)	-120.7
AICC (smaller is better)	-119.5
BIC (smaller is better)	-102.2

Charles River: Adult Males
Outlier Screens
Entire Prostate Weight (g)

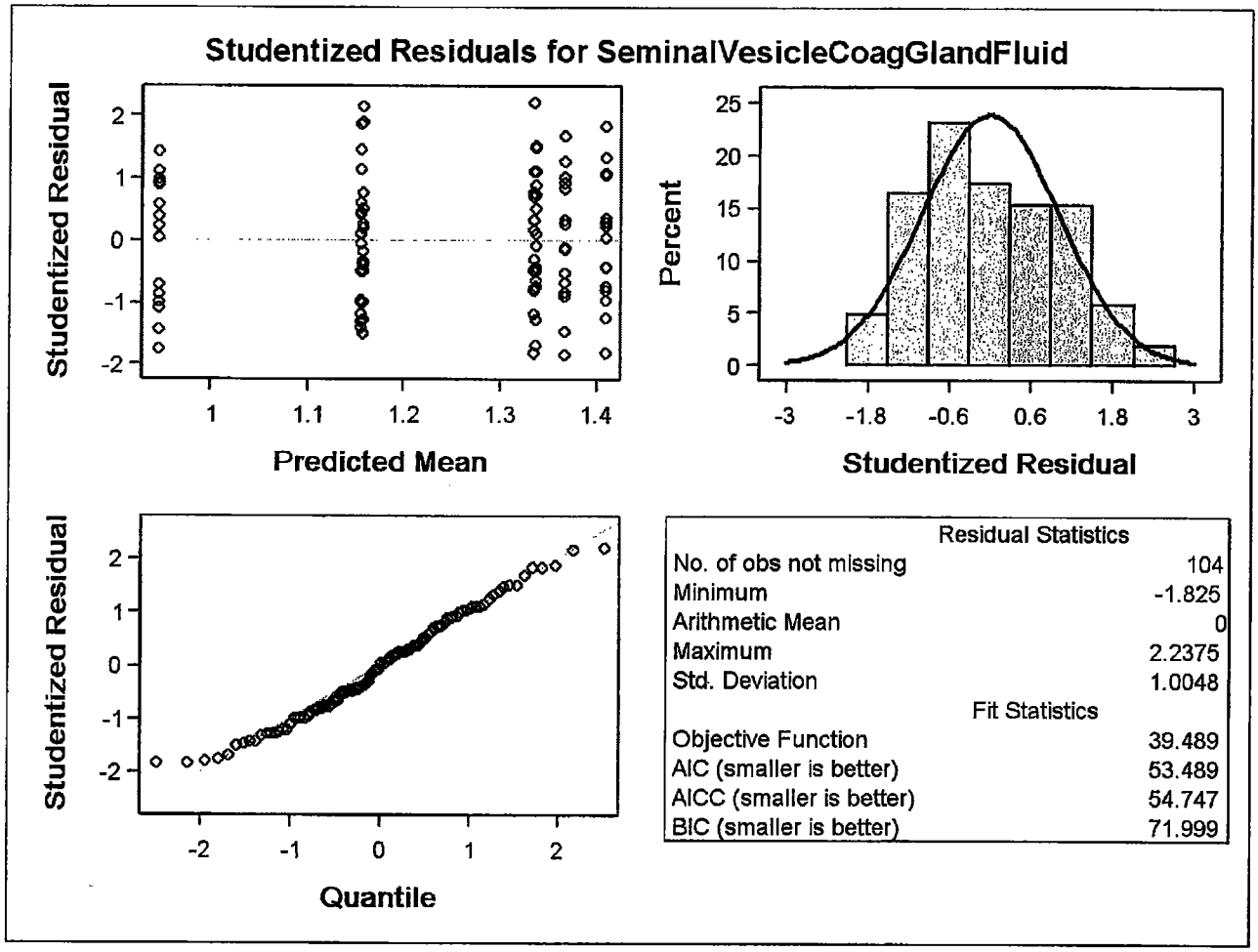
The Mixed Procedure



Residual Statistics	
No. of obs not missing	104
Minimum	-2.664
Arithmetic Mean	0
Maximum	2.4962
Std. Deviation	1.0048
Fit Statistics	
Objective Function	-4.945
AIC (smaller is better)	9.0554
AICC (smaller is better)	10.314
BIC (smaller is better)	27.566

Charles River: Adult Males
Outlier Screens
Seminal Vesicles with Fluid and Coagulating Gland Weight (g)

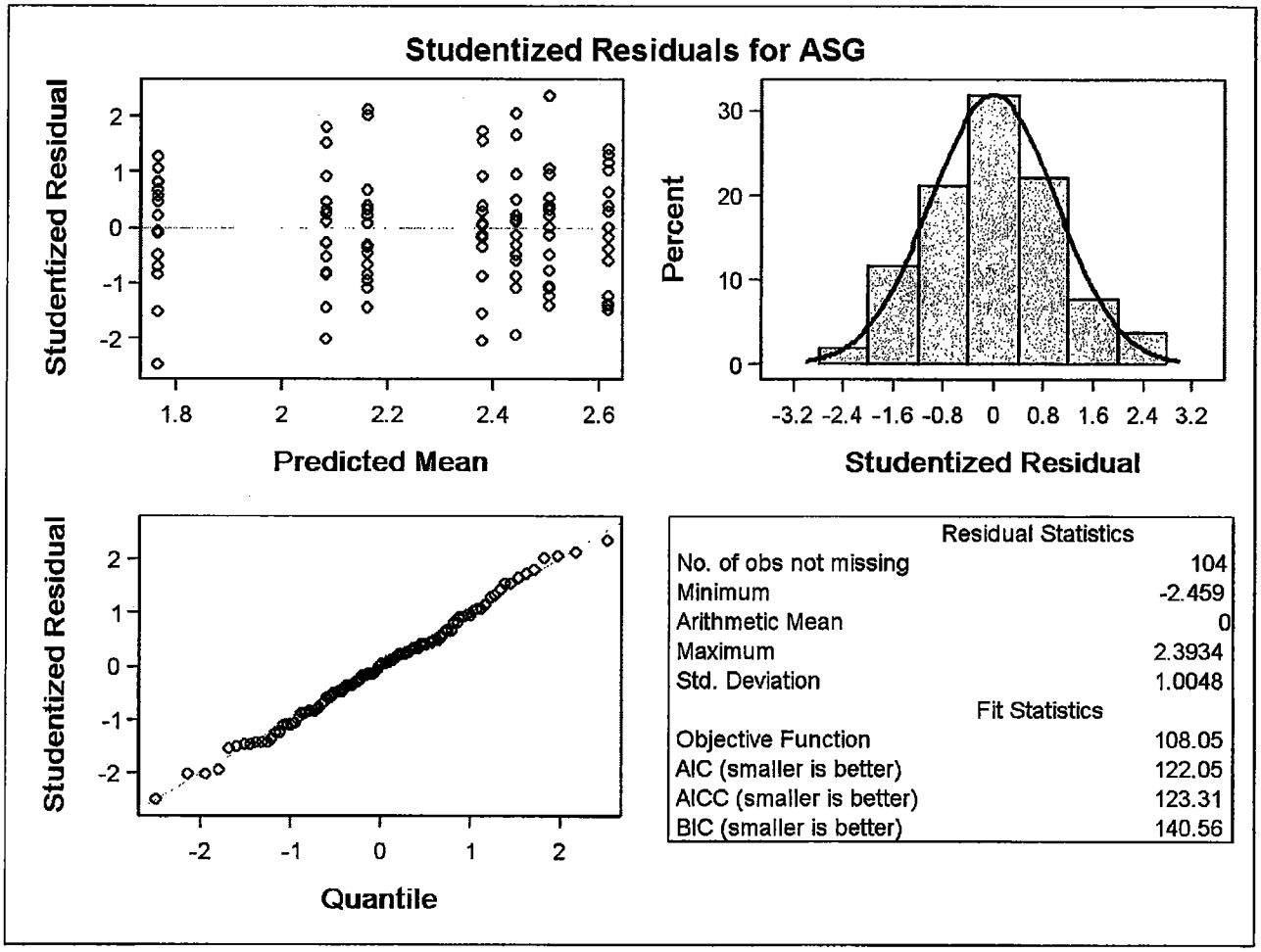
The Mixed Procedure



Residual Statistics	
No. of obs not missing	104
Minimum	-1.825
Arithmetic Mean	0
Maximum	2.2375
Std. Deviation	1.0048
Fit Statistics	
Objective Function	39.489
AIC (smaller is better)	53.489
AICC (smaller is better)	54.747
BIC (smaller is better)	71.999

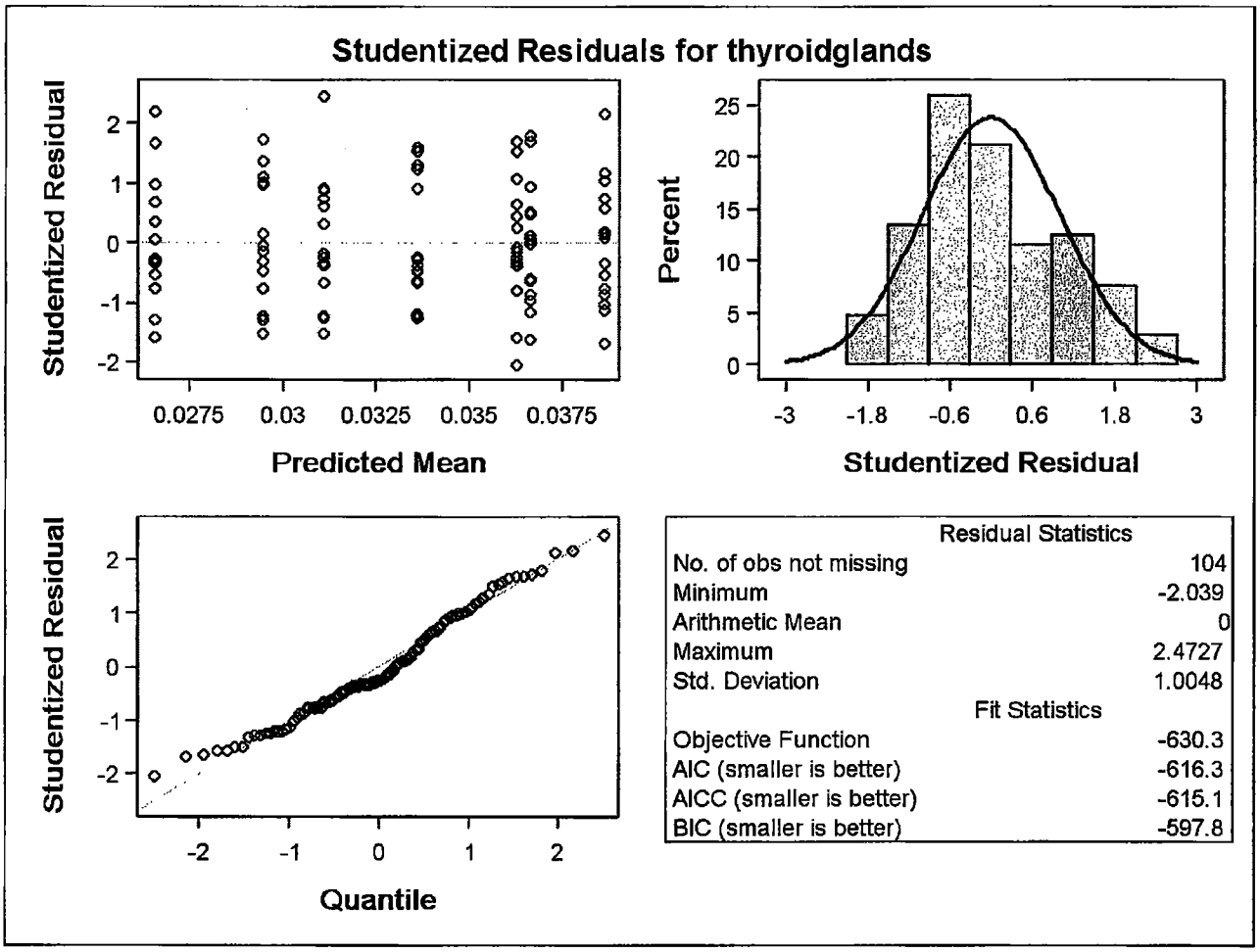
Charles River: Adult Males
Outlier Screens
Accessory Sex Gland Weight (g)

The Mixed Procedure



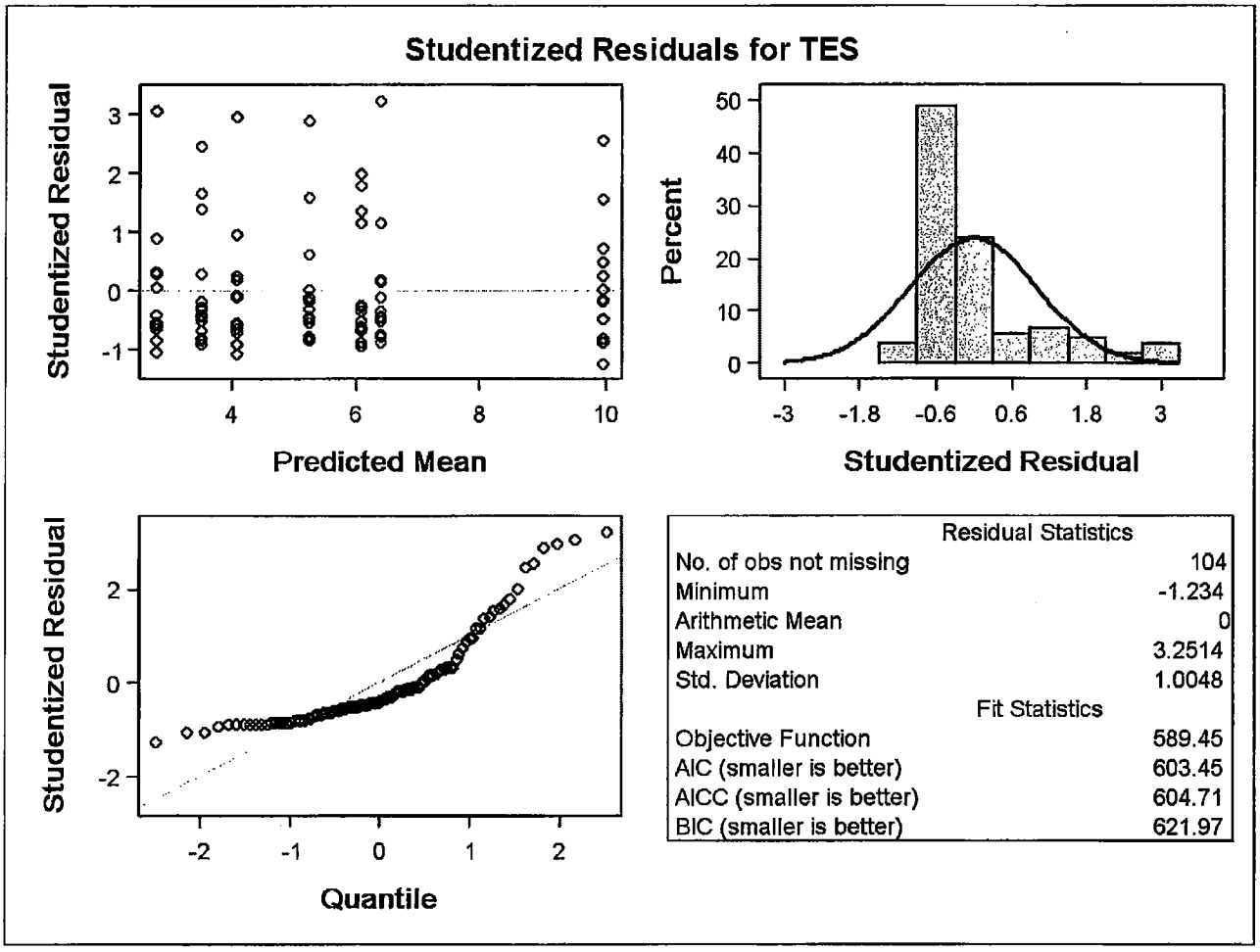
Residual Statistics	
No. of obs not missing	104
Minimum	-2.459
Arithmetic Mean	0
Maximum	2.3934
Std. Deviation	1.0048
Fit Statistics	
Objective Function	108.05
AIC (smaller is better)	122.05
AICC (smaller is better)	123.31
BIC (smaller is better)	140.56

The Mixed Procedure



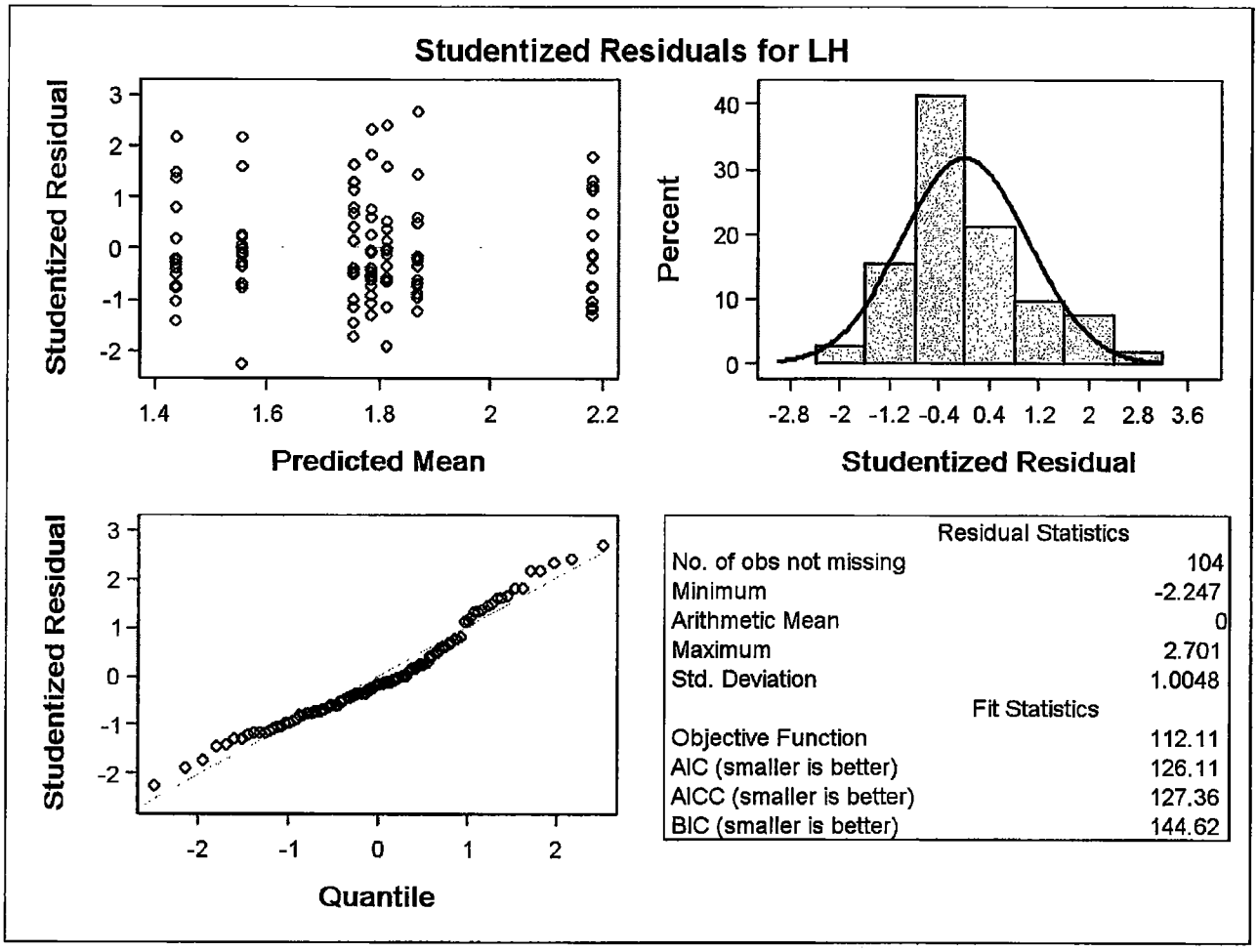
Residual Statistics	
No. of obs not missing	104
Minimum	-2.039
Arithmetic Mean	0
Maximum	2.4727
Std. Deviation	1.0048
Fit Statistics	
Objective Function	-630.3
AIC (smaller is better)	-616.3
AICC (smaller is better)	-615.1
BIC (smaller is better)	-597.8

The Mixed Procedure



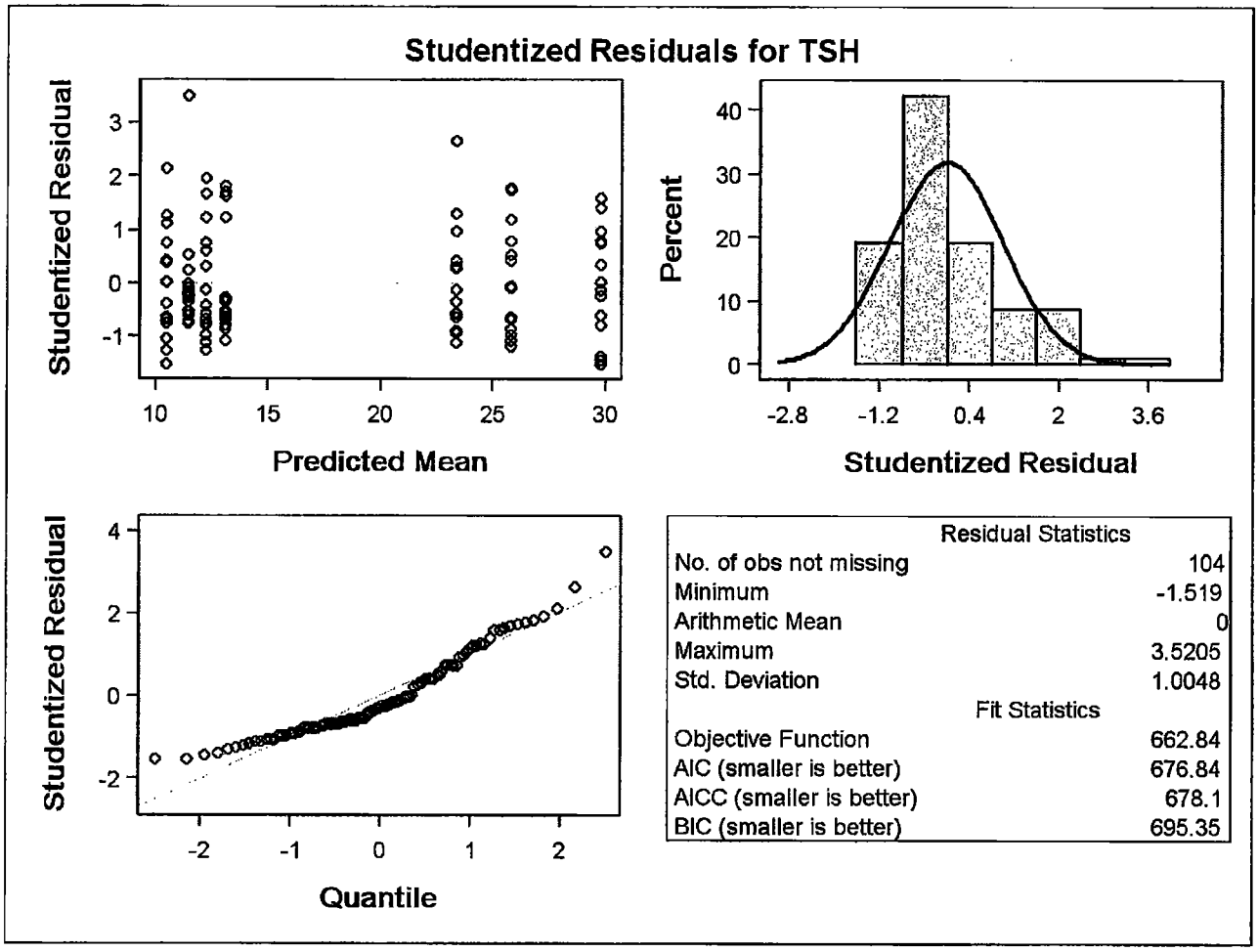
Residual Statistics	
No. of obs not missing	104
Minimum	-1.234
Arithmetic Mean	0
Maximum	3.2514
Std. Deviation	1.0048
Fit Statistics	
Objective Function	589.45
AIC (smaller is better)	603.45
AICC (smaller is better)	604.71
BIC (smaller is better)	621.97

The Mixed Procedure



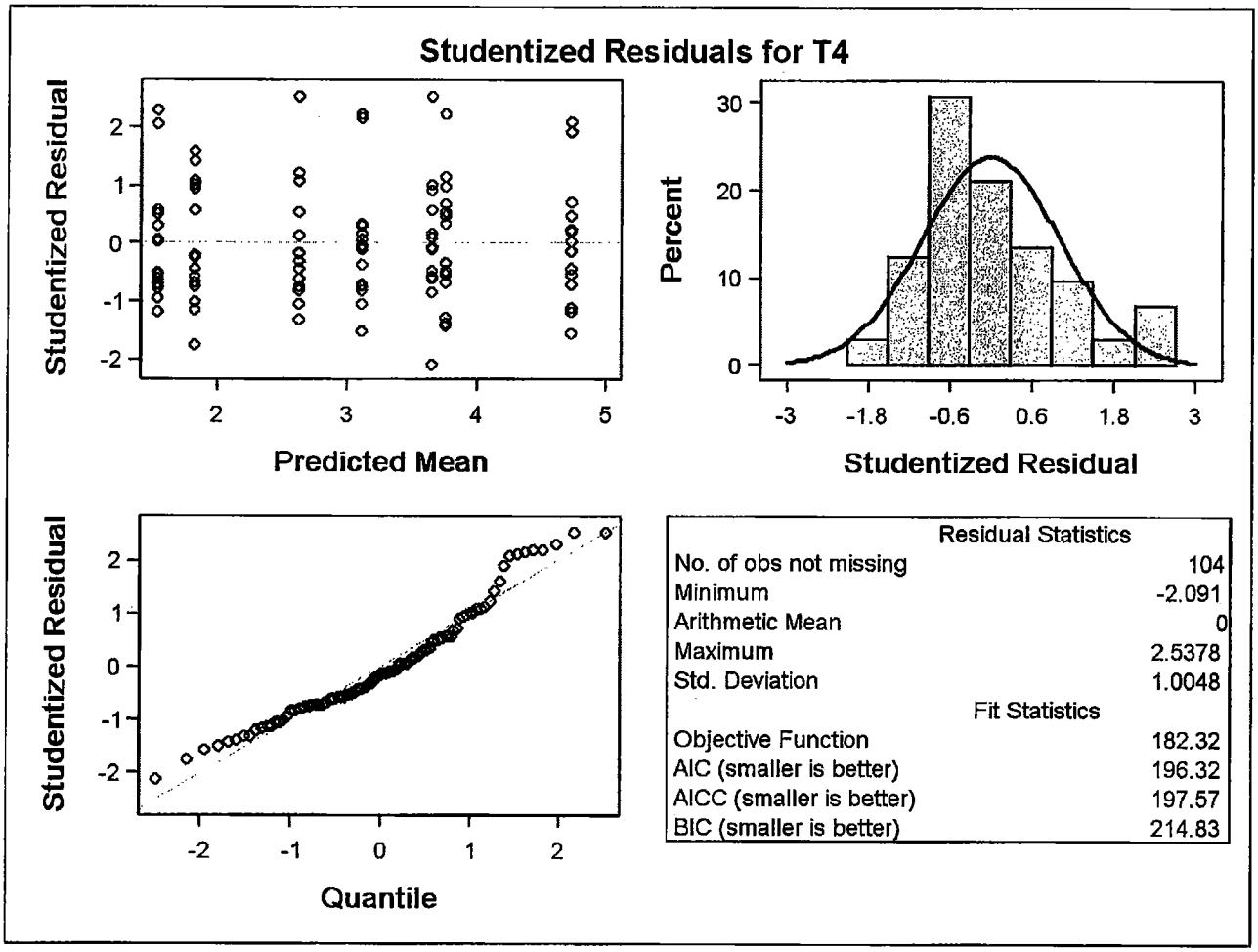
Residual Statistics	
No. of obs not missing	104
Minimum	-2.247
Arithmetic Mean	0
Maximum	2.701
Std. Deviation	1.0048
Fit Statistics	
Objective Function	112.11
AIC (smaller is better)	126.11
AICC (smaller is better)	127.36
BIC (smaller is better)	144.62

The Mixed Procedure



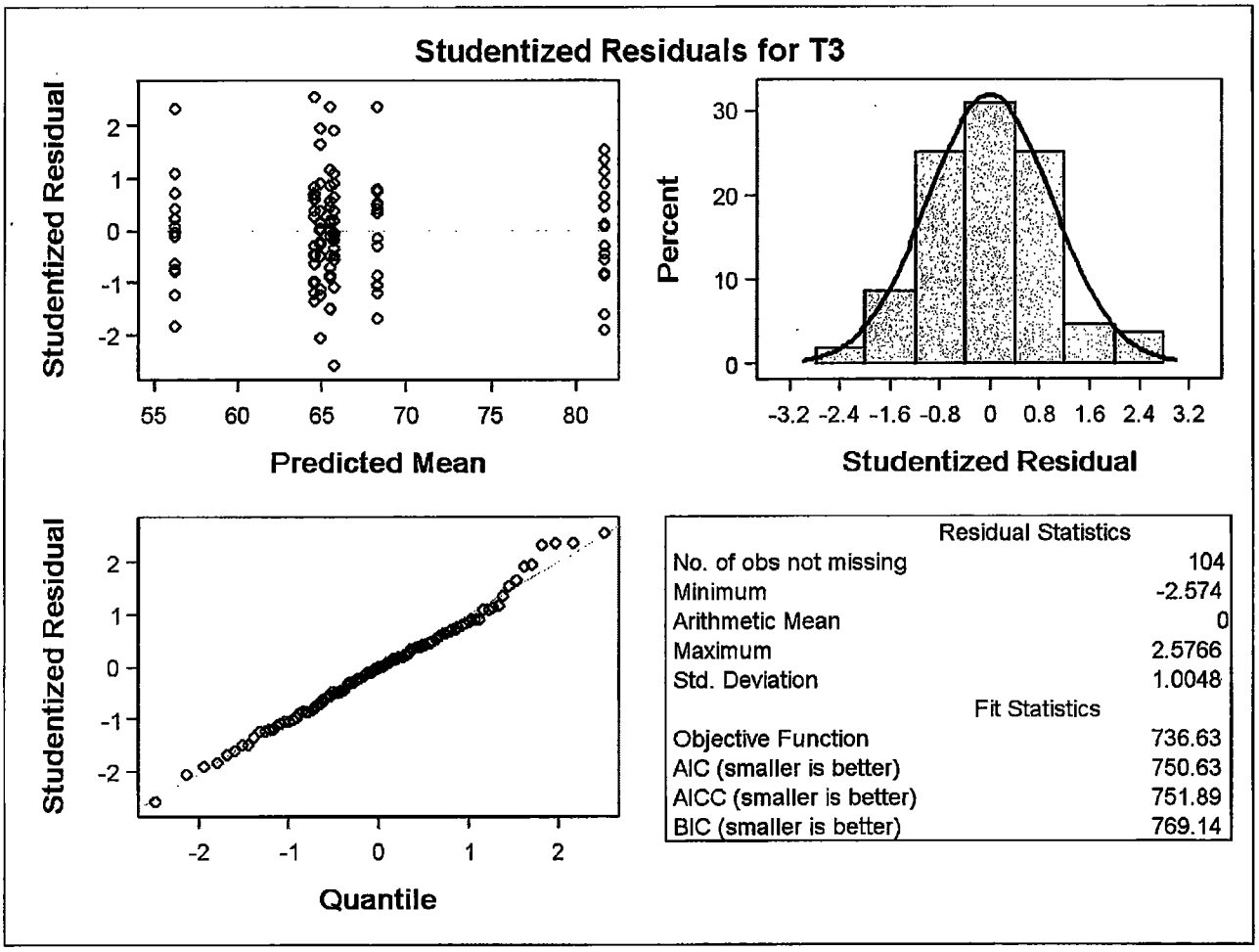
Residual Statistics	
No. of obs not missing	104
Minimum	-1.519
Arithmetic Mean	0
Maximum	3.5205
Std. Deviation	1.0048
Fit Statistics	
Objective Function	662.84
AIC (smaller is better)	676.84
AICC (smaller is better)	678.1
BIC (smaller is better)	695.35

The Mixed Procedure



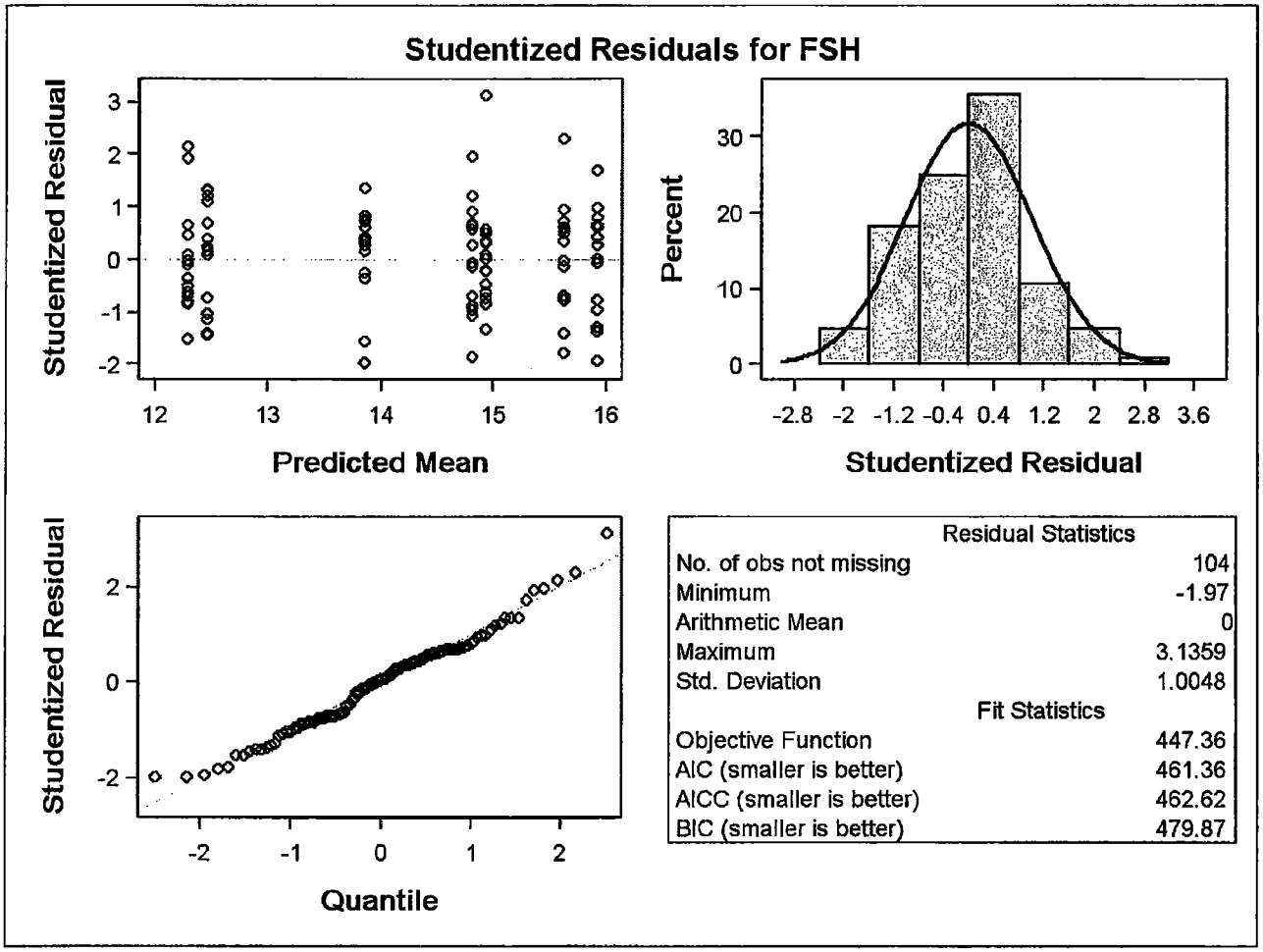
Residual Statistics	
No. of obs not missing	104
Minimum	-2.091
Arithmetic Mean	0
Maximum	2.5378
Std. Deviation	1.0048
Fit Statistics	
Objective Function	182.32
AIC (smaller is better)	196.32
AICC (smaller is better)	197.57
BIC (smaller is better)	214.83

The Mixed Procedure



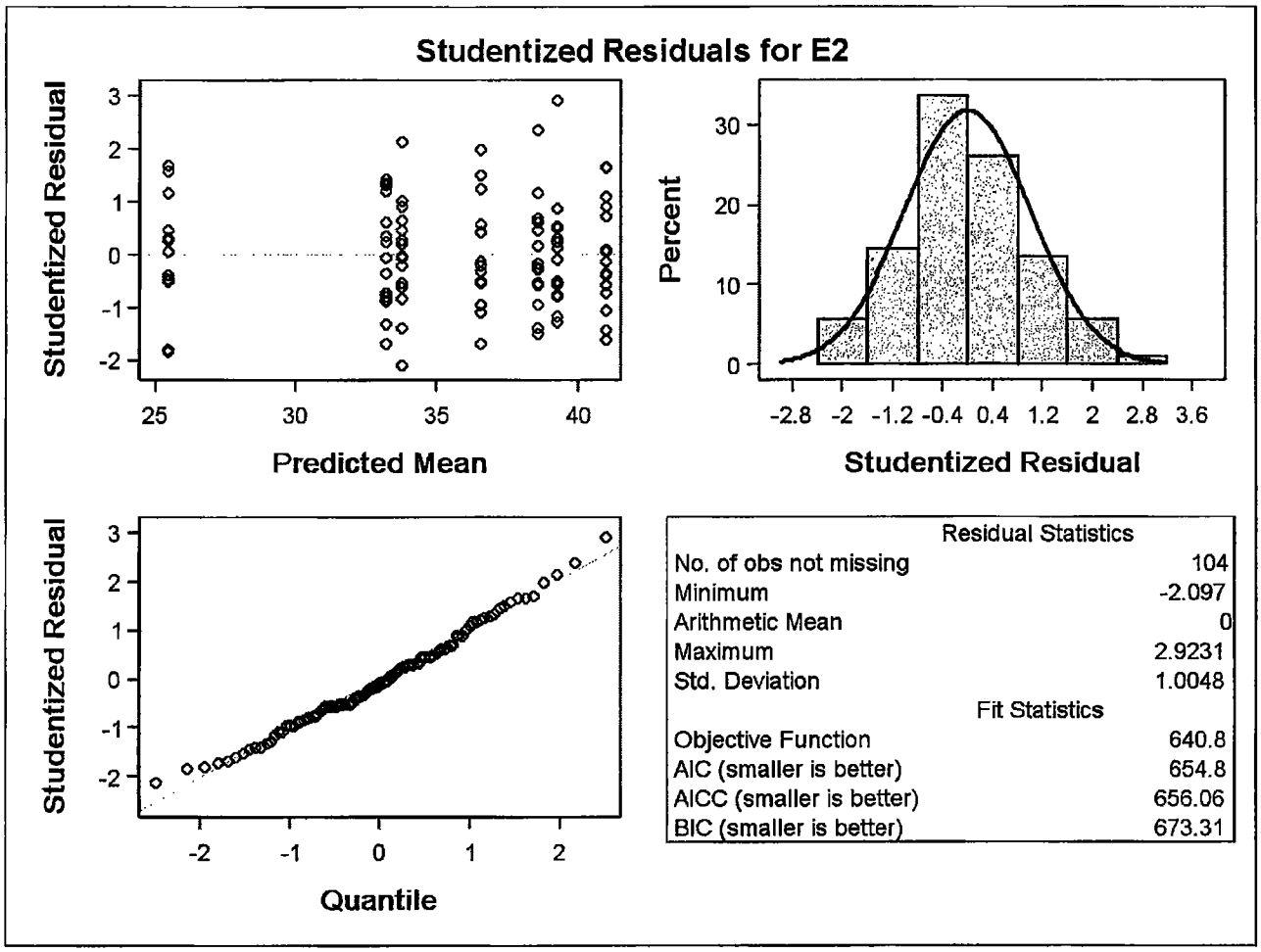
Residual Statistics	
No. of obs not missing	104
Minimum	-2.574
Arithmetic Mean	0
Maximum	2.5766
Std. Deviation	1.0048
Fit Statistics	
Objective Function	736.63
AIC (smaller is better)	750.63
AICC (smaller is better)	751.89
BIC (smaller is better)	769.14

The Mixed Procedure



Residual Statistics	
No. of obs not missing	104
Minimum	-1.97
Arithmetic Mean	0
Maximum	3.1359
Std. Deviation	1.0048
Fit Statistics	
Objective Function	447.36
AIC (smaller is better)	461.36
AICC (smaller is better)	462.62
BIC (smaller is better)	479.87

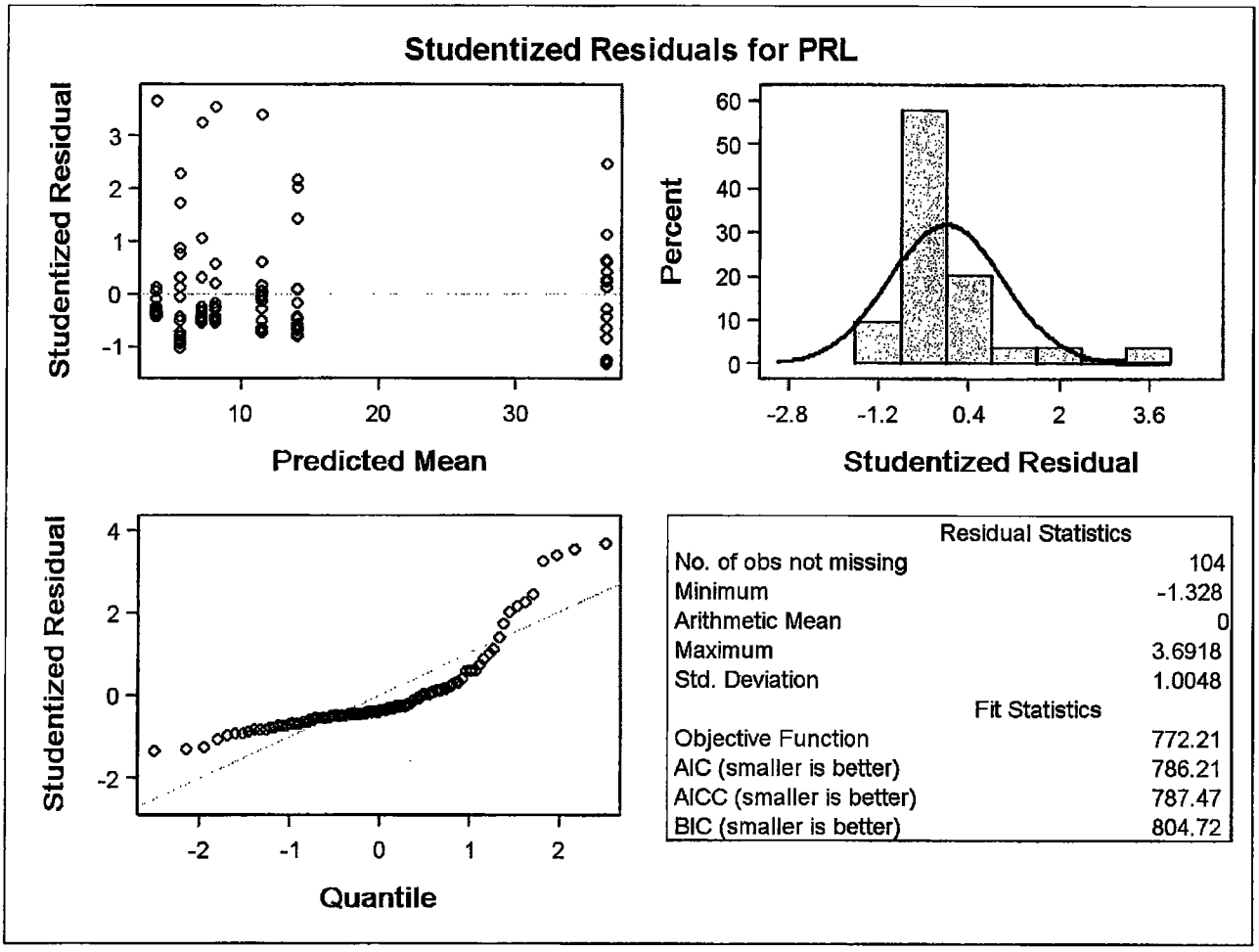
The Mixed Procedure



Residual Statistics	
No. of obs not missing	104
Minimum	-2.097
Arithmetic Mean	0
Maximum	2.9231
Std. Deviation	1.0048
Fit Statistics	
Objective Function	640.8
AIC (smaller is better)	654.8
AICC (smaller is better)	656.06
BIC (smaller is better)	673.31

Charles River: Adult Males
Outlier Screens
Prolactin (ng/ml)

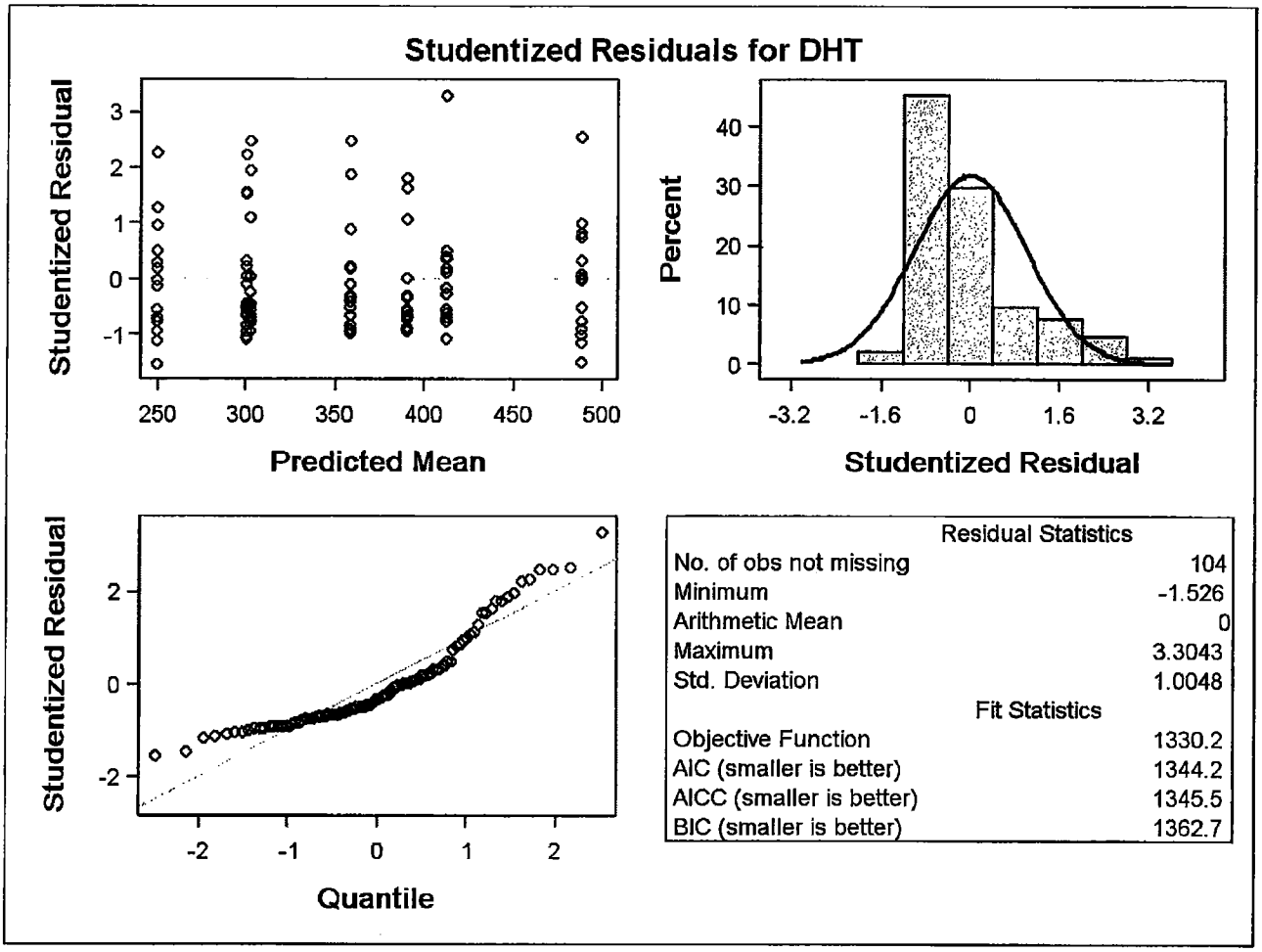
The Mixed Procedure



Residual Statistics	
No. of obs not missing	104
Minimum	-1.328
Arithmetic Mean	0
Maximum	3.6918
Std. Deviation	1.0048
Fit Statistics	
Objective Function	772.21
AIC (smaller is better)	786.21
AICC (smaller is better)	787.47
BIC (smaller is better)	804.72

Charles River: Adult Males
Outlier Screens
DHT (pg/ml)

The Mixed Procedure



Residual Statistics	
No. of obs not missing	104
Minimum	-1.526
Arithmetic Mean	0
Maximum	3.3043
Std. Deviation	1.0048
Fit Statistics	
Objective Function	1330.2
AIC (smaller is better)	1344.2
AICC (smaller is better)	1345.5
BIC (smaller is better)	1362.7

Appendix B

Potential Outliers Flagged by the Outlier Detection Procedure. Flag Value if Absolute Studentized Residual ≥2.84. Disposition of Flagged Values in Analysis.

Parameter	Test Chemical	Dosage Level	Animal Number	Observed	Predicted	Residual	Student
Values Included In Analysis[1]							
Body Weight Change (GD8-GD0) (g/day)	Vehicle	0	10302	11.8571	5.7619	6.0952	2.9079
	Linuron	100	10336	-5.8571	-1.0857	-4.7714	-2.9342
Body Weight Change (GD15-GD8) (g/day)	Linuron	50	10325	-0.1429	2.56190	-2.7048	-2.8697
	Linuron	150	10356	-3.4286	2.43810	-5.8667	-2.9830
Body Weight Change (GD15-GD0) (g/day)	Vehicle	0	10302	10.2143	5.0667	5.1476	2.9123
	Linuron	100	10336	-3.3571	0.6143	-3.9714	-2.9376
Food Consumption (GD8-GD1) (g/kg/day)	Linuron	100	10336	27.4973	48.4479	-20.951	-3.0294
Food Consumption (GD15-GD1) (g/kg/day)	Linuron	100	10336	29.0746	49.4132	-20.339	-3.1060
Right Testis Weight (g)	Linuron	50	10324	2.05240	1.72564	0.3268	2.8879
Left Testis Weight (g)	Linuron	50	10324	2.11440	1.73902	0.3754	2.8615
Paired Testes Weight (g)	Linuron	50	10324	4.16680	3.46466	0.7021	2.8911
Values Completely Excluded[2]							
Testosterone (ng/ml)	Linuron	50	10324	28.3700	6.39600	21.9740	3.2514
	Linuron	100	10332	22.7300	5.23067	17.4993	2.9319
	Linuron	150	10354	15.1600	4.06867	11.0913	2.9887
	Phenobarbital	100	10402	10.3900	2.77714	7.6129	3.0720
FSH (ng/ml)	Linuron	50	10324	33.1400	11.3967	21.743	3.5205
LSH (ng/ml)	Linuron	50	10324	24.0400	14.9200	9.1200	3.1359
Estradiol (pg/ml)	Linuron	150	10351	59.7900	39.2100	20.580	2.9231
Prolactin (ng/ml)	Linuron	50	10328	54.310	8.0400	46.270	3.5790
	Linuron	150	10346	28.610	3.7920	24.818	3.6918
	Phenobarbital	50	10384	58.200	11.4593	46.741	3.4405
	Phenobarbital	100	10403	44.860	7.1186	37.741	3.2650
DHT (pg/ml)	Linuron	50	10324	1342.30	412.394	929.91	3.3043

[1] Potential outliers judged to be valid data and included in analyses.
[2] Potential outliers judged to be invalid data and excluded from analyses.

Appendix C

Preliminary Summary Results for Growth and Body Weight, Food Consumption, Organ Weights, Organ Weight to Body Ratios (Adj. Organ Weights), and Hormonal Analysis Endpoints.

od	parm	TestChemical	DosageLevel	N	Mean	Std	CV	Min	Max
1	Body Weight Change TD8-TD1 (g/day)	Linuron	50	15	0.124	2.037	1645.64	-3.143	3.29
		Linuron	100	15	-1.086	1.683	-155.03	-5.857	1.29
		Linuron	150	15	-4.657	2.554	-54.85	-9.429	-1.14
		Phenobarbital	25	15	5.971	1.971	33.00	2.857	9.43
		Phenobarbital	50	15	5.286	1.553	29.38	2.714	8.29
		Phenobarbital	100	15	2.124	2.287	107.68	-1.857	5.29
		Vehicle	0	15	5.762	2.170	37.66	3.857	11.86
2	Body Weight Change TD15-TD8 (g/day)	Linuron	50	15	2.562	0.976	38.08	-0.143	3.57
		Linuron	100	15	2.314	1.780	76.93	-0.857	4.57
		Linuron	150	15	2.438	2.036	83.50	-3.429	5.14
		Phenobarbital	25	15	4.800	1.235	25.72	2.714	7.29
		Phenobarbital	50	15	4.790	1.494	31.19	2.571	8.71
		Phenobarbital	100	14	4.490	1.501	33.43	2.714	7.14
		Vehicle	0	15	4.371	1.765	40.38	0.714	8.57
3	Body Weight Change TD15-TD1 (g/day)	Linuron	50	15	1.343	1.188	88.46	-0.429	3.14
		Linuron	100	15	0.614	1.399	227.81	-3.357	2.43
		Linuron	150	15	-1.110	1.513	-136.37	-4.071	0.86
		Phenobarbital	25	15	5.386	1.554	28.86	2.929	8.36
		Phenobarbital	50	15	5.038	1.324	26.29	2.857	8.50
		Phenobarbital	100	14	3.337	1.210	36.25	1.071	5.79
		Vehicle	0	15	5.067	1.830	36.11	2.286	10.21
4	Final Body Weight (g)	Linuron	50	15	353.067	25.753	7.29	302.000	390.00
		Linuron	100	15	344.533	19.276	5.59	306.000	380.00
		Linuron	150	15	321.200	23.072	7.18	283.000	351.00
		Phenobarbital	25	15	412.000	33.295	8.08	352.000	475.00
		Phenobarbital	50	15	407.467	29.081	7.14	351.000	470.00
		Phenobarbital	100	14	381.357	25.200	6.61	320.000	417.00
		Vehicle	0	15	403.400	32.965	8.17	363.000	476.00
5	Food Consumption TD8-TD1 (g/kg/day)	Linuron	50	13	54.128	8.032	14.84	37.326	67.27
		Linuron	100	15	48.448	7.159	14.78	27.497	59.26
		Linuron	150	14	37.530	10.325	27.51	22.168	55.05
		Phenobarbital	25	15	71.532	6.159	8.61	59.222	80.82
		Phenobarbital	50	15	71.374	5.119	7.17	62.759	79.14
		Phenobarbital	100	15	61.116	8.108	13.27	46.231	74.15

od	parm	TestChemical	DosageLevel	N	Mean	Std	CV	Min	Max
		Vehicle	0	15	71.229	5.139	7.21	64.032	80.18
6	Food Consumption TD15-TD8 (g/kg/day)	Linuron	50	15	55.057	7.057	12.82	38.438	65.28
		Linuron	100	15	50.378	7.879	15.64	30.652	61.04
		Linuron	150	14	48.751	9.601	19.69	34.431	73.51
		Phenobarbital	25	14	60.411	3.935	6.51	54.466	67.88
		Phenobarbital	50	15	62.464	3.333	5.34	55.632	70.82
		Phenobarbital	100	14	62.902	6.766	10.76	52.271	80.16
		Vehicle	0	15	61.851	4.918	7.95	53.344	70.92
7	Food Consumption TD15-TD1 (g/kg/day)	Linuron	50	13	54.386	6.893	12.67	42.203	63.90
		Linuron	100	15	49.413	6.778	13.72	29.075	58.92
		Linuron	150	13	42.347	8.027	18.95	30.128	56.86
		Phenobarbital	25	14	65.640	4.457	6.79	57.162	72.98
		Phenobarbital	50	15	66.919	3.810	5.69	59.196	74.20
		Phenobarbital	100	14	61.920	3.282	5.30	56.344	68.03
		Vehicle	0	15	66.540	4.672	7.02	58.688	75.55
8	Liver Weight (g)	Linuron	50	15	11.946	1.131	9.46	9.707	13.45
		Linuron	100	15	12.335	1.402	11.36	9.466	14.88
		Linuron	150	15	12.239	1.495	12.22	9.754	14.90
		Phenobarbital	25	15	18.182	1.980	10.89	15.192	23.23
		Phenobarbital	50	15	18.906	2.150	11.37	14.919	23.43
		Phenobarbital	100	14	20.430	2.378	11.64	15.245	24.47
		Vehicle	0	15	14.051	1.590	11.32	11.873	17.17
9	Right Testis Weight (g)	Linuron	50	15	1.726	0.117	6.79	1.581	2.05
		Linuron	100	15	1.666	0.125	7.52	1.501	1.95
		Linuron	150	15	1.645	0.156	9.49	1.332	1.86
		Phenobarbital	25	15	1.677	0.131	7.82	1.444	1.90
		Phenobarbital	50	15	1.711	0.132	7.70	1.447	1.98
		Phenobarbital	100	14	1.713	0.138	8.04	1.514	1.94
		Vehicle	0	15	1.657	0.092	5.53	1.494	1.80
10	Left Testis Weight (g)	Linuron	50	15	1.739	0.136	7.81	1.555	2.11
		Linuron	100	15	1.680	0.110	6.54	1.522	1.92
		Linuron	150	15	1.667	0.143	8.58	1.339	1.87
		Phenobarbital	25	15	1.708	0.118	6.93	1.438	1.94
		Phenobarbital	50	15	1.690	0.123	7.25	1.401	1.92

Charles River Adult Males
Descriptive Statistics

Code	Parm	Test Chemical	Dosage Level	N	Mean	Std	CV	Min	Max
		Phenobarbital	100	14	1.734	0.131	7.53	1.568	2.03
		Vehicle	0	15	1.680	0.099	5.87	1.496	1.83
11	Testes Paired Weight (g)	Linuron	50	15	3.465	0.251	7.26	3.136	4.17
		Linuron	100	15	3.347	0.231	6.90	3.061	3.88
		Linuron	150	15	3.312	0.293	8.84	2.671	3.73
		Phenobarbital	25	15	3.385	0.241	7.12	2.883	3.84
		Phenobarbital	50	15	3.401	0.248	7.28	2.848	3.90
		Phenobarbital	100	14	3.447	0.260	7.54	3.134	3.97
		Vehicle	0	15	3.337	0.186	5.57	2.989	3.61
12	Epididymides Weight (g)	Linuron	50	15	1.156	0.104	8.99	1.004	1.44
		Linuron	100	15	1.136	0.105	9.28	0.958	1.32
		Linuron	150	15	1.058	0.134	12.64	0.880	1.26
		Phenobarbital	25	15	1.190	0.089	7.44	1.054	1.35
		Phenobarbital	50	15	1.235	0.117	9.51	1.049	1.40
		Phenobarbital	100	14	1.255	0.139	11.10	1.056	1.45
		Vehicle	0	15	1.223	0.091	7.46	1.075	1.39
13	Entire Prostate Weight (g)	Linuron	50	15	1.002	0.155	15.52	0.612	1.22
		Linuron	100	15	0.928	0.192	20.66	0.435	1.27
		Linuron	150	15	0.818	0.231	28.23	0.323	1.35
		Phenobarbital	25	15	1.139	0.200	17.56	0.911	1.59
		Phenobarbital	50	15	1.206	0.227	18.85	0.804	1.65
		Phenobarbital	100	14	1.042	0.239	22.93	0.691	1.62
		Vehicle	0	15	1.106	0.277	25.04	0.647	1.55
14	Seminal Vesicles with Fluid and Coagulating Gland Weight (g)	Linuron	50	15	1.157	0.301	26.04	0.727	1.79
		Linuron	100	15	1.155	0.370	32.06	0.655	1.82
		Linuron	150	15	0.944	0.257	27.20	0.515	1.30
		Phenobarbital	25	15	1.366	0.265	19.37	0.900	1.80
		Phenobarbital	50	15	1.409	0.191	13.54	1.079	1.75
		Phenobarbital	100	14	1.335	0.344	25.78	0.739	2.08
		Vehicle	0	15	1.336	0.209	15.63	1.000	1.65
15	Accessory Sex Gland Weight (g)	Linuron	50	15	2.159	0.374	17.32	1.644	2.93
		Linuron	100	15	2.083	0.499	23.96	1.122	2.96
		Linuron	150	15	1.762	0.389	22.07	0.839	2.25
		Phenobarbital	25	15	2.505	0.336	13.43	2.052	3.28

Charles River Adult Males
Descriptive Statistics

pathno	TestChemical	DoseLevel	N	Mean	Std	CoV	Min	Max
	Phenobarbital	50	15	2.615	0.352	13.46	2.122	3.11
	Phenobarbital	100	14	2.377	0.424	17.83	1.556	3.10
	Vehicle	0	15	2.442	0.335	13.73	1.817	3.12
16 Thyroid Weight (g)	Linuron	50	15	0.034	0.014	42.78	0.016	0.06
	Linuron	100	15	0.031	0.007	21.66	0.021	0.05
	Linuron	150	15	0.027	0.010	38.68	0.011	0.05
	Phenobarbital	25	15	0.039	0.008	20.21	0.026	0.06
	Phenobarbital	50	15	0.036	0.008	22.21	0.020	0.05
	Phenobarbital	100	14	0.037	0.008	22.55	0.024	0.05
	Vehicle	0	15	0.029	0.006	21.44	0.020	0.04
17 Liver Weight to Body Weight Ratio (%)	Linuron	50	15	3.386	0.243	7.16	2.966	3.77
	Linuron	100	15	3.578	0.334	9.35	3.089	4.22
	Linuron	150	15	3.802	0.286	7.52	3.338	4.31
	Phenobarbital	25	15	4.408	0.198	4.50	4.139	4.90
	Phenobarbital	50	15	4.634	0.307	6.62	3.934	5.15
	Phenobarbital	100	14	5.349	0.382	7.14	4.761	5.98
	Vehicle	0	15	3.480	0.204	5.85	3.113	3.94
18 Right Testis Weight to Body Weight Ratio (%)	Linuron	50	15	0.491	0.047	9.50	0.420	0.57
	Linuron	100	15	0.484	0.036	7.47	0.431	0.57
	Linuron	150	15	0.513	0.045	8.85	0.429	0.60
	Phenobarbital	25	15	0.409	0.040	9.67	0.353	0.49
	Phenobarbital	50	15	0.421	0.034	8.03	0.381	0.52
	Phenobarbital	100	14	0.450	0.029	6.42	0.409	0.50
	Vehicle	0	15	0.412	0.031	7.45	0.365	0.48
19 Left Testis Weight to Body Weight Ratio (%)	Linuron	50	15	0.495	0.054	10.85	0.413	0.59
	Linuron	100	15	0.489	0.038	7.80	0.426	0.57
	Linuron	150	15	0.520	0.043	8.25	0.431	0.61
	Phenobarbital	25	15	0.417	0.038	9.03	0.351	0.48
	Phenobarbital	50	15	0.416	0.032	7.76	0.372	0.51
	Phenobarbital	100	14	0.456	0.038	8.25	0.393	0.53
	Vehicle	0	15	0.418	0.032	7.59	0.369	0.49
20 Testes Paired Weight to Body Weight Ratio (%)	Linuron	50	15	0.986	0.100	10.16	0.833	1.16

Charles River Adult Males
Descriptive Statistics

od	parm	TestChemical	DosageLevel	N	Mean	Std	CV	Min	Max
		Linuron	100	15	0.973	0.073	7.50	0.857	1.14
		Linuron	150	15	1.033	0.086	8.31	0.860	1.21
		Phenobarbital	25	15	0.825	0.075	9.13	0.704	0.97
		Phenobarbital	50	15	0.837	0.064	7.71	0.762	1.03
		Phenobarbital	100	14	0.906	0.064	7.11	0.803	1.03
		Vehicle	0	15	0.831	0.062	7.42	0.735	0.97
21	Epididymides Weight to Body Weight Ratio (%)	Linuron	50	15	0.329	0.038	11.41	0.274	0.40
		Linuron	100	15	0.330	0.033	9.96	0.280	0.40
		Linuron	150	15	0.330	0.037	11.14	0.255	0.40
		Phenobarbital	25	15	0.291	0.033	11.38	0.242	0.36
		Phenobarbital	50	15	0.305	0.036	11.71	0.248	0.37
		Phenobarbital	100	14	0.330	0.042	12.83	0.272	0.42
		Vehicle	0	15	0.305	0.031	10.13	0.238	0.36
22	Entire Prostate Weight to Body Weight Ratio (%)	Linuron	50	15	0.285	0.050	17.45	0.157	0.39
		Linuron	100	15	0.269	0.051	18.95	0.131	0.34
		Linuron	150	15	0.253	0.064	25.44	0.114	0.39
		Phenobarbital	25	15	0.279	0.062	22.05	0.223	0.45
		Phenobarbital	50	15	0.298	0.063	21.30	0.195	0.40
		Phenobarbital	100	14	0.275	0.069	24.99	0.172	0.45
		Vehicle	0	15	0.278	0.080	28.72	0.146	0.41
23	Seminal Vesicles with Fluid and Coagulating Gland Weight to Body Weight	Linuron	50	15	0.327	0.077	23.48	0.194	0.50
		Linuron	100	15	0.333	0.101	30.17	0.202	0.55
		Linuron	150	15	0.294	0.076	26.01	0.173	0.39
		Phenobarbital	25	15	0.336	0.081	24.11	0.211	0.48
		Phenobarbital	50	15	0.349	0.063	18.02	0.252	0.46
		Phenobarbital	100	14	0.349	0.081	23.35	0.226	0.52
		Vehicle	0	15	0.333	0.061	18.39	0.248	0.45
24	Accessory Sex Gland Weight to Body Weight Ratio (%)	Linuron	50	15	0.613	0.101	16.52	0.473	0.80
		Linuron	100	15	0.602	0.131	21.81	0.338	0.85
		Linuron	150	15	0.547	0.110	20.19	0.296	0.69
		Phenobarbital	25	15	0.615	0.120	19.52	0.472	0.93

Charles River Adult Males
Descriptive Statistics

	parm	Test Chemical	Dosage Level	N	Mean	Std	CV	Min	Max
		Phenobarbital	50	15	0.647	0.112	17.37	0.468	0.85
		Phenobarbital	100	14	0.624	0.107	17.22	0.420	0.83
		Vehicle	0	15	0.611	0.113	18.46	0.452	0.82
25	Thyroid Weight to Body Weight Ratio (%)	Linuron	50	15	0.010	0.004	42.02	0.004	0.02
		Linuron	100	15	0.009	0.002	19.87	0.006	0.01
		Linuron	150	15	0.008	0.003	40.35	0.003	0.02
		Phenobarbital	25	15	0.009	0.002	20.54	0.007	0.01
		Phenobarbital	50	15	0.009	0.002	22.20	0.005	0.01
		Phenobarbital	100	14	0.010	0.002	20.50	0.006	0.01
		Vehicle	0	15	0.007	0.002	23.06	0.005	0.01
26	Testosterone (ng/ml)	Linuron	50	15	6.396	6.996	109.37	0.630	28.37
		Linuron	100	15	5.231	6.178	118.11	0.230	22.73
		Linuron	150	15	4.069	3.841	94.41	0.200	15.16
		Phenobarbital	25	15	6.072	4.693	77.30	1.890	15.23
		Phenobarbital	50	15	3.495	2.799	80.09	1.130	10.21
		Phenobarbital	100	14	2.777	2.572	92.60	0.200	10.39
		Vehicle	0	15	9.927	7.254	73.07	1.280	28.11
27	LH (ng/ml)	Linuron	50	15	1.753	0.370	21.09	1.150	2.35
		Linuron	100	15	1.782	0.529	29.71	1.130	2.99
		Linuron	150	15	1.865	0.692	37.10	1.060	3.67
		Phenobarbital	25	15	1.811	0.381	21.03	1.120	2.70
		Phenobarbital	50	15	1.435	0.210	14.61	1.150	1.88
		Phenobarbital	100	14	1.555	0.256	16.48	1.000	2.10
		Vehicle	0	15	2.178	0.492	22.57	1.570	3.04
28	TSH (ng/ml)	Linuron	50	15	11.397	6.393	56.10	6.770	33.14
		Linuron	100	15	12.212	5.881	48.16	5.180	23.42
		Linuron	150	15	10.463	3.355	32.07	5.540	17.47
		Phenobarbital	25	15	23.349	11.144	47.73	11.300	52.10
		Phenobarbital	50	15	25.741	7.839	30.45	16.820	39.37
		Phenobarbital	100	14	29.727	8.489	28.56	17.380	42.84
		Vehicle	0	15	13.095	6.512	49.73	6.490	24.73
29	T4 (ug/dl)	Linuron	50	15	3.099	0.658	21.22	2.150	4.51
		Linuron	100	15	1.819	0.439	24.13	1.080	2.50
		Linuron	150	15	1.537	0.474	30.83	1.000	2.59

od	parm	TestChemical	DosageLevel	N	Mean	Std	CV	Min	Max
		Phenobarbital	25	15	3.751	0.532	14.18	3.030	4.89
		Phenobarbital	50	15	3.642	0.501	13.75	2.630	4.87
		Phenobarbital	100	14	2.623	0.611	23.28	1.860	4.11
		Vehicle	0	15	4.729	0.799	16.89	3.540	6.36
30	T3 (ng/dl)	Linuron	50	15	68.196	7.405	10.86	56.430	85.20
		Linuron	100	15	65.621	9.924	15.12	40.940	84.34
		Linuron	150	15	64.469	8.606	13.35	53.440	85.89
		Phenobarbital	25	15	64.847	9.890	15.25	45.370	83.72
		Phenobarbital	50	15	65.408	12.417	18.98	47.770	94.14
		Phenobarbital	100	14	56.146	9.410	16.76	39.560	77.61
		Vehicle	0	15	81.653	11.712	14.34	60.360	99.32
31	FSH (ng/ml)	Linuron	50	15	14.920	3.010	20.18	11.080	24.04
		Linuron	100	15	15.622	3.156	20.20	10.240	22.66
		Linuron	150	15	15.909	2.423	15.23	11.400	19.96
		Phenobarbital	25	15	13.841	1.845	13.33	10.330	16.30
		Phenobarbital	50	15	12.459	1.382	11.09	10.560	14.26
		Phenobarbital	100	14	12.278	1.946	15.85	9.430	16.32
		Vehicle	0	15	14.807	2.184	14.75	10.960	18.99
32	Estradiol (pg/ml)	Linuron	50	15	33.199	5.229	15.75	24.650	40.56
		Linuron	100	15	40.946	7.639	18.66	29.110	53.24
		Linuron	150	15	39.210	7.288	18.59	30.380	59.79
		Phenobarbital	25	15	33.709	5.687	16.87	22.190	45.48
		Phenobarbital	50	15	36.525	6.252	17.12	26.350	48.59
		Phenobarbital	100	14	38.523	7.328	19.02	27.990	55.36
		Vehicle	0	15	25.403	3.618	14.24	19.010	31.35
33	Prolactin (ng/ml)	Linuron	50	15	8.040	13.382	166.44	1.020	54.31
		Linuron	100	15	5.566	4.607	82.77	0.980	15.79
		Linuron	150	15	3.792	6.958	183.50	1.010	28.61
		Phenobarbital	25	15	14.050	15.819	112.59	1.730	47.41
		Phenobarbital	50	15	11.459	14.062	122.71	1.540	58.20
		Phenobarbital	100	14	7.119	11.996	168.51	1.090	44.86
		Vehicle	0	15	36.476	27.109	74.32	1.690	101.52
34	DHT (pg/ml)	Linuron	50	15	412.394	291.300	70.64	112.900	1342.30
		Linuron	100	15	357.769	269.498	75.33	109.970	1010.60

Charles River Adult Males
Descriptive Statistics

od	parm	TestChemical	DosageLevel	N	Mean	Std	CV	Min	Max
		Linuron	150	15	299.866	170.458	56.84	127.770	670.40
		Phenobarbital	25	15	389.625	213.305	54.75	201.190	764.62
		Phenobarbital	50	15	301.385	202.637	67.24	122.920	787.39
		Phenobarbital	100	14	248.849	116.286	46.73	77.900	505.90
		Vehicle	0	15	487.734	245.057	50.24	140.210	1093.40

APPENDIX 10 - QUALITY ASSURANCE STATEMENT

CHARLES RIVER
LABORATORIES
Preclinical Services

QUALITY ASSURANCE STATEMENT

Protocol: RTP00004

This study has been inspected by the Quality Assurance Unit to assure conformance with the Good Laboratory Practice (GLP) regulations promulgated by the EPA (FIFRA/TSCA); the Organisation for Economic Co-operation and Development; and the Japanese Ministry of Agriculture, Forestry and Fisheries. Reports were submitted in accordance with Standard Operating Procedures as follows:

QA INSPECTION DATES

Dates of Inspection	Phase(s) Inspected	Dates Findings Submitted to:	
		Study Director	Study Director Management
05 OCT 05 06 OCT 05 07 OCT 05	Protocol	05 OCT 05 06 OCT 05 07 OCT 05	05 OCT 05 06 OCT 05 07 OCT 05
18 OCT 05	Test Substance Preparation	18 OCT 05	18 OCT 05
25 OCT 05	Test Substance Administration	31 OCT 05	31 OCT 05
08 NOV 05	Blood Collection	14 NOV 05	14 NOV 05
08 NOV 05	Sacrifice	14 NOV 05	14 NOV 05
17-20 NOV 05	In-Life Data	21 NOV 05	21 NOV 05
20 NOV 05	Necropsy Data	21 NOV 05	21 NOV 05
01 DEC 05	Formulation Data	01 DEC 05	01 DEC 05
07-08 DEC 05	Report Tables	08 DEC 05	08 DEC 05
09-10 &12 JAN 06 16 JAN 06	Methods	12 JAN 06 16 JAN 06	12 JAN 06 16 JAN 06
13 & 16 JAN 06 30 JAN 06	Results	16 JAN 06 30 JAN 06	16 JAN 06 30 JAN 06
31 JAN 06	Summary	31 JAN 06	31 JAN 06
07 MAR 06 21& 24 APR 06 01 MAY 06	Revised Report	07 MAR 06 24 APR 06 01 MAY 06	07 MAR 06 24 APR 06 01 MAY 06

The final report has been reviewed to assure that it accurately describes the materials and methods, and the reported results accurately reflect the raw data.

Lisa A. Zaborowski 5-2-06

Lisa A. Zaborowski, B.S. Date
Senior Quality Assurance Auditor
Principal Auditor