FINAL REPORT

# Inter-Laboratory Validation of the 15-Day Adult Intact Male Rat Assay with Linuron and Phenobarbital


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Alan H. Staple, M.Sc. Date
Vice President
Health Sciences
RTI International

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## APPENDICES

RTI Project No.: 65U-08055.004.040
RTI Protocol No.: RTI-956

# Inter-Laboratory Validation of the 15-Day Adult Intact Male Rat Assay with Linuron and Phenobarbital

## 1.0 EXECUTIVE SUMMARY

This study was done, with RTI International as the lead laboratory and two other participating laboratories, as an inter-laboratory validation of the 15-Day Adult Intact Male Rat Assay as an alternative Endocrine Disruptor Tier I screening assay (EDSTAC, 1998). The transferability or standardization of the protocol and the practicality or sensitivity of this *in vivo* assay were evaluated using linuron and phenobarbital, two chemical compounds known to affect the endocrine system through different pathways and/or mechanisms of action. This assay is expected to detect estrogenic-, androgenic- and thyroid-like activity based on compound-related changes in target organ weight and systemic circulating hormones.

Adult male rats were dosed daily for 15 days from Test Day (TD) 1 through 15 via oral gavage. Dose levels were administered on a mg/kg body weight basis according to the most recent body weight. Aqueous methylcellulose (0.25%) was administered as the vehicle control. The dosing volume was 5 ml/kg for all but one animal for one dose and all animals were dosed between 0609 and 0916 hours with what is believed to be the correct concentration with stable and homogenous suspensions. A percentage (3.11%) of the dosing occurred outside the desired dosing period between 0600 and 0800.

The blood samples were collected prior to necropsy on TD 15 for hormone analyses and all assay results were considered reliable according to acceptable performance criteria for each assay.

Body weights were recorded on TD 1 through 14 before dosing and clinical observations were recorded daily within two hours after dosing in the morning and again in the afternoon for TD 3 or 4 through TD 14. Food consumption was determined for TD 1, 8, and 15 when possible.

Necropsy was performed on TD 15 between 0824 and 1229 hours, with 29.4% of the animals necropsied outside the desired 0800 to 1100 period, which would have been within two

to three hours of final dosing. Trunk blood was collected after decapitation with not more than 60 seconds of $CO_2$ anesthetic, target organs were collected and weighed and histopathology was done on the testes, epididymides and thyroids from the animals in the control and high dose groups.

The study design for the experiment was as follows for the two chemicals, linuron and phenobarbital:

**Table 1. Study Design, Test Chemicals, and Target Doses**

| Group No.[c] | No. Males | Chemical | Dose (mg/kg/day)[b] | Concentration (mg/ml) | Dose Volume (ml/kg) |
|---|---|---|---|---|---|
| 0 | 15 | Vehicle Control[a] | 0 | 0.0 | 5 |
| 1 | 15 | Phenobarbital | 25 | 5.0 | 5 |
| 2 | 15 | Phenobarbital | 50 | 10.0 | 5 |
| 3 | 15 | Phenobarbital | 100 | 20.0 | 5 |
| 4 | 15 | Linuron | 50 | 10.0 | 5 |
| 5 | 15 | Linuron | 100 | 20.0 | 5 |
| 6 | 15 | Linuron | 150 | 30.0 | 5 |

[a] 0.25% aqueous methylcellulose
[b] Test compounds administered once daily by gavage on Test Days 1 through 15.
[c] Groups 0-6 are equivalent to groups 1-7 as listed in protocol.

- **Linuron**

There was one animal receiving 150 mg/kg linuron (no. 203) that was found moribund on the study and was euthanized on TD 7. There was no significant difference in the mean body weights across all groups on TD 1. At TD 15, the body weights of the treatment groups were significantly ($p<0.006$) lower than those of the control group (Table 2 and Figure 3). The body weight change (g/day) for the treated groups was significantly decreased ($p<0.006$) in a dose related manner for TD 1-8 and 1-15 (Table 2 and Figures 4 and 6). The body weight changes for TD 1-8 and TD 1-15 displayed a significant decreasing linear dose-related trend ($p<0.006$) (Table 2). The body weight change for TD 8-15 was significantly decreased ($p<0.05$) only for the 100 mg/kg/day group (Table 2). The mean body weight change on TD 8-15 was lower at 150 mg/kg/day, but was not statistically identified.

Feed consumption (g/kg/day) of all the treated groups was significantly decreased on TD 1-8 (all treated groups at the $p<0.006$ level) and 1-15 (50 and 100 mg/kg/day at the $p<0.05$ level and 150 mg/kg/day at the $p<0.006$ level) from the control group (Table 2 and Figures 7 and 9), but was not significantly decreased for any treated group for TD 8-15. The linear trends showed significantly ($p<0.006$) decreased dose-related feed consumption for the TD 1-15 and TD 1-8 time periods.

Clinical observations in control animals included alopecia (n=1), broken toe nail (n=2), and efflux of the dosing compound (n=2). The animals in the 50 mg/kg/day group had the following clinical observations: lethargy (n=1), and nose chromodacryorrhea (n=1). The animals in the 100 mg/kg/day group exhibited nose chromodacryorrhea (n=8), and piloerection (n=1). The animals in the 150 mg/kg/day group showed ataxia (n=3), eye(s) chromodacyorrhea (n=2), hypothermia to the touch (n=1), lethargy (n=5), nose chromodacryorrhea (n=12), piloerection (n=1), prone (n=1), and brown urine (n=1). The prone animal, no. 203, was sacrificed on TD 7. Only the clinical observations at 150 mg/kg/day appear to be the most treatment related findings.

The final body weights of the animals in all treated groups were significantly decreased from those of the control group and showed a significant linear trend ($p< 0.006$). The liver, paired epididymides, prostate, and accessory sex gland unit absolute weights in the two highest dose groups were significantly lower than those in the control group (Figures 11, 15, 16, and 18). In the 100 and 150 mg/kg/day groups, the absolute paired epididymides and absolute liver weight were significantly lower than the control at the $p<0.006$ level, as were the linear trends for these organs, and the prostate and accessory sex gland absolute weight were significant at the $p<0.05$ level for the 100 mg/kg/day group and at the $p<0.006$ level for the 150 mg/kg/day group and the linear trend (Tables 5 and 13). The absolute weight of the seminal vesicles with fluid and coagulating glands was significantly decreased ($p<0.006$) at only the 150 mg/kg/day dose level but there was a significant ($p<0.006$) linear trend (Table 5 and Figure 17). The absolute thyroid and absolute paired testes weights of treated animals were not significantly different from the controls at any dose (Tables 5 and 13).

When relative weights (% of final body weight) were considered, the relative thyroid weight was significantly higher ($p<0.05$) than the control for the 50 and 100 mg/kg/day groups.

The 150 mg/kg/day group and the linear trend were significant at the $p<0.006$ level (Table 14). The relative paired epididymides weight was only significantly higher ($p<0.006$) in the 150 mg/kg/day group, although there was a significant linear dose-related trend ($p<0.006$) for the weight increasing with increasing dose levels (Table 14). The relative paired testis weight was significantly increased in the treated animals. The 50 mg/kg/day group was significantly increased at the $p<0.05$ level, whereas the 100 and 150 mg/kg/day groups were increased significantly at the $p<0.006$ level as was the linear trend (Table 6). The relative liver and prostate weights in all treated groups were not significantly different from the control group (Tables 6 and 14).

The serum estradiol level was significantly ($p<0.006$) higher than the controls in all treated groups. This increase showed a dose related linear trend ($p<0.006$). Thyroxine level was significantly lower ($p<0.006$) in all treatment groups from the control and showed a dose-related decreasing linear trend ($p<0.006$). Thyroid stimulating hormone (TSH) was significantly ($p<0.05$) lower in the 100 mg/kg/day group. Triiodothyronine was significantly lower than controls in the two highest dose groups. Follicle stimulating hormone (FSH) was significantly higher in the 100 mg/kg/day group and there was a significant increasing linear trend with increasing doses of linuron (Table 16). Serum testosterone, dihydrotestosterone (DHT), prolactin and luteinizing hormones (LH) values were similar in all groups (Table 16).

In the gross necropsy findings, one control animal had hydronephrosis in the right kidney and there was a similar finding in one animal in the highest dose level of linuron (common occurrence in male CD (SD) rats). The 50 mg/kg/day group had one animal with alopecia on the limbs and one animal in the 100 mg/kg/day had reduced size of the seminal vesicles and coagulating glands bilaterally (Table II-6). Administration of 150 mg/kg/day linuron was associated with a slight increased incidence of seminiferous tubule degeneration within the testes.

- **Phenobarbital**

There were two animals that received 100 mg/kg/day phenobarbital, that were found moribund on the study. Animal no. 51 was sacrificed on TD 3 and animal no. 165 was sacrificed on TD 5. There was no significant difference in the mean body weights across all

groups on TD 0. The body weight on TD 15 and the body weight changes for TD 1-8 and TD 1-15 were significantly lower ($p<0.006$) than the control for only the 100 mg/kg/day group although there was a significant ($p<0.006$) linear dose-related trend for decreasing body weight for TD 15 and for the body weight changes for TD 1-8 and TD 1-15. The body weight change for TD 8-15 was not significant for any dose group.

The feed consumption for the TD 1-8 interval was significantly lower for the animals in the 100 mg/kg/day dose group. The linear trend also is significant for this interval. The feed consumption (g/kg/day), without outlier values, was significantly higher for the 100 mg/kg/day group than the control group for the interval TD 8-15 ($p<0.05$). The linear trend for the TD 8-15 period was significant at $p<0.006$ (Table 9).

Clinical observations in control animals included alopecia (n=1), broken toe nail (n=2), and efflux of the dosing compound (n=2). The phenobarbital 25 mg/kg/day group had one animal with eye chromodacryorrhea, 5 animals with efflux of the dosing compound, and other observations that did not appear to be treatment related. The animals in the 50 mg/kg/day group exhibited efflux of the dosing compound (n=5), eye chromodacryorrhea (n=3), lethargy (n=3), nose chromodacryorrhea (n=1), and nasal discharge (n=1). The animals in the 100 mg/kg/day group showed ataxia (n=15), ear damaged (n=1), efflux of the dosing compound (n=7), eye(s) chromodacyorrhea (n=13), eye discharge (n=1), hind limb: apparent paralysis right (n=1), lethargy (n=15), loss of righting reflex (n=2), minimal response to physical or auditory stimulus (n=2), nasal discharge: red (n=2), nose chromodacryorrhea (n=6), piloerection (n=1), prone (n=8), rough coat (n=3), and salivation post dosing (n=1).

At necropsy, there were significant increases in all dosed groups in absolute thyroid weight. Without outliers, the 25 mg/kg/day group was significant at the $p<0.05$ level and the other two groups were at the $p<0.006$ level as was the linear trend showing a dose-related increase in absolute thyroid weight (Table 11). The absolute paired epididymides weight and prostate weight showed no significant difference from the control group values (Table 11). The relative weights of the thyroid showed the same pattern as the absolute weights with dose-related increases in a significant upward trend (Table 12). However, the relative paired epididymides weight and relative prostate weight were significantly increased for the

100 mg/kg/day group as were the linear trends at $p<0.006$ (Table 12). The relative paired epididymides and prostate both showed increased weights in this dose group. The absolute liver weight was significantly increased ($p<0.006$) in all the treated dose groups and there was a significant upward dose-related linear trend ($p<0.006$) shown in Table 3. There was no difference in the paired testis weight between groups (Table 3). The seminal vesicles with coagulating gland and fluid weights were not significantly different between groups. The accessory sex gland weight was significantly ($p<0.05$) increased in the 100 mg/kg/day group and showed a significant ($p<0.05$) linear dose-related upward trend (Table 3).

The relative weights of the liver and paired testis were significantly increased from the control in the highest dose group and showed significant linear trends at the $p<0.006$ level (Table 4). The relative liver weight was also significantly increased at the other two dose levels at $p<0.006$ (Table 4). The relative thyroid weight was also significantly increased at the 25 mg/kg/day dose level at $p<0.05$ and at the 50 and 100 mg/kg/day levels at $p<0.006$ (Table 12).

The mean serum thyroxine, triiodothyronine, and FSH levels of all of the treatment groups were significantly lower than the control group values. The thyroxine and FSH levels were decreased at a significant level for all dose groups ($p<0.006$) and showed a significant dose related decreasing linear trend with increasing dose groups ($p<0.006$). The triiodothyronine levels were significantly lowered in the 25 mg/kg/day dose group ($p<0.05$) and the other two higher groups were significantly lowered at $p<0.006$. There was also a significant dose related decreasing linear trend in triiodothyronine levels with increasing dose groups ($p<0.006$). The estradiol levels of the 50 and 100 mg/kg/day groups were significantly higher than that of the control group. The TSH level of the 50 mg/kg/day group was significantly higher than the control group level. Mean serum testosterone, DHT, LH, and prolactin values were similar in all groups including controls.

The animal found moribund and sacrificed on TD 3 (no. 51) had a urinary bladder cyst and the one found moribund and sacrificed on TD 5 (no.165) had a right kidney with hydronephrosis and no food in its stomach. Both of these animals received the highest dose level of phenobarbital. Administration of 100 mg/kg/day phenobarbital was also associated

with the presence and increased severity of mononuclear cell infiltration within the epididymides (bilateral).

In conclusion:

- Fifteen days of gavage with linuron resulted in decreased body weight, decreased feed consumption, and clinical signs such as lethargy. There was toxicity in terms of body weight decrease (at least a 10% decrease from the control group) in the two highest dose groups. Decreased sacrifice body weight, liver weight, accessory sex gland unit weight, and prostate and seminal vesicles with coagulating glands weights were observed at higher doses. The estradiol values increased with increasing doses whereas the thyroxine value decreased. TSH decreased with increasing doses, only statistically significantly at 100 mg/kg/day. Triiodothyronine was decreased at the two highest dose levels. FSH was increased with increasing dose levels but only statistically significant at the 100 mg/kg/day level.

- Fifteen days of gavage with phenobarbital resulted in decreased body weight, decreased feed consumption on Days 1-8, and clinical signs such as lethargy, being prone after dosing, and chromodacryorrhea. Phenobarbital caused increased liver weight indicative of enzyme induction. The thyroid is the target organ for phenobarbital and the thyroid weight was increased in all treated groups. The thyroxine and triiodothyronine levels were decreased with increasing phenobarbital levels. There were no histopathological changes in the thyroid. The TSH level was increased but only significantly at the 50 mg/kg/day level. Estradiol was increased with increasing dose levels of phenobarbital. FSH levels decreased at all dose levels.

- The 100 mg/kg/day phenobarbital group had 61.5% of the animals with a greater than 10% decrease in body weight from the control mean body weight (n=15) on TD 15, whereas the other two dose groups had none. The linuron treated animals, however, had 20.0% of the animals with a greater than 10% decrease in body weight from the control group at the 50 mg/kg/day dose group, 66.7% in the 100 mg/kg/day dose group, and 92.9% in the 150 mg/kg/day group. Fifty percent of the animals in the high dose linuron group had greater than 20% decreases in body weight. The phenobarbital dosing appears to be within the

maximum tolerated dose (MTD) and the results could be interpreted endocrinologically with minimal toxicological effects confounding the interpretations except in the highest dose group where two moribund animals had to be euthanized. However, the linuron dosing for the two higher dose groups was more than the MTD (overexposed) and, therefore, interpretation of those results should be on a toxicological basis only.

- The protocol for the adult male assay resulted in significant findings in body weights, organ weights and hormone levels that appeared to be dose related and comparable to those of previous studies for both linuron and phenobarbital.

## 2.0 BACKGROUND AND OBJECTIVES

The objectives of this study were:

1) To determine if similar results for each endpoint can be obtained among three different laboratories using a similar protocol, compounds, and dose levels.

2) To determine if the observed results in the present studies are comparable to the results observed in earlier studies using the same protocol.

3) 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.

The Food Quality Protection Act of 1996 requires the EPA to develop and implement a screening program using valid tests for determining the potential in humans for estrogenic effects from pesticides. This program has been expanded on the advice of the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) to include androgenic (and anti-androgenic) effects, antiestrogenic effects, and effects from thyroid-hormone (TH)-like (and anti-TH) substances (EDSTAC, 1998). EPA proposed a two-tiered screening and testing program in a Federal Register notice in 1998 (63 FR 71542-71568, Dec. 28, 1998) that covered not only pesticides but also commercial chemicals subject to regulation under the Toxic Substances Control Act (TSCA; 15 USC 2601) and environmental and drinking water contaminants. One of the assays recommended as an alternative for potential inclusion in the Tier 1 screening battery is a short term 15-day-adult intact male rat assay. The adult male assay was developed by DuPont to identify compounds that have the potential to act as agonists or antagonists to the estrogen, androgen, progesterone, or dopamine receptor; 5α-reductase inhibitor; steroid biosynthesis inhibitors; and compounds that alter thyroid function. Results from this assay using oral and/or intraperitoneal (ip) injection as the routes of administration, and other assays with a similar purpose, have been reported (O'Connor et al., 1996, 1999, 2002a,b).

## 3.0 METHODS

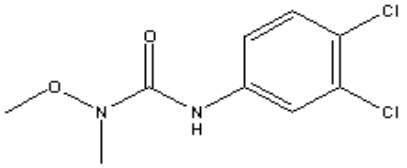
### 3.1 Test Substances

**Linuron**

CAS Number: 330-55-2

Synonyms: Linuron ; N-(3,4-Dichlorophenyl)-N'-methoxy-N'-methylurea; Linuron; Lorox; Afalon; Linurex; N'-(3,4-Dichlorophenyl)-N-methoxy-N-methylurea; methoxydiuron; du Pont Herbicide 326; Hoe 2810; Linorox; Sarclex; Aflon; Linex 4L; Lorox 4L; Lorox 50W; Lorox DF; Lorox L; Lorox Plus; Alafon; Linex; Lorax; 1-Methoxy-1-methyl-3-(3,4-dichlorophenyl)urea; 3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea; Urea, 1-(3,4-dichlorophenyl)-3-methoxy-3-methyl-; Garnitan; Methoxy-1-methyl-3-(3,4-dichlorophenyl)urea; Premalin; Cephalon; 3-(3,4-Dichlorophenyl)-1-methoxy(methyl)urea;

Structure:

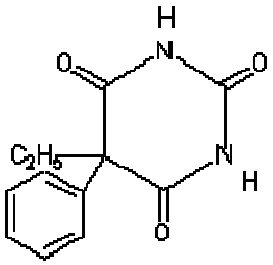



| | |
|---|---|
| Supplier: | Chem Services |
| Lot Number: | 348-8A |
| Purity: | 99.5% |
| Appearance: | Crystalline solid |
| Suspension/Solution: | Suspension |
| Molecular Formula: | $C_9H_{10}Cl_2N_2O_2$ |
| Molecular Weight: | 249.1 g/mole |
| Storage, Bulk Chemical: | Room Temperature |
| Storage, Test Suspension: | 4º C |

**Phenobarbital**

CAS Number: 50-06-6

Structure:




| | |
|---|---|
| Supplier: | Sigma-Aldrich |
| Lot Number: | 104K2600 |
| Purity: | 99.1% |
| Appearance: | White powder |
| Molecular Formula: | $C_{12}H_{12}N_2O_3$ |
| Molecular Weight: | 232.2 g/mole |
| Solution/Suspension: | Suspension |
| Storage, Bulk Chemical: | Room Temperature |
| Storage, Test Solution: | 4º C |

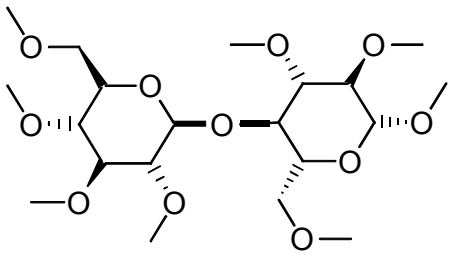
**Vehicle: Methylcellulose**

CAS Number: 9004-67-5

Synonyms: Cologel; Celevac; tylose mh20; tylose mh50; tylose mh300; tylose mh1000; tylose mh2000; tylose mh4000; tylose mh300p; tylose sap; tylose sl; tylose sl 100; tylose sl 400; tylose sl 600; tylose twa; methylcellulose; viscol; viscontran l52; viscosol; walsroder mc 20000s; methyl ether cellulose; adulsin; bagolax; bufapto methalose; bulkaloid; celacol m; celacol m20; celacol m450; celacol mm; celacol mm 10p; celacol m 20p; cellapret; cellogran; cellothyl; cellulose methyl; cellulose methylate; cellumeth; cethylose; cethytin; culminal k 42; edisol m; hydrolose; mapolose m25; mapolose 60sh50; mco 8000; mc 4000 cp; mc 20000s; mellose; methocel 10; methocel 15; methocel 181; methocel 400; methocel 4000; methocel a; methocel chg; methocel 400cps; methocel 4000cps; methocel mc; methocel mc 25; methocel mc4000; methocel mc 8000; methocel sm 100; methulose; methyl cellulose-a; methyl cellulose ether;

metolose mc 8000; metolose 60sh; metolose 60sh400; metolose sm 15; metolose sm 100; metolose sm 4000; mmts-btr; napolone; Nicel; rhomellose; syncelose; tylose 444; tylose A4S; tylose mf; tylose mh; Cellulose, methyl ether; Citrucel; Methyl cellulose (viscosity: ca 15 cP [2% solution in water]); Methylcel MC;

Structure:



| | |
|---|---|
| Supplier: | Sigma-Aldrich |
| Lot Number: | 062KO144 |
| Appearance: | Off white powder |
| Molecular Formula: | $(C_7H_{14}O_5)_x$ (polymer) |
| Molecular Weight: | 40,000 to 180,000 (polymer) |
| Storage, Bulk Chemical: | Room Temperature |
| Storage, Vehicle Solution: | 4ºC |

### 3.2 Dose Formulation and Analysis

The dosing formulations were prepared twice, on September 21, 2005 and October 5, 2005. All dosing formulations for both chemicals were stable for at least two weeks. Linuron's stability, in 0.25% methylcellulose, was established by Battelle Memorial Institute to be up to 21 days (see Appendix VII). The phenobarbital stability, in 0.25% methylcellulose was determined to be less than three weeks (see Appendix VII). The neat chemicals were shipped to RTI from Battelle Memorial Institute along with a supply of already prepared 0.25% methylcellulose.

The dosing bottles were identified at RTI by a five-digit random number Rx code, a color code and the compound name. The study technicians were blinded for dose. The dose code had to be broken for the study director, at the direction of the sponsor, when the first animal was found moribund. The dosing formulations were stored at refrigerated temperatures. Prior to dosing each day, the formulations were removed from the refrigerator. All dose levels, including the control, were brought to room temperature while being stirred for two hours on individual magnetic stirrers before dosing. Each dose formulation was stirred continuously throughout the

daily dosing period and when sampling for analysis. The vehicle control was 0.25% methyl-cellulose and the route of administration was oral gavage.

Samples of each dose formulation were collected in duplicate (one for dose analysis and one for back-up) on Test Days 1 and 15 (of Group 1) for the formulations prepared on September 21, 2005 and on Test Day 15 (of Group 2) for the formulations prepared on October 5, 2005. They were stored according to Battelle Memorial Institute's instructions until dose analysis (i.e., homogeneity and concentration verification). RTI analyzed the concentration and homogeneity of these samples according to instructions from Battelle Memorial Institute. Results of these analyses are in Appendix VII. Formulations were analyzed for the first day of dosing (September 29, 2005) for the formulations prepared on September 21, 2005 and for the last day of dosing (October 11, 2005) for the formulations prepared on September 21, 2005, and for the last day of dosing (October 13, 2005) for the formulations prepared on October 05, 2005.

The percent of nominal for linuron varied from 65 to 96% for the September 21, 2005 formulation analyzed on October 11-12, 2005, whereas it was 85.5 to 106 % of nominal when analyzed on September 24-26, 2005. Homogeneity samples did not differ more than 16% for any sample. The October 5, 2005 formulation analyzed on October 5-6, 2005 varied from 86.2 to 97.5% of nominal and on October 17-18, 2005, it varied from 101 to 110% of nominal. Homogeneity samples did not differ more than 4%. The dose formulations were prepared properly, were stable for the period of use and homogenous. The animals appeared to be properly exposed to the two lower dose levels of linuron. The highest level of exposure resulted in large body weight differences from the control animals and one animal had to be euthanized when found moribund.

For phenobarbital, the percent of nominal varied from 85.1 to 98.5% for the September 21, 2005 formulation analyzed on September 29-30, 2005. When analyzed on October 11-12, 2005, the formulation varied from 76.7 to 109%. The formulation from October 5, 2005, analyzed on October 5-6, 2005, varied from 85.9 to 90.3% of the nominal and on October 17-18, 2005 values varied from 97.8 to 120 % of nominal. Homogeneity samples varied from 0.7% to 18%. The Dose formulations were prepared properly, were stable for the period of use and homogenous. The animals at the two lower doses were properly exposed to the dose

formulation. The highest level of exposure resulted in large body weight differences from the control group and two animals had to be euthanized when they were found moribund.

### 3.3 Test Animals

The test animals were the Sprague Dawley Derived Outbred Albino Rat Crl:CD®(SD) supplied by Charles River Laboratories, Inc., Raleigh, NC.

The use of live animals was requested by the Sponsor. Alternative test systems are not available for the assessment of effects of chemicals on reproduction and development in intact mammals for determining the potential risk for humans from endocrine-mediated effects of pesticides and other chemicals. The Charles River CD® rat has been the subject of choice on reproductive and developmental toxicology contracts at RTI since 1976, and has been used for other toxicology studies with these test materials. Large historical data bases for growth, food, and water consumption are available from the supplier. The Crl:CD®(SD) rat was selected on the basis of extensive experience with this strain and its suitability with respect to sensitivity to endocrine modulators. This study did not unnecessarily duplicate any previous study, but it is a validation of an assay considered for the Endocrine Disruptor Screening Program (EDSP) which was conducted in multiple laboratories.

Adult male Crl:CD®(SD) rats, approximately 9 weeks of age were purchased specifically for this study. Animals randomized on September 26, 2005 ranged from 313.33 to 358.19 grams and those randomized on September 27, 2005 ranged from 317.50 to 369.99 grams and both groups were approximately 10 weeks of age at the start of dose administration. A total of 105 rats were placed on the study (see section 3.6 for study design).

The shipment of males was quarantined on arrival, and evaluation was initiated that day. Animal Health testing records from Charles River Laboratories, Inc., Raleigh, were kept with the study records. Animals were housed under the conditions of the study with deionized water and low phytoestrogen feed except for Purina Chow 5002 being fed on the day of arrival, September 19, 2005.

Since animals were not housed at RTI International for more than one month, no sentinel animals were necessary. The male rats were quarantined for approximately one week, with the prior concurrence of the RTI Animal Research Facility (ARF) veterinarian. They were observed daily for general health status and ability to adapt to the ARF husbandry conditions. The animals

were weighed three times [Test Day (TD)-5, -3 and 0] during this period and observed with respect to weight gain and any gross signs of disease or injury.

They were released from quarantine by the attending ARF veterinarian on September 26, 2005.

### 3.4 Housing, Feed, and Water

Males were singly housed in solid-bottom, polycarbonate cages (8"x19"x10.5") fitted with stainless steel wire lids (Laboratory Products, Rochelle Park, NJ). Sani-Chip® cage bedding (P.J. Murphy, Forest Products, Inc., Montville, NJ) were used in all cages. Powdered feed, Teklad 2018 CM diet, 18% protein (low phytoestrogen, lot no. 082605 MA, analyzed August 26, 2005, expiration date February 26, 2006) was fed in glass containers with stainless steel lids. Deionized water, produced at RTI from tap water from the Durham, NC water system, was available *ad libitum* in plastic bottles with stainless steel sipper tubes throughout the quarantine and study periods. The analyses of the Purina 5002 and Teklad diet for chemical composition and possible chemical contamination, and analysis of Durham City water were provided by the suppliers and maintained in the study records. Water samples were analyzed for total bacterial counts, and the presence of coliforms. Feed samples were found to have bacteria and fungi present but this was not observed to have an effect on the study. Samples from freshly washed cage racks were analyzed to ensure adequate sanitation by the cage washers and showed no growth. In addition, the lot number of Teklad 2018 diet used was analyzed by the supplier for concentrations of the phytoestrogens genistein, daidzein, and glycitein. The diet was stored at approximately 60-70°F, and the period of use did not exceed six months from the milling date. At all times, animals were housed, handled, and used according to the NRC Guide (NRC, 1996).

### 3.5 Environmental Conditions

Environmental conditions in the ARF were continuously monitored, recorded, and controlled during the course of the study by an automated system (Siebe/Barber-Colman Network 8000 System) with Version 4.4.1 Signal® software (Siebe Environmental Controls (SEC)/Barber-Colman Company, Loves Park, IL). Animal rooms used for this study were maintained on a 12:12 hour light:dark cycle. Lights came on at approximately 6:00 AM and went off at approximately 6:00 PM. Target conditions for temperature and relative humidity in the animal rooms were between 64-79°F (18-26°C) and 30-70%, respectively, with 10-15 air

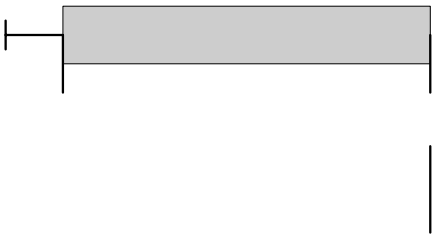
changes per hour (NRC, 1996). No readings were outside these specified ranges. The room temperature ranged from 71.44 – 74.43º F with 44.2 – 59.05% humidity during the course of the study.

All male rats were individually identified by ear tag after arrival at RTI on Test Day -6. In addition, all males assigned to the study were given an animal study number. All data generated during the course of this study were tracked by these numbers.

Some toxicity was caused by exposure at the high doses of each test material. The three animals (one for linuron, two for phenobarbital) that were severely debilitated or moribund, were humanely terminated.

### 3.6 Study Design, Test Chemicals, and Dose Selection

The study was conducted in two components with start dates staggered by one day, and consisted of three dose groups each of two chemicals, and a vehicle control group (total of 7 groups). Part of each group, including controls, started on each start day. Each group was comprised of 15 males which were randomized across treatment groups, based on body weight taken on TD 0 (the day before experimental start). The study males were dosed by gavage once daily for 15 consecutive days. Table 1 presents the study design and target doses of the test chemicals. A graphical representation of the study design is presented in Figure 1 below.



Key: Q = quarantine, TD = test day, N = necropsy

**Figure 1. Study Design for the 15-day Adult Male Assay**

The U.S. EPA selected two test chemicals for evaluation. The two test chemicals and their target/mechanism of action are as follows: (1) linuron, an anti-androgen; which competitively binds to the androgen receptor, and (2) phenobarbital which alters thyroid function.

**Study Dates**:

| | |
|---|---|
| Males arrived at RTI: | September 19, 2005 |
| Release of males from quarantine: | September 26, 2005 |
| Experimental Start Dates: | September 27 and 28, 2005 |
| Experimental Termination Dates: | October 11 and 12, 2005 |
| Submission of audited draft final report: | December 15, 2005 |

**Table 1. Study Design, Test Chemicals, and Target Doses**

| Group No.[c] | No. Males | Chemical | Dose (mg/kg/day) [b] | Concentration (mg/mL) | Dose Volume (mL/kg) |
|---|---|---|---|---|---|
| 0 | 15 | Vehicle Control[a] | 0 | 0.0 | 5 |
| 1 | 15 | Phenobarbital | 25 | 5.0 | 5 |
| 2 | 15 | Phenobarbital | 50 | 10.0 | 5 |
| 3 | 15 | Phenobarbital | 100 | 20.0 | 5 |
| 4 | 15 | Linuron | 50 | 10.0 | 5 |
| 5 | 15 | Linuron | 100 | 20.0 | 5 |
| 6 | 15 | Linuron | 150 | 30.0 | 5 |

[a] 0.25% aqueous methylcellulose, vehicle only
[b] Test compounds administered once daily by gavage on Test Days 1 through 15
[c] Groups 0-6 are equivalent to groups 1-7 as listed in protocol.

## 3.7 Treatment of Adult Males

Beginning on Test Day 1, each male was dosed with one of the test materials at one of the dose levels or the vehicle control (0.25 % aqueous methylcellulose). Each animal was weighed daily on Test Days 1 to 14 prior to treatment and, on Test Day 15 after treatment, and the body weight recorded. Vehicle or dose formulations were administered daily by oral gavage at a dosing volume of 5 ml/kg body weight based on body weight prior to dosing from Test Days 1 – 14. Gavage dosing was used since it is part of the established protocol for this assay.

Oral administration is the usual route for phenobarbital and is a possible exposure route for linuron. For gavage dosing, a 16-gauge, two-inch curved dosing needle fitted with a plastic (disposable) syringe of the appropriate volume for each treatment group was used. The dose volume was 5 mL/kg except in the case of one animal which was misdosed with a lower dose and the proper dosage was achieved by increasing the total dosing volume for that one day. Another animal received 1.8 mL instead of 1.9 mL of the dosing formulation.

The daily dosage for each compound was administered starting at approximately 0600 daily so that animals could have blood collected and be necropsied between 0800 and 1100 hr on Test Day 15 (2-3 hr after the final dose). During the 15 days of dosing, animals were gavaged between 0609 and 0916 hours, with 3.11% of the dosing occurring after 0800. The treatments were administered on an mg/kg body weight basis, adjusted based on the most recent body weight, with the exception of Test Day 15, when the dose was based on the body weight taken on Test Day 14. On Test Days 1-14, the body weights were taken and recorded, feed weights recorded on appropriate days, dosing performed, and clinical observations recorded. On Test Day 15, the animals were dosed, body weights taken, feed measured, and clinical observations recorded. The volume of the dose administered was recorded each day.

### 3.8 Clinical Observations

Clinical observations of male study animals were documented at least once daily during quarantine, and at least twice daily, at dosing and one to two hours post dosing, on TD 1-14. Clinical observations were also made after 2 PM from Test Days 4-14 for Group 1 and on TD 3-14 for group 2. This was done since positive clinical signs were observed at 6-8 hours after dosing. On TD 15, clinical observations were made at dosing. The examining technicians were unaware of the dose levels. Observations were made for (but not limited to):

A. Any response with respect to body position, activity, coordination, or gait
B. Any unusual behavior such as head flicking, compulsive biting or licking, circling, etc.
C. The presence of:
    1. Convulsions, tremors, or ataxia
    2. Lethargy
    3. Increased lacrimation or red-colored tears (chromodacryorrhea)
    4. Increased or decreased urination or defecation (including diarrhea)

5. Piloerection
6. Mydriasis or miosis (enlarged or constricted pupils)
7. Unusual respirations (fast, slow, labored, audible, or gasping)

Cage-side examinations to detect moribund or dead rats were conducted twice daily throughout the study by the Animal Research Facility staff. Moribund rats were sacrificed. Moribund rats were given a gross pathological evaluation. At every weighing, each rat was individually handled and examined for abnormal behavior and appearance as part of the clinical observations.

### 3.9 Male Body Weights and Feed Consumption

All study males were weighed in the morning of Test Day 0 (prior to assignment to treatment groups), and every day in the morning on Test Day 1-14, for adjustment of dosing volume based on the most recent body weight. They were also weighed on Test Day 15 for calculation of food consumption. Male body weight and weight change were calculated and analyzed for TD 1-8, 8-15 and 1-15. Feed weights for the individually-housed males were recorded on TD 1, 8, and 15, and feed consumption was reported as g/kg body weight/day.

### 3.10 Gross Necropsy, Blood Collection, and Organ Weights

No rats were found dead or sacrificed moribund prior to experimental start. After experimental start, there were three rats found moribund (no.203, one of the high dose linuron, and nos. 51 and 165, two of the high dose of phenobarbital) and they were euthanized and necropsied. Gross lesions and target organs (as described below) were saved but did not have histopathological evaluation. Blood was taken from the trunk of animal nos. 165 and 203 but no blood could be collected from animal no. 51 due to its very low blood pressure and respiration. All remaining rats were sacrificed as designed on Test Day 15.

Animals were dosed in the animal room on Test Day 15, based on the body weight of Test Day 14, and then moved to the necropsy area approximately 1 hour before necropsy to minimize stress-induced changes in hormone levels related to cage transport. Animals were not fasted prior to necropsy. Rats were euthanized by decapitation with prior anesthesia using $CO_2$ for no more than 60 seconds and exsanguinated via the site of decapitation. Rapid euthanasia was necessary because of the likelihood that undue stress associated with the administration of

anesthesia alone could interfere with the accurate measurement of the various hormones that are essential endpoints of this assay. On the first day of the necropsies, the time of necropsy ranged from 0824 to 1229 with 30 animals being necropsied outside of the desired 0800 to1100 time period. This delay was due to only having two prosectors available instead of three. Time of death was recorded for all animals. On Test Day 15 for the second day of the necropsies, all necropsies occurred between the hours of 0800 – 1100, within 2-3 hours of the last administered dose. This means that 29.4% of the animals were necropsied outside of the desired 0800 to 1100 range.

Animals were evaluated for gross observations of toxicity, organ weights, and serum hormone concentrations. The testes, thyroid gland, and epididymides were evaluated microscopically.

Final body, liver, thyroid gland, left and right testis, entire prostate, paired epididymides, and seminal vesicles and coagulating gland (with fluid) weights were taken. Total weight of the testes was the combined weight of the left and right testis, and accessory sex gland unit weight was the combined weight of the entire prostate and seminal vesicles and coagulating gland with fluid weights. All organs were weighed to four decimal places (0.0001g). Relative organ weights (% of live weight on Test Day 15) were calculated.

### 3.11 Histology and Pathology

The liver and epididymides from each rat were placed in formalin fixative, and then embedded in paraffin. The thyroid glands and surrounding tissue were removed and placed into formalin fixative for at least 48 hrs prior to trimming, weighing, and embedding in paraffin. Following fixation, final dissection of the thyroid was performed by one individual in order to reduce the variability of the dissection procedure, and hence, reduce the variability of the thyroid weights. Testes were placed in Bouin's fixative for 24 hours after which they were rinsed and stored in 70% alcohol until embedded in paraffin. The testes (left and right), epididymides (left and right), and thyroid were evaluated microscopically. The embedded tissues were sectioned at 3-5 microns and stained with hematoxylin and eosin (H and E). Microscopic evaluations were performed on control and high dose animals for both compounds. Stained sections of the control and high dose groups were evaluated by a Board Certified veterinary pathologist for pathologic abnormalities and potential treatment-related effects. Thyroids were evaluated for morphologic

changes such as altered follicular epithelial height, the relative number and staining characteristics of colloid, the extent of thyroid vascular supply, and the density, size, and shape of the thyroid follicles. The testes and epididymides were evaluated for spermatogenesis, spermiogenesis, status of seminiferous tubules in the testis, and sperm in the epididymis, as well as the structural integrity of these organs.

## 3.12 Hormone Evaluation

Blood was collected from the trunk of the animal at the time of sacrifice from all animals. The blood was placed in a centrifuge tube until the serum was prepared. The blood was allowed to clot and centrifuged under refrigeration at a setting of approximately 1400 x g for approximately ten minutes. Aliquots of serum were made based on the number of different assays run to minimize the potential freeze and thaw effect on hormone concentrations. Serum was 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. Each assay included all samples from the control group and each dose level for both chemicals. 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. Proteinaceous rat hormones were obtained from the National Hormone and Pituitary Program and the steroids were purchased from commercial suppliers. All laboratories used the same sources. All assays at RTI International were counted in a Packard Biosciences Cobra II Series Model 5002 gamma counter using RIASMART software, version 1.0.

### 3.12.1 Estradiol Radioimmunoassay Procedure

The estradiol (17-β estradiol) radioimmunoassay (RIA) used was a no-extraction, double antibody $^{125}$I RIA (catalog #DSL 39100, 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. This kit was designed for use with human serum. No modification to

the kit was necessary, however, controls in rat serum were assayed to confirm the validity of the results. 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 2 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 4ºC for 4 hours. The $^{125}$I-estradiol (100 µL) was added, and the tubes were vortexed and incubated at 4ºC for approximately 21 hours. After overnight incubation, cold precipitating solution (1 mL) was added and the tubes 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 2.

**Table 2. 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** | 0/20.6%<br>7.5/13.0%<br>25/15.0% | 5/25.5% (68.7-79.9%)[d]<br>33.3/19.9% (25.1-32.1%)[d] |
| **Inter-assay Variation[a]** | | |
| **No. of assays** | 1 | 1 |
| **Mass added** | N/A | N/A |
| **% recovery of added mass[b]** | 7.5/90.1%<br>25/87.3% | 5/107.9%<br>33.3/98.7% |
| **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 # 134 (pooled from 3 rats).
[f] Estradiol from kit calibrators.
N/A = Not applicable

**Table 2A. Estradiol Standard Curve Values; Assay Date 10-26-05**

| Defined Dose (pg/mL) | Average Calculated Dose (pg/mL) | % Bound |
|---|---|---|
| 1.5 | 1.52 | 93.62, 90.08 |
| 5.0 | 4.86 | 72.31, 71.14 |
| 15.0 | 15.75 | 41.30, 41.29 |
| 50.0 | 46.09 | 21.55, 20.39 |
| 150.0 | 163.49 | 11.34, 11.10 |

### 3.12.2 Rat Follicle Stimulating Hormone Radioimmunoassay Procedure

The rat follicle stimulating hormone (rat (r)FSH) RIA used was a no-extraction, double antibody $^{125}$I RIA (catalog #RPA.550, 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. This kit was designed for use with rat serum samples. 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 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 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 18 hours. After overnight incubation, cold precipitating solution (400 µL) 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 3.

**Table 3. Parameters for FSH RIAs Used for Male Rat Hormone Determinations**

| Parameter | Rat Serum Controls[f] | Zero Calibrator Controls[e] |
|---|---|---|
| **Units** | (ng/mL) | (ng/mL) |
| **Intra-assay Variation[a]** | | |
| **Mass added** | 0/9.3%<br>6.25/3.3%<br>25/5.8% | 7.5/1.5% (66.6-67.4%)[d]<br>30/9.9% (27.1-32.3%)[d] |
| **Inter-assay Variation[a]** | | |
| **No. of assays** | 1 | 1 |
| **Mass added** | N/A | N/A |
| **% recovery of added mass[b]** | 6.25/96.0%<br>25/87.7% | 7.5/197.0%<br>30/170.9% |
| **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] NIDDK-rFSH-RP2 from the National Hormone and Pituitary Program (Torrance, CA).
[f] Male CD rat serum RTI Lot # 134 (3 rats), FSH from kit calibrators.
N/A = Not applicable

**Table 3A. Rat FSH Standard Curve Values; Assay Date 10-10-05**

| Defined Dose (ng/mL) | Average Calculated Dose (ng/mL) | % Bound |
|---|---|---|
| 1.6 | 1.79 | 98.97, 95.22 |
| 3.1 | 2.59 | 93.79, 96.85 |
| 6.2 | 6.57 | 84.27, 86.68 |
| 12.5 | 12.22 | 71.77, 72.40 |
| 25.0 | 25.54 | 47.74, 52.26 |
| 50.0 | 50.94 | 29.07, 32.01 |
| 100.0 | 95.25 | 18.48, 19.34 |

### 3.12.3 Rat Luteinizing Hormone Radioimmunoassay Procedure

The rat luteinizing hormone (rLH) RIA used was a no-extraction, double antibody $^{125}$I RIA (catalog #RPA.552, 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. This kit was designed for use with rat serum samples. 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 4 below). All samples were analyzed in one assay. The sensitivity of the assay was 0.9 ng/mL as reported by Amersham Biosciences. Not all samples read within curve range of 0.8 to 50 ng/mL. For the control group, 2 values were below the assay detection limits (BDL); for phenobarbital 25 mg/kg/day, 1 value was BDL; for phenobarbital 50 mg/kg/day, 2 values were BDL; and for phenobarbital 100 mg/kg/day, 1 value was BDL. For linuron 50 mg/kg/day, there were no values BDL; for linuron 100 mg/kg/day; there were 3 values BDL; and for linuron 150 mg/kg/day, there were 2 values BDL. 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 21 hours. After overnight incubation, cold precipitating solution (400 µL) 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 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** | 0/11.2%<br>3.1/7.5%<br>12.5/5.0% | 3.75/6.8% (67.8-72.6%)[d]<br>15/6.6% (22.1-25.4%)[d] |
| **Inter-assay Variation[a]** | | |
| **No. of assays** | 1 | 1 |
| **Mass added** | N/A | N/A |
| **% recovery of added mass[b]** | 3.1/88.0%<br>12.5/92.3% | 3.75/140.7%<br>15/132.6% |
| **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 # 134 (3 rats), LH from kit calibrators.
[f] NIDDK-rLH-RP3 from the National Hormone and Pituitary Program (Torrance, CA).
N/A = Not applicable

**Table 4A. Rat LH Standard Curve Values; Assay Date 10-26-05**

| Defined Dose (ng/mL) | Average Calculated Dose (ng/mL) | % Bound |
|---|---|---|
| 0.8 | 0.77 | 98.44, 97.01 |
| 1.6 | 1.69 | 91.59, 92.53 |
| 3.1 | 3.01 | 81.36, 85.23 |
| 6.2 | 6.15 | 63.19, 66.76 |
| 12.5 | 12.81 | 40.45, 42.07 |
| 25.0 | 24.68 | 23.11, 24.57 |
| 50.0 | 49.44 | 12.58, 13.41 |

### 3.12.4 Rat Prolactin Radioimmunoassay Procedure

The rat prolactin (rPRL) RIA used was a no-extraction, double antibody $^{125}$I RIA (catalog #RPA.553, 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. This kit was designed for use with rat serum samples. 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 interassay coefficient of variation, percent recovery, and index of parallelism for the assays was determined (see Table 5 below). 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 4-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 17 - 22 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 5.

**Table 5. 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** | 0/5.7%, 3.0%, 10.2%<br>3.1/3.7%, 1.4%, 1.7%<br>12.5/2.3%, 2.6%, 0.8% | 2.5/14.2%, 1.2%, 2.0% (62.7-70.7%)[d]<br>10/23.4%, 6.7%, 4.1% (26.9-37.3%)[d] |
| **Inter-assay Variation[a]** | | |
| **No. of assays** | 3 | 3 |
| **Mass added** | 0/6.8%<br>3.1/5.2%<br>12.5/0.1% | 2.5/6.1%<br>10/10.7% |
| **% recovery of added mass[b]** | 3.1/114.7-136.9%<br>12.5/102.9-135.8% | 2.5/195.5-220.2%<br>10/180.3-219.9% |
| **Index of parallelism[c]** | 95.0% | 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 # 134 (3 rats), prolactin from kit calibrators.
[f] NIDDK-rPRL-RP3 from the National Hormone and Pituitary Program (Torrance, CA).
N/A = Not applicable

**Table 5A. Rat Prolactin Standard Curve Values; Assay Date 10-06-05**

| Defined Dose (ng/mL) | Average Calculated Dose (ng/mL) | % Bound |
|---|---|---|
| 0.8 | 0.77 | 98.60, 97.65 |
| 1.6 | 1.62 | 88.42, 92.59 |
| 3.1 | 3.13 | 80.37, 78.63 |
| 6.2 | 6.16 | 62.73, 64.57 |
| 12.5 | 12.60 | 43.74, 44.90 |
| 25.0 | 24.93 | 27.38, 27.43 |
| 50.0 | 49.76 | 15.11, 14.30 |

### 3.12.5 Rat Thyroid Stimulating Hormone Radioimmunoassay Procedure

The rat thyroid stimulating hormone (rTSH) RIA used was a no-extraction, double antibody $^{125}$I RIA (catalog #RPA.554, 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. This kit was designed for use with rat serum samples. 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 for the assay was determined (see Table 6 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 20 hours. After overnight incubation, cold precipitating solution (400µL) was added and the tubes 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 6.

**Table 6. 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** | 0/7.8%<br>4/7.3%<br>16/2.2% | 2.5/1.8% (69.9-71.14%)[d]<br>10/3.9% (28.7-30.8%)[d] |
| **Inter-assay Variation[a]** | | |
| **No. of assays** | 1 | 1 |
| **Mass added** | N/A | N/A |
| **% recovery of added mass[b]** | 4/98.1%<br>16/135.3% | 2.5/240.7%<br>10/219.5% |
| **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 # 134 (3 rats), TSH from kit calibrators.
[f] NIDDK-rTSH-RP3 from the National Hormone and Pituitary Program (Torrance, CA).
N/A = Not applicable

**Table 6A. Rat TSH Standard Curve Values; Assay Date 10-06-05**

| Defined Dose (ng/mL) | Average Calculated Dose (ng/mL) | % Bound |
|---|---|---|
| 1.0 | 0.94 | 94.73, 92.77 |
| 2.0 | 2.10 | 88.25, 85.97 |
| 4.0 | 3.95 | 76.40, 76.84 |
| 8.0 | 7.91 | 58.12, 58.74 |
| 16.0 | 16.54 | 35.06, 36.07 |
| 32.0 | 30.87 | 17.77, 21.55 |
| 64.0 | 64.91 | 10.48, 5.71 |

### 3.12.6 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 (catalog #TKTT5, Diagnostic Products Corporation [DPC], Los Angeles, CA). This kit was designed for use with human serum. No modification to the kit was necessary, however, controls in rat serum were assayed to confirm the validity of the results. 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 interassay coefficient of variation, and percent recovery for the assays was determined (see Table 7 below). The sensitivity of the assay was 0.04 ng/mL as reported by DPC. 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 37ºC 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 7.

**Table 7. 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** | 0.5/7.0% and 1.8%<br>4/9.8% and 9.4%<br>8/2.2% and 5.2% | 0.5/10.0% and 12.1% (68.5-73.6%) [d]<br>4/5.7% and 4.0% (29.8-34.2%) [d] |
| **Inter-assay Variation[a]** | | |
| **No. of assays** | 2 | 2 |
| **Mass added** | 0.5/9.4%<br>4/2.2%<br>8/5.1% | 0.5/13.1%<br>4/7.6% |
| **% recovery of added mass[b]** | 0.5/70.0-79.0%<br>4/94.5-97.4%<br>8/97.4-104.6% | 0.5/88.5-105.0%<br>4/96.3-107.3% |
| **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 # 135 (10 rats).
[f] Testosterone from kit calibrators.
N/A = Not applicable

**Table 7A. Total Testosterone Standard Curve Values; Assay Date 10-19-05**

| Defined Dose (ng/mL) | Average Calculated Dose (ng/mL) | % Bound |
|---|---|---|
| 0.2 | 0.18 | 87.55, 88.17 |
| 1.0 | 1.09 | 58.84, 57.26 |
| 4.0 | 4.10 | 31.92, 33.45 |
| 8.0 | 7.61 | 22.93, 23.12 |
| 16.0 | 15.48 | 14.22, 14.64 |

### 3.12.7 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 (catalog #TKT35, DPC, Los Angeles, CA). This kit was designed for use with human serum. No modification to the kit was necessary, however, controls in rat serum were assayed to confirm the validity of the results. 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 8 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 37ºC 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 8.

**Table 8. Parameters for Triiodothyronine (T3) RIAs Used for Male Rat Hormone Determinations**

| Parameter | Rat Serum Controls[e,f] | Zero Calibrator Controls |
|---|---|---|
| **Units** | (ng/dL) | (ng/dL) |
| **Intra-assay Variation[a]** | | |
| **Mass added** | 0/16.1%<br>50/11.6%<br>300/5.6% | 50/4.1% (72.9-74.6%)[d]<br>300/5.6% (25.5-27.8%)[d] |
| **Inter-assay Variation[a]** | | |
| **No. of assays** | 1 | 1 |
| **Mass added** | N/A | N/A |
| **% recovery of added mass[b]** | 50/105.1%<br>300/115.4% | 50/88.3%<br>300/108.6% |
| **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 # 134 (3 rats)
[f] T3 from kit calibrators.

N/A = Not applicable

**Table 8A. Total Triiodothyronine Standard Curve Values; Assay Date 10-24-05**

| Defined Dose (ng/dL) | Average Calculated Dose (ng/dL) | % Bound |
|---|---|---|
| 20.0 | 19.04 | 86.72, 88.12 |
| 50.0 | 51.49 | 70.88, 71.81 |
| 100.0 | 99.07 | 54.50, 56.18 |
| 200.0 | 205.11 | 35.89, 36.55 |
| 600.0 | 580.14 | 15.93, 15.91 |

### 3.12.8 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 4 calibrators for the standard curve (catalog #TKT45, DPC, Los Angeles, CA). This kit was designed for use with human serum. No modification to the kit was necessary, however, controls in rat serum were assayed to confirm the validity of the results. 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 interassay coefficient of variation and percent recovery for the assays were determined (see Table 9 below). The sensitivity of the assay was 0.25 µg/dL as reported by DPC. 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 37ºC 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 9.

**Table 9. Parameters for Thyroxine (T4) RIAs Used for Male Rat Hormone Determinations**

| Parameter | Rat Serum Controls[e,f] | Zero Calibrator Controls |
|---|---|---|
| **Units** | (µg/dL) | (µg/dL) |
| **Intra-assay Variation[a]** | | |
| **Mass added** | 0/10.3% and 15.7%<br>5/8.6% and 10.0%<br>8/10.0% and 14.5%<br>12/7.3% and 16.1% | 2.67/7.5% and 3.7% (66.5-70.3%)[d]<br>16/7.0% and 8.2% (27.5-30.2%)[d] |
| **Inter-assay Variation[a]** | | |
| **No. of assays** | 2 | 2 |
| **Mass added** | 0/23.8%<br>5/1.0%<br>8/2.4%<br>12/4.4% | 2.67/2.8%<br>16/0.5% |
| **% recovery of added mass[b]** | 5/97.6-110.4%<br>8/97.2-102.3%<br>12/99.4-100.5% | 2.67/103.2-107.4%<br>16/100.8-101.5% |
| **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 # 134 (3 rats).
[f] T4 from kit calibrators.
N/A = Not applicable

**Table 9A. Total Thyroxine Standard Curve Values; Assay Date 10-24-05**

| Defined Dose (µg/dL) | Average Calculated Dose (µg/dL) | % Bound |
|---|---|---|
| 1.0 | 0.93 | 91.95, 91.67 |
| 4.0 | 4.25 | 60.87, 62.67 |
| 10.0 | 10.44 | 38.48, 41.30 |
| 16.0 | 15.25 | 29.95, 32.58 |
| 24.0 | 23.48 | 22.23, 22.82 |

### 3.12.9 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 (catalog #DSL-9600, 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 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. This kit was designed for use with human serum. No modification to the kit was necessary, however, controls in rat serum were assayed to confirm the validity of the results. 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 10 below). All samples were analyzed in one assay. 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. 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 10.

**Table 10. Parameters for Dihydrotestosterone (DHT) 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** | 0/33.7%<br>100/7.3%<br>400/N/A | 64/9.5% (63.5-68.7%)[d]<br>320/8.3% (25.5-29.1%)[d] |
| **Inter-assay Variation[a]** | | |
| **No. of assays** | 1 | 1 |
| **Mass added** | N/A | N/A |
| **% recovery of added mass[b]** | 100/48.7%<br>400/111.6% | 64/110.8%<br>320/105.3% |
| **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.
[f] DHT from kit calibrators.
N/A = Not applicable

**Table 10A. Dihydrotestosterone Standard Curve Values; Assay Date 11-29-05**

| Defined Dose (pg/mL) | Average Calculated Dose (pg/mL) | % Bound |
|---|---|---|
| 12.5 | 13.83 | 89.15, 90.38 |
| 25.0 | 21.97 | 83.89, 86.78 |
| 50.0 | 49.18 | 70.80, 75.69 |
| 100.0 | 106.48 | 56.11, 56.82 |
| 200.0 | 202.16 | 42.48, 39.51 |
| 400.0 | 392.02 | 25.69, 28.00 |
| 800.0 | 781.60 | 15.86, 16.43 |

## 3.13 Statistical Analyses

A common statistical plan was used for this study by the three individual laboratories. This plan is attached to this report as Appendix VI.

## 3.14 Personnel

This study was conducted at RTI International, Research Triangle Park, NC, under contract to Battelle, Columbus, OH. Dr. D. P. Houchens, EDSP Program Manager, was the Sponsor's Representative. Dr. R.W. Tyl served as Principal Investigator. Ms. B.T. Hamby was

the Project Manager. Ms. C.S. Sloan was the Work Assignment Leader and Study Director. Reproductive and Developmental Toxicology and Laboratory of Reproductive and Endocrine Toxicology personnel included Ms. M.C. Marr, Ms. C.B. Myers (Reproductive Toxicity Study Supervisor and Data Analyst), Ms. V.I. Wilson, Ms. S.W. Pearce, Ms. K.D. Vick, Ms. N.A. Ostin, Mr. C.G. Leach, and Mr. T.W. Wiley. Chemical stability data were provided by the Sponsor through Dr. E.A. Crecelius, PNNL, Battelle Marine Sciences Laboratory, Sequim, WA. Mr. M.M. Veselica (Supervisor, RTI Materials Handling Facility), Mr. D.L. Hubbard, Mr. J. E. Larson, and Mr. R.A. Price provided receipt of the bulk chemicals and preparation of dose formulations at RTI. Dose formulation analysis was provided by Ms. N.P. Castillo, Mr. D.J. Watkins, and Ms. J.B. Whitaker. Chemical vault storage and shipping and receiving arrangements were provided by Mr. V.L. Parker. Animal care was provided by Dr. L. Heath, DVM, ACLAM, RTI Veterinarian, J. Scott-Emuakpor, attending Veterinarian and Mr. R.S. Silverstein, Manager of RTI Animal Research Facility. RTI Quality Assurance personnel were Ms. D.A. Drissel, Ms. C.A. Ingalls, Ms. C.D. Keller, Ms. M.M. Oh, and Ms. K.C. Collier.

The final report was prepared by Ms. C.D. Sloan, with assistance from Dr. R.W. Tyl, Dr. J.D. George, Ms. B.T. Hamby, Ms. C.B. Myers, Ms. M.C. Marr and Ms. S.W. Pearce. Ms. S.W. Pearce conducted all of the radioimmunoassays. Ms. C.B. Myers was responsible for data compilation and statistical analyses, and Mr. T.W. Wiley was responsible for data entry. Ms. M.C. Marr, Ms. N.A. Ostin, and Ms. S.W. Pearce were responsible for all activities concerning organization and custody of the study records and for archiving the study records. Ms. D.B. Bynum and Ms. V.M. McCall provided secretarial assistance.

### 3.15 Compliance

All in-life data was collected electronically by the validated Toxicology Analysis System Customized (TASC). QC and quality assurance (QA) procedures follow those outlined in the Quality Assurance Project Plan (QAPP) prepared for this study. The study is under OECD GLP Principles, Japanese (MAFF) and EPA FIFRA GLP Regulations. All specimens will be disposed of after they no longer afford evaluation. All records, which remain the responsibility of RTI, will be retained in the RTI archives for the life of the contract.

## 4.0 RESULTS

### 4.1 Linuron Results

One animal at 150 mg/kg/day linuron, was found moribund on the study and was euthanized on TD 7. There was no significant difference in the mean body weights across all groups on TD 1. At TD 15, the body weights of the control group were significantly higher ($p<0.006$) than those of all the treated groups (Table 2 and Figure 3). The body weight change (g/day) for the treated groups was significantly decreased ($p<0.006$) in a dose related manner for TD 1-8 and 1-15 (Table 2 and Figures 4 and 6). The body weight changes for TD 1-8 and TD 1-15 displayed a significant linear decreasing trend ($p<0.006$) related directly to increases in dose levels (Table 2). The body weight change for TD 8-15 was significantly decreased ($p<0.05$) for only the 100 mg/kg/day group but there was a significant decreasing linear trend for this time period (<0.05) (Table 2).

Feed consumption (g/kg/day) of all the treated groups was significantly decreased on TD 1-8 (all treated groups at the $p<0.006$ level) and 1-15 (50 and 100 mg/kg/day at the $p<0.05$ level and 150 mg/kg/day at the $p<0.006$ level) from the control group (Table 2 and Figures 7 and 9) but was not significantly decreased for any treated group for TD 8-15 (Table 2 and Figure 8). The linear trends showed significantly ($p<0.006$) decreased feed consumption for the TD 1-15 and TD 1-8 time periods.

Clinical observations in control animals included alopecia (n=1), broken toe nail (n=2), and efflux of the dosing compound (n=2). The animals in the 50 mg/kg/day group had the following clinical observations: alopecia (n=2), efflux of the dosing compound (n=4), lethargy (n=1), nose chromodacryorrhea (n=1), and sores (n=1). The animals in the 100 mg/kg/day group exhibited alopecia (n=2), nose chromodacryorrhea (n=8), piloerection (n=1) and struggling during dosing (n=1). The animals in the 150 mg/kg/day group showed alopecia (n=1), ataxia (n=3), efflux of the dosing compound (n=4), eye(s) chromodacyorrhea (n=2), gasping post dosing (n=1), hypothermia to the touch (n=1), lethargy (n=5), nose chromodacryorrhea (n=12), piloerection (n=1), prone (n=1), rust colored fur (n=1), struggling during dosing (n=1), and brown urine (n=1). The prone animal, no. 203, was sacrificed on TD 7.

The final body weights (Table 2) of the animals in all treated groups were significantly decreased from those of the control group and showed a significant decreasing linear trend

($p< 0.006$). The liver, paired epididymides, prostate, and accessory sex gland unit absolute weights of the two highest dose groups were significantly lower than those of the control group (Figures 11, 15, 16, and 18). The absolute paired epididymides and absolute liver weight were significantly lower for the 100 and 150 mg/kg/day groups than the control at the $p<0.006$ level, as were the linear trends (Tables 5 and 13 and Figures 11 and 15), and the prostate and accessory sex gland absolute weights were significant at the $p<0.05$ level for the 100 mg/kg/day group and at the $p<0.006$ level for the 150 mg/kg/day group and the dose-related decreasing linear trend (Tables 5 and 13 and Figures 16 and 18). The absolute weight of the seminal vesicles with fluid and coagulating glands was significantly decreased ($p<0.006$) at only the 150 mg/kg/day dose level but there was a significant ($p<0.006$) dose-related decreasing linear trend (Table 5 and Figure 17). The absolute thyroid and absolute paired testes weights of treated animals were not significantly different from the controls (Tables 5 and 13, and Figures 10 and 14).

When relative weights (% of final body weight) were considered, the relative thyroid weight was significantly higher ($p<0.05$) than the control at the 50 and 100 mg/kg/day levels and at $p<0.006$ for the 150 mg/kg/day group and the increasing dose-related linear trend (Table 14). The relative paired epididymides weight was only significantly higher ($p<0.006$) in the 150 mg/kg/day group, although there was a significant dose-related increasing linear trend ($p<0.006$) (Table 14). The relative paired testis weight was significantly increased in the treated animals. The 50 mg/kg/day group was significantly increased at the $p<0.05$ level, whereas the 100 and 150 mg/kg/day groups were increased significantly at the $p<0.006$ level as was the dose-related increasing linear trend (Table 6). The relative liver and prostate weights were not significantly different from the control group (Tables 6 and 14).

The serum estradiol level was significantly ($p<0.006$) higher than the controls in all treated groups (Table 8 and Figure 25). This increase showed a dose related increasing linear trend ($p<0.006$). Thyroxine level was significantly lower ($p<0.006$) in all treatment groups from the control and showed a dose related decreasing linear trend ($p<0.006$) (Table 8 and Figure 22). TSH was significantly ($p<0.05$) lower in the 100 mg/kg/day group (Table 16 and Figure 21). Triiodothyronine was significantly lower than controls in the two highest dose groups ($p<0.05$) and there was a dose-related decreasing linear trend ($p<0.006$) (Table 8 and Figure 23). Follicle stimulating hormone was significantly higher in the 100 mg/kg/day group and there was a dose-related increasing linear trend ($p<0.05$) (Table 16 and Figure 24). Serum testosterone

(Figure 19), DHT (Figure 27), prolactin (Figure 26) (Table 16 for all three) and LH values (Table 8 and Figure 20) were similar in all groups.

In the gross necropsy findings, one control animal had hydronephrosis in the right kidney and there was a similar finding in one animal in the highest dose level of linuron. The 50 mg/kg/day group had one animal with alopecia on the limbs and one animal in the 100 mg/kg/day had reduced size of the seminal vesicles and coagulating glands bilaterally (Table II-6).

Administration of 150 mg/kg/day linuron was associated with a slight increased incidence of seminiferous tubule degeneration within the testes. This change was characterized by a bilateral (2 out of 3 cases) degeneration of seminiferous tubules which was minimal in severity and consisted of one or more tubules which had thinning of spermatogenic epithelium and/or vacuolization within the spermatogenic epithelium which was accompanied by a few degenerative spermatogonia cells. This change involved only a few tubules. No decrease in sperm or desquamated degenerative spermatogonial cells was noted in the epididymal tubules. No related histopathology was detected in the thyroids, testes or epididymides which could account for the weight changes observed.

### 4.2 Phenobarbital Results

There were two animals that received phenobarbital, 100 mg/kg/day that were found moribund on the study. Animal no. 51 was sacrificed on TD 3 and animal no. 165 was sacrificed on TD 5. There was no significant difference in the mean body weights across all groups on TD 0. The body weight on TD 15 and the body weight changes for TD 1-8 and TD 1-15 were significantly lower ($p<0.006$) than the control for only the 100 mg/kg/day group although there was a significant ($p<0.006$) dose-related decreasing linear trend for both TD 15 and the body weight changes for TD 1-8 and TD 1-15 (Table 1). The body weight change for TD 8-15 was not significant for any dose group (Table 9).

The feed consumption (g/kg/day) without outlier values was significantly higher for the 100 mg/kg/day group than the control group for the interval TD 8-15 ($p<0.05$). The increasing linear trend for the TD 8-15 period was significant at $p<0.006$ (Table 9). Feed consumption for TD 1 to 8 for 100 mg/kg/day was significantly lower than the control values as was the dose-related decreasing linear trend ($p<0.006$) (Table 1).

Clinical observations in control animals included alopecia (n=1), broken toe nail (n=2), and efflux of the dosing compound (n=2). The animals in the phenobarbital 25 mg/kg/day group had the following clinical observations: alopecia (n=2), broken toenail (n=1), efflux of the dosing compound (n=5), eye chromodacryorrhea (n=1), and struggling during dosing (n=1). The animals in the 50 mg/kg/day group exhibited efflux of the dosing compound (n=5), eye chromodacryorrhea (n=3), gasping post dosing (n=1), lethargy (n=3), nasal discharge: red (n=1), nose chromodacryorrhea (n=1), and struggling during dosing (n=1). The animals in the 100 mg/kg/day group showed ataxia (n=15), ear damaged (n=1), efflux of the dosing compound (n=7), eye(s) chromodacyorrhea (n=13), eye discharge (n=1), gasping post dosing (n=1), hind limb: apparent paralysis right (n=1), lethargy (n=15), loss of righting reflex (n=2), minimal response to physical or auditory stimulus (n=2), nasal discharge: red (n=2), nose chromodacryorrhea (n=6), piloerection (n=1), prone (n=8), rough coat (n=3), struggling during dosing (n=2), and salivation post dosing (n=1).

At necropsy, there were significant increases in all dose groups in absolute thyroid weight. Without potential outliers, the 25 mg/kg/day group was significant at the $p<0.05$ level and the other two groups were at the $p<0.006$ level as was the dose-related increasing linear trend (Table 11 and Figure 10). The paired epididymides weight and prostate weight showed no significant difference from the control group (Table 11 and Figures 15 and 16). The relative weights of the thyroid showed the same pattern as the absolute weights. However, the relative paired epididymides weight and relative prostate weight were significantly increased for only the 100 mg/kg/day group as were the increasing linear trends at $p<0.006$ (Table 12). The absolute liver weight was significantly increased ($p<0.006$) in all the treated dose groups and there was a significant increasing linear trend at $p<0.006$ (Table 3 and Figure 11). There was no difference in the absolute paired testis weight between groups (Table 3 and Figure 14). The seminal vesicles with coagulating gland and fluid weights were not significantly different between groups (Table 3 and Figure 17). The accessory sex gland weight was significantly ($p<0.05$) increased in the 100 mg/kg/day group and showed a significant ($p<0.05$) dose-related increasing linear trend (Table 3 and Figure 18).

The relative weights of the thyroid, liver, paired testis, and paired epididymides all were significantly increased from the control in the highest dose group and showed significant increasing linear trends at the $p<0.006$ level (Tables 4 and 12). The relative liver weight was

also significantly increased at the two lower dose levels and had a dose-related increasing linear trend at $p<0.006$ (Table 4). The relative thyroid weight was also significantly increased at the 25 mg/kg/day dose level at $p<0.05$ and at the 50 and 100 mg/kg/day levels and there was a dose-related increasing linear trend at $p<0.006$ (Table 12). The relative prostate ($p<0.006$) (Table 12), seminal vesicles and coagulating glands ($p<0.05$), and accessory sex gland weights ($p<0.006$) (Table 4) were all significantly increased in the 100 mg/kg/day group and had significantly increasing ($p<0.006$) linear trends (Tables 4 and 12).

Other necropsy findings included a control rat with right kidney hydronephrosis, 2 animals with alopecia on their limbs in the 25 mg/kg/day group, one rat in the 100 mg/kg/day group with the ventral prostate missing the left lobe, and one rat from the 50 mg/kg/day group had the seminal vesicles with coagulating glands nicked during necropsy and fluid leaked.

The mean serum thyroxine, triiodothyronine, and FSH levels of all of the treatment groups were significantly lower than the control group values (Tables 7 and 15, Figure 22, 23, and 24). The thyroxine and FSH levels (Figures 22 and 24) were decreased at a significant level for all dose groups ($p<0.006$) and showed a significant dose-related decreasing linear trend ($p<0.006$). The triiodothyronine levels were significantly lowered in the 25 mg/kg/day dose group ($p<0.05$) and the other two higher groups were significantly lowered at $p<0.006$ (Figure 23). There was also a significant dose-related decreasing linear trend in triiodothyronine levels ($p<0.006$). The estradiol levels of the 50 and 100 mg/kg/day groups were significantly higher than that of the control group and there was a significant dose-related increasing linear trend ($p<0.006$) (Figure 25). The TSH level of the 50 mg/kg/day group was significantly higher than the control group level. The TSH level at 100 mg/kg/day was approximately equal to the level at 50 mg/kg/day but not found to be significant, although probably treatment related (Figure 21). Serum testosterone, DHT, LH, and prolactin values were similar in all groups including controls (Figures 19, 20, 26, and 27).

The animal found moribund and sacrificed on TD 3 had a urinary bladder cyst and the one found moribund and sacrificed on TD 5 had a right kidney with hydronephrosis and no food in its stomach. Both of these animals received the highest dose level of phenobarbital.

Administration of 100 mg/kg/day phenobarbital was associated with the presence and increased severity of mononuclear cell infiltration within the epididymides (bilateral). Normally,

the epididymis may have one or two small foci of perivascular, mononuclear inflammatory cell infiltrates which are generally not recorded. However, in over half of the high dose animals, a minimal to mild increased severity was noted. In these cases, the perivascular foci were more numerous, larger and widely distributed. In the mild cases, interstitial edema was also observed, particularly in the cauda portion of the epididymis. No changes of the epididymal tubules or epithelium were noted. The pathogenesis of this change was unclear. Treatment associated lesions were not observed in the testes or thyroid glands.

## 5.0 DISCUSSION AND CONCLUSIONS

### 5.1 Linuron

Linuron administration for 15 days to adult male rats resulted in significantly lowered body weights and changes in specific organ weights as well as specific circulating hormone levels. The 150 mg/kg/day group showed a final mean body weight that was 81% that of the control group with 13 of the 14 animals having body weights at least 10% below the mean of the control group. The low and mid linuron treated groups had mean body weights that were 6 and 13% decreases respectively from the control. If 20% decrease in body weight is used as the determinant, the 100 mg/kg/day would be considered the MTD and the 150 mg/kg/day group would be considered overexposed. However, there were 10 of the 15 animals in the 100 mg/kg/day that still had TD 15 body weights at least 10% below the mean of the control group and the 50 mg/kg/day group had 3 of the 15 animals with body weights lowered by at least 10%. The three lightest animals of this dose group differed from the control group mean by 11, 12, and 17%. The overall body weight change for the 50 mg/kg/day dose group was 6.4% which would not be considered overexposed. As early as TD 6, there was a direct dose-related effect on the body weight decrease over time. The animals showed increasing degrees of lethargy and decreased food consumption which was reflected in the body weight changes in the animals as the dose of linuron increased. The animal that had to be euthanized on TD 7 had lost 95.3 grams by this day of treatment and had a one day body weight loss of 35.3 grams the day before it was euthanized. These animals can be considered overexposed to the chemical in terms of body weight changes. Body weight is certainly an important endpoint for this study with this chemical, but one should not only consider the group means but what happens to the individual animals and proportions of animals that have certain body weight decreases. For instance, 80% of the low dose groups had body weight decreases ≤10%, in the mid-level dose, 33% had body weight decreases ≤10%, and 33% had body weight decreases from 11-15%. However, there were few clinical observations in either of these groups. Most people interpreting these results would conclude that the MTD would be between 50 and 100 mg/kg/day.

The significant absolute decrease in liver weight at the two higher doses likely reflects the metabolic consequences of the decreased food consumption and body weight decreases. The significantly decreased absolute prostate weight at the two highest doses and the significantly

decreased absolute seminal vesicles and absolute coagulating gland weight at the highest dose and the resulting significantly lowered accessory sex gland weight, all are consistent with the changes in the hormone levels. Estradiol levels were significantly higher for all of the treated groups and the FSH level was significantly higher in the 100 mg/kg/day group. These values show the anti-androgenic effect of the linuron on the adult male rat. The absolute thyroid weights were not significantly different in the treated groups compared to the control group value. In contrast, the thyroid related hormones did show significant changes. The TSH level was significantly lowered in the 100 mg/kg/day group and the triiodothyronine was significantly lowered in the two highest dose groups, whereas the thyroxine was significantly lowered in all treated groups.

It is difficult to interpret the endocrine results in terms of the large body weight decreases seen in individual animals in the two highest groups but certainly the thyroid related hormones agree with the body weight decreases. Based on the combined information from the body weight decreases, circulating hormone levels and clinical observations, the MTD would likely be estimated to fall between the 50 and 100 mg/kg/day doses.

### 5.2 Phenobarbital

The mean body weight of the 100 mg/kg/day group was decreased from the control group from TD 3 through TD15. The mean body weight on TD 15 was 90% for this group compared with the control group. On TD 15, the 100 mg/kg/day group had 8 of the remaining 13 animals with body weights at least 10% below the body weight mean of the control group. The animals in this group were often described as lethargic and prone after dosing each day, although this effect tended to decrease in the later days of treatment. These clinical observations are consistent with sedation by phenobarbital. The feed consumption was also decreased for this group. The two animals that had to be euthanized, after they became moribund, showed one day body weight losses of 36.6 and 36.0 grams.

Phenobarbital is known to alter thyroid function. The thyroid weights of the treated groups were all significantly increased. The thyroxine and triiodothyronine levels were significantly decreased in all treated groups. The TSH level was significantly increased in the 50 mg/kg/day group. These values are consistent with an endocrine disruptor that acts on the thyroid.

The FSH concentrations were significantly decreased at all dose levels and the estradiol concentrations were increased statistically significantly at the two highest doses.

The liver weight (absolute and relative) was significantly increased in all of the treated groups compared to the control group. This indicates enzyme induction occurring due to the administration of phenobarbital. The only other significant increase in organ weight was seen in the accessory sex gland weight, which increased with increasing dose and was significant at the highest dose level.

## 5.3 Overall Conclusions and Discussion

The body weight decreases in the animals was one of the most significant findings for these studies as can be seen from the following table.

**Table 11. 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,%) |
|---|---|---|---|---|---|---|
| 1 | Phenobarbital 25 mg/kg/day | 15 | 15 (100.00) | | | |
| 2 | Phenobarbital 50 mg/kg/day | 15 | 15 (100.00) | | | |
| 3 | Phenobarbital 100 mg/kg/day | 13 | 5 (38.46) | 6 (46.15) | 2 (15.38) | |
| 4 | Linuron 50 mg/kg/day | 15 | 12 (80.00) | 2 (13.33) | 1 (6.67) | |
| 5 | Linuron 100 mg/kg/day | 15 | 5 (33.33) | 5 (33.33) | 3 (20.00) | 2 (13.33) |
| 6 | Linuron 150 mg/kg/day | 14 | 1 (7.14) | 3 (21.43) | 3 (21.43) | 7 (50.00) |

As Table 11 shows, the 100 mg/kg/day phenobarbital group had 8 animals (61.5%) with a greater than 10% decrease in body weight from the control mean body weight (n=15) on TD 15, whereas the other two dose groups had none. The linuron treated animals; however, had 3 of 15 animals (20.0%) with a greater than 10% decrease in body weight from the control group at the 50 mg/kg/day dose group, 10 of 15 animals (66.7%) in the 100 mg/kg/day dose group, and 13 of 14 animals (92.9%) in the 150 mg/kg/day group. Fifty percent of the animals in the high

dose linuron group had greater than 20% decreases in body weight. The phenobarbital dosing appears to be within the MTD and the results could be interpreted endocrinologically with minimal toxicological effects confounding the interpretations except in the highest dose group where two moribund animals had to be euthanized. However, the two higher linuron doses were more than the MTD and, therefore, interpretation of the results of these doses should be on a toxicological basis with support from the endocrine parameters.

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## REGULATORY CITATIONS

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| SOP Deviations | | |
|---|---|---|
| **SOP Number/Deviation** | **Effect on Study** | **Reason** |
| SOP LAS-VET-001, The Director of LAS or Attending veterinarian did not date the Quarantine Evaluation Form and after it was dated in front of the study director, it was misplaced. | None | Veterinarian error and inappropriate filing. |
| SOP LAS-ARF-003, The animal room was not recorded as being sanitized on one weekday, 10-04-05 | None | Caretaker error |
| SOP LAS-GEN-003, If an animal has feed outside of the feed jar, the weight is not recorded. We recorded the weight of unspilled feed. | None | The study director asked that the remaining feed weight be recorded since most of the animals had a slight amount of feed outside the jar. This was probably due to the ataxia that some of the animals exhibited after treatment. |
| SOP RET-END-007, Standards prepared in the laboratory were used instead of the kit standards and the 4 parameter logistics curve fit was used | None | This curve provided more accurate values for the validation studies. |

| Protocol Deviations | | |
|---|---|---|
| **Deviation** | **Effect on Study** | **Reason** |
| The phenobarbital animals were dosed one day beyond the determined stability of two weeks for the compound. | None observed | The stability for phenobarbital had not been determined when the study was being conducted. The compound was determined to not be stable at three weeks and therefore animals were inadvertently dosed beyond the two week stability period. |
| Animals received the wrong feed. | None observed | The Teklad diet had not arrived on the day of the animal arrival and so the animals received Purina 5002 pellets for less than 24 hours of their first day in quarantine. They were fed the proper diet for the next 7 days before the study began and for the remainder of the study. |
| Some of the dosing bottles were coded with the compound. Later the sponsor asked that the codes be broken entirely. | None, just that study director was no longer blinded | The Materials Handling Facility mistakenly labeled some dosing bottles with the compound name. After an animal had to be euthanized moribund, the sponsor requested that the dosing codes be broken. |
| No animals were designated as sentinels. | None | Since the animals were not at RTI for at least a month, no sentinels were necessary. |
| One animal (no. 91) received a greater dose volume on one day than 5 mL/kg. | None observed | One animal was under-dosed and a second dosing to correct for this resulted in a larger volume being administered. |
| The right and left testis for one unscheduled death animal (no.203) were weighed together instead of separately. | None | Technician error |
| One animal (no. 193) received a dose volume of 1.8 mL instead of 1.9 mL on TD 15. | None observed | Technician error |
| The initial lot number of the vehicle provided by the sponsor was incorporated into the protocol and the actual lot sent was a different lot number. | None | Sponsor error |
| Thirty animals were necropsied outside the 0800 to 1100 window | None | Inadequate number of prosectors |

In the Study Director's professional opinion, these deviations did not affect the study integrity, performance, or interpretation, and are presented for completeness.

Carol S. Sloan — 4-13-06

Carol S. Sloan
Study Director

Date

RTI INTERNATIONAL

# Quality Assurance Statement

**Study Title:** **Inter-Laboratory Validation of the 15-Day Adult Intact Male Rat Assay with Linuron and Phenobarbital**

**Sponsor:** **Battelle Memorial Institute**

**Study Code:** **RTI-956; Rt05-ED09; WA 5-15; 08055.004.040**

This study was audited by the Sciences and Engineering – Quality Assurance Unit and the results of the inspections and audits were reported to the 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 Study Director and Management |
|---|---|---|
| Protocol | September 13, 2005 | September 14, 2005 |
| Protocol Amendment | September 21, 2005 | September 21, 2005 |
| Sample Preparation | September 29-30, 2005 | September 30, 2005 |
| Dosing, Body Weights, Clinical Observations | September 28, 2005 | October 04, 2005 |
| Necropsy | October 12, 2005 | October 12, 2005 |
| Assay Sample Receipt | November 15, 2005 | November 15, 2005 |
| DHT Hormone Assays | November 28, 2005 | November 30, 2005 |
| Data Audit (In Life) | November 16-18, 2005 | November 21, 2005 |
| Data Audit (Hormone Assays) | November 21-23, 2005 | November 23, 2005 |
| Data/Report Audit (Analytical) | November 22-23, 2005 | November 28, 2005 |
| Data Audit | December 08, 2005 | December 08, 2005 |
| Data/Report Audit | December 12-13, 2005 | December 14, 2005 |
| Draft Final Report Audit | December 02-06, 2005 | December 06, 2005 |
| Report Audit | January 17-19, 2006 | January 19, 2006 |

| | | |
|---|---|---|
| Report Audit | February 16-18, 2006 | February 20, 2006 |
| Report Audit | April 10-13, 2006 | April 13, 2006 |

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4/13/06
Date

## Appendix Table of Contents

## Appendix III Figures

# Appendix I

# Individual Tables

Table I-1. Individual Male Body Weights (g) During the Dosing Period (page 1 of 4)

| Group[a] | Animal ID | Test Day 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 1 | 327.54 | 333.30 | 337.87 | 340.39 | 349.25 | 351.85 | 356.63 | 361.90 | 368.56 | 373.43 | 377.97 | 384.32 | 385.49 | 388.75 | 393.41 |
| | 3 | 339.66 | 344.50 | 339.46 | 347.72 | 351.80 | 354.47 | 359.83 | 364.71 | 369.98 | 364.32 | 371.19 | 375.85 | 381.85 | 385.42 | 390.09 |
| | 5 | 334.36 | 332.63 | 338.35 | 341.55 | 341.44 | 341.95 | 344.16 | 350.25 | 353.65 | 358.18 | 364.78 | 373.12 | 377.87 | 384.38 | 389.32 |
| | 7 | 342.68 | 345.14 | 342.22 | 343.81 | 350.63 | 355.02 | 356.40 | 361.06 | 365.30 | 369.34 | 369.21 | 370.75 | 371.66 | 374.83 | 379.88 |
| | 9 | 340.14 | 350.94 | 360.16 | 366.28 | 369.85 | 376.49 | 385.09 | 392.60 | 397.24 | 403.00 | 407.16 | 415.70 | 417.22 | 424.18 | 422.41 |
| | 11 | 349.90 | 358.70 | 363.48 | 364.73 | 369.52 | 372.65 | 374.72 | 379.10 | 382.96 | 402.34 | 386.46 | 390.65 | 389.45 | 390.99 | 392.11 |
| | 13 | 351.05 | 352.89 | 360.49 | 372.79 | 375.85 | 380.65 | 392.19 | 390.75 | 395.94 | 408.74 | 406.48 | 409.48 | 413.29 | 422.64 | 416.92 |
| | 15 | 347.99 | 355.28 | 355.33 | 363.58 | 373.17 | 383.86 | 390.52 | 395.79 | 401.50 | 409.30 | 412.12 | 418.86 | 417.55 | 424.59 | 428.02 |
| | 107 | 327.73 | 329.98 | 339.33 | 343.71 | 348.83 | 353.86 | 360.38 | 366.92 | 370.93 | 378.51 | 383.62 | 384.23 | 389.05 | 398.16 | 402.21 |
| | 109 | 331.65 | 340.71 | 347.78 | 353.64 | 364.19 | 372.29 | 374.60 | 382.34 | 385.06 | 389.87 | 392.68 | 392.42 | 399.51 | 397.89 | 401.46 |
| | 111 | 335.46 | 339.88 | 346.11 | 353.10 | 360.91 | 368.52 | 374.37 | 377.00 | 379.64 | 384.53 | 388.20 | 393.08 | 395.41 | 396.58 | 398.80 |
| | 113 | 346.05 | 351.27 | 353.93 | 358.21 | 364.20 | 368.51 | 374.79 | 378.68 | 378.19 | 380.25 | 385.15 | 387.36 | 393.83 | 393.12 | 395.67 |
| | 115 | 348.52 | 347.68 | 359.90 | 366.13 | 372.12 | 372.90 | 377.85 | 378.45 | 384.36 | 387.87 | 394.49 | 401.62 | 405.34 | 405.62 | 399.26 |
| | 117 | 355.99 | 359.06 | 367.49 | 373.07 | 382.83 | 386.73 | 393.57 | 398.32 | 401.75 | 406.24 | 415.57 | 416.27 | 422.04 | 426.43 | 426.42 |
| | 119 | 363.64 | 367.94 | 375.13 | 377.83 | 383.35 | 388.26 | 394.35 | 398.80 | 398.87 | 401.21 | 409.63 | 417.23 | 418.37 | 427.28 | 422.83 |
| 1 | 17 | 324.50 | 332.70 | 333.14 | 340.33 | 343.18 | 346.05 | 349.41 | 339.56 | 342.56 | 349.29 | 354.79 | 361.23 | 362.11 | 366.05 | 371.77 |
| | 19 | 323.31 | 341.45 | 348.55 | 350.14 | 351.25 | 360.90 | 359.16 | 358.19 | 364.31 | 366.77 | 373.23 | 374.37 | 378.28 | 382.01 | 385.07 |
| | 21 | 345.76 | 351.09 | 360.38 | 361.61 | 367.44 | 368.64 | 373.87 | 375.00 | 378.58 | 385.50 | 387.99 | 387.77 | 391.64 | 394.37 | 397.74 |
| | 23 | 337.00 | 345.89 | 352.80 | 356.87 | 363.71 | 365.94 | 369.69 | 372.98 | 378.37 | 381.45 | 388.12 | 389.43 | 392.39 | 396.00 | 395.79 |
| | 25 | 342.85 | 346.84 | 359.38 | 358.83 | 369.27 | 372.14 | 376.05 | 384.16 | 381.31 | 390.68 | 393.28 | 391.07 | 396.26 | 401.17 | 401.69 |
| | 27 | 342.41 | 356.14 | 357.52 | 370.04 | 378.48 | 384.21 | 386.45 | 395.20 | 403.19 | 405.86 | 417.69 | 422.68 | 422.96 | 436.05 | 433.73 |
| | 29 | 352.91 | 366.34 | 370.99 | 379.50 | 386.50 | 396.72 | 401.36 | 391.38 | 414.67 | 427.26 | 432.56 | 441.01 | 438.09 | 449.91 | 448.52 |
| | 121 | 331.79 | 344.30 | 357.61 | 363.00 | 365.23 | 368.43 | 374.11 | 375.35 | 383.09 | 387.59 | 393.44 | 394.83 | 398.77 | 401.80 | 404.43 |
| | 123 | 329.89 | 326.31 | 336.86 | 341.34 | 344.01 | 348.36 | 351.85 | 351.23 | 356.21 | 358.79 | 359.15 | 360.02 | 363.08 | 366.88 | 364.52 |
| | 125 | 326.52 | 336.66 | 345.35 | 351.77 | 359.64 | 361.88 | 359.99 | 366.23 | 370.75 | 377.27 | 379.72 | 383.35 | 385.61 | 388.58 | 391.22 |
| | 127 | 337.27 | 353.53 | 365.21 | 371.67 | 371.96 | 377.03 | 381.45 | 381.67 | 391.53 | 393.12 | 396.71 | 399.39 | 402.18 | 410.05 | 411.02 |
| | 129 | 345.85 | 354.50 | 360.39 | 366.15 | 367.19 | 373.39 | 375.53 | 377.82 | 387.02 | 388.70 | 394.89 | 394.70 | 399.44 | 404.77 | 405.84 |
| | 131 | 352.56 | 361.60 | 367.60 | 373.50 | 378.63 | 384.92 | 385.71 | 388.98 | 391.94 | 398.55 | 399.03 | 403.20 | 403.69 | 409.57 | 412.42 |
| | 133 | 357.68 | 370.68 | 376.31 | 383.66 | 386.74 | 391.34 | 395.42 | 401.87 | 396.95 | 406.78 | 409.27 | 409.73 | 412.79 | 413.74 | 419.29 |
| | 135 | 363.87 | 377.49 | 384.89 | 394.68 | 398.40 | 402.47 | 401.15 | 411.93 | 415.57 | 420.08 | 426.20 | 429.58 | 439.41 | 444.54 | 443.26 |

Table I-1. Individual Male Body Weights (g) During the Dosing Period (page 2 of 4)

| | | Test Day | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group[a] | Animal ID | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| 2 | 31 | 321.89 | 328.10 | 330.30 | 336.15 | 337.44 | 341.68 | 345.39 | 348.35 | 350.51 | 355.39 | 357.97 | 362.30 | 361.27 | 367.14 | 374.13 |
| | 33 | 333.56 | 345.08 | 354.35 | 357.54 | 363.46 | 367.91 | 368.21 | 369.00 | 380.75 | 383.46 | 389.02 | 389.28 | 390.45 | 395.48 | 399.57 |
| | 35 | 339.91 | 343.25 | 347.99 | 357.11 | 357.66 | 359.41 | 361.38 | 364.17 | 374.46 | 375.58 | 382.66 | 380.29 | 379.79 | 387.46 | 385.50 |
| | 37 | 344.48 | 352.88 | 357.77 | 364.60 | 371.25 | 374.10 | 379.04 | 385.71 | 391.24 | 399.24 | 404.04 | 410.10 | 411.44 | 419.10 | 418.67 |
| | 39 | 341.77 | 347.50 | 354.89 | 348.05 | 355.61 | 360.48 | 365.87 | 369.29 | 380.70 | 380.47 | 390.75 | 398.14 | 403.32 | 402.44 | 407.17 |
| | 41 | 348.19 | 356.16 | 363.71 | 365.46 | 370.53 | 370.43 | 372.16 | 375.63 | 385.07 | 383.12 | 388.42 | 390.32 | 393.26 | 397.29 | 401.11 |
| | 43 | 356.69 | 362.64 | 369.75 | 380.86 | 387.49 | 393.71 | 401.86 | 404.52 | 414.07 | 415.93 | 426.04 | 429.85 | 434.01 | 440.10 | 442.08 |
| | 45 | 354.93 | 364.90 | 375.48 | 378.36 | 382.82 | 385.02 | 386.91 | 390.50 | 402.55 | 409.00 | 399.94 | 413.76 | 416.20 | 423.98 | 423.85 |
| | 137 | 327.39 | 338.73 | 341.40 | 343.77 | 348.20 | 349.33 | 349.93 | 354.14 | 352.98 | 358.74 | 359.94 | 361.72 | 365.48 | 368.32 | 369.98 |
| | 139 | 333.88 | 337.69 | 343.13 | 349.78 | 350.59 | 350.03 | 352.20 | 358.00 | 358.94 | 357.85 | 363.19 | 367.66 | 373.59 | 379.83 | 379.82 |
| | 141 | 344.89 | 346.50 | 352.35 | 356.78 | 361.54 | 360.27 | 363.47 | 366.53 | 370.52 | 377.71 | 378.45 | 381.00 | 385.18 | 389.91 | 392.03 |
| | 143 | 337.15 | 343.84 | 352.94 | 359.83 | 362.62 | 362.67 | 363.65 | 365.46 | 370.73 | 374.62 | 372.36 | 374.61 | 377.36 | 377.61 | 380.04 |
| | 145 | 350.38 | 362.20 | 371.68 | 378.56 | 380.20 | 380.00 | 379.33 | 382.99 | 390.44 | 393.12 | 400.39 | 404.08 | 407.96 | 414.16 | 414.23 |
| | 147 | 359.29 | 363.01 | 365.60 | 367.99 | 373.85 | 379.18 | 383.41 | 386.56 | 390.33 | 398.84 | 399.01 | 404.31 | 409.12 | 413.33 | 416.49 |
| | 149 | 362.94 | 367.19 | 378.91 | 387.21 | 395.23 | 400.49 | 407.61 | 415.51 | 422.71 | 430.70 | 436.83 | 438.62 | 447.20 | 452.39 | 449.54 |
| 3 | 47 | 329.87 | 332.47 | 327.39 | 331.09 | 331.01 | 332.08 | 327.97 | 325.67 | 331.99 | 332.66 | 328.60 | 333.61 | 338.81 | 341.98 | 345.69 |
| | 49 | 335.30 | 336.88 | 342.39 | 348.93 | 348.69 | 348.38 | 344.81 | 343.97 | 345.92 | 346.44 | 342.80 | 342.41 | 342.09 | 344.90 | 345.65 |
| | 51 | 336.64 | 353.96 | 317.38 | .[b] | | | | | | | | | | | |
| | 53 | 346.71 | 344.19 | 338.12 | 336.93 | 335.54 | 334.41 | 334.99 | 339.46 | 342.89 | 352.02 | 358.28 | 361.20 | 363.50 | 366.83 | 372.54 |
| | 55 | 339.37 | 347.15 | 333.73 | 330.35 | 323.55 | 321.75 | 322.98 | 324.59 | 334.44 | 334.17 | 334.70 | 338.56 | 344.27 | 345.07 | 348.67 |
| | 57 | 341.59 | 353.28 | 330.57 | 331.78 | 332.95 | 346.52 | 352.81 | 362.31 | 372.76 | 381.68 | 387.72 | 395.36 | 396.75 | 400.28 | 404.79 |
| | 59 | 346.35 | 358.50 | 357.62 | 347.67 | 343.88 | 336.11 | 336.09 | 340.97 | 343.45 | 352.38 | 346.44 | 347.51 | 346.36 | 350.38 | 354.75 |
| | 151 | 330.03 | 335.45 | 326.85 | 319.48 | 317.10 | 324.55 | 331.42 | 333.46 | 339.03 | 337.01 | 338.25 | 340.74 | 345.21 | 348.26 | 346.44 |
| | 153 | 321.57 | 328.85 | 311.65 | 305.57 | 295.15 | 296.62 | 301.90 | 311.53 | 327.22 | 326.01 | 326.21 | 326.90 | 327.64 | 331.68 | 331.50 |
| | 155 | 332.02 | 331.79 | 324.39 | 317.75 | 302.34 | 301.97 | 309.95 | 314.89 | 321.47 | 333.58 | 331.73 | 334.64 | 334.55 | 335.29 | 339.21 |
| | 157 | 343.90 | 349.42 | 324.38 | 312.56 | 303.19 | 312.87 | 317.41 | 325.62 | 343.14 | 347.02 | 342.82 | 346.63 | 352.98 | 356.38 | 356.67 |
| | 159 | 349.16 | 348.96 | 336.13 | 342.44 | 345.21 | 339.69 | 340.59 | 344.60 | 346.85 | 354.64 | 353.94 | 356.06 | 357.20 | 364.84 | 369.37 |
| | 161 | 358.53 | 356.15 | 360.76 | 365.32 | 366.70 | 368.15 | 368.14 | 372.26 | 378.69 | 379.36 | 384.23 | 389.88 | 391.67 | 392.65 | 395.38 |
| | 163 | 360.40 | 364.40 | 353.72 | 346.27 | 345.72 | 359.13 | 368.22 | 375.31 | 381.31 | 378.65 | 382.71 | 386.84 | 388.40 | 392.26 | 392.31 |
| | 165 | 363.40 | 386.42 | 370.44 | 334.46 | 324.63 | .[c] | | | | | | | | | |

Table I-1. Individual Male Body Weights (g) During the Dosing Period (page 3 of 4)

| Group[a] | Animal ID | Test Day 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | 61 | 320.87 | 319.77 | 314.62 | 318.33 | 326.34 | 329.83 | 333.10 | 337.61 | 340.15 | 342.34 | 345.91 | 354.94 | 358.20 | 361.83 | 367.64 |
| | 63 | 325.82 | 326.46 | 321.08 | 321.39 | 317.11 | 319.19 | 325.68 | 326.92 | 327.93 | 336.89 | 340.41 | 348.46 | 349.66 | 350.71 | 359.40 |
| | 65 | 334.40 | 346.82 | 341.19 | 338.48 | 342.00 | 347.47 | 347.21 | 342.70 | 350.72 | 360.82 | 363.82 | 358.52 | 374.67 | 365.21 | 376.85 |
| | 67 | 339.92 | 335.89 | 339.32 | 341.88 | 347.13 | 351.10 | 356.09 | 357.38 | 363.84 | 364.50 | 372.95 | 371.53 | 376.86 | 375.71 | 383.92 |
| | 69 | 341.28 | 349.52 | 345.32 | 346.16 | 349.53 | 359.72 | 359.59 | 364.56 | 369.53 | 375.33 | 381.46 | 376.79 | 378.37 | 384.41 | 386.24 |
| | 71 | 349.11 | 339.06 | 337.24 | 344.35 | 352.61 | 362.84 | 363.86 | 358.34 | 368.60 | 365.36 | 377.65 | 381.14 | 385.12 | 383.84 | 392.12 |
| | 73 | 350.80 | 352.34 | 351.73 | 354.13 | 359.65 | 365.61 | 365.26 | 365.59 | 363.64 | 369.93 | 378.85 | 386.66 | 385.07 | 385.71 | 383.73 |
| | 75 | 356.72 | 352.97 | 347.60 | 352.15 | 357.65 | 361.95 | 366.34 | 360.15 | 365.46 | 378.25 | 387.35 | 384.56 | 379.87 | 387.55 | 386.05 |
| | 167 | 324.78 | 317.66 | 313.53 | 308.60 | 314.88 | 313.85 | 308.72 | 306.27 | 319.01 | 327.04 | 316.06 | 311.58 | 324.14 | 325.42 | 334.40 |
| | 169 | 332.27 | 328.82 | 327.66 | 333.77 | 336.46 | 336.50 | 341.59 | 346.88 | 352.28 | 353.13 | 353.58 | 353.07 | 359.24 | 360.17 | 366.59 |
| | 171 | 339.55 | 344.07 | 337.95 | 341.48 | 353.93 | 353.74 | 347.44 | 357.01 | 359.60 | 368.24 | 376.90 | 376.18 | 383.30 | 389.21 | 390.90 |
| | 173 | 339.84 | 343.09 | 343.71 | 351.25 | 346.34 | 351.88 | 354.92 | 354.58 | 362.65 | 368.51 | 368.25 | 370.70 | 369.40 | 377.12 | 380.16 |
| | 175 | 347.28 | 341.25 | 337.05 | 330.75 | 334.57 | 333.01 | 333.48 | 338.90 | 347.73 | 351.19 | 353.34 | 353.95 | 354.15 | 357.45 | 357.09 |
| | 177 | 352.09 | 351.35 | 342.23 | 355.36 | 359.75 | 361.04 | 369.15 | 374.49 | 374.37 | 388.18 | 388.88 | 392.53 | 396.35 | 395.47 | 399.24 |
| | 179 | 363.09 | 362.57 | 357.75 | 356.40 | 360.40 | 361.59 | 364.11 | 376.82 | 382.76 | 389.08 | 396.04 | 397.61 | 400.75 | 403.38 | 408.05 |
| 5 | 77 | 327.58 | 308.40 | 289.10 | 293.16 | 300.30 | 299.77 | 301.58 | 297.49 | 297.16 | 303.40 | 306.62 | 305.55 | 311.15 | 320.21 | 319.79 |
| | 79 | 334.20 | 319.03 | 313.17 | 320.12 | 325.56 | 326.16 | 333.03 | 333.63 | 337.39 | 336.39 | 340.75 | 344.26 | 351.28 | 339.30 | 349.13 |
| | 81 | 337.50 | 327.51 | 331.93 | 330.23 | 341.09 | 342.52 | 347.17 | 352.01 | 359.70 | 361.76 | 362.58 | 368.77 | 372.83 | 377.05 | 377.49 |
| | 83 | 336.77 | 317.75 | 308.20 | 323.13 | 320.79 | 333.90 | 337.84 | 338.97 | 333.38 | 329.86 | 327.46 | 340.87 | 348.31 | 352.03 | 344.56 |
| | 85 | 343.36 | 323.05 | 312.04 | 313.66 | 325.16 | 334.54 | 338.27 | 341.00 | 346.53 | 351.26 | 353.61 | 361.01 | 359.99 | 366.88 | 373.19 |
| | 87 | 345.26 | 339.78 | 337.48 | 337.42 | 342.83 | 339.22 | 348.86 | 348.45 | 351.76 | 347.58 | 357.09 | 352.62 | 354.27 | 356.32 | 355.08 |
| | 89 | 350.30 | 325.45 | 309.48 | 320.24 | 323.06 | 331.92 | 338.10 | 332.78 | 348.44 | 341.63 | 351.99 | 358.66 | 346.70 | 351.26 | 356.24 |
| | 181 | 329.74 | 319.39 | 318.00 | 322.11 | 319.07 | 323.31 | 321.03 | 326.85 | 326.56 | 330.01 | 331.62 | 333.97 | 335.83 | 335.61 | 338.10 |
| | 183 | 334.04 | 319.12 | 307.14 | 304.02 | 298.11 | 298.30 | 288.64 | 287.03 | 291.42 | 288.87 | 283.92 | 298.78 | 292.93 | 298.45 | 304.00 |
| | 185 | 341.18 | 331.40 | 323.47 | 335.55 | 339.22 | 336.27 | 338.71 | 345.79 | 343.38 | 343.57 | 345.95 | 346.91 | 342.38 | 354.68 | 352.93 |
| | 187 | 340.88 | 323.05 | 315.80 | 313.04 | 321.10 | 332.78 | 335.78 | 340.92 | 341.48 | 346.53 | 356.74 | 355.18 | 363.70 | 364.52 | 366.06 |
| | 189 | 351.70 | 330.90 | 315.86 | 322.67 | 327.41 | 330.31 | 334.55 | 341.23 | 345.51 | 348.01 | 350.93 | 354.45 | 357.05 | 364.37 | 365.01 |
| | 191 | 359.22 | 345.65 | 327.77 | 332.14 | 326.89 | 340.00 | 332.93 | 328.12 | 343.02 | 343.21 | 339.13 | 348.61 | 362.12 | 360.61 | 357.77 |
| | 193 | 357.90 | 346.63 | 336.39 | 335.83 | 345.07 | 343.72 | 346.50 | 356.71 | 357.15 | 365.84 | 365.78 | 361.24 | 370.58 | 375.40 | 374.89 |
| | 195 | 362.01 | 343.18 | 331.07 | 331.49 | 332.60 | 335.49 | 327.82 | 334.40 | 335.49 | 338.34 | 326.58 | 315.02 | 319.90 | 320.39 | 331.20 |

Table I-1. Individual Male Body Weights (g) During the Dosing Period (page 4 of 4)

| | | Test Day | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group[a] | Animal ID | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| 6 | 91 | 321.75 | 312.82 | 300.62 | 295.62 | 308.53 | 311.16 | 311.62 | 314.70 | 314.40 | 319.58 | 322.11 | 323.95 | 329.75 | 332.38 | 338.87 |
| | 93 | 335.11 | 330.19 | 328.34 | 332.78 | 326.66 | 326.89 | 328.56 | 332.94 | 335.44 | 339.42 | 337.00 | 337.27 | 339.64 | 349.91 | 350.53 |
| | 95 | 339.13 | 317.85 | 311.78 | 304.87 | 306.39 | 300.16 | 299.97 | 304.00 | 295.16 | 301.50 | 298.96 | 303.72 | 312.48 | 305.22 | 293.61 |
| | 97 | 337.94 | 318.59 | 312.23 | 312.80 | 310.43 | 309.53 | 308.48 | 305.58 | 307.47 | 304.59 | 297.57 | 303.80 | 305.96 | 307.97 | 308.57 |
| | 99 | 345.45 | 333.08 | 320.88 | 314.94 | 312.71 | 313.73 | 314.39 | 317.04 | 317.49 | 311.72 | 309.05 | 307.35 | 306.43 | 311.34 | 318.72 |
| | 101 | 352.91 | 334.10 | 329.02 | 326.08 | 331.18 | 333.35 | 335.63 | 336.40 | 339.48 | 335.99 | 345.39 | 346.44 | 348.23 | 355.60 | 366.85 |
| | 103 | 340.33 | 321.50 | 309.23 | 305.86 | 290.17 | 292.68 | 289.49 | 289.70 | 286.35 | 285.24 | 294.92 | 298.82 | 309.58 | 302.64 | 298.92 |
| | 105 | 354.09 | 334.46 | 324.02 | 310.08 | 305.96 | 304.41 | 313.02 | 299.65 | 291.85 | 287.48 | 306.41 | 312.56 | 317.19 | 309.87 | 321.30 |
| | 197 | 324.82 | 318.66 | 322.23 | 318.69 | 315.27 | 313.54 | 310.69 | 308.06 | 304.84 | 306.89 | 313.05 | 318.76 | 313.75 | 320.38 | 326.00 |
| | 199 | 324.98 | 312.94 | 302.73 | 289.87 | 288.51 | 288.16 | 289.29 | 291.92 | 294.45 | 294.99 | 298.68 | 293.85 | 300.74 | 304.93 | 303.86 |
| | 201 | 327.20 | 321.98 | 308.32 | 296.96 | 287.08 | 276.90 | 267.71 | 259.36 | 294.26 | 303.18 | 300.12 | 309.30 | 315.40 | 317.63 | 316.14 |
| | 203 | 332.81 | 322.22 | 305.30 | 293.20 | 290.94 | 255.63 | 237.46 | .[d] | | | | | | | |
| | 205 | 337.44 | 327.85 | 320.16 | 314.64 | 318.01 | 322.81 | 317.13 | 320.68 | 320.81 | 328.49 | 323.40 | 326.83 | 324.68 | 332.79 | 329.25 |
| | 207 | 352.30 | 345.18 | 343.48 | 337.12 | 340.38 | 335.60 | 338.00 | 338.86 | 339.14 | 339.82 | 335.83 | 351.75 | 352.72 | 356.00 | 355.82 |
| | 209 | 359.46 | 346.77 | 335.90 | 331.58 | 327.87 | 326.55 | 327.20 | 328.85 | 340.16 | 343.91 | 344.85 | 347.36 | 340.59 | 343.78 | 351.54 |

[a]Groups are 0 is control; 1 is 25 mg/kg/day of Phenobarbital; 2 is 50 mg/kg/day of Phenobarbital; 3 is 100 mg/kg/day of Phenobarbital; 4 is 50 mg/kg/day of Linuron; 5 is 100 mg/kg/day of Linuron; and 6 is 150 mg/kg/day of Linuron.
[b]Male was euthanized moribund on test day 3.
[c]Male was euthanized moribund on test day 5.
[d]Male was euthanized moribund on test day 7.

Table I-2. Individual Male Feed Consumption (g/day) During the Dosing Period[a] (page 1 of 3)

| Group[b] | Animal ID | Test Days 1-8 | Test Days 8-15 | Test Days 1-15 |
|---|---|---|---|---|
| 0 | 1 | 26.1 | 25.4 | 25.8 |
| | 3 | 24.3 | 22.7 | 23.5 |
| | 5 | 19.9 | 22.4 | 21.1 |
| | 7 | 25.2 | 23.0 | 24.1 |
| | 9 | 28.4 | 27.9 | 28.2 |
| | 11 | 30.2 | 24.7 | 27.4 |
| | 13 | 28.1 | 25.1 | 26.6 |
| | 15 | 28.8 | 30.3 | 29.6 |
| | 107 | 27.3 | 28.5 | 27.9 |
| | 109 | 27.0 | 25.6 | 26.3 |
| | 111 | 24.5 | 23.1 | 23.8 |
| | 113 | 26.5 | 23.3 | 24.9 |
| | 115 | 25.1 | 25.4 | 25.3 |
| | 117 | 26.4 | 25.5 | 26.0 |
| | 119 | 31.8 | 32.1 | 31.9 |
| 1 | 17 | 23.0 | 22.1 | 22.5 |
| | 19 | .[c] | 22.3 | .[d] |
| | 21 | 27.8 | 24.6 | 26.2 |
| | 23 | 26.6 | 26.3 | 26.4 |
| | 25 | 26.2 | 24.6 | 25.4 |
| | 27 | 31.4 | 30.1 | 30.7 |
| | 29 | .[e] | 33.9 | .[d] |
| | 121 | .[e] | 25.5 | .[d] |
| | 123 | 23.9 | 21.7 | 22.8 |
| | 125 | 24.6 | 22.7 | 23.6 |
| | 127 | 26.4 | 23.8 | 25.1 |
| | 129 | .[e] | 24.9 | .[d] |
| | 131 | .[e] | 26.8 | .[d] |
| | 133 | 28.6 | 25.3 | 27.0 |
| | 135 | .[e] | 29.8 | .[d] |
| 2 | 31 | 28.4 | 25.6 | 27.0 |
| | 33 | .[c] | 25.9 | .[d] |
| | 35 | 26.6 | 24.0 | 25.3 |
| | 37 | 27.8 | 26.9 | 27.4 |
| | 39 | 28.0 | 32.1 | 30.0 |
| | 41 | 31.2 | 26.4 | 28.8 |
| | 43 | 30.7 | 29.2 | 30.0 |
| | 45 | 29.1 | 13.8 | 21.5 |
| | 137 | .[e] | 22.2 | .[d] |
| | 139 | 26.1 | 23.2 | 24.7 |
| | 141 | .[e] | 26.8 | .[d] |
| | 143 | .[e] | 25.5 | .[d] |
| | 145 | 29.9 | 29.5 | 29.7 |
| | 147 | 27.0 | 26.5 | 26.7 |
| | 149 | 29.0 | 28.6 | 28.8 |

Table I-2. Individual Male Feed Consumption (g/day) During the Dosing Period[a] (page 2 of 3)

| Group[b] | Animal ID | Test Days 1-8 | Test Days 8-15 | Test Days 1-15 |
|---|---|---|---|---|
| 3 | 47 | 22.3 | 22.6 | 22.5 |
| | 49 | 23.5 | 19.5 | 21.5 |
| | 51 | .[f] | | |
| | 53 | 18.3 | 27.9 | 23.1 |
| | 55 | 14.4 | 24.0 | 19.2 |
| | 57 | 20.9 | 30.5 | 25.7 |
| | 59 | 21.2 | 23.9 | 22.5 |
| | 151 | 21.2 | 24.0 | 22.6 |
| | 153 | 14.2 | 22.0 | 18.1 |
| | 155 | 16.0 | 29.2 | 22.6 |
| | 157 | .[e] | 28.7 | .[d] |
| | 159 | .[e] | 22.3 | .[d] |
| | 161 | 26.1 | 28.5 | 27.3 |
| | 163 | .[e] | 26.0 | .[d] |
| | 165 | .[g] | | |
| 4 | 61 | 23.7 | 23.1 | 23.4 |
| | 63 | 17.6 | 21.5 | 19.6 |
| | 65 | 19.9 | 21.2 | 20.5 |
| | 67 | 22.5 | 23.1 | 22.8 |
| | 69 | 26.3 | 27.0 | 26.7 |
| | 71 | 21.5 | 23.3 | 22.4 |
| | 73 | 22.5 | 22.6 | 22.5 |
| | 75 | 20.4 | 4.5 | 12.5 |
| | 167 | 13.4 | 17.9 | 15.7 |
| | 169 | .[e] | 26.9 | .[d] |
| | 171 | 23.1 | 24.2 | 23.6 |
| | 173 | 22.7 | 25.9 | 24.3 |
| | 175 | .[e] | 24.6 | .[d] |
| | 177 | 25.6 | 27.1 | 26.3 |
| | 179 | 23.0 | 26.5 | 24.7 |
| 5 | 77 | 10.8 | 16.6 | 13.7 |
| | 79 | 17.7 | 27.8 | 22.7 |
| | 81 | 22.5 | 22.8 | 22.6 |
| | 83 | 17.8 | 17.5 | 17.6 |
| | 85 | 17.3 | 24.6 | 20.9 |
| | 87 | 23.8 | 29.5 | 26.6 |
| | 89 | 18.7 | 20.5 | 19.6 |
| | 181 | .[e] | 19.1 | .[d] |
| | 183 | .[e] | 14.8 | .[d] |
| | 185 | .[e] | 34.5 | .[d] |
| | 187 | .[e] | 20.8 | .[d] |
| | 189 | .[e] | 22.6 | .[d] |
| | 191 | .[e] | 24.3 | .[d] |
| | 193 | 19.6 | 22.0 | 20.8 |
| | 195 | .[e] | 21.3 | .[d] |

Table I-2. Individual Male Feed Consumption (g/day) During the Dosing Period[a] (page 3 of 3)

| Group[b] | Animal ID | Test Days 1-8 | Test Days 8-15 | Test Days 1-15 |
|---|---|---|---|---|
| 6 | 91 | 15.9 | 18.3 | 17.1 |
| | 93 | 17.9 | 16.6 | 17.2 |
| | 95 | .[e] | 13.7 | .[d] |
| | 97 | 13.8 | 13.9 | 13.9 |
| | 99 | .[e] | 13.3 | .[d] |
| | 101 | .[e] | 22.3 | .[d] |
| | 103 | .[e] | 26.4 | .[d] |
| | 105 | .[e] | 17.8 | .[d] |
| | 197 | .[e] | 19.8 | .[d] |
| | 199 | 10.1 | 16.2 | 13.2 |
| | 201 | .[e] | 21.2 | .[d] |
| | 203 | .[h] | | |
| | 205 | 15.2 | 16.4 | 15.8 |
| | 207 | .[e] | 23.1 | .[d] |
| | 209 | 16.6 | 22.6 | 19.6 |

[a]These data represent the difference between the new feed jar weights (beginning weight; full feed jar) and the old feed jar (end weight; after the animal has fed for the measurement interval) weights divided by the number of days in the measurement interval.

[b]Groups are 0 is control; 1 is 25 mg/kg/day of Phenobarbital; 2 is 50 mg/kg/day of Phenobarbital; 3 is 100 mg/kg/day of Phenobarbital; 4 is 50 mg/kg/day of Linuron; 5 is 100 mg/kg/day of Linuron; and 6 is 150 mg/kg/day of Linuron.

[c]Animal placed large amounts of bedding in his feed jar and therefore an accurate feed weight could not be obtained.

[d]Interim feed consumption value(s) is missing and therefore the overall feed consumption value could not be calculated.

[e]Animal pulled the feed out of the feed jar and into the cage and therefore an accurate feed weight could not be obtained.

[f]Male was euthanized moribund on test day 3.

[g]Male was euthanized moribund on test day 5.

[h]Male was euthanized moribund on test day 7.

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 1 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 0 | 1 | 1 | 6:21 | within normal limits |
| | | | 8:33 | within normal limits |
| | | 2 | 6:15 | within normal limits |
| | | | 8:01 | within normal limits |
| | | 3 | 6:18 | within normal limits |
| | | | 8:05 | within normal limits |
| | | 4 | 6:19 | within normal limits |
| | | | 8:02 | within normal limits |
| | | | 14:36 | within normal limits |
| | | 5 | 6:17 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:04 | within normal limits |
| | | 6 | 6:18 | within normal limits |
| | | | 7:55 | within normal limits |
| | | | 14:23 | within normal limits |
| | | 7 | 6:19 | within normal limits |
| | | | 8:04 | within normal limits |
| | | | 14:00 | within normal limits |
| | | 8 | 6:21 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:25 | within normal limits |
| | | 9 | 6:20 | within normal limits |
| | | | 8:16 | within normal limits |
| | | | 14:02 | within normal limits |
| | | 10 | 6:20 | within normal limits |
| | | | 7:50 | within normal limits |
| | | | 15:18 | within normal limits |
| | | 11 | 6:19 | within normal limits |
| | | | 8:05 | within normal limits |
| | | | 14:29 | within normal limits |
| | | 12 | 6:20 | within normal limits |
| | | | 8:32 | within normal limits |
| | | | 14:02 | within normal limits |
| | | 13 | 6:20 | within normal limits |
| | | | 7:51 | within normal limits |
| | | | 14:16 | within normal limits |
| | | 14 | 6:27 | within normal limits |
| | | | 8:15 | within normal limits |
| | | | 15:08 | within normal limits |
| | | 15 | 6:09 | within normal limits |
| | | | 6:09 | scheduled sacrifice |
| | 3 | 1 | 6:22 | within normal limits |
| | | | 8:34 | within normal limits |
| | | 2 | 6:16 | within normal limits |
| | | | 8:01 | within normal limits |
| | | 3 | 6:20 | efflux of the dosing compound |
| | | | 8:05 | within normal limits |
| | | 4 | 6:20 | within normal limits |
| | | | 8:03 | within normal limits |
| | | | 14:36 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 2 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 0 | 3 | 5 | 6:18 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:04 | within normal limits |
| | | 6 | 6:18 | within normal limits |
| | | | 7:55 | within normal limits |
| | | | 14:23 | within normal limits |
| | | 7 | 6:20 | within normal limits |
| | | | 8:04 | within normal limits |
| | | | 14:01 | within normal limits |
| | | 8 | 6:22 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:25 | within normal limits |
| | | 9 | 6:22 | within normal limits |
| | | | 8:16 | within normal limits |
| | | | 14:02 | within normal limits |
| | | 10 | 6:21 | within normal limits |
| | | | 7:50 | within normal limits |
| | | | 15:18 | within normal limits |
| | | 11 | 6:20 | within normal limits |
| | | | 8:05 | within normal limits |
| | | | 14:29 | within normal limits |
| | | 12 | 6:20 | within normal limits |
| | | | 8:32 | within normal limits |
| | | | 14:02 | within normal limits |
| | | 13 | 6:21 | within normal limits |
| | | | 7:51 | within normal limits |
| | | | 14:16 | within normal limits |
| | | 14 | 6:27 | within normal limits |
| | | | 8:15 | within normal limits |
| | | | 15:08 | within normal limits |
| | | 15 | 6:21 | within normal limits |
| | | | 6:21 | scheduled sacrifice |
| | 5 | 1 | 6:23 | within normal limits |
| | | | 8:34 | within normal limits |
| | | 2 | 6:17 | broken toenail |
| | | | 8:01 | within normal limits |
| | | 3 | 6:20 | within normal limits |
| | | | 8:05 | within normal limits |
| | | 4 | 6:21 | within normal limits |
| | | | 8:03 | within normal limits |
| | | | 14:36 | within normal limits |
| | | 5 | 6:19 | broken toenail |
| | | | 8:07 | broken toenail |
| | | | 14:05 | within normal limits |
| | | 6 | 6:19 | within normal limits |
| | | | 7:55 | within normal limits |
| | | | 14:23 | within normal limits |
| | | 7 | 6:24 | within normal limits |
| | | | 8:04 | within normal limits |
| | | | 14:01 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 3 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 0 | 5 | 8 | 6:23 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:26 | within normal limits |
| | | 9 | 6:23 | within normal limits |
| | | | 8:16 | within normal limits |
| | | | 14:02 | within normal limits |
| | | 10 | 6:22 | within normal limits |
| | | | 7:50 | within normal limits |
| | | | 15:18 | within normal limits |
| | | 11 | 6:21 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:29 | within normal limits |
| | | 12 | 6:21 | within normal limits |
| | | | 8:33 | within normal limits |
| | | | 14:03 | within normal limits |
| | | 13 | 6:21 | within normal limits |
| | | | 7:51 | within normal limits |
| | | | 14:16 | within normal limits |
| | | 14 | 6:28 | within normal limits |
| | | | 8:15 | within normal limits |
| | | | 15:08 | within normal limits |
| | | 15 | 6:42 | within normal limits |
| | | | 6:42 | scheduled sacrifice |
| | 7 | 1 | 6:25 | efflux of the dosing compound |
| | | | 6:26 | received partial dose: half |
| | | | 8:34 | within normal limits |
| | | 2 | 6:18 | efflux of the dosing compound |
| | | | 8:01 | within normal limits |
| | | 3 | 6:21 | within normal limits |
| | | | 8:05 | within normal limits |
| | | 4 | 6:23 | within normal limits |
| | | | 8:03 | within normal limits |
| | | | 14:36 | within normal limits |
| | | 5 | 6:20 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:05 | within normal limits |
| | | 6 | 6:20 | within normal limits |
| | | | 7:55 | within normal limits |
| | | | 14:23 | within normal limits |
| | | 7 | 6:25 | within normal limits |
| | | | 8:04 | within normal limits |
| | | | 14:01 | within normal limits |
| | | 8 | 6:24 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:26 | within normal limits |
| | | 9 | 6:24 | within normal limits |
| | | | 8:16 | within normal limits |
| | | | 14:02 | within normal limits |
| | | 10 | 6:22 | within normal limits |
| | | | 7:50 | within normal limits |
| | | | 15:18 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 4 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 0 | 7 | 11 | 6:21 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:29 | within normal limits |
| | | 12 | 6:22 | within normal limits |
| | | | 8:33 | within normal limits |
| | | | 14:03 | within normal limits |
| | | 13 | 6:23 | within normal limits |
| | | | 7:51 | within normal limits |
| | | | 14:16 | within normal limits |
| | | 14 | 6:29 | within normal limits |
| | | | 8:15 | within normal limits |
| | | | 15:08 | within normal limits |
| | | 15 | 7:00 | within normal limits |
| | | | 7:00 | scheduled sacrifice |
| | 9 | 1 | 6:27 | within normal limits |
| | | | 8:34 | within normal limits |
| | | 2 | 6:19 | within normal limits |
| | | | 8:01 | within normal limits |
| | | 3 | 6:22 | within normal limits |
| | | | 8:05 | within normal limits |
| | | 4 | 6:24 | within normal limits |
| | | | 8:03 | within normal limits |
| | | | 14:36 | within normal limits |
| | | 5 | 6:21 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:05 | within normal limits |
| | | 6 | 6:20 | within normal limits |
| | | | 7:55 | within normal limits |
| | | | 14:23 | within normal limits |
| | | 7 | 6:25 | within normal limits |
| | | | 8:04 | within normal limits |
| | | | 14:01 | within normal limits |
| | | 8 | 6:25 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:26 | within normal limits |
| | | 9 | 6:24 | within normal limits |
| | | | 8:16 | within normal limits |
| | | | 14:02 | within normal limits |
| | | 10 | 6:24 | within normal limits |
| | | | 7:50 | within normal limits |
| | | | 15:18 | within normal limits |
| | | 11 | 6:22 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:30 | within normal limits |
| | | 12 | 6:23 | within normal limits |
| | | | 8:33 | within normal limits |
| | | | 14:03 | within normal limits |
| | | 13 | 6:24 | within normal limits |
| | | | 7:51 | within normal limits |
| | | | 14:16 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 5 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 0 | 9 | 14 | 6:29 | within normal limits |
| | | | 8:15 | within normal limits |
| | | | 15:08 | within normal limits |
| | | 15 | 7:21 | within normal limits |
| | | | 7:21 | scheduled sacrifice |
| | 11 | 1 | 6:28 | within normal limits |
| | | | 8:34 | within normal limits |
| | | 2 | 6:21 | within normal limits |
| | | | 8:01 | within normal limits |
| | | 3 | 6:22 | within normal limits |
| | | | 8:05 | within normal limits |
| | | 4 | 6:25 | within normal limits |
| | | | 8:03 | within normal limits |
| | | | 14:36 | within normal limits |
| | | 5 | 6:22 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:05 | within normal limits |
| | | 6 | 6:21 | within normal limits |
| | | | 7:55 | within normal limits |
| | | | 14:23 | within normal limits |
| | | 7 | 6:26 | within normal limits |
| | | | 8:04 | within normal limits |
| | | | 14:01 | within normal limits |
| | | 8 | 6:25 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:26 | within normal limits |
| | | 9 | 6:25 | within normal limits |
| | | | 8:18 | within normal limits |
| | | | 14:02 | within normal limits |
| | | 10 | 6:24 | within normal limits |
| | | | 7:50 | within normal limits |
| | | | 15:18 | within normal limits |
| | | 11 | 6:23 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:30 | within normal limits |
| | | 12 | 6:23 | within normal limits |
| | | | 8:33 | within normal limits |
| | | | 14:03 | within normal limits |
| | | 13 | 6:24 | within normal limits |
| | | | 7:51 | within normal limits |
| | | | 14:16 | within normal limits |
| | | 14 | 6:31 | within normal limits |
| | | | 8:15 | within normal limits |
| | | | 15:08 | within normal limits |
| | | 15 | 7:42 | within normal limits |
| | | | 7:42 | scheduled sacrifice |
| | 13 | 1 | 6:29 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 9:45 | broken toenail |
| | | 2 | 6:21 | within normal limits |
| | | | 8:01 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 6 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 0 | 11 | 3 | 6:23 | within normal limits |
| | | | 8:05 | within normal limits |
| | | 4 | 6:25 | within normal limits |
| | | | 8:03 | within normal limits |
| | | | 14:36 | within normal limits |
| | | 5 | 6:22 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:06 | within normal limits |
| | | 6 | 6:21 | within normal limits |
| | | | 7:55 | within normal limits |
| | | | 14:23 | within normal limits |
| | | 7 | 6:27 | within normal limits |
| | | | 8:04 | within normal limits |
| | | | 14:01 | within normal limits |
| | | 8 | 6:26 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:26 | within normal limits |
| | | 9 | 6:25 | within normal limits |
| | | | 8:18 | within normal limits |
| | | | 14:02 | within normal limits |
| | | 10 | 6:25 | within normal limits |
| | | | 7:50 | within normal limits |
| | | | 15:18 | within normal limits |
| | | 11 | 6:24 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:30 | within normal limits |
| | | 12 | 6:24 | within normal limits |
| | | | 8:33 | within normal limits |
| | | | 14:03 | within normal limits |
| | | 13 | 6:25 | within normal limits |
| | | | 7:52 | within normal limits |
| | | | 14:16 | within normal limits |
| | | 14 | 6:31 | within normal limits |
| | | | 8:15 | within normal limits |
| | | | 15:08 | within normal limits |
| | | 15 | 8:04 | within normal limits |
| | | | 8:04 | scheduled sacrifice |
| | 15 | 1 | 6:30 | within normal limits |
| | | | 8:34 | within normal limits |
| | | 2 | 6:22 | within normal limits |
| | | | 8:01 | within normal limits |
| | | 3 | 6:25 | within normal limits |
| | | | 8:06 | within normal limits |
| | | 4 | 6:26 | within normal limits |
| | | | 8:03 | within normal limits |
| | | | 14:36 | within normal limits |
| | | 5 | 6:23 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:06 | within normal limits |

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 0 | 15 | 6 | 6:22 | within normal limits |
| | | | 7:55 | within normal limits |
| | | | 14:24 | within normal limits |
| | | 7 | 6:27 | within normal limits |
| | | | 8:04 | within normal limits |
| | | | 14:01 | within normal limits |
| | | 8 | 6:26 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:26 | within normal limits |
| | | 9 | 6:26 | within normal limits |
| | | | 8:18 | within normal limits |
| | | | 14:02 | within normal limits |
| | | 10 | 6:25 | within normal limits |
| | | | 7:50 | within normal limits |
| | | | 15:18 | within normal limits |
| | | 11 | 6:24 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:30 | within normal limits |
| | | 12 | 6:25 | within normal limits |
| | | | 8:33 | within normal limits |
| | | | 14:03 | within normal limits |
| | | 13 | 6:25 | within normal limits |
| | | | 7:52 | within normal limits |
| | | | 14:17 | within normal limits |
| | | 14 | 6:32 | within normal limits |
| | | | 8:15 | within normal limits |
| | | | 15:08 | within normal limits |
| | | 15 | 8:25 | within normal limits |
| | | | 8:25 | scheduled sacrifice |
| | 107 | 1 | 6:23 | within normal limits |
| | | | 8:02 | within normal limits |
| | | 2 | 6:26 | within normal limits |
| | | | 8:06 | within normal limits |
| | | 3 | 6:26 | within normal limits |
| | | | 8:03 | within normal limits |
| | | | 14:36 | within normal limits |
| | | 4 | 6:24 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:06 | within normal limits |
| | | 5 | 6:23 | within normal limits |
| | | | 7:56 | within normal limits |
| | | | 14:24 | within normal limits |
| | | 6 | 6:28 | within normal limits |
| | | | 8:04 | within normal limits |
| | | | 14:02 | within normal limits |
| | | 7 | 6:27 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:26 | within normal limits |
| | | 8 | 6:26 | within normal limits |
| | | | 8:18 | within normal limits |
| | | | 14:02 | within normal limits |

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 0 | 107 | 9 | 6:25 | within normal limits |
| | | | 7:50 | within normal limits |
| | | | 15:19 | within normal limits |
| | | 10 | 6:26 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:30 | within normal limits |
| | | 11 | 6:25 | within normal limits |
| | | | 8:33 | within normal limits |
| | | | 14:03 | within normal limits |
| | | 12 | 6:26 | within normal limits |
| | | | 7:52 | within normal limits |
| | | | 14:17 | within normal limits |
| | | 13 | 6:33 | within normal limits |
| | | | 8:17 | within normal limits |
| | | | 15:08 | within normal limits |
| | | 14 | 6:19 | within normal limits |
| | | | 7:32 | within normal limits |
| | | | 15:21 | within normal limits |
| | | 15 | 6:09 | within normal limits |
| | | | 6:09 | scheduled sacrifice |
| | 109 | 1 | 6:24 | within normal limits |
| | | | 8:02 | within normal limits |
| | | 2 | 6:27 | within normal limits |
| | | | 8:06 | within normal limits |
| | | 3 | 6:27 | within normal limits |
| | | | 8:03 | within normal limits |
| | | | 14:36 | within normal limits |
| | | 4 | 6:24 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:06 | within normal limits |
| | | 5 | 6:23 | within normal limits |
| | | | 7:56 | within normal limits |
| | | | 14:24 | within normal limits |
| | | 6 | 6:29 | within normal limits |
| | | | 8:04 | within normal limits |
| | | | 14:02 | within normal limits |
| | | 7 | 6:28 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:29 | within normal limits |
| | | 8 | 6:27 | within normal limits |
| | | | 8:18 | within normal limits |
| | | | 14:02 | within normal limits |
| | | 9 | 6:26 | within normal limits |
| | | | 7:50 | within normal limits |
| | | | 15:19 | within normal limits |
| | | 10 | 6:26 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:30 | within normal limits |
| | | 11 | 6:26 | within normal limits |
| | | | 8:33 | within normal limits |
| | | | 14:03 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 9 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 0 | 109 | 12 | 6:27 | within normal limits |
| | | | 7:52 | within normal limits |
| | | | 14:17 | within normal limits |
| | | 13 | 6:34 | within normal limits |
| | | | 8:17 | within normal limits |
| | | | 15:08 | within normal limits |
| | | 14 | 6:19 | within normal limits |
| | | | 7:32 | within normal limits |
| | | | 15:21 | within normal limits |
| | | 15 | 6:18 | within normal limits |
| | | | 6:18 | scheduled sacrifice |
| | 111 | 1 | 6:25 | within normal limits |
| | | | 8:02 | within normal limits |
| | | 2 | 6:27 | within normal limits |
| | | | 8:06 | within normal limits |
| | | 3 | 6:28 | within normal limits |
| | | | 8:03 | within normal limits |
| | | | 14:36 | within normal limits |
| | | 4 | 6:25 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:06 | within normal limits |
| | | 5 | 6:24 | within normal limits |
| | | | 7:56 | within normal limits |
| | | | 14:24 | within normal limits |
| | | 6 | 6:29 | within normal limits |
| | | | 8:04 | within normal limits |
| | | | 14:02 | within normal limits |
| | | 7 | 6:28 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:29 | within normal limits |
| | | 8 | 6:28 | within normal limits |
| | | | 8:18 | within normal limits |
| | | | 14:02 | within normal limits |
| | | 9 | 6:27 | within normal limits |
| | | | 7:50 | within normal limits |
| | | | 15:19 | within normal limits |
| | | 10 | 6:27 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:30 | within normal limits |
| | | 11 | 6:27 | within normal limits |
| | | | 8:33 | within normal limits |
| | | | 14:03 | within normal limits |
| | | 12 | 6:27 | within normal limits |
| | | | 7:52 | within normal limits |
| | | | 14:17 | within normal limits |
| | | 13 | 6:35 | within normal limits |
| | | | 8:17 | within normal limits |
| | | | 15:08 | within normal limits |
| | | 14 | 6:20 | within normal limits |
| | | | 7:32 | within normal limits |
| | | | 15:21 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 10 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 0 | 111 | 15 | 6:36 | within normal limits |
| | | | 6:36 | scheduled sacrifice |
| | 113 | 1 | 6:25 | within normal limits |
| | | | 8:02 | within normal limits |
| | | 2 | 6:28 | within normal limits |
| | | | 8:06 | within normal limits |
| | | 3 | 6:28 | within normal limits |
| | | | 8:03 | within normal limits |
| | | | 14:36 | within normal limits |
| | | 4 | 6:25 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:06 | within normal limits |
| | | 5 | 6:24 | within normal limits |
| | | | 7:56 | within normal limits |
| | | | 14:24 | within normal limits |
| | | 6 | 6:30 | within normal limits |
| | | | 8:04 | within normal limits |
| | | | 14:02 | within normal limits |
| | | 7 | 6:29 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:29 | within normal limits |
| | | 8 | 6:28 | within normal limits |
| | | | 8:18 | within normal limits |
| | | | 14:02 | within normal limits |
| | | 9 | 6:27 | within normal limits |
| | | | 7:50 | within normal limits |
| | | | 15:19 | within normal limits |
| | | 10 | 6:28 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:30 | within normal limits |
| | | 11 | 6:27 | within normal limits |
| | | | 8:33 | within normal limits |
| | | | 14:03 | within normal limits |
| | | 12 | 6:28 | within normal limits |
| | | | 7:52 | within normal limits |
| | | | 14:17 | within normal limits |
| | | 13 | 6:36 | within normal limits |
| | | | 8:17 | within normal limits |
| | | | 15:08 | within normal limits |
| | | 14 | 6:22 | within normal limits |
| | | | 7:33 | within normal limits |
| | | | 15:21 | within normal limits |
| | | 15 | 6:52 | within normal limits |
| | | | 6:52 | scheduled sacrifice |
| | 115 | 1 | 6:27 | within normal limits |
| | | | 8:02 | within normal limits |
| | | 2 | 6:29 | within normal limits |
| | | | 8:06 | within normal limits |
| | | 3 | 6:29 | within normal limits |
| | | | 8:03 | within normal limits |
| | | | 14:36 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 11 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 0 | 115 | 4 | 6:26 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:07 | within normal limits |
| | | 5 | 6:25 | within normal limits |
| | | | 7:56 | within normal limits |
| | | | 14:24 | within normal limits |
| | | 6 | 6:31 | within normal limits |
| | | | 8:04 | within normal limits |
| | | | 14:02 | within normal limits |
| | | 7 | 6:29 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:29 | within normal limits |
| | | 8 | 6:29 | within normal limits |
| | | | 8:18 | within normal limits |
| | | | 14:02 | within normal limits |
| | | 9 | 6:28 | within normal limits |
| | | | 7:50 | within normal limits |
| | | | 15:19 | within normal limits |
| | | 10 | 6:29 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:30 | within normal limits |
| | | 11 | 6:28 | within normal limits |
| | | | 8:33 | within normal limits |
| | | | 14:03 | within normal limits |
| | | 12 | 6:29 | within normal limits |
| | | | 7:52 | within normal limits |
| | | | 14:17 | within normal limits |
| | | 13 | 6:37 | within normal limits |
| | | | 8:17 | within normal limits |
| | | | 15:08 | within normal limits |
| | | 14 | 6:23 | within normal limits |
| | | | 7:33 | within normal limits |
| | | | 15:21 | within normal limits |
| | | 15 | 7:07 | within normal limits |
| | | | 7:07 | scheduled sacrifice |
| | 117 | 1 | 6:28 | within normal limits |
| | | | 8:02 | within normal limits |
| | | 2 | 6:30 | within normal limits |
| | | | 8:06 | within normal limits |
| | | 3 | 6:30 | within normal limits |
| | | | 8:03 | within normal limits |
| | | | 14:36 | within normal limits |
| | | 4 | 6:27 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:07 | within normal limits |
| | | 5 | 6:26 | within normal limits |
| | | | 7:56 | within normal limits |
| | | | 14:24 | within normal limits |
| | | 6 | 6:32 | within normal limits |
| | | | 8:04 | within normal limits |
| | | | 14:02 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 12 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 0 | 117 | 7 | 6:30 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:30 | within normal limits |
| | | 8 | 6:30 | within normal limits |
| | | | 8:18 | within normal limits |
| | | | 14:02 | within normal limits |
| | | 9 | 6:29 | within normal limits |
| | | | 7:50 | within normal limits |
| | | | 15:19 | within normal limits |
| | | 10 | 6:29 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:30 | within normal limits |
| | | 11 | 6:29 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 14:03 | within normal limits |
| | | 12 | 6:29 | within normal limits |
| | | | 7:53 | within normal limits |
| | | | 14:17 | within normal limits |
| | | 13 | 6:38 | within normal limits |
| | | | 8:17 | within normal limits |
| | | | 15:08 | within normal limits |
| | | 14 | 6:25 | within normal limits |
| | | | 7:33 | within normal limits |
| | | | 15:21 | within normal limits |
| | | 15 | 7:25 | within normal limits |
| | | | 7:25 | scheduled sacrifice |
| | 119 | 1 | 6:28 | within normal limits |
| | | | 8:02 | within normal limits |
| | | 2 | 6:30 | alopecia: limb(s) |
| | | | 8:06 | alopecia: limb(s) |
| | | 3 | 0:00 | alopecia: limb(s) |
| | | | 6:30 | alopecia: limb(s) |
| | | | 8:03 | alopecia: limb(s) |
| | | 4 | 6:28 | alopecia: limb(s) |
| | | | 8:07 | alopecia: limb(s) |
| | | | 14:07 | alopecia: limb(s) |
| | | 5 | 6:27 | alopecia: limb(s) |
| | | | 7:56 | alopecia: limb(s) |
| | | | 14:25 | alopecia: limb(s) |
| | | 6 | 6:32 | alopecia: limb(s) |
| | | | 8:04 | alopecia: limb(s) |
| | | | 14:03 | alopecia: limb(s) |
| | | 7 | 6:31 | alopecia: limb(s) |
| | | | 8:24 | alopecia: limb(s) |
| | | | 14:30 | alopecia: limb(s) |
| | | 8 | 6:30 | alopecia: limb(s) |
| | | | 8:18 | alopecia: limb(s) |
| | | | 14:02 | alopecia: limb(s) |
| | | 9 | 6:29 | alopecia: limb(s) |
| | | | 7:51 | alopecia: limb(s) |
| | | | 15:19 | alopecia: limb(s) |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 13 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 0 | 119 | 10 | 6:30 | alopecia: limb(s) |
| | | | 8:06 | alopecia: limb(s) |
| | | | 14:30 | alopecia: limb(s) |
| | | 11 | 6:29 | alopecia: limb(s) |
| | | | 8:34 | alopecia: limb(s) |
| | | | 14:03 | alopecia: limb(s) |
| | | 12 | 6:30 | alopecia: limb(s) |
| | | | 7:53 | alopecia: limb(s) |
| | | | 14:17 | alopecia: limb(s) |
| | | 13 | 6:38 | alopecia: limb(s) |
| | | | 8:17 | alopecia: limb(s) |
| | | | 15:09 | alopecia: limb(s) |
| | | 14 | 6:26 | alopecia: limb(s) |
| | | | 7:33 | alopecia: limb(s) |
| | | | 15:21 | alopecia: limb(s) |
| | | 15 | 7:42 | alopecia: limb(s) |
| | | | 7:42 | scheduled sacrifice |
| 1 | 17 | 1 | 6:33 | within normal limits |
| | | | 8:35 | within normal limits |
| | | 2 | 6:31 | within normal limits |
| | | | 8:02 | within normal limits |
| | | 3 | 6:33 | within normal limits |
| | | | 8:07 | within normal limits |
| | | 4 | 6:32 | within normal limits |
| | | | 8:04 | within normal limits |
| | | | 14:37 | within normal limits |
| | | 5 | 6:30 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:07 | within normal limits |
| | | 6 | 6:28 | within normal limits |
| | | | 7:59 | within normal limits |
| | | | 14:25 | within normal limits |
| | | 7 | 6:36 | within normal limits |
| | | | 8:05 | within normal limits |
| | | | 14:03 | within normal limits |
| | | 8 | 6:32 | within normal limits |
| | | | 8:25 | within normal limits |
| | | | 14:30 | within normal limits |
| | | 9 | 6:32 | efflux of the dosing compound |
| | | | 6:32 | received partial dose: half |
| | | | 8:18 | within normal limits |
| | | | 14:04 | within normal limits |
| | | 10 | 6:31 | within normal limits |
| | | | 7:51 | within normal limits |
| | | | 15:19 | within normal limits |
| | | 11 | 6:31 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:31 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 14 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 1 | 17 | 12 | 6:30 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 14:04 | within normal limits |
| | | 13 | 6:31 | within normal limits |
| | | | 7:53 | within normal limits |
| | | | 14:17 | within normal limits |
| | | 14 | 6:40 | within normal limits |
| | | | 8:19 | within normal limits |
| | | | 15:09 | within normal limits |
| | | 15 | 6:11 | within normal limits |
| | | | 6:11 | scheduled sacrifice |
| | 19 | 1 | 6:33 | within normal limits |
| | | | 8:35 | within normal limits |
| | | 2 | 6:33 | within normal limits |
| | | | 8:02 | within normal limits |
| | | 3 | 6:34 | within normal limits |
| | | | 8:07 | within normal limits |
| | | 4 | 6:33 | within normal limits |
| | | | 8:04 | within normal limits |
| | | | 14:37 | within normal limits |
| | | 5 | 6:31 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:07 | within normal limits |
| | | 6 | 6:29 | within normal limits |
| | | | 7:59 | within normal limits |
| | | | 14:25 | within normal limits |
| | | 7 | 6:36 | within normal limits |
| | | | 8:05 | within normal limits |
| | | | 14:03 | within normal limits |
| | | 8 | 6:33 | within normal limits |
| | | | 8:25 | within normal limits |
| | | | 14:30 | within normal limits |
| | | 9 | 6:33 | within normal limits |
| | | | 8:18 | within normal limits |
| | | | 14:04 | within normal limits |
| | | 10 | 6:31 | within normal limits |
| | | | 7:51 | within normal limits |
| | | | 15:19 | within normal limits |
| | | 11 | 6:31 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:31 | within normal limits |
| | | 12 | 6:31 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 14:04 | within normal limits |
| | | 13 | 6:31 | within normal limits |
| | | | 7:53 | within normal limits |
| | | | 14:17 | within normal limits |
| | | 14 | 6:41 | within normal limits |
| | | | 8:19 | within normal limits |
| | | | 15:09 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 15 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 1 | 19 | 15 | 6:24 | within normal limits |
| | | | 6:25 | scheduled sacrifice |
| | 21 | 1 | 6:34 | within normal limits |
| | | | 8:35 | within normal limits |
| | | 2 | 6:34 | within normal limits |
| | | | 8:02 | within normal limits |
| | | 3 | 6:34 | within normal limits |
| | | | 8:07 | within normal limits |
| | | 4 | 6:34 | within normal limits |
| | | | 8:04 | within normal limits |
| | | | 14:37 | within normal limits |
| | | 5 | 6:32 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:07 | within normal limits |
| | | 6 | 6:29 | within normal limits |
| | | | 7:59 | within normal limits |
| | | | 14:25 | within normal limits |
| | | 7 | 6:37 | within normal limits |
| | | | 8:05 | within normal limits |
| | | | 14:03 | within normal limits |
| | | 8 | 6:33 | within normal limits |
| | | | 8:25 | within normal limits |
| | | | 14:30 | within normal limits |
| | | 9 | 6:33 | within normal limits |
| | | | 8:22 | within normal limits |
| | | | 14:04 | within normal limits |
| | | 10 | 6:32 | within normal limits |
| | | | 7:51 | within normal limits |
| | | | 15:20 | within normal limits |
| | | 11 | 6:32 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:31 | within normal limits |
| | | 12 | 6:32 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 14:04 | within normal limits |
| | | 13 | 6:32 | within normal limits |
| | | | 7:53 | within normal limits |
| | | | 14:18 | within normal limits |
| | | 14 | 6:41 | within normal limits |
| | | | 8:19 | within normal limits |
| | | | 15:09 | within normal limits |
| | | 15 | 6:46 | within normal limits |
| | | | 6:46 | scheduled sacrifice |
| | 23 | 1 | 6:35 | within normal limits |
| | | | 8:35 | within normal limits |
| | | 2 | 6:35 | within normal limits |
| | | | 8:02 | within normal limits |
| | | 3 | 6:35 | within normal limits |
| | | | 8:07 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 16 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 1 | 23 | 4 | 6:34 | within normal limits |
| | | | 8:04 | eye(s): chromodacryorrhea |
| | | | 14:37 | eye(s): chromodacryorrhea |
| | | 5 | 6:33 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:08 | within normal limits |
| | | 6 | 6:30 | within normal limits |
| | | | 7:59 | within normal limits |
| | | | 14:25 | within normal limits |
| | | 7 | 6:38 | within normal limits |
| | | | 8:05 | within normal limits |
| | | | 14:03 | within normal limits |
| | | 8 | 6:34 | within normal limits |
| | | | 8:25 | within normal limits |
| | | | 14:30 | within normal limits |
| | | 9 | 6:34 | within normal limits |
| | | | 8:22 | within normal limits |
| | | | 14:04 | within normal limits |
| | | 10 | 6:32 | within normal limits |
| | | | 7:51 | within normal limits |
| | | | 15:20 | within normal limits |
| | | 11 | 6:32 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:31 | within normal limits |
| | | 12 | 6:32 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 14:04 | within normal limits |
| | | 13 | 6:33 | within normal limits |
| | | | 7:58 | within normal limits |
| | | | 14:18 | within normal limits |
| | | 14 | 6:42 | within normal limits |
| | | | 8:19 | within normal limits |
| | | | 15:09 | within normal limits |
| | | 15 | 7:03 | within normal limits |
| | | | 7:03 | scheduled sacrifice |
| | 25 | 1 | 6:37 | struggling during dosing |
| | | | 8:35 | within normal limits |
| | | 2 | 6:37 | within normal limits |
| | | | 8:02 | within normal limits |
| | | 3 | 6:36 | within normal limits |
| | | | 8:07 | within normal limits |
| | | 4 | 6:35 | within normal limits |
| | | | 8:05 | within normal limits |
| | | | 14:37 | within normal limits |
| | | 5 | 6:33 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:08 | within normal limits |
| | | 6 | 6:30 | within normal limits |
| | | | 7:59 | within normal limits |
| | | | 14:25 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 17 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 1 | 25 | 7 | 6:38 | within normal limits |
| | | | 8:05 | within normal limits |
| | | | 14:03 | within normal limits |
| | | 8 | 6:35 | within normal limits |
| | | | 8:25 | within normal limits |
| | | | 14:30 | within normal limits |
| | | 9 | 6:34 | within normal limits |
| | | | 8:22 | within normal limits |
| | | | 14:04 | within normal limits |
| | | 10 | 6:32 | within normal limits |
| | | | 7:51 | within normal limits |
| | | | 15:20 | within normal limits |
| | | 11 | 6:34 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:31 | within normal limits |
| | | 12 | 6:32 | within normal limits |
| | | | 8:35 | within normal limits |
| | | | 14:04 | within normal limits |
| | | 13 | 6:33 | within normal limits |
| | | | 7:58 | within normal limits |
| | | | 14:18 | within normal limits |
| | | 14 | 6:42 | within normal limits |
| | | | 8:19 | within normal limits |
| | | | 15:09 | within normal limits |
| | | 15 | 7:26 | within normal limits |
| | | | 7:26 | scheduled sacrifice |
| | 27 | 1 | 6:38 | within normal limits |
| | | | 8:35 | within normal limits |
| | | 2 | 6:38 | within normal limits |
| | | | 8:02 | within normal limits |
| | | 3 | 6:37 | within normal limits |
| | | | 8:07 | within normal limits |
| | | 4 | 6:36 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:37 | within normal limits |
| | | 5 | 6:34 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:08 | within normal limits |
| | | 6 | 6:31 | within normal limits |
| | | | 7:59 | within normal limits |
| | | | 14:25 | within normal limits |
| | | 7 | 6:39 | within normal limits |
| | | | 8:05 | within normal limits |
| | | | 14:03 | within normal limits |
| | | 8 | 6:35 | within normal limits |
| | | | 8:25 | within normal limits |
| | | | 14:30 | within normal limits |
| | | 9 | 6:35 | within normal limits |
| | | | 8:22 | within normal limits |
| | | | 14:04 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 18 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 1 | 27 | 10 | 6:33 | within normal limits |
| | | | 7:51 | within normal limits |
| | | | 15:20 | within normal limits |
| | | 11 | 6:34 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:31 | within normal limits |
| | | 12 | 6:34 | within normal limits |
| | | | 8:35 | within normal limits |
| | | | 14:04 | within normal limits |
| | | 13 | 6:34 | within normal limits |
| | | | 7:58 | within normal limits |
| | | | 14:18 | within normal limits |
| | | 14 | 6:43 | within normal limits |
| | | | 8:19 | within normal limits |
| | | | 15:09 | within normal limits |
| | | 15 | 7:47 | within normal limits |
| | | | 7:47 | scheduled sacrifice |
| | 29 | 1 | 6:39 | within normal limits |
| | | | 8:35 | within normal limits |
| | | 2 | 6:40 | within normal limits |
| | | | 8:02 | within normal limits |
| | | 3 | 6:38 | alopecia: limb(s) |
| | | | 8:07 | alopecia: limb(s) |
| | | 4 | 6:36 | alopecia: limb(s) |
| | | | 8:06 | alopecia: limb(s) |
| | | | 14:37 | alopecia: limb(s) |
| | | 5 | 6:35 | alopecia: limb(s) |
| | | | 6:35 | efflux of the dosing compound |
| | | | 6:35 | received partial dose: half |
| | | | 8:08 | alopecia: limb(s) |
| | | | 14:08 | alopecia: limb(s) |
| | | 6 | 6:32 | alopecia: limb(s) |
| | | | 7:59 | alopecia: limb(s) |
| | | | 14:26 | alopecia: limb(s) |
| | | 7 | 6:40 | alopecia: limb(s) |
| | | | 8:05 | alopecia: limb(s) |
| | | | 14:04 | alopecia: limb(s) |
| | | 8 | 0:00 | alopecia: limb(s) |
| | | | 8:25 | alopecia: limb(s) |
| | | | 14:31 | alopecia: limb(s) |
| | | 9 | 6:36 | alopecia: limb(s) |
| | | | 8:23 | alopecia: limb(s) |
| | | | 14:04 | alopecia: limb(s) |
| | | 10 | 6:34 | alopecia: limb(s) |
| | | | 7:52 | alopecia: limb(s) |
| | | | 15:20 | alopecia: limb(s) |
| | | 11 | 6:35 | alopecia: limb(s) |
| | | | 8:07 | alopecia: limb(s) |
| | | | 14:31 | alopecia: limb(s) |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 19 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 1 | 29 | 12 | 6:34 | alopecia: limb(s) |
| | | | 8:35 | alopecia: limb(s) |
| | | | 14:04 | alopecia: limb(s) |
| | | 13 | 6:34 | alopecia: limb(s) |
| | | | 7:58 | alopecia: limb(s) |
| | | | 14:18 | alopecia: limb(s) |
| | | 14 | 6:43 | alopecia: limb(s) |
| | | | 8:19 | alopecia: limb(s) |
| | | | 15:09 | alopecia: limb(s) |
| | | 15 | 8:05 | alopecia: limb(s) |
| | | | 8:05 | scheduled sacrifice |
| | 121 | 1 | 6:41 | within normal limits |
| | | | 8:03 | within normal limits |
| | | 2 | 6:38 | within normal limits |
| | | | 8:08 | within normal limits |
| | | 3 | 6:37 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:38 | within normal limits |
| | | 4 | 6:36 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:08 | within normal limits |
| | | 5 | 6:32 | within normal limits |
| | | | 7:59 | within normal limits |
| | | | 14:26 | within normal limits |
| | | 6 | 6:41 | within normal limits |
| | | | 8:05 | within normal limits |
| | | | 14:04 | within normal limits |
| | | 7 | 6:41 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 14:31 | within normal limits |
| | | 8 | 6:36 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:04 | within normal limits |
| | | 9 | 6:34 | within normal limits |
| | | | 7:52 | within normal limits |
| | | | 15:20 | within normal limits |
| | | 10 | 6:36 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:32 | within normal limits |
| | | 11 | 6:35 | within normal limits |
| | | | 8:35 | within normal limits |
| | | | 14:04 | within normal limits |
| | | 12 | 6:35 | within normal limits |
| | | | 7:58 | within normal limits |
| | | | 14:18 | within normal limits |
| | | 13 | 6:44 | within normal limits |
| | | | 8:22 | within normal limits |
| | | | 15:09 | within normal limits |
| | | 14 | 6:28 | within normal limits |
| | | | 7:35 | within normal limits |
| | | | 15:21 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 20 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 1 | 121 | 15 | 6:10 | within normal limits |
| | | | 6:10 | scheduled sacrifice |
| | 123 | 1 | 6:42 | broken toenail |
| | | | 8:03 | broken toenail |
| | | 2 | 6:39 | within normal limits |
| | | | 8:08 | within normal limits |
| | | 3 | 6:38 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:38 | within normal limits |
| | | 4 | 6:37 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:09 | within normal limits |
| | | 5 | 6:33 | within normal limits |
| | | | 7:59 | within normal limits |
| | | | 14:26 | within normal limits |
| | | 6 | 6:41 | within normal limits |
| | | | 8:05 | within normal limits |
| | | | 14:04 | within normal limits |
| | | 7 | 6:41 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 14:31 | within normal limits |
| | | 8 | 6:37 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:04 | within normal limits |
| | | 9 | 6:35 | within normal limits |
| | | | 7:52 | within normal limits |
| | | | 15:20 | within normal limits |
| | | 10 | 6:36 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:32 | within normal limits |
| | | 11 | 6:35 | within normal limits |
| | | | 8:35 | within normal limits |
| | | | 14:04 | within normal limits |
| | | 12 | 6:35 | within normal limits |
| | | | 7:58 | within normal limits |
| | | | 14:18 | within normal limits |
| | | 13 | 6:47 | within normal limits |
| | | | 8:22 | within normal limits |
| | | | 15:09 | within normal limits |
| | | 14 | 6:28 | within normal limits |
| | | | 7:35 | within normal limits |
| | | | 15:21 | within normal limits |
| | | 15 | 6:20 | within normal limits |
| | | | 6:20 | scheduled sacrifice |
| | 125 | 1 | 6:43 | within normal limits |
| | | | 8:03 | within normal limits |
| | | 2 | 6:40 | within normal limits |
| | | | 8:08 | within normal limits |
| | | 3 | 6:38 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:38 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 21 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 1 | 125 | 4 | 6:38 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:09 | within normal limits |
| | | 5 | 6:33 | within normal limits |
| | | | 7:59 | within normal limits |
| | | | 14:27 | within normal limits |
| | | 6 | 6:42 | within normal limits |
| | | | 8:05 | within normal limits |
| | | | 14:04 | within normal limits |
| | | 7 | 6:43 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 14:31 | within normal limits |
| | | 8 | 6:38 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:04 | within normal limits |
| | | 9 | 6:35 | within normal limits |
| | | | 7:52 | within normal limits |
| | | | 15:20 | within normal limits |
| | | 10 | 6:37 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:32 | within normal limits |
| | | 11 | 6:36 | within normal limits |
| | | | 8:35 | within normal limits |
| | | | 14:04 | within normal limits |
| | | 12 | 6:36 | within normal limits |
| | | | 7:58 | within normal limits |
| | | | 14:18 | within normal limits |
| | | 13 | 6:47 | within normal limits |
| | | | 8:22 | within normal limits |
| | | | 15:10 | within normal limits |
| | | 14 | 6:29 | within normal limits |
| | | | 7:35 | within normal limits |
| | | | 15:21 | within normal limits |
| | | 15 | 6:38 | within normal limits |
| | | | 6:38 | scheduled sacrifice |
| | 127 | 1 | 6:45 | within normal limits |
| | | | 8:03 | within normal limits |
| | | 2 | 6:41 | within normal limits |
| | | | 8:08 | within normal limits |
| | | 3 | 6:39 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:38 | within normal limits |
| | | 4 | 6:39 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:09 | within normal limits |
| | | 5 | 6:34 | within normal limits |
| | | | 7:59 | within normal limits |
| | | | 14:27 | within normal limits |
| | | 6 | 6:43 | alopecia: limb(s) |
| | | | 8:06 | alopecia: limb(s) |
| | | | 14:04 | alopecia: limb(s) |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 22 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 1 | 127 | 7 | 6:43 | alopecia: limb(s) |
| | | | 8:26 | alopecia: limb(s) |
| | | | 14:31 | alopecia: limb(s) |
| | | 8 | 6:38 | alopecia: limb(s) |
| | | | 6:38 | efflux of the dosing compound |
| | | | 8:24 | alopecia: limb(s) |
| | | | 14:04 | alopecia: limb(s) |
| | | 9 | 6:36 | alopecia: limb(s) |
| | | | 7:52 | alopecia: limb(s) |
| | | | 15:21 | alopecia: limb(s) |
| | | 10 | 6:38 | alopecia: limb(s) |
| | | | 8:07 | alopecia: limb(s) |
| | | | 14:32 | alopecia: limb(s) |
| | | 11 | 6:37 | alopecia: limb(s) |
| | | | 8:35 | alopecia: limb(s) |
| | | | 14:04 | alopecia: limb(s) |
| | | 12 | 6:36 | alopecia: limb(s) |
| | | | 7:59 | alopecia: limb(s) |
| | | | 14:18 | alopecia: limb(s) |
| | | 13 | 0:00 | alopecia: limb(s) |
| | | | 6:48 | alopecia: limb(s) |
| | | | 15:10 | alopecia: limb(s) |
| | | 14 | 6:31 | alopecia: limb(s) |
| | | | 7:35 | alopecia: limb(s) |
| | | | 15:21 | alopecia: limb(s) |
| | | 15 | 6:55 | alopecia: limb(s) |
| | | | 6:55 | scheduled sacrifice |
| | 129 | 1 | 6:46 | within normal limits |
| | | | 8:03 | within normal limits |
| | | 2 | 6:41 | within normal limits |
| | | | 8:08 | within normal limits |
| | | 3 | 6:40 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:38 | within normal limits |
| | | 4 | 6:39 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:09 | within normal limits |
| | | 5 | 6:34 | within normal limits |
| | | | 7:59 | within normal limits |
| | | | 14:27 | within normal limits |
| | | 6 | 6:44 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:05 | within normal limits |
| | | 7 | 6:44 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 14:31 | within normal limits |
| | | 8 | 6:39 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:04 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 23 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 1 | 129 | 9 | 6:36 | within normal limits |
| | | | 7:52 | within normal limits |
| | | | 15:21 | within normal limits |
| | | 10 | 6:38 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:32 | within normal limits |
| | | 11 | 6:37 | within normal limits |
| | | | 8:35 | within normal limits |
| | | | 14:04 | within normal limits |
| | | 12 | 6:37 | within normal limits |
| | | | 7:59 | within normal limits |
| | | | 14:18 | within normal limits |
| | | 13 | 6:49 | within normal limits |
| | | | 8:22 | within normal limits |
| | | | 15:10 | within normal limits |
| | | 14 | 6:32 | within normal limits |
| | | | 7:35 | within normal limits |
| | | | 15:21 | within normal limits |
| | | 15 | 7:10 | within normal limits |
| | | | 7:10 | scheduled sacrifice |
| | 131 | 1 | 6:47 | within normal limits |
| | | | 8:03 | within normal limits |
| | | 2 | 6:42 | within normal limits |
| | | | 8:08 | within normal limits |
| | | 3 | 6:40 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:38 | within normal limits |
| | | 4 | 6:40 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:09 | within normal limits |
| | | 5 | 6:35 | within normal limits |
| | | | 7:59 | within normal limits |
| | | | 14:27 | within normal limits |
| | | 6 | 6:44 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:05 | within normal limits |
| | | 7 | 6:46 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 14:31 | within normal limits |
| | | 8 | 6:40 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:04 | within normal limits |
| | | 9 | 6:37 | within normal limits |
| | | | 7:52 | within normal limits |
| | | | 15:21 | within normal limits |
| | | 10 | 6:39 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:32 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 24 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 1 | 131 | 11 | 6:38 | within normal limits |
| | | | 8:35 | within normal limits |
| | | | 14:04 | within normal limits |
| | | 12 | 6:38 | within normal limits |
| | | | 7:59 | within normal limits |
| | | | 14:19 | within normal limits |
| | | 13 | 6:50 | within normal limits |
| | | | 8:22 | within normal limits |
| | | | 15:10 | within normal limits |
| | | 14 | 6:32 | within normal limits |
| | | | 7:35 | within normal limits |
| | | | 15:21 | within normal limits |
| | | 15 | 7:27 | within normal limits |
| | | | 7:27 | scheduled sacrifice |
| | 133 | 1 | 6:49 | within normal limits |
| | | | 8:03 | within normal limits |
| | | 2 | 6:43 | within normal limits |
| | | | 8:08 | within normal limits |
| | | 3 | 6:41 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:38 | within normal limits |
| | | 4 | 6:40 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:13 | within normal limits |
| | | 5 | 6:36 | within normal limits |
| | | | 8:00 | within normal limits |
| | | | 14:27 | within normal limits |
| | | 6 | 6:45 | efflux of the dosing compound |
| | | | 8:06 | within normal limits |
| | | | 14:05 | within normal limits |
| | | 7 | 8:26 | within normal limits |
| | | | 9:15 | within normal limits |
| | | | 11:05 | within normal limits |
| | | | 14:32 | within normal limits |
| | | 8 | 6:40 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:04 | within normal limits |
| | | 9 | 6:38 | within normal limits |
| | | | 7:52 | within normal limits |
| | | | 15:21 | within normal limits |
| | | 10 | 6:39 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:32 | within normal limits |
| | | 11 | 6:38 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:04 | within normal limits |
| | | 12 | 6:38 | within normal limits |
| | | | 7:59 | within normal limits |
| | | | 14:19 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 25 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 1 | 133 | 13 | 6:50 | within normal limits |
| | | | 8:22 | within normal limits |
| | | | 15:10 | within normal limits |
| | | 14 | 6:34 | within normal limits |
| | | | 7:35 | within normal limits |
| | | | 15:21 | within normal limits |
| | | 15 | 7:46 | within normal limits |
| | | | 7:46 | scheduled sacrifice |
| | 135 | 1 | 6:49 | within normal limits |
| | | | 8:03 | within normal limits |
| | | 2 | 6:44 | efflux of the dosing compound |
| | | | 6:44 | received partial dose: half |
| | | | 8:08 | within normal limits |
| | | 3 | 6:42 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:38 | within normal limits |
| | | 4 | 6:41 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:13 | within normal limits |
| | | 5 | 6:36 | within normal limits |
| | | | 8:00 | within normal limits |
| | | | 14:27 | within normal limits |
| | | 6 | 6:48 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:05 | within normal limits |
| | | 7 | 8:27 | within normal limits |
| | | | 9:16 | within normal limits |
| | | | 11:05 | within normal limits |
| | | | 14:32 | within normal limits |
| | | 8 | 6:41 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:05 | within normal limits |
| | | 9 | 6:38 | within normal limits |
| | | | 7:52 | within normal limits |
| | | | 15:21 | within normal limits |
| | | 10 | 6:40 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:33 | within normal limits |
| | | 11 | 6:39 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:04 | within normal limits |
| | | 12 | 6:39 | within normal limits |
| | | | 7:59 | within normal limits |
| | | | 14:19 | within normal limits |
| | | 13 | 6:51 | within normal limits |
| | | | 8:22 | within normal limits |
| | | | 15:10 | within normal limits |
| | | 14 | 6:35 | within normal limits |
| | | | 7:36 | within normal limits |
| | | | 15:21 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 26 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 1 | 135 | 15 | 8:02 | within normal limits |
| | | | 8:02 | scheduled sacrifice |
| 2 | 31 | 1 | 6:40 | within normal limits |
| | | | 8:36 | within normal limits |
| | | 2 | 6:51 | within normal limits |
| | | | 8:04 | within normal limits |
| | | 3 | 6:47 | within normal limits |
| | | | 8:08 | within normal limits |
| | | 4 | 6:44 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:41 | within normal limits |
| | | 5 | 6:43 | within normal limits |
| | | | 8:10 | within normal limits |
| | | | 14:13 | within normal limits |
| | | 6 | 6:38 | within normal limits |
| | | | 8:01 | within normal limits |
| | | | 14:27 | within normal limits |
| | | 7 | 6:50 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:05 | within normal limits |
| | | 8 | 7:08 | within normal limits |
| | | | 8:27 | within normal limits |
| | | | 14:32 | within normal limits |
| | | 9 | 6:42 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 14:08 | within normal limits |
| | | 10 | 6:40 | within normal limits |
| | | | 7:54 | within normal limits |
| | | | 15:21 | within normal limits |
| | | 11 | 6:41 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:33 | within normal limits |
| | | 12 | 6:40 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:06 | within normal limits |
| | | 13 | 6:41 | within normal limits |
| | | | 8:00 | within normal limits |
| | | | 14:19 | within normal limits |
| | | 14 | 6:52 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 15:11 | within normal limits |
| | | 15 | 6:12 | within normal limits |
| | | | 6:12 | scheduled sacrifice |
| | 33 | 1 | 6:41 | within normal limits |
| | | | 8:36 | within normal limits |
| | | 2 | 6:52 | within normal limits |
| | | | 8:04 | within normal limits |

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 2 | 33 | 3 | 6:48 | efflux of the dosing compound |
| | | | 8:08 | within normal limits |
| | | 4 | 6:45 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:41 | within normal limits |
| | | 5 | 6:43 | within normal limits |
| | | | 8:10 | within normal limits |
| | | | 14:13 | within normal limits |
| | | 6 | 6:39 | within normal limits |
| | | | 8:01 | within normal limits |
| | | | 14:27 | within normal limits |
| | | 7 | 6:51 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:05 | within normal limits |
| | | 8 | 7:09 | within normal limits |
| | | | 8:27 | within normal limits |
| | | | 14:32 | within normal limits |
| | | 9 | 6:44 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 14:08 | within normal limits |
| | | 10 | 6:40 | within normal limits |
| | | | 7:54 | within normal limits |
| | | | 15:22 | within normal limits |
| | | 11 | 6:41 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:33 | within normal limits |
| | | 12 | 6:43 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:06 | within normal limits |
| | | 13 | 6:41 | within normal limits |
| | | | 8:00 | within normal limits |
| | | | 14:19 | within normal limits |
| | | 14 | 6:52 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 15:11 | within normal limits |
| | | 15 | 6:27 | within normal limits |
| | | | 6:27 | scheduled sacrifice |
| | 35 | 1 | 6:42 | within normal limits |
| | | | 8:36 | within normal limits |
| | | 2 | 6:53 | within normal limits |
| | | | 8:04 | within normal limits |
| | | 3 | 6:50 | within normal limits |
| | | | 8:08 | within normal limits |
| | | 4 | 6:45 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:41 | within normal limits |
| | | 5 | 6:44 | within normal limits |
| | | | 8:10 | within normal limits |
| | | | 14:14 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 28 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 2 | 35 | 6 | 6:39 | within normal limits |
| | | | 8:01 | nose: chromodacryorrhea |
| | | | 14:28 | within normal limits |
| | | 7 | 6:52 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:06 | within normal limits |
| | | 8 | 7:09 | within normal limits |
| | | | 8:27 | within normal limits |
| | | | 14:32 | within normal limits |
| | | 9 | 6:44 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 14:08 | within normal limits |
| | | 10 | 6:41 | within normal limits |
| | | | 7:54 | within normal limits |
| | | | 15:22 | within normal limits |
| | | 11 | 6:42 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:33 | within normal limits |
| | | 12 | 6:43 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:06 | within normal limits |
| | | 13 | 6:42 | within normal limits |
| | | | 8:00 | within normal limits |
| | | | 14:19 | within normal limits |
| | | 14 | 6:53 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 15:11 | within normal limits |
| | | 15 | 6:49 | within normal limits |
| | | | 6:49 | scheduled sacrifice |
| | 37 | 1 | 6:43 | within normal limits |
| | | | 8:36 | within normal limits |
| | | 2 | 6:54 | within normal limits |
| | | | 8:04 | within normal limits |
| | | 3 | 6:50 | within normal limits |
| | | | 8:09 | within normal limits |
| | | 4 | 6:46 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:41 | within normal limits |
| | | 5 | 6:44 | within normal limits |
| | | | 8:10 | within normal limits |
| | | | 14:14 | within normal limits |
| | | 6 | 6:40 | within normal limits |
| | | | 8:01 | within normal limits |
| | | | 14:28 | eye(s): chromodacryorrhea |
| | | 7 | 6:52 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:06 | within normal limits |
| | | 8 | 7:10 | within normal limits |
| | | | 8:27 | within normal limits |
| | | | 14:32 | within normal limits |

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 2 | 37 | 9 | 6:45 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 14:08 | within normal limits |
| | | 10 | 6:41 | within normal limits |
| | | | 7:54 | within normal limits |
| | | | 15:22 | within normal limits |
| | | 11 | 6:42 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:33 | within normal limits |
| | | 12 | 6:44 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:06 | within normal limits |
| | | 13 | 6:43 | within normal limits |
| | | | 8:00 | within normal limits |
| | | | 14:19 | within normal limits |
| | | 14 | 6:55 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 15:11 | within normal limits |
| | | 15 | 7:06 | within normal limits |
| | | | 7:06 | scheduled sacrifice |
| | 39 | 1 | 6:43 | within normal limits |
| | | | 8:36 | within normal limits |
| | | 2 | 6:55 | within normal limits |
| | | | 8:04 | within normal limits |
| | | 3 | 6:51 | within normal limits |
| | | | 8:09 | within normal limits |
| | | 4 | 6:47 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 14:41 | within normal limits |
| | | 5 | 6:45 | within normal limits |
| | | | 8:10 | within normal limits |
| | | | 14:14 | within normal limits |
| | | 6 | 6:41 | within normal limits |
| | | | 8:01 | within normal limits |
| | | | 14:29 | within normal limits |
| | | 7 | 6:53 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:06 | within normal limits |
| | | 8 | 7:11 | within normal limits |
| | | | 8:27 | within normal limits |
| | | | 14:32 | within normal limits |
| | | 9 | 6:46 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 14:08 | within normal limits |
| | | 10 | 6:42 | within normal limits |
| | | | 7:54 | within normal limits |
| | | | 15:22 | within normal limits |
| | | 11 | 6:43 | efflux of the dosing compound |
| | | | 8:08 | within normal limits |
| | | | 14:33 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 30 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 2 | 39 | 12 | 6:45 | within normal limits |
| | | | 8:37 | within normal limits |
| | | | 14:06 | within normal limits |
| | | 13 | 6:43 | within normal limits |
| | | | 8:01 | within normal limits |
| | | | 14:19 | within normal limits |
| | | 14 | 6:56 | within normal limits |
| | | | 8:25 | within normal limits |
| | | | 15:11 | within normal limits |
| | | 15 | 7:28 | within normal limits |
| | | | 7:28 | scheduled sacrifice |
| | 41 | 1 | 6:44 | within normal limits |
| | | | 8:36 | within normal limits |
| | | 2 | 6:55 | within normal limits |
| | | | 8:04 | within normal limits |
| | | 3 | 6:52 | within normal limits |
| | | | 8:09 | within normal limits |
| | | 4 | 6:48 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:41 | within normal limits |
| | | 5 | 6:46 | within normal limits |
| | | | 8:10 | within normal limits |
| | | | 14:15 | within normal limits |
| | | 6 | 6:41 | within normal limits |
| | | | 8:01 | within normal limits |
| | | | 14:29 | within normal limits |
| | | 7 | 6:54 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:06 | within normal limits |
| | | 8 | 7:12 | within normal limits |
| | | | 8:27 | within normal limits |
| | | | 14:32 | within normal limits |
| | | 9 | 6:46 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 14:08 | within normal limits |
| | | 10 | 6:43 | within normal limits |
| | | | 7:54 | within normal limits |
| | | | 15:22 | within normal limits |
| | | 11 | 6:44 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:33 | within normal limits |
| | | 12 | 6:45 | within normal limits |
| | | | 8:37 | within normal limits |
| | | | 14:06 | within normal limits |
| | | 13 | 6:44 | within normal limits |
| | | | 8:01 | within normal limits |
| | | | 14:19 | within normal limits |
| | | 14 | 6:57 | within normal limits |
| | | | 8:25 | within normal limits |
| | | | 15:12 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 31 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 2 | 41 | 15 | 7:48 | within normal limits |
| | | | 7:48 | scheduled sacrifice |
| | 43 | 1 | 6:44 | within normal limits |
| | | | 8:36 | within normal limits |
| | | 2 | 6:56 | within normal limits |
| | | | 8:05 | within normal limits |
| | | 3 | 6:52 | within normal limits |
| | | | 8:09 | within normal limits |
| | | 4 | 6:48 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:41 | within normal limits |
| | | 5 | 6:46 | within normal limits |
| | | | 8:10 | within normal limits |
| | | | 14:15 | within normal limits |
| | | 6 | 6:42 | within normal limits |
| | | | 8:01 | within normal limits |
| | | | 14:29 | within normal limits |
| | | 7 | 6:54 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:06 | within normal limits |
| | | 8 | 7:12 | within normal limits |
| | | | 8:27 | within normal limits |
| | | | 14:32 | within normal limits |
| | | 9 | 6:47 | efflux of the dosing compound |
| | | | 8:26 | within normal limits |
| | | | 14:08 | nasal discharge: red |
| | | 10 | 6:43 | within normal limits |
| | | | 7:54 | within normal limits |
| | | | 15:22 | within normal limits |
| | | 11 | 6:44 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:34 | within normal limits |
| | | 12 | 6:46 | within normal limits |
| | | | 8:37 | within normal limits |
| | | | 14:06 | within normal limits |
| | | 13 | 6:44 | within normal limits |
| | | | 8:01 | within normal limits |
| | | | 14:19 | within normal limits |
| | | 14 | 6:58 | within normal limits |
| | | | 8:25 | within normal limits |
| | | | 15:12 | within normal limits |
| | | 15 | 8:10 | within normal limits |
| | | | 8:10 | scheduled sacrifice |
| | 45 | 1 | 6:46 | struggling during dosing |
| | | | 6:46 | gasping: post dosing |
| | | | 8:36 | within normal limits |
| | | 2 | 6:57 | within normal limits |
| | | | 8:05 | within normal limits |
| | | 3 | 6:53 | within normal limits |
| | | | 8:09 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 32 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 2 | 45 | 4 | 6:49 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:41 | within normal limits |
| | | 5 | 6:47 | within normal limits |
| | | | 8:10 | within normal limits |
| | | | 14:15 | within normal limits |
| | | 6 | 6:42 | within normal limits |
| | | | 8:01 | within normal limits |
| | | | 14:29 | within normal limits |
| | | 7 | 6:55 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:06 | within normal limits |
| | | 8 | 7:13 | within normal limits |
| | | | 8:28 | within normal limits |
| | | | 14:33 | within normal limits |
| | | 9 | 6:48 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 14:09 | within normal limits |
| | | 10 | 6:44 | within normal limits |
| | | | 7:54 | within normal limits |
| | | | 15:22 | within normal limits |
| | | 11 | 6:45 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:34 | within normal limits |
| | | 12 | 6:46 | within normal limits |
| | | | 8:37 | within normal limits |
| | | | 14:06 | within normal limits |
| | | 13 | 6:45 | within normal limits |
| | | | 8:01 | within normal limits |
| | | | 14:20 | within normal limits |
| | | 14 | 6:58 | within normal limits |
| | | | 8:25 | within normal limits |
| | | | 15:12 | within normal limits |
| | | 15 | 8:27 | within normal limits |
| | | | 8:27 | scheduled sacrifice |
| | 137 | 1 | 6:58 | within normal limits |
| | | | 8:05 | within normal limits |
| | | 2 | 6:54 | within normal limits |
| | | | 8:09 | within normal limits |
| | | 3 | 6:50 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:41 | within normal limits |
| | | 4 | 6:48 | within normal limits |
| | | | 8:10 | within normal limits |
| | | | 14:16 | within normal limits |
| | | 5 | 6:43 | within normal limits |
| | | | 8:02 | within normal limits |
| | | | 14:30 | within normal limits |
| | | 6 | 6:56 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:07 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 33 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 2 | 137 | 7 | 7:13 | within normal limits |
| | | | 8:28 | within normal limits |
| | | | 14:33 | within normal limits |
| | | 8 | 6:50 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 14:09 | within normal limits |
| | | 9 | 6:45 | within normal limits |
| | | | 7:54 | within normal limits |
| | | | 15:22 | within normal limits |
| | | 10 | 6:45 | within normal limits |
| | | | 8:08 | Lethargic |
| | | | 14:34 | within normal limits |
| | | 11 | 6:47 | within normal limits |
| | | | 8:37 | within normal limits |
| | | | 14:06 | within normal limits |
| | | 12 | 6:45 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:20 | within normal limits |
| | | 13 | 6:59 | within normal limits |
| | | | 8:30 | Lethargic |
| | | | 15:12 | within normal limits |
| | | 14 | 6:37 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 15:22 | within normal limits |
| | | 15 | 6:12 | within normal limits |
| | | | 6:12 | scheduled sacrifice |
| | 139 | 1 | 6:59 | within normal limits |
| | | | 8:05 | within normal limits |
| | | 2 | 6:54 | within normal limits |
| | | | 8:09 | within normal limits |
| | | 3 | 6:50 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:41 | eye(s): chromodacryorrhea |
| | | 4 | 6:48 | within normal limits |
| | | | 8:10 | within normal limits |
| | | | 14:16 | within normal limits |
| | | 5 | 6:43 | within normal limits |
| | | | 8:02 | within normal limits |
| | | | 14:30 | within normal limits |
| | | 6 | 6:56 | within normal limits |
| | | | 8:07 | Lethargic |
| | | | 14:07 | within normal limits |
| | | 7 | 7:14 | within normal limits |
| | | | 8:28 | within normal limits |
| | | | 14:33 | within normal limits |
| | | 8 | 6:50 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 14:09 | within normal limits |
| | | 9 | 6:45 | within normal limits |
| | | | 7:54 | within normal limits |
| | | | 15:22 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 34 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 2 | 139 | 10 | 6:46 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:34 | within normal limits |
| | | 11 | 6:48 | within normal limits |
| | | | 8:37 | within normal limits |
| | | | 14:06 | within normal limits |
| | | 12 | 6:46 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:20 | within normal limits |
| | | 13 | 6:59 | within normal limits |
| | | | 8:30 | within normal limits |
| | | | 15:12 | within normal limits |
| | | 14 | 6:38 | within normal limits |
| | | | 8:06 | within normal limits |
| | | | 15:23 | within normal limits |
| | | 15 | 6:22 | efflux of the dosing compound |
| | | | 6:22 | scheduled sacrifice |
| | 141 | 1 | 6:59 | within normal limits |
| | | | 8:05 | within normal limits |
| | | 2 | 6:55 | within normal limits |
| | | | 8:09 | within normal limits |
| | | 3 | 6:51 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:41 | within normal limits |
| | | 4 | 6:49 | within normal limits |
| | | | 8:10 | within normal limits |
| | | | 14:16 | within normal limits |
| | | 5 | 6:44 | within normal limits |
| | | | 8:02 | within normal limits |
| | | | 14:30 | within normal limits |
| | | 6 | 6:58 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:07 | within normal limits |
| | | 7 | 7:15 | within normal limits |
| | | | 8:28 | within normal limits |
| | | | 14:33 | within normal limits |
| | | 8 | 6:51 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 14:09 | within normal limits |
| | | 9 | 6:46 | within normal limits |
| | | | 7:54 | within normal limits |
| | | | 15:22 | within normal limits |
| | | 10 | 6:46 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:34 | within normal limits |
| | | 11 | 6:48 | within normal limits |
| | | | 8:37 | within normal limits |
| | | | 14:06 | within normal limits |
| | | 12 | 6:47 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:20 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 35 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 2 | 141 | 13 | 7:00 | within normal limits |
| | | | 8:30 | within normal limits |
| | | | 15:12 | within normal limits |
| | | 14 | 6:39 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 15:23 | within normal limits |
| | | 15 | 6:40 | within normal limits |
| | | | 6:40 | scheduled sacrifice |
| | 143 | 1 | 7:00 | within normal limits |
| | | | 8:05 | within normal limits |
| | | 2 | 6:56 | within normal limits |
| | | | 8:09 | within normal limits |
| | | 3 | 6:51 | efflux of the dosing compound |
| | | | 6:51 | received partial dose: half |
| | | | 8:08 | within normal limits |
| | | | 14:41 | within normal limits |
| | | 4 | 6:49 | within normal limits |
| | | | 8:10 | within normal limits |
| | | | 14:17 | eye(s): chromodacryorrhea |
| | | 5 | 6:44 | within normal limits |
| | | | 8:02 | within normal limits |
| | | | 14:30 | within normal limits |
| | | 6 | 6:59 | within normal limits |
| | | | 8:08 | Lethargic |
| | | | 14:07 | Lethargic |
| | | 7 | 7:15 | within normal limits |
| | | | 8:28 | within normal limits |
| | | | 14:33 | within normal limits |
| | | 8 | 6:51 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 14:09 | within normal limits |
| | | 9 | 6:46 | within normal limits |
| | | | 7:54 | within normal limits |
| | | | 15:22 | within normal limits |
| | | 10 | 6:47 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:34 | within normal limits |
| | | 11 | 6:49 | within normal limits |
| | | | 8:37 | within normal limits |
| | | | 14:06 | within normal limits |
| | | 12 | 6:47 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:20 | within normal limits |
| | | 13 | 7:01 | within normal limits |
| | | | 8:30 | Lethargic |
| | | | 15:12 | within normal limits |
| | | 14 | 6:41 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 15:23 | within normal limits |
| | | 15 | 6:57 | within normal limits |
| | | | 6:57 | scheduled sacrifice |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 36 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 2 | 145 | 1 | 7:01 | within normal limits |
| | | | 8:05 | within normal limits |
| | | 2 | 6:56 | within normal limits |
| | | | 8:09 | within normal limits |
| | | 3 | 6:53 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:41 | within normal limits |
| | | 4 | 6:50 | within normal limits |
| | | | 8:10 | within normal limits |
| | | | 14:18 | within normal limits |
| | | 5 | 6:45 | within normal limits |
| | | | 8:02 | within normal limits |
| | | | 14:30 | within normal limits |
| | | 6 | 7:01 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:08 | within normal limits |
| | | 7 | 7:16 | within normal limits |
| | | | 8:28 | within normal limits |
| | | | 14:33 | within normal limits |
| | | 8 | 6:52 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 14:09 | within normal limits |
| | | 9 | 6:47 | within normal limits |
| | | | 7:54 | within normal limits |
| | | | 15:23 | within normal limits |
| | | 10 | 6:47 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:35 | within normal limits |
| | | 11 | 6:50 | within normal limits |
| | | | 8:37 | within normal limits |
| | | | 14:06 | within normal limits |
| | | 12 | 6:48 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 14:20 | within normal limits |
| | | 13 | 7:03 | within normal limits |
| | | | 8:30 | within normal limits |
| | | | 15:12 | within normal limits |
| | | 14 | 6:41 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 15:23 | within normal limits |
| | | 15 | 7:12 | within normal limits |
| | | | 7:12 | scheduled sacrifice |
| | 147 | 1 | 7:02 | within normal limits |
| | | | 8:05 | within normal limits |
| | | 2 | 6:57 | within normal limits |
| | | | 8:09 | within normal limits |
| | | 3 | 6:53 | within normal limits |
| | | | 8:09 | within normal limits |
| | | | 14:41 | within normal limits |

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 2 | 147 | 4 | 6:51 | within normal limits |
| | | | 8:10 | within normal limits |
| | | | 14:19 | within normal limits |
| | | 5 | 6:45 | within normal limits |
| | | | 8:02 | within normal limits |
| | | | 14:31 | within normal limits |
| | | 6 | 7:03 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:08 | within normal limits |
| | | 7 | 7:17 | within normal limits |
| | | | 8:28 | within normal limits |
| | | | 14:33 | within normal limits |
| | | 8 | 6:53 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 14:09 | within normal limits |
| | | 9 | 6:48 | within normal limits |
| | | | 7:54 | within normal limits |
| | | | 15:23 | within normal limits |
| | | 10 | 6:49 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:35 | within normal limits |
| | | 11 | 6:50 | within normal limits |
| | | | 8:38 | within normal limits |
| | | | 14:06 | within normal limits |
| | | 12 | 6:48 | within normal limits |
| | | | 8:10 | within normal limits |
| | | | 14:21 | within normal limits |
| | | 13 | 7:04 | within normal limits |
| | | | 8:30 | within normal limits |
| | | | 15:12 | within normal limits |
| | | 14 | 6:43 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 15:23 | within normal limits |
| | | 15 | 7:30 | within normal limits |
| | | | 7:30 | scheduled sacrifice |
| | 149 | 1 | 7:03 | within normal limits |
| | | | 8:05 | within normal limits |
| | | 2 | 6:58 | within normal limits |
| | | | 8:09 | within normal limits |
| | | 3 | 6:54 | within normal limits |
| | | | 8:09 | within normal limits |
| | | | 14:41 | within normal limits |
| | | 4 | 6:51 | within normal limits |
| | | | 8:10 | within normal limits |
| | | | 14:19 | within normal limits |
| | | 5 | 6:46 | within normal limits |
| | | | 8:02 | within normal limits |
| | | | 14:31 | within normal limits |
| | | 6 | 7:03 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 14:08 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 38 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 2 | 149 | 7 | 7:17 | within normal limits |
| | | | 8:28 | within normal limits |
| | | | 14:33 | within normal limits |
| | | 8 | 6:53 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 14:09 | within normal limits |
| | | 9 | 6:48 | within normal limits |
| | | | 7:54 | within normal limits |
| | | | 15:23 | within normal limits |
| | | 10 | 6:49 | within normal limits |
| | | | 8:09 | within normal limits |
| | | | 14:35 | within normal limits |
| | | 11 | 6:51 | within normal limits |
| | | | 8:38 | within normal limits |
| | | | 14:06 | within normal limits |
| | | 12 | 6:49 | within normal limits |
| | | | 8:11 | within normal limits |
| | | | 14:21 | within normal limits |
| | | 13 | 7:04 | within normal limits |
| | | | 8:30 | within normal limits |
| | | | 15:12 | within normal limits |
| | | 14 | 6:44 | within normal limits |
| | | | 8:07 | within normal limits |
| | | | 15:23 | within normal limits |
| | | 15 | 7:48 | within normal limits |
| | | | 7:48 | scheduled sacrifice |
| 3 | 47 | 1 | 6:49 | within normal limits |
| | | | 8:37 | within normal limits |
| | | 2 | 7:06 | within normal limits |
| | | | 9:06 | within normal limits |
| | | 3 | 7:00 | efflux of the dosing compound |
| | | | 7:00 | eye(s): chromodacryorrhea |
| | | | 8:10 | within normal limits |
| | | 4 | 6:56 | within normal limits |
| | | | 8:10 | Lethargic |
| | | | 14:41 | Lethargic |
| | | 5 | 6:53 | within normal limits |
| | | | 8:10 | within normal limits |
| | | | 14:20 | Lethargic |
| | | 6 | 6:48 | within normal limits |
| | | | 8:03 | within normal limits |
| | | | 14:32 | Ataxia |
| | | 7 | 7:07 | efflux of the dosing compound |
| | | | 8:24 | within normal limits |
| | | | 14:08 | within normal limits |
| | | 8 | 7:19 | within normal limits |
| | | | 8:29 | within normal limits |
| | | | 14:34 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 39 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 3 | 47 | 9 | 6:55 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 14:10 | Lethargic |
| | | 10 | 6:50 | within normal limits |
| | | | 7:55 | within normal limits |
| | | | 15:23 | Lethargic |
| | | 11 | 6:51 | within normal limits |
| | | | 8:09 | Lethargic |
| | | | 14:35 | Lethargic |
| | | 12 | 6:52 | within normal limits |
| | | | 8:38 | within normal limits |
| | | | 14:08 | Lethargic |
| | | 13 | 6:51 | within normal limits |
| | | | 8:11 | within normal limits |
| | | | 14:21 | Lethargic |
| | | 14 | 7:06 | within normal limits |
| | | | 8:31 | within normal limits |
| | | | 15:12 | within normal limits |
| | | 15 | 6:14 | within normal limits |
| | | | 6:14 | scheduled sacrifice |
| | 49 | 1 | 6:50 | within normal limits |
| | | | 8:37 | within normal limits |
| | | 2 | 7:07 | efflux of the dosing compound |
| | | | 9:06 | within normal limits |
| | | 3 | 7:01 | efflux of the dosing compound |
| | | | 8:10 | within normal limits |
| | | 4 | 6:56 | within normal limits |
| | | | 8:10 | within normal limits |
| | | | 14:42 | Lethargic |
| | | | 14:42 | Ataxia |
| | | 5 | 6:54 | within normal limits |
| | | | 8:10 | within normal limits |
| | | | 14:21 | within normal limits |
| | | 6 | 0:00 | Lethargic |
| | | | 6:48 | within normal limits |
| | | | 14:33 | Lethargic |
| | | | 14:33 | Ataxia |
| | | 7 | 7:08 | within normal limits |
| | | | 8:24 | Lethargic |
| | | | 14:09 | Prone |
| | | 8 | 7:19 | within normal limits |
| | | | 8:29 | Lethargic |
| | | | 14:34 | Lethargic |
| | | 9 | 6:55 | within normal limits |
| | | | 8:26 | Lethargic |
| | | | 14:10 | Lethargic |
| | | 10 | 6:51 | within normal limits |
| | | | 7:55 | Lethargic |
| | | | 15:23 | Lethargic |

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 3 | 49 | 11 | 6:51 | within normal limits |
| | | | 8:09 | Ataxia |
| | | | 14:36 | Lethargic |
| | | 12 | 6:53 | within normal limits |
| | | | 8:38 | Lethargic |
| | | | 14:08 | Lethargic |
| | | 13 | 6:51 | efflux of the dosing compound |
| | | | 8:12 | Lethargic |
| | | | 8:12 | Ataxia |
| | | | 14:21 | Lethargic |
| | | 14 | 7:06 | within normal limits |
| | | | 8:31 | Lethargic |
| | | | 15:12 | Lethargic |
| | | 15 | 6:30 | within normal limits |
| | | | 6:30 | scheduled sacrifice |
| | 51 | 1 | 6:51 | within normal limits |
| | | | 8:37 | within normal limits |
| | | 2 | 7:08 | within normal limits |
| | | | 9:07 | Lethargic |
| | | 3 | 0:00 | rough coat |
| | | | 0:00 | eye(s): chromodacryorrhea |
| | | | 0:00 | Ataxia |
| | | | 0:00 | Piloerection |
| | | | 0:00 | Prone |
| | | | 0:00 | eye(s): discharge |
| | | | 0:00 | hindlimb: apparent paralysis, right; veterinary finding upon observation |
| | | | 0:00 | minimal response to physical or auditory stimulus; veterinary finding upon observation |
| | | | 0:00 | euthanized moribund |
| | | | 7:03 | Lethargic |
| | | | 8:12 | Prone |
| | | | 8:13 | eye(s): chromodacryorrhea |
| | | | 8:13 | rough coat |
| | 53 | 1 | 6:52 | within normal limits |
| | | | 8:37 | within normal limits |
| | | 2 | 7:08 | within normal limits |
| | | | 9:07 | within normal limits |
| | | 3 | 7:06 | within normal limits |
| | | | 8:14 | Lethargic |
| | | | 8:15 | Ataxia |
| | | 4 | 6:57 | within normal limits |
| | | | 8:10 | Lethargic |
| | | | 14:43 | Lethargic |
| | | | 14:43 | Ataxia |
| | | | 14:43 | Prone |
| | | 5 | 6:55 | within normal limits |
| | | | 8:11 | Lethargic |
| | | | 14:23 | Prone |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 41 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 3 | 53 | 6 | 6:50 | eye(s): chromodacryorrhea |
| | | | 6:50 | nose: chromodacryorrhea |
| | | | 8:05 | Lethargic |
| | | | 14:34 | Lethargic |
| | | | 14:34 | Ataxia |
| | | | 14:34 | nose: chromodacryorrhea |
| | | 7 | 7:09 | efflux of the dosing compound |
| | | | 8:25 | within normal limits |
| | | | 14:10 | within normal limits |
| | | 8 | 7:20 | efflux of the dosing compound |
| | | | 8:29 | within normal limits |
| | | | 14:34 | within normal limits |
| | | 9 | 6:56 | within normal limits |
| | | | 8:28 | Lethargic |
| | | | 14:11 | Lethargic |
| | | | 14:11 | Ataxia |
| | | 10 | 6:51 | within normal limits |
| | | | 7:55 | within normal limits |
| | | | 15:24 | within normal limits |
| | | 11 | 6:52 | within normal limits |
| | | | 8:10 | Lethargic |
| | | | 14:36 | Lethargic |
| | | 12 | 6:54 | within normal limits |
| | | | 8:39 | Lethargic |
| | | | 14:08 | Lethargic |
| | | 13 | 6:54 | salivating: post dosing |
| | | | 8:12 | Lethargic |
| | | | 14:22 | Lethargic |
| | | 14 | 7:07 | within normal limits |
| | | | 8:31 | Lethargic |
| | | | 15:13 | Lethargic |
| | | 15 | 7:09 | within normal limits |
| | | | 7:09 | scheduled sacrifice |
| | 55 | 1 | 6:53 | within normal limits |
| | | | 8:37 | within normal limits |
| | | 2 | 7:09 | within normal limits |
| | | | 9:08 | Lethargic |
| | | 3 | 7:08 | efflux of the dosing compound |
| | | | 7:08 | gasping: post dosing |
| | | | 8:15 | eye(s): chromodacryorrhea |
| | | | 8:16 | Lethargic |
| | | 4 | 6:58 | eye(s): chromodacryorrhea |
| | | | 8:11 | Lethargic |
| | | | 8:11 | eye(s): chromodacryorrhea |
| | | | 14:47 | Lethargic |
| | | | 14:47 | eye(s): chromodacryorrhea |
| | | | 14:47 | Prone |
| | | | 14:47 | Ataxia |
| | | | 14:47 | loss of righting reflex |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 42 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 3 | 55 | 5 | 6:56 | eye(s): chromodacryorrhea |
| | | | 6:56 | nose: chromodacryorrhea |
| | | | 8:13 | Lethargic |
| | | | 8:13 | eye(s): chromodacryorrhea |
| | | | 8:13 | nose: chromodacryorrhea |
| | | | 14:27 | Prone |
| | | | 14:27 | eye(s): chromodacryorrhea |
| | | | 14:27 | nose: chromodacryorrhea |
| | | 6 | 6:51 | eye(s): chromodacryorrhea |
| | | | 6:51 | nose: chromodacryorrhea |
| | | | 8:05 | Lethargic |
| | | | 8:05 | eye(s): chromodacryorrhea |
| | | | 8:05 | nose: chromodacryorrhea |
| | | | 14:35 | Prone |
| | | | 14:35 | eye(s): chromodacryorrhea |
| | | | 14:35 | nose: chromodacryorrhea |
| | | 7 | 7:10 | within normal limits |
| | | | 8:25 | Lethargic |
| | | | 8:25 | eye(s): chromodacryorrhea |
| | | | 14:10 | Lethargic |
| | | | 14:10 | Ataxia |
| | | | 14:10 | eye(s): chromodacryorrhea |
| | | 8 | 7:21 | within normal limits |
| | | | 8:30 | within normal limits |
| | | | 14:35 | Prone |
| | | 9 | 6:56 | within normal limits |
| | | | 8:28 | Lethargic |
| | | | 14:11 | Lethargic |
| | | 10 | 6:52 | within normal limits |
| | | | 7:55 | Lethargic |
| | | | 15:24 | Lethargic |
| | | 11 | 6:53 | efflux of the dosing compound |
| | | | 6:53 | received partial dose: half |
| | | | 8:10 | Lethargic |
| | | | 14:36 | Lethargic |
| | | 12 | 6:54 | within normal limits |
| | | | 8:39 | Lethargic |
| | | | 14:08 | Lethargic |
| | | 13 | 6:55 | within normal limits |
| | | | 8:13 | Lethargic |
| | | | 14:22 | Lethargic |
| | | 14 | 7:08 | within normal limits |
| | | | 8:31 | Lethargic |
| | | | 15:13 | Lethargic |
| | | 15 | 7:30 | within normal limits |
| | | | 7:30 | scheduled sacrifice |
| | 57 | 1 | 6:54 | struggling during dosing |
| | | | 8:38 | Lethargic |
| | | 2 | 7:10 | within normal limits |
| | | | 9:08 | Lethargic |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 43 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 3 | 57 | 3 | 7:11 | eye(s): chromodacryorrhea |
| | | | 8:16 | eye(s): chromodacryorrhea |
| | | 4 | 6:59 | within normal limits |
| | | | 8:12 | Lethargic |
| | | | 14:48 | Lethargic |
| | | | 14:48 | Ataxia |
| | | 5 | 6:57 | within normal limits |
| | | | 8:13 | Lethargic |
| | | | 14:28 | Lethargic |
| | | | 14:28 | Ataxia |
| | | 6 | 6:51 | within normal limits |
| | | | 8:07 | Ataxia |
| | | | 8:07 | Lethargic |
| | | | 14:35 | Lethargic |
| | | 7 | 7:11 | within normal limits |
| | | | 8:26 | Lethargic |
| | | | 14:11 | Lethargic |
| | | 8 | 7:22 | within normal limits |
| | | | 8:30 | within normal limits |
| | | | 14:35 | Lethargic |
| | | 9 | 6:57 | within normal limits |
| | | | 8:28 | Lethargic |
| | | | 14:11 | Lethargic |
| | | 10 | 6:53 | within normal limits |
| | | | 7:56 | Lethargic |
| | | | 15:25 | Lethargic |
| | | 11 | 6:54 | within normal limits |
| | | | 8:10 | Lethargic |
| | | | 14:36 | Lethargic |
| | | 12 | 6:55 | within normal limits |
| | | | 8:39 | Lethargic |
| | | | 14:08 | Lethargic |
| | | 13 | 6:56 | within normal limits |
| | | | 8:13 | Lethargic |
| | | | 14:22 | Lethargic |
| | | 14 | 7:08 | within normal limits |
| | | | 8:32 | Lethargic |
| | | | 15:13 | Lethargic |
| | | 15 | 7:51 | within normal limits |
| | | | 7:51 | scheduled sacrifice |
| | 59 | 1 | 6:56 | struggling during dosing |
| | | | 8:39 | Lethargic |
| | | 2 | 7:11 | within normal limits |
| | | | 9:08 | eye(s): chromodacryorrhea |
| | | 3 | 7:13 | eye(s): chromodacryorrhea |
| | | | 8:17 | Lethargic |
| | | | 8:17 | eye(s): chromodacryorrhea |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 44 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 3 | 59 | 4 | 7:00 | eye(s): chromodacryorrhea |
| | | | 8:12 | Lethargic |
| | | | 8:12 | eye(s): chromodacryorrhea |
| | | | 14:49 | Lethargic |
| | | | 14:49 | eye(s): chromodacryorrhea |
| | | | 14:49 | Prone |
| | | | 14:49 | Ataxia |
| | | 5 | 6:58 | eye(s): chromodacryorrhea |
| | | | 8:13 | Lethargic |
| | | | 8:13 | eye(s): chromodacryorrhea |
| | | | 14:29 | Lethargic |
| | | | 14:29 | Ataxia |
| | | | 14:29 | eye(s): chromodacryorrhea |
| | | 6 | 6:52 | eye(s): chromodacryorrhea |
| | | | 8:07 | Lethargic |
| | | | 14:36 | Lethargic |
| | | | 14:36 | Ataxia |
| | | 7 | 7:13 | eye(s): chromodacryorrhea |
| | | | 8:26 | Lethargic |
| | | | 8:26 | eye(s): chromodacryorrhea |
| | | | 14:12 | Lethargic |
| | | | 14:12 | Ataxia |
| | | | 14:12 | eye(s): chromodacryorrhea |
| | | 8 | 7:22 | within normal limits |
| | | | 8:30 | Lethargic |
| | | | 14:35 | Prone |
| | | 9 | 6:57 | within normal limits |
| | | | 8:28 | Lethargic |
| | | | 14:11 | Lethargic |
| | | 10 | 6:53 | within normal limits |
| | | | 7:56 | Lethargic |
| | | | 7:56 | eye(s): chromodacryorrhea |
| | | | 15:25 | Lethargic |
| | | 11 | 6:55 | within normal limits |
| | | | 8:10 | Lethargic |
| | | | 14:36 | Lethargic |
| | | 12 | 6:56 | within normal limits |
| | | | 8:39 | Lethargic |
| | | | 14:08 | Lethargic |
| | | 13 | 6:57 | within normal limits |
| | | | 8:13 | Lethargic |
| | | | 14:22 | Lethargic |
| | | 14 | 7:09 | within normal limits |
| | | | 8:32 | Lethargic |
| | | | 15:13 | Lethargic |
| | | 15 | 8:15 | ear(s): damaged |
| | | | 8:16 | scheduled sacrifice |
| | 151 | 1 | 7:12 | within normal limits |
| | | | 9:08 | within normal limits |
| | | 2 | 7:13 | within normal limits |
| | | | 8:35 | eye(s): chromodacryorrhea |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 45 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 3 | 151 | 3 | 7:01 | within normal limits |
| | | | 8:14 | Lethargic |
| | | | 8:14 | Ataxia |
| | | | 14:50 | Ataxia |
| | | | 14:50 | Lethargic |
| | | | 14:50 | eye(s): chromodacryorrhea |
| | | 4 | 6:59 | within normal limits |
| | | | 8:15 | Lethargic |
| | | | 8:15 | eye(s): chromodacryorrhea |
| | | | 8:15 | nose: chromodacryorrhea |
| | | | 14:30 | Lethargic |
| | | | 14:30 | Ataxia |
| | | | 14:30 | eye(s): chromodacryorrhea |
| | | | 14:30 | nose: chromodacryorrhea |
| | | 5 | 6:53 | within normal limits |
| | | | 8:08 | Lethargic |
| | | | 14:39 | Lethargic |
| | | | 14:39 | Ataxia |
| | | 6 | 7:14 | within normal limits |
| | | | 8:26 | Lethargic |
| | | | 14:13 | Ataxia |
| | | 7 | 7:23 | within normal limits |
| | | | 8:30 | within normal limits |
| | | | 14:36 | Ataxia |
| | | | 14:36 | Lethargic |
| | | 8 | 6:58 | within normal limits |
| | | | 8:28 | Lethargic |
| | | | 14:12 | Lethargic |
| | | 9 | 6:54 | within normal limits |
| | | | 8:09 | Lethargic |
| | | | 15:25 | Lethargic |
| | | 10 | 6:55 | within normal limits |
| | | | 8:11 | Lethargic |
| | | | 8:11 | Ataxia |
| | | | 14:37 | Lethargic |
| | | 11 | 6:56 | within normal limits |
| | | | 8:40 | Lethargic |
| | | | 14:09 | Lethargic |
| | | 12 | 6:58 | within normal limits |
| | | | 8:20 | Lethargic |
| | | | 14:22 | Lethargic |
| | | 13 | 7:10 | within normal limits |
| | | | 8:33 | Lethargic |
| | | | 15:14 | Lethargic |
| | | 14 | 6:45 | within normal limits |
| | | | 8:08 | within normal limits |
| | | | 15:24 | Lethargic |
| | | 15 | 6:13 | within normal limits |
| | | | 6:13 | scheduled sacrifice |
| | 153 | 1 | 7:13 | within normal limits |
| | | | 9:09 | Lethargic |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 46 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 3 | 153 | 2 | 7:15 | efflux of the dosing compound |
| | | | 7:15 | nasal discharge: red |
| | | | 8:36 | within normal limits |
| | | 3 | 7:01 | within normal limits |
| | | | 8:14 | Lethargic |
| | | | 8:14 | Ataxia |
| | | | 14:50 | Ataxia |
| | | | 14:50 | Lethargic |
| | | 4 | 7:00 | eye(s): chromodacryorrhea |
| | | | 8:15 | Lethargic |
| | | | 14:31 | Lethargic |
| | | | 14:31 | Ataxia |
| | | | 14:31 | eye(s): chromodacryorrhea |
| | | 5 | 6:54 | within normal limits |
| | | | 8:08 | Lethargic |
| | | | 14:41 | Lethargic |
| | | | 14:41 | Ataxia |
| | | 6 | 7:15 | within normal limits |
| | | | 8:26 | Lethargic |
| | | | 14:13 | Lethargic |
| | | | 14:13 | Ataxia |
| | | 7 | 7:24 | within normal limits |
| | | | 8:30 | within normal limits |
| | | | 14:37 | Ataxia |
| | | | 14:37 | Lethargic |
| | | 8 | 6:59 | within normal limits |
| | | | 8:29 | within normal limits |
| | | | 14:12 | Lethargic |
| | | 9 | 6:55 | within normal limits |
| | | | 8:09 | Lethargic |
| | | | 15:25 | Lethargic |
| | | 10 | 6:56 | within normal limits |
| | | | 8:11 | Lethargic |
| | | | 14:37 | Lethargic |
| | | 11 | 6:57 | within normal limits |
| | | | 8:40 | Lethargic |
| | | | 14:09 | Lethargic |
| | | 12 | 6:58 | efflux of the dosing compound |
| | | | 8:20 | within normal limits |
| | | | 14:22 | Lethargic |
| | | 13 | 7:11 | within normal limits |
| | | | 8:33 | within normal limits |
| | | | 15:14 | Lethargic |
| | | 14 | 6:47 | efflux of the dosing compound |
| | | | 6:48 | received partial dose: half |
| | | | 8:09 | within normal limits |
| | | | 15:24 | Lethargic |
| | | 15 | 6:25 | received partial dose: half |
| | | | 6:25 | efflux of the dosing compound |
| | | | 6:25 | scheduled sacrifice |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 47 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 3 | 155 | 1 | 7:15 | within normal limits |
| | | | 9:09 | within normal limits |
| | | 2 | 7:16 | within normal limits |
| | | | 8:36 | eye(s): chromodacryorrhea |
| | | | 8:36 | nose: chromodacryorrhea |
| | | 3 | 7:02 | eye(s): chromodacryorrhea |
| | | | 7:02 | nose: chromodacryorrhea |
| | | | 8:14 | eye(s): chromodacryorrhea |
| | | | 8:14 | Lethargic |
| | | | 8:14 | Ataxia |
| | | | 14:51 | Ataxia |
| | | | 14:51 | Lethargic |
| | | | 14:51 | eye(s): chromodacryorrhea |
| | | | 14:51 | nose: chromodacryorrhea |
| | | 4 | 7:01 | eye(s): chromodacryorrhea |
| | | | 8:15 | Lethargic |
| | | | 8:15 | eye(s): chromodacryorrhea |
| | | | 8:15 | nose: chromodacryorrhea |
| | | | 14:32 | eye(s): chromodacryorrhea |
| | | | 14:32 | Prone |
| | | 5 | 6:55 | eye(s): chromodacryorrhea |
| | | | 6:55 | rough coat |
| | | | 8:09 | Lethargic |
| | | | 8:09 | Ataxia |
| | | | 8:09 | eye(s): chromodacryorrhea |
| | | | 14:41 | Ataxia |
| | | | 14:41 | Lethargic |
| | | | 14:41 | eye(s): chromodacryorrhea |
| | | 6 | 7:16 | rough coat |
| | | | 8:27 | Lethargic |
| | | | 14:14 | Prone |
| | | 7 | 7:24 | within normal limits |
| | | | 8:30 | Lethargic |
| | | | 14:37 | Lethargic |
| | | | 14:37 | Prone |
| | | 8 | 6:59 | within normal limits |
| | | | 8:29 | within normal limits |
| | | | 14:12 | Lethargic |
| | | | 14:12 | Ataxia |
| | | 9 | 6:55 | within normal limits |
| | | | 8:09 | Lethargic |
| | | | 15:25 | Lethargic |
| | | 10 | 6:57 | within normal limits |
| | | | 8:11 | Lethargic |
| | | | 14:37 | Lethargic |
| | | 11 | 6:58 | within normal limits |
| | | | 8:40 | Lethargic |
| | | | 14:09 | Ataxia |
| | | 12 | 6:59 | within normal limits |
| | | | 8:20 | Lethargic |
| | | | 14:22 | Lethargic |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 48 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 3 | 155 | 13 | 7:12 | within normal limits |
| | | | 8:33 | Lethargic |
| | | | 15:14 | Lethargic |
| | | 14 | 6:51 | within normal limits |
| | | | 8:09 | within normal limits |
| | | | 8:21 | Lethargic |
| | | | 15:24 | Lethargic |
| | | 15 | 6:43 | within normal limits |
| | | | 6:43 | scheduled sacrifice |
| | 157 | 1 | 7:15 | within normal limits |
| | | | 9:09 | Lethargic |
| | | 2 | 7:17 | within normal limits |
| | | | 8:38 | Lethargic |
| | | 3 | 7:05 | Lethargic |
| | | | 7:05 | eye(s): chromodacryorrhea |
| | | | 7:05 | nose: chromodacryorrhea |
| | | | 8:16 | Lethargic |
| | | | 14:52 | Lethargic |
| | | | 14:52 | eye(s): chromodacryorrhea |
| | | | 14:52 | nose: chromodacryorrhea |
| | | | 14:52 | Prone |
| | | | 14:52 | Ataxia |
| | | | 14:52 | loss of righting reflex |
| | | 4 | 7:01 | eye(s): chromodacryorrhea |
| | | | 8:16 | Lethargic |
| | | | 8:16 | eye(s): chromodacryorrhea |
| | | | 8:16 | nose: chromodacryorrhea |
| | | | 14:34 | Prone |
| | | | 14:34 | eye(s): chromodacryorrhea |
| | | | 14:34 | nose: chromodacryorrhea |
| | | 5 | 6:56 | Lethargic |
| | | | 6:56 | Ataxia |
| | | | 6:56 | eye(s): chromodacryorrhea |
| | | | 6:56 | nose: chromodacryorrhea |
| | | | 6:56 | rough coat |
| | | | 8:09 | Lethargic |
| | | | 8:09 | eye(s): chromodacryorrhea |
| | | | 8:09 | nose: chromodacryorrhea |
| | | | 14:43 | Prone |
| | | | 14:43 | eye(s): chromodacryorrhea |
| | | | 14:43 | nose: chromodacryorrhea |
| | | 6 | 7:17 | eye(s): chromodacryorrhea |
| | | | 8:27 | Lethargic |
| | | | 8:27 | eye(s): chromodacryorrhea |
| | | | 14:15 | Lethargic |
| | | | 14:15 | Ataxia |
| | | 7 | 7:25 | within normal limits |
| | | | 8:31 | Lethargic |
| | | | 14:43 | Lethargic |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 49 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 3 | 157 | 8 | 7:00 | within normal limits |
| | | | 8:30 | Lethargic |
| | | | 14:13 | Lethargic |
| | | 9 | 6:56 | within normal limits |
| | | | 8:10 | Lethargic |
| | | | 15:26 | Lethargic |
| | | 10 | 6:57 | within normal limits |
| | | | 8:12 | Lethargic |
| | | | 14:38 | Lethargic |
| | | 11 | 6:59 | within normal limits |
| | | | 8:40 | Lethargic |
| | | | 14:10 | Lethargic |
| | | 12 | 7:00 | within normal limits |
| | | | 8:21 | Lethargic |
| | | | 14:23 | Lethargic |
| | | 13 | 7:12 | within normal limits |
| | | | 8:33 | Lethargic |
| | | | 15:14 | Lethargic |
| | | 14 | 6:53 | within normal limits |
| | | | 8:22 | Lethargic |
| | | | 15:24 | Lethargic |
| | | 15 | 7:00 | within normal limits |
| | | | 7:00 | scheduled sacrifice |
| | 159 | 1 | 7:16 | within normal limits |
| | | | 9:09 | within normal limits |
| | | 2 | 7:19 | efflux of the dosing compound |
| | | | 7:19 | nasal discharge: red |
| | | | 8:38 | within normal limits |
| | | 3 | 7:06 | within normal limits |
| | | | 8:16 | Lethargic |
| | | | 8:16 | Ataxia |
| | | | 14:54 | Ataxia |
| | | | 14:54 | Lethargic |
| | | 4 | 7:03 | within normal limits |
| | | | 8:17 | Lethargic |
| | | | 14:35 | within normal limits |
| | | 5 | 6:57 | eye(s): chromodacryorrhea |
| | | | 8:10 | Lethargic |
| | | | 14:43 | Lethargic |
| | | | 14:43 | eye(s): chromodacryorrhea |
| | | 6 | 7:18 | within normal limits |
| | | | 8:27 | Lethargic |
| | | | 14:15 | Lethargic |
| | | 7 | 7:26 | within normal limits |
| | | | 8:31 | within normal limits |
| | | | 14:44 | within normal limits |
| | | 8 | 7:01 | within normal limits |
| | | | 8:30 | within normal limits |
| | | | 14:13 | Lethargic |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 50 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 3 | 159 | 9 | 6:56 | within normal limits |
| | | | 8:10 | Lethargic |
| | | | 15:26 | Lethargic |
| | | 10 | 6:58 | within normal limits |
| | | | 8:12 | Lethargic |
| | | | 14:39 | Lethargic |
| | | 11 | 7:00 | within normal limits |
| | | | 8:40 | Lethargic |
| | | | 14:10 | Lethargic |
| | | 12 | 7:01 | within normal limits |
| | | | 8:21 | within normal limits |
| | | | 14:23 | Lethargic |
| | | 13 | 7:13 | within normal limits |
| | | | 8:33 | Lethargic |
| | | | 15:14 | Lethargic |
| | | 14 | 6:53 | within normal limits |
| | | | 8:28 | Lethargic |
| | | | 15:24 | Lethargic |
| | | 15 | 7:15 | within normal limits |
| | | | 7:15 | scheduled sacrifice |
| | 161 | 1 | 7:17 | within normal limits |
| | | | 9:10 | Lethargic |
| | | 2 | 7:19 | within normal limits |
| | | | 8:41 | within normal limits |
| | | 3 | 7:06 | within normal limits |
| | | | 8:16 | Lethargic |
| | | | 14:54 | Lethargic |
| | | | 14:55 | Ataxia |
| | | 4 | 7:04 | within normal limits |
| | | | 8:17 | Lethargic |
| | | | 14:36 | Lethargic |
| | | | 14:36 | Ataxia |
| | | 5 | 6:58 | within normal limits |
| | | | 8:11 | Lethargic |
| | | | 14:44 | Lethargic |
| | | | 14:44 | Ataxia |
| | | 6 | 7:19 | within normal limits |
| | | | 8:28 | Lethargic |
| | | | 14:16 | Lethargic |
| | | | 14:16 | Ataxia |
| | | 7 | 7:27 | within normal limits |
| | | | 8:31 | within normal limits |
| | | | 14:45 | Ataxia |
| | | | 14:45 | Lethargic |
| | | 8 | 7:01 | within normal limits |
| | | | 8:30 | within normal limits |
| | | | 14:13 | Lethargic |
| | | 9 | 6:57 | within normal limits |
| | | | 8:10 | Lethargic |
| | | | 15:26 | Lethargic |

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 3 | 161 | 10 | 6:59 | within normal limits |
| | | | 8:13 | Lethargic |
| | | | 14:39 | Lethargic |
| | | 11 | 7:02 | within normal limits |
| | | | 8:41 | Lethargic |
| | | | 14:10 | Lethargic |
| | | 12 | 7:01 | within normal limits |
| | | | 8:21 | Lethargic |
| | | | 14:23 | Lethargic |
| | | 13 | 7:14 | within normal limits |
| | | | 8:33 | Lethargic |
| | | | 15:14 | Lethargic |
| | | 14 | 6:56 | within normal limits |
| | | | 8:28 | Lethargic |
| | | | 15:24 | Lethargic |
| | | 15 | 7:32 | within normal limits |
| | | | 7:32 | scheduled sacrifice |
| | 163 | 1 | 7:18 | within normal limits |
| | | | 9:10 | within normal limits |
| | | 2 | 7:21 | efflux of the dosing compound |
| | | | 8:41 | Lethargic |
| | | 3 | 7:07 | within normal limits |
| | | | 8:17 | Lethargic |
| | | | 8:17 | Ataxia |
| | | | 14:56 | Ataxia |
| | | | 14:56 | Lethargic |
| | | 4 | 7:05 | eye(s): chromodacryorrhea |
| | | | 8:19 | Lethargic |
| | | | 8:19 | eye(s): chromodacryorrhea |
| | | | 14:38 | Lethargic |
| | | | 14:38 | Ataxia |
| | | | 14:38 | eye(s): chromodacryorrhea |
| | | 5 | 6:59 | eye(s): chromodacryorrhea |
| | | | 8:11 | Lethargic |
| | | | 8:11 | eye(s): chromodacryorrhea |
| | | | 14:44 | Lethargic |
| | | | 14:44 | Ataxia |
| | | | 14:44 | eye(s): chromodacryorrhea |
| | | 6 | 7:19 | within normal limits |
| | | | 8:28 | within normal limits |
| | | | 14:17 | Ataxia |
| | | | 14:17 | Lethargic |
| | | 7 | 7:27 | within normal limits |
| | | | 8:31 | Lethargic |
| | | | 14:45 | Lethargic |
| | | 8 | 7:02 | within normal limits |
| | | | 8:30 | within normal limits |
| | | | 14:13 | Lethargic |
| | | 9 | 6:58 | within normal limits |
| | | | 8:10 | Lethargic |
| | | | 15:26 | Lethargic |

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 3 | 163 | 10 | 6:59 | within normal limits |
| | | | 8:13 | Lethargic |
| | | | 14:39 | Lethargic |
| | | 11 | 7:02 | within normal limits |
| | | | 8:41 | Lethargic |
| | | | 14:10 | Lethargic |
| | | 12 | 7:02 | within normal limits |
| | | | 8:21 | Lethargic |
| | | | 14:23 | Lethargic |
| | | 13 | 7:14 | within normal limits |
| | | | 8:33 | Lethargic |
| | | | 15:14 | Lethargic |
| | | 14 | 6:56 | within normal limits |
| | | | 8:28 | Lethargic |
| | | | 15:24 | Lethargic |
| | | 15 | 7:51 | within normal limits |
| | | | 7:51 | scheduled sacrifice |
| | 165 | 1 | 7:19 | within normal limits |
| | | | 9:10 | Lethargic |
| | | 2 | 7:22 | within normal limits |
| | | | 8:41 | Lethargic |
| | | 3 | 7:08 | eye(s): chromodacryorrhea |
| | | | 8:17 | Lethargic |
| | | | 8:17 | eye(s): chromodacryorrhea |
| | | | 14:56 | Lethargic |
| | | | 14:56 | eye(s): chromodacryorrhea |
| | | | 14:57 | Ataxia |
| | | 4 | 7:06 | Ataxia |
| | | | 7:06 | Lethargic |
| | | | 7:06 | eye(s): chromodacryorrhea |
| | | | 8:19 | Lethargic |
| | | | 8:19 | eye(s): chromodacryorrhea |
| | | | 8:19 | nose: chromodacryorrhea |
| | | | 14:38 | eye(s): chromodacryorrhea |
| | | | 14:39 | Prone |
| | | 5 | 7:00 | Prone |
| | | | 7:00 | eye(s): chromodacryorrhea |
| | | | 7:00 | not dosed: moribund |
| | | | 7:01 | minimal response to physical or auditory stimulus |
| | | | 7:02 | euthanized moribund |
| 4 | 61 | 1 | 6:58 | within normal limits |
| | | | 8:40 | within normal limits |
| | | 2 | 7:21 | within normal limits |
| | | | 9:10 | within normal limits |
| | | 3 | 7:24 | within normal limits |
| | | | 8:43 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 53 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 4 | 61 | 4 | 7:10 | within normal limits |
| | | | 8:19 | within normal limits |
| | | | 14:58 | within normal limits |
| | | 5 | 7:10 | within normal limits |
| | | | 8:29 | within normal limits |
| | | | 14:39 | within normal limits |
| | | 6 | 7:04 | efflux of the dosing compound |
| | | | 8:12 | within normal limits |
| | | | 14:45 | within normal limits |
| | | 7 | 7:21 | efflux of the dosing compound |
| | | | 8:29 | within normal limits |
| | | | 14:17 | within normal limits |
| | | 8 | 7:30 | within normal limits |
| | | | 9:18 | within normal limits |
| | | | 14:45 | within normal limits |
| | | 9 | 7:04 | within normal limits |
| | | | 8:31 | within normal limits |
| | | | 14:14 | within normal limits |
| | | 10 | 7:00 | within normal limits |
| | | | 8:12 | within normal limits |
| | | | 15:27 | within normal limits |
| | | 11 | 7:01 | within normal limits |
| | | | 8:14 | within normal limits |
| | | | 14:39 | within normal limits |
| | | 12 | 7:04 | within normal limits |
| | | | 8:41 | within normal limits |
| | | | 14:11 | within normal limits |
| | | 13 | 7:04 | within normal limits |
| | | | 8:22 | within normal limits |
| | | | 14:23 | within normal limits |
| | | 14 | 7:16 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 15:15 | within normal limits |
| | | 15 | 6:15 | within normal limits |
| | | | 6:16 | scheduled sacrifice |
| | 63 | 1 | 6:58 | within normal limits |
| | | | 8:40 | within normal limits |
| | | 2 | 7:22 | within normal limits |
| | | | 9:10 | within normal limits |
| | | 3 | 7:24 | within normal limits |
| | | | 8:43 | within normal limits |
| | | 4 | 7:11 | within normal limits |
| | | | 8:19 | within normal limits |
| | | | 14:58 | within normal limits |
| | | 5 | 7:10 | within normal limits |
| | | | 8:29 | within normal limits |
| | | | 14:39 | within normal limits |
| | | 6 | 7:05 | within normal limits |
| | | | 8:12 | within normal limits |
| | | | 14:45 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 54 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 4 | 63 | 7 | 7:22 | within normal limits |
| | | | 8:29 | within normal limits |
| | | | 14:17 | within normal limits |
| | | 8 | 7:31 | within normal limits |
| | | | 9:18 | within normal limits |
| | | | 14:45 | within normal limits |
| | | 9 | 7:05 | within normal limits |
| | | | 8:31 | within normal limits |
| | | | 14:14 | within normal limits |
| | | 10 | 7:00 | within normal limits |
| | | | 8:12 | within normal limits |
| | | | 15:27 | within normal limits |
| | | 11 | 7:02 | within normal limits |
| | | | 8:14 | within normal limits |
| | | | 14:39 | within normal limits |
| | | 12 | 7:05 | within normal limits |
| | | | 8:41 | within normal limits |
| | | | 14:11 | within normal limits |
| | | 13 | 7:04 | within normal limits |
| | | | 8:22 | within normal limits |
| | | | 14:24 | within normal limits |
| | | 14 | 7:17 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 15:15 | within normal limits |
| | | 15 | 6:33 | within normal limits |
| | | | 6:33 | scheduled sacrifice |
| | 65 | 1 | 6:59 | within normal limits |
| | | | 8:40 | within normal limits |
| | | 2 | 7:23 | efflux of the dosing compound |
| | | | 9:11 | within normal limits |
| | | 3 | 7:25 | within normal limits |
| | | | 8:43 | within normal limits |
| | | 4 | 7:12 | within normal limits |
| | | | 8:19 | nose: chromodacryorrhea |
| | | | 14:58 | within normal limits |
| | | 5 | 7:11 | within normal limits |
| | | | 8:29 | within normal limits |
| | | | 14:40 | within normal limits |
| | | 6 | 7:06 | within normal limits |
| | | | 8:12 | within normal limits |
| | | | 14:45 | within normal limits |
| | | 7 | 7:22 | within normal limits |
| | | | 8:29 | within normal limits |
| | | | 14:17 | within normal limits |
| | | 8 | 7:31 | within normal limits |
| | | | 9:19 | within normal limits |
| | | | 14:46 | within normal limits |
| | | 9 | 7:05 | within normal limits |
| | | | 8:31 | within normal limits |
| | | | 14:14 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 55 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 4 | 65 | 10 | 7:01 | within normal limits |
| | | | 8:12 | within normal limits |
| | | | 15:27 | within normal limits |
| | | 11 | 7:03 | within normal limits |
| | | | 8:14 | within normal limits |
| | | | 14:39 | within normal limits |
| | | 12 | 7:05 | within normal limits |
| | | | 8:41 | within normal limits |
| | | | 14:11 | within normal limits |
| | | 13 | 7:05 | within normal limits |
| | | | 8:22 | within normal limits |
| | | | 14:24 | within normal limits |
| | | 14 | 7:18 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 15:15 | within normal limits |
| | | 15 | 6:51 | within normal limits |
| | | | 6:52 | scheduled sacrifice |
| | 67 | 1 | 7:00 | within normal limits |
| | | | 8:40 | within normal limits |
| | | 2 | 7:25 | within normal limits |
| | | | 9:11 | within normal limits |
| | | 3 | 7:26 | within normal limits |
| | | | 8:43 | within normal limits |
| | | 4 | 7:12 | within normal limits |
| | | | 8:19 | within normal limits |
| | | | 14:58 | within normal limits |
| | | 5 | 7:12 | within normal limits |
| | | | 8:29 | within normal limits |
| | | | 14:40 | within normal limits |
| | | 6 | 7:06 | within normal limits |
| | | | 8:12 | within normal limits |
| | | | 14:45 | within normal limits |
| | | 7 | 7:23 | within normal limits |
| | | | 8:29 | within normal limits |
| | | | 14:17 | within normal limits |
| | | 8 | 7:32 | within normal limits |
| | | | 9:19 | within normal limits |
| | | | 14:57 | within normal limits |
| | | 9 | 7:06 | within normal limits |
| | | | 8:31 | Lethargic |
| | | | 14:14 | within normal limits |
| | | 10 | 7:01 | within normal limits |
| | | | 8:12 | within normal limits |
| | | | 15:27 | within normal limits |
| | | 11 | 7:03 | within normal limits |
| | | | 8:14 | within normal limits |
| | | | 14:39 | within normal limits |
| | | 12 | 7:06 | within normal limits |
| | | | 8:41 | within normal limits |
| | | | 14:11 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 56 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 4 | 67 | 13 | 7:05 | within normal limits |
| | | | 8:22 | within normal limits |
| | | | 14:24 | within normal limits |
| | | 14 | 7:19 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 15:15 | within normal limits |
| | | 15 | 7:12 | within normal limits |
| | | | 7:12 | scheduled sacrifice |
| | 69 | 1 | 7:01 | within normal limits |
| | | | 8:40 | within normal limits |
| | | 2 | 7:26 | within normal limits |
| | | | 9:11 | within normal limits |
| | | 3 | 7:27 | within normal limits |
| | | | 8:43 | within normal limits |
| | | 4 | 7:13 | within normal limits |
| | | | 8:19 | within normal limits |
| | | | 14:58 | within normal limits |
| | | 5 | 7:13 | within normal limits |
| | | | 8:29 | within normal limits |
| | | | 14:40 | within normal limits |
| | | 6 | 7:07 | within normal limits |
| | | | 8:12 | within normal limits |
| | | | 14:46 | within normal limits |
| | | 7 | 7:23 | within normal limits |
| | | | 8:29 | within normal limits |
| | | | 14:17 | within normal limits |
| | | 8 | 7:33 | within normal limits |
| | | | 9:19 | within normal limits |
| | | | 14:57 | within normal limits |
| | | 9 | 7:07 | within normal limits |
| | | | 8:31 | within normal limits |
| | | | 14:14 | within normal limits |
| | | 10 | 7:02 | within normal limits |
| | | | 8:12 | within normal limits |
| | | | 15:27 | within normal limits |
| | | 11 | 7:04 | within normal limits |
| | | | 8:14 | within normal limits |
| | | | 14:39 | within normal limits |
| | | 12 | 7:08 | within normal limits |
| | | | 8:41 | within normal limits |
| | | | 14:11 | within normal limits |
| | | 13 | 7:06 | within normal limits |
| | | | 8:22 | within normal limits |
| | | | 14:24 | within normal limits |
| | | 14 | 7:19 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 15:15 | within normal limits |
| | | 15 | 7:34 | within normal limits |
| | | | 7:34 | scheduled sacrifice |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 57 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 4 | 71 | 1 | 7:02 | within normal limits |
| | | | 8:40 | within normal limits |
| | | 2 | 7:27 | within normal limits |
| | | | 9:11 | within normal limits |
| | | 3 | 7:28 | within normal limits |
| | | | 8:43 | within normal limits |
| | | 4 | 7:14 | within normal limits |
| | | | 8:19 | within normal limits |
| | | | 14:58 | within normal limits |
| | | 5 | 7:14 | within normal limits |
| | | | 8:29 | within normal limits |
| | | | 14:40 | within normal limits |
| | | 6 | 7:07 | within normal limits |
| | | | 8:12 | within normal limits |
| | | | 14:46 | within normal limits |
| | | 7 | 7:24 | within normal limits |
| | | | 8:29 | within normal limits |
| | | | 14:18 | within normal limits |
| | | 8 | 7:33 | within normal limits |
| | | | 9:19 | within normal limits |
| | | | 14:57 | within normal limits |
| | | 9 | 7:08 | within normal limits |
| | | | 8:31 | within normal limits |
| | | | 14:14 | within normal limits |
| | | 10 | 7:02 | within normal limits |
| | | | 8:12 | within normal limits |
| | | | 15:27 | within normal limits |
| | | 11 | 7:04 | within normal limits |
| | | | 8:14 | within normal limits |
| | | | 14:39 | within normal limits |
| | | 12 | 7:09 | within normal limits |
| | | | 8:41 | within normal limits |
| | | | 14:11 | within normal limits |
| | | 13 | 7:07 | within normal limits |
| | | | 8:22 | within normal limits |
| | | | 14:24 | within normal limits |
| | | 14 | 7:20 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 15:16 | within normal limits |
| | | 15 | 7:55 | within normal limits |
| | | | 7:55 | scheduled sacrifice |
| | 73 | 1 | 7:03 | within normal limits |
| | | | 8:40 | within normal limits |
| | | 2 | 7:28 | within normal limits |
| | | | 9:11 | within normal limits |
| | | 3 | 7:29 | within normal limits |
| | | | 8:43 | within normal limits |
| | | 4 | 7:15 | within normal limits |
| | | | 8:19 | within normal limits |
| | | | 14:58 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 58 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 4 | 73 | 5 | 7:15 | within normal limits |
| | | | 8:29 | within normal limits |
| | | | 14:41 | within normal limits |
| | | 6 | 7:08 | within normal limits |
| | | | 8:12 | within normal limits |
| | | | 14:46 | within normal limits |
| | | 7 | 7:25 | within normal limits |
| | | | 8:29 | within normal limits |
| | | | 14:18 | within normal limits |
| | | 8 | 7:34 | within normal limits |
| | | | 9:19 | within normal limits |
| | | | 14:57 | within normal limits |
| | | 9 | 7:08 | within normal limits |
| | | | 8:31 | within normal limits |
| | | | 14:14 | within normal limits |
| | | 10 | 7:03 | within normal limits |
| | | | 8:12 | within normal limits |
| | | | 15:27 | within normal limits |
| | | 11 | 7:05 | within normal limits |
| | | | 8:14 | within normal limits |
| | | | 14:40 | within normal limits |
| | | 12 | 7:10 | within normal limits |
| | | | 8:41 | within normal limits |
| | | | 14:11 | within normal limits |
| | | 13 | 7:08 | within normal limits |
| | | | 8:22 | within normal limits |
| | | | 14:24 | within normal limits |
| | | 14 | 7:21 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 15:16 | within normal limits |
| | | 15 | 0:00 | scheduled sacrifice |
| | | | 8:17 | within normal limits |
| | 75 | 1 | 7:04 | sore(s): head |
| | | | 8:40 | sore(s): head |
| | | 2 | 7:29 | sore(s): head |
| | | | 9:11 | sore(s): head |
| | | 3 | 7:29 | sore(s): head |
| | | | 8:43 | sore(s): head |
| | | 4 | 7:16 | sore(s): head |
| | | | 8:19 | sore(s): head |
| | | | 14:58 | sore(s): head |
| | | 5 | 7:16 | sore(s): head |
| | | | 8:30 | sore(s): head |
| | | | 14:41 | sore(s): head |
| | | 6 | 7:09 | sore(s): head |
| | | | 8:12 | sore(s): head |
| | | | 14:46 | sore(s): head |
| | | 7 | 7:26 | sore(s): head |
| | | | 8:29 | sore(s): head |
| | | | 14:18 | sore(s): head |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 59 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 4 | 75 | 8 | 7:35 | alopecia: head |
| | | | 9:19 | alopecia: head |
| | | | 14:57 | alopecia: head |
| | | 9 | 7:09 | alopecia: head |
| | | | 8:31 | alopecia: head |
| | | | 14:14 | alopecia: head |
| | | 10 | 7:04 | alopecia: head |
| | | | 8:12 | alopecia: head |
| | | | 15:27 | alopecia: head |
| | | 11 | 7:06 | alopecia: head |
| | | | 8:14 | alopecia: head |
| | | | 14:40 | alopecia: head |
| | | 12 | 7:11 | alopecia: head |
| | | | 8:42 | alopecia: head |
| | | | 14:11 | alopecia: head |
| | | 13 | 7:09 | alopecia: head |
| | | | 8:22 | alopecia: head |
| | | | 14:24 | alopecia: head |
| | | 14 | 7:22 | alopecia: head |
| | | | 8:34 | alopecia: head |
| | | | 15:16 | alopecia: head |
| | | 15 | 8:30 | alopecia: head |
| | | | 8:30 | scheduled sacrifice |
| | 167 | 1 | 7:31 | within normal limits |
| | | | 9:11 | within normal limits |
| | | 2 | 7:31 | within normal limits |
| | | | 8:44 | within normal limits |
| | | 3 | 7:17 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:58 | within normal limits |
| | | 4 | 7:16 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 14:41 | within normal limits |
| | | 5 | 7:10 | within normal limits |
| | | | 8:13 | within normal limits |
| | | | 14:46 | within normal limits |
| | | 6 | 7:27 | within normal limits |
| | | | 8:29 | within normal limits |
| | | | 14:18 | within normal limits |
| | | 7 | 7:36 | within normal limits |
| | | | 9:19 | within normal limits |
| | | | 14:57 | within normal limits |
| | | 8 | 7:10 | within normal limits |
| | | | 8:32 | within normal limits |
| | | | 14:14 | within normal limits |
| | | 9 | 7:04 | within normal limits |
| | | | 8:12 | within normal limits |
| | | | 15:27 | within normal limits |
| | | 10 | 7:07 | within normal limits |
| | | | 8:14 | within normal limits |
| | | | 14:40 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 60 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 4 | 167 | 11 | 7:12 | within normal limits |
| | | | 8:42 | within normal limits |
| | | | 14:11 | within normal limits |
| | | 12 | 7:10 | within normal limits |
| | | | 8:23 | within normal limits |
| | | | 14:25 | within normal limits |
| | | 13 | 7:23 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 15:16 | within normal limits |
| | | 14 | 6:59 | within normal limits |
| | | | 8:38 | within normal limits |
| | | | 15:26 | within normal limits |
| | | 15 | 6:14 | within normal limits |
| | | | 6:14 | scheduled sacrifice |
| | 169 | 1 | 7:32 | within normal limits |
| | | | 9:11 | within normal limits |
| | | 2 | 7:32 | within normal limits |
| | | | 8:44 | within normal limits |
| | | 3 | 7:17 | within normal limits |
| | | | 8:25 | within normal limits |
| | | | 14:58 | within normal limits |
| | | 4 | 7:17 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 14:41 | within normal limits |
| | | 5 | 7:10 | within normal limits |
| | | | 8:13 | within normal limits |
| | | | 14:46 | within normal limits |
| | | 6 | 7:27 | within normal limits |
| | | | 8:29 | within normal limits |
| | | | 14:18 | within normal limits |
| | | 7 | 7:37 | within normal limits |
| | | | 9:19 | within normal limits |
| | | | 14:58 | within normal limits |
| | | 8 | 7:10 | within normal limits |
| | | | 8:32 | within normal limits |
| | | | 14:14 | within normal limits |
| | | 9 | 7:06 | within normal limits |
| | | | 8:12 | within normal limits |
| | | | 15:27 | within normal limits |
| | | 10 | 7:07 | within normal limits |
| | | | 8:14 | within normal limits |
| | | | 14:40 | within normal limits |
| | | 11 | 7:13 | within normal limits |
| | | | 8:42 | within normal limits |
| | | | 14:11 | within normal limits |
| | | 12 | 7:10 | within normal limits |
| | | | 8:23 | within normal limits |
| | | | 14:25 | within normal limits |
| | | 13 | 7:23 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 15:16 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 61 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 4 | 169 | 14 | 7:02 | within normal limits |
| | | | 8:38 | within normal limits |
| | | | 15:26 | within normal limits |
| | | 15 | 6:27 | within normal limits |
| | | | 6:27 | scheduled sacrifice |
| | 171 | 1 | 7:33 | within normal limits |
| | | | 9:11 | within normal limits |
| | | 2 | 7:32 | within normal limits |
| | | | 8:44 | within normal limits |
| | | 3 | 7:18 | within normal limits |
| | | | 8:25 | within normal limits |
| | | | 14:58 | within normal limits |
| | | 4 | 7:18 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 14:41 | within normal limits |
| | | 5 | 7:12 | within normal limits |
| | | | 8:13 | within normal limits |
| | | | 14:46 | within normal limits |
| | | 6 | 7:28 | within normal limits |
| | | | 8:29 | within normal limits |
| | | | 14:18 | within normal limits |
| | | 7 | 7:38 | within normal limits |
| | | | 9:19 | within normal limits |
| | | | 14:58 | within normal limits |
| | | 8 | 7:11 | within normal limits |
| | | | 8:32 | within normal limits |
| | | | 14:14 | within normal limits |
| | | 9 | 7:06 | within normal limits |
| | | | 8:12 | within normal limits |
| | | | 15:27 | within normal limits |
| | | 10 | 7:08 | within normal limits |
| | | | 8:14 | within normal limits |
| | | | 14:40 | within normal limits |
| | | 11 | 7:14 | within normal limits |
| | | | 8:42 | within normal limits |
| | | | 14:11 | within normal limits |
| | | 12 | 7:11 | within normal limits |
| | | | 8:23 | within normal limits |
| | | | 14:25 | within normal limits |
| | | 13 | 7:24 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 15:16 | within normal limits |
| | | 14 | 7:02 | within normal limits |
| | | | 8:38 | within normal limits |
| | | | 15:27 | within normal limits |
| | | 15 | 6:45 | within normal limits |
| | | | 6:45 | scheduled sacrifice |
| | 173 | 1 | 7:34 | within normal limits |
| | | | 9:11 | within normal limits |
| | | 2 | 7:33 | alopecia: limb(s) |
| | | | 8:44 | alopecia: limb(s) |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 62 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 4 | 173 | 3 | 7:19 | alopecia: limb(s) |
| | | | 8:25 | alopecia: limb(s) |
| | | | 14:58 | alopecia: limb(s) |
| | | 4 | 7:19 | alopecia: limb(s) |
| | | | 8:34 | alopecia: limb(s) |
| | | | 14:42 | alopecia: limb(s) |
| | | 5 | 7:12 | alopecia: limb(s) |
| | | | 8:13 | alopecia: limb(s) |
| | | | 14:47 | alopecia: limb(s) |
| | | 6 | 7:29 | alopecia: limb(s) |
| | | | 8:29 | alopecia: limb(s) |
| | | | 14:19 | alopecia: limb(s) |
| | | 7 | 7:40 | alopecia: limb(s) |
| | | | 9:19 | alopecia: limb(s) |
| | | | 14:58 | alopecia: limb(s) |
| | | 8 | 7:12 | alopecia: limb(s) |
| | | | 8:32 | alopecia: limb(s) |
| | | | 14:14 | alopecia: limb(s) |
| | | 9 | 7:07 | alopecia: limb(s) |
| | | | 8:12 | alopecia: limb(s) |
| | | | 15:28 | alopecia: limb(s) |
| | | 10 | 7:09 | alopecia: limb(s) |
| | | | 8:14 | alopecia: limb(s) |
| | | | 14:40 | alopecia: limb(s) |
| | | 11 | 7:15 | alopecia: limb(s) |
| | | | 8:42 | alopecia: limb(s) |
| | | | 14:11 | alopecia: limb(s) |
| | | 12 | 7:11 | alopecia: limb(s) |
| | | | 8:23 | alopecia: limb(s) |
| | | | 14:25 | alopecia: limb(s) |
| | | 13 | 7:25 | alopecia: limb(s) |
| | | | 8:34 | alopecia: limb(s) |
| | | | 15:16 | alopecia: limb(s) |
| | | 14 | 7:04 | alopecia: limb(s) |
| | | | 8:38 | alopecia: limb(s) |
| | | | 15:27 | alopecia: limb(s) |
| | | 15 | 7:02 | alopecia: limb(s) |
| | | | 7:02 | scheduled sacrifice |
| | 175 | 1 | 7:36 | within normal limits |
| | | | 9:11 | within normal limits |
| | | 2 | 7:34 | within normal limits |
| | | | 8:45 | within normal limits |
| | | 3 | 7:20 | within normal limits |
| | | | 8:25 | within normal limits |
| | | | 14:58 | within normal limits |
| | | 4 | 7:20 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 14:42 | within normal limits |
| | | 5 | 7:13 | within normal limits |
| | | | 8:13 | within normal limits |
| | | | 14:47 | within normal limits |

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 4 | 175 | 6 | 7:29 | within normal limits |
| | | | 8:29 | within normal limits |
| | | | 14:19 | within normal limits |
| | | 7 | 7:41 | within normal limits |
| | | | 9:19 | within normal limits |
| | | | 14:58 | within normal limits |
| | | 8 | 7:12 | within normal limits |
| | | | 8:32 | within normal limits |
| | | | 14:15 | within normal limits |
| | | 9 | 7:08 | within normal limits |
| | | | 8:12 | within normal limits |
| | | | 15:28 | within normal limits |
| | | 10 | 7:09 | efflux of the dosing compound |
| | | | 8:14 | within normal limits |
| | | | 14:40 | within normal limits |
| | | 11 | 7:16 | within normal limits |
| | | | 8:42 | within normal limits |
| | | | 14:11 | within normal limits |
| | | 12 | 7:12 | within normal limits |
| | | | 8:23 | within normal limits |
| | | | 14:25 | within normal limits |
| | | 13 | 7:26 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 15:16 | within normal limits |
| | | 14 | 7:05 | within normal limits |
| | | | 8:38 | within normal limits |
| | | | 15:27 | within normal limits |
| | | 15 | 7:17 | within normal limits |
| | | | 7:17 | scheduled sacrifice |
| | 177 | 1 | 7:37 | within normal limits |
| | | | 9:11 | within normal limits |
| | | 2 | 7:35 | within normal limits |
| | | | 8:45 | within normal limits |
| | | 3 | 7:20 | within normal limits |
| | | | 8:25 | within normal limits |
| | | | 14:59 | within normal limits |
| | | 4 | 7:20 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 14:42 | within normal limits |
| | | 5 | 7:13 | within normal limits |
| | | | 8:13 | within normal limits |
| | | | 14:47 | within normal limits |
| | | 6 | 7:30 | within normal limits |
| | | | 8:29 | within normal limits |
| | | | 14:19 | within normal limits |
| | | 7 | 7:42 | within normal limits |
| | | | 9:19 | within normal limits |
| | | | 14:58 | within normal limits |
| | | 8 | 7:13 | within normal limits |
| | | | 8:32 | within normal limits |
| | | | 14:15 | within normal limits |

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 4 | 177 | 9 | 7:08 | within normal limits |
| | | | 8:12 | within normal limits |
| | | | 15:28 | within normal limits |
| | | 10 | 7:10 | within normal limits |
| | | | 8:15 | within normal limits |
| | | | 14:41 | within normal limits |
| | | 11 | 7:16 | within normal limits |
| | | | 8:42 | within normal limits |
| | | | 14:11 | within normal limits |
| | | 12 | 7:13 | within normal limits |
| | | | 8:23 | within normal limits |
| | | | 14:25 | within normal limits |
| | | 13 | 7:26 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 15:16 | within normal limits |
| | | 14 | 7:07 | within normal limits |
| | | | 8:39 | within normal limits |
| | | | 15:27 | within normal limits |
| | | 15 | 7:35 | efflux of the dosing compound |
| | | | 7:35 | scheduled sacrifice |
| | 179 | 1 | 7:38 | within normal limits |
| | | | 9:11 | within normal limits |
| | | 2 | 7:36 | within normal limits |
| | | | 8:45 | within normal limits |
| | | 3 | 7:21 | within normal limits |
| | | | 8:25 | within normal limits |
| | | | 14:59 | within normal limits |
| | | 4 | 7:21 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 14:43 | within normal limits |
| | | 5 | 7:14 | within normal limits |
| | | | 8:14 | within normal limits |
| | | | 14:47 | within normal limits |
| | | 6 | 7:31 | within normal limits |
| | | | 8:29 | within normal limits |
| | | | 14:19 | within normal limits |
| | | 7 | 7:42 | within normal limits |
| | | | 9:19 | within normal limits |
| | | | 14:58 | within normal limits |
| | | 8 | 7:13 | within normal limits |
| | | | 8:32 | within normal limits |
| | | | 14:15 | within normal limits |
| | | 9 | 7:09 | within normal limits |
| | | | 8:12 | within normal limits |
| | | | 15:28 | within normal limits |
| | | 10 | 7:11 | within normal limits |
| | | | 8:15 | within normal limits |
| | | | 14:41 | within normal limits |
| | | 11 | 7:18 | within normal limits |
| | | | 8:42 | within normal limits |
| | | | 14:11 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 65 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 4 | 179 | 12 | 7:15 | within normal limits |
| | | | 8:23 | within normal limits |
| | | | 14:25 | within normal limits |
| | | 13 | 7:27 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 15:16 | within normal limits |
| | | 14 | 7:10 | within normal limits |
| | | | 8:39 | within normal limits |
| | | | 15:27 | within normal limits |
| | | 15 | 7:52 | within normal limits |
| | | | 7:52 | scheduled sacrifice |
| 5 | 77 | 1 | 7:07 | within normal limits |
| | | | 8:41 | within normal limits |
| | | 2 | 7:41 | within normal limits |
| | | | 9:12 | within normal limits |
| | | 3 | 7:38 | within normal limits |
| | | | 8:49 | within normal limits |
| | | 4 | 7:22 | within normal limits |
| | | | 8:31 | within normal limits |
| | | | 15:00 | within normal limits |
| | | 5 | 7:23 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:43 | within normal limits |
| | | 6 | 7:16 | within normal limits |
| | | | 8:25 | within normal limits |
| | | | 14:47 | within normal limits |
| | | 7 | 7:32 | within normal limits |
| | | | 8:40 | within normal limits |
| | | | 14:19 | within normal limits |
| | | 8 | 7:45 | within normal limits |
| | | | 9:20 | within normal limits |
| | | | 14:58 | within normal limits |
| | | 9 | 7:15 | within normal limits |
| | | | 8:33 | within normal limits |
| | | | 14:16 | within normal limits |
| | | 10 | 7:10 | within normal limits |
| | | | 8:25 | within normal limits |
| | | | 15:28 | within normal limits |
| | | 11 | 7:12 | within normal limits |
| | | | 8:22 | within normal limits |
| | | | 14:41 | within normal limits |
| | | 12 | 7:19 | within normal limits |
| | | | 8:43 | within normal limits |
| | | | 14:12 | within normal limits |
| | | 13 | 7:16 | nose: chromodacryorrhea |
| | | | 8:27 | within normal limits |
| | | | 14:25 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 66 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 5 | 77 | 14 | 7:28 | within normal limits |
| | | | 8:46 | within normal limits |
| | | | 15:18 | within normal limits |
| | | 15 | 6:17 | within normal limits |
| | | | 6:17 | scheduled sacrifice |
| | 79 | 1 | 7:08 | within normal limits |
| | | | 8:41 | within normal limits |
| | | 2 | 7:42 | within normal limits |
| | | | 9:12 | within normal limits |
| | | 3 | 7:39 | within normal limits |
| | | | 8:49 | within normal limits |
| | | 4 | 7:23 | within normal limits |
| | | | 8:31 | within normal limits |
| | | | 15:01 | within normal limits |
| | | 5 | 7:23 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:43 | within normal limits |
| | | 6 | 7:16 | within normal limits |
| | | | 8:25 | within normal limits |
| | | | 14:47 | within normal limits |
| | | 7 | 7:33 | within normal limits |
| | | | 8:40 | within normal limits |
| | | | 14:19 | within normal limits |
| | | 8 | 7:45 | within normal limits |
| | | | 9:20 | within normal limits |
| | | | 14:58 | within normal limits |
| | | 9 | 7:16 | within normal limits |
| | | | 8:33 | within normal limits |
| | | | 14:16 | within normal limits |
| | | 10 | 7:11 | within normal limits |
| | | | 8:25 | within normal limits |
| | | | 15:28 | within normal limits |
| | | 11 | 7:13 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:41 | within normal limits |
| | | 12 | 7:20 | within normal limits |
| | | | 8:43 | within normal limits |
| | | | 14:12 | within normal limits |
| | | 13 | 7:17 | within normal limits |
| | | | 8:27 | within normal limits |
| | | | 14:25 | within normal limits |
| | | 14 | 7:28 | within normal limits |
| | | | 8:46 | within normal limits |
| | | | 15:18 | within normal limits |
| | | 15 | 6:36 | within normal limits |
| | | | 6:36 | scheduled sacrifice |
| | 81 | 1 | 7:10 | within normal limits |
| | | | 8:41 | within normal limits |
| | | 2 | 7:43 | within normal limits |
| | | | 9:12 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 67 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 5 | 81 | 3 | 7:40 | within normal limits |
| | | | 8:49 | within normal limits |
| | | 4 | 7:24 | within normal limits |
| | | | 8:31 | within normal limits |
| | | | 15:01 | within normal limits |
| | | 5 | 7:24 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:43 | within normal limits |
| | | 6 | 7:17 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 14:47 | within normal limits |
| | | 7 | 7:33 | within normal limits |
| | | | 8:40 | within normal limits |
| | | | 14:19 | within normal limits |
| | | 8 | 7:46 | within normal limits |
| | | | 9:20 | within normal limits |
| | | | 14:58 | within normal limits |
| | | 9 | 7:16 | within normal limits |
| | | | 8:33 | within normal limits |
| | | | 14:16 | within normal limits |
| | | 10 | 7:11 | within normal limits |
| | | | 8:25 | within normal limits |
| | | | 15:28 | within normal limits |
| | | 11 | 7:13 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:41 | within normal limits |
| | | 12 | 7:21 | within normal limits |
| | | | 8:43 | within normal limits |
| | | | 14:12 | within normal limits |
| | | 13 | 7:17 | within normal limits |
| | | | 8:28 | within normal limits |
| | | | 14:25 | within normal limits |
| | | 14 | 7:29 | within normal limits |
| | | | 8:46 | within normal limits |
| | | | 15:18 | within normal limits |
| | | 15 | 6:54 | within normal limits |
| | | | 6:55 | scheduled sacrifice |
| | 83 | 1 | 7:11 | within normal limits |
| | | | 8:41 | within normal limits |
| | | 2 | 7:44 | within normal limits |
| | | | 9:12 | within normal limits |
| | | 3 | 7:41 | within normal limits |
| | | | 8:50 | within normal limits |
| | | 4 | 7:25 | within normal limits |
| | | | 8:31 | within normal limits |
| | | | 15:01 | within normal limits |
| | | 5 | 7:25 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:43 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 68 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 5 | 83 | 6 | 7:18 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 14:48 | within normal limits |
| | | 7 | 7:34 | within normal limits |
| | | | 8:40 | within normal limits |
| | | | 14:20 | within normal limits |
| | | 8 | 7:47 | within normal limits |
| | | | 9:20 | within normal limits |
| | | | 14:58 | within normal limits |
| | | 9 | 7:17 | within normal limits |
| | | | 8:33 | within normal limits |
| | | | 14:16 | within normal limits |
| | | 10 | 7:12 | within normal limits |
| | | | 8:25 | within normal limits |
| | | | 15:28 | within normal limits |
| | | 11 | 7:14 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:41 | within normal limits |
| | | 12 | 7:21 | within normal limits |
| | | | 8:43 | within normal limits |
| | | | 14:12 | within normal limits |
| | | 13 | 7:18 | within normal limits |
| | | | 8:28 | within normal limits |
| | | | 14:25 | within normal limits |
| | | 14 | 7:30 | within normal limits |
| | | | 8:46 | within normal limits |
| | | | 15:18 | within normal limits |
| | | 15 | 7:15 | within normal limits |
| | | | 7:15 | scheduled sacrifice |
| | 85 | 1 | 7:12 | within normal limits |
| | | | 8:41 | within normal limits |
| | | 2 | 7:45 | within normal limits |
| | | | 9:12 | within normal limits |
| | | 3 | 7:41 | within normal limits |
| | | | 8:50 | within normal limits |
| | | 4 | 7:26 | within normal limits |
| | | | 8:31 | within normal limits |
| | | | 15:01 | within normal limits |
| | | 5 | 7:26 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:43 | within normal limits |
| | | 6 | 7:18 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 14:48 | within normal limits |
| | | 7 | 7:35 | within normal limits |
| | | | 8:40 | within normal limits |
| | | | 14:20 | within normal limits |
| | | 8 | 7:47 | within normal limits |
| | | | 9:20 | within normal limits |
| | | | 14:58 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 69 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 5 | 85 | 9 | 7:17 | within normal limits |
| | | | 8:33 | within normal limits |
| | | | 14:16 | within normal limits |
| | | 10 | 7:12 | within normal limits |
| | | | 8:25 | within normal limits |
| | | | 15:28 | within normal limits |
| | | 11 | 7:15 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:41 | within normal limits |
| | | 12 | 7:22 | within normal limits |
| | | | 8:43 | within normal limits |
| | | | 14:12 | within normal limits |
| | | 13 | 7:18 | within normal limits |
| | | | 8:28 | within normal limits |
| | | | 14:26 | within normal limits |
| | | 14 | 7:31 | within normal limits |
| | | | 8:46 | within normal limits |
| | | | 15:18 | within normal limits |
| | | 15 | 7:38 | within normal limits |
| | | | 7:38 | scheduled sacrifice |
| | 87 | 1 | 7:13 | struggling during dosing |
| | | | 7:14 | efflux of the dosing compound |
| | | | 8:41 | within normal limits |
| | | 2 | 7:46 | efflux of the dosing compound |
| | | | 9:12 | within normal limits |
| | | 3 | 7:42 | within normal limits |
| | | | 8:50 | within normal limits |
| | | 4 | 7:26 | within normal limits |
| | | | 8:31 | within normal limits |
| | | | 15:01 | within normal limits |
| | | 5 | 7:27 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:44 | within normal limits |
| | | 6 | 7:19 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 14:48 | within normal limits |
| | | 7 | 7:36 | within normal limits |
| | | | 8:41 | within normal limits |
| | | | 14:20 | within normal limits |
| | | 8 | 7:48 | within normal limits |
| | | | 9:20 | within normal limits |
| | | | 14:59 | within normal limits |
| | | 9 | 7:18 | efflux of the dosing compound |
| | | | 8:33 | within normal limits |
| | | | 14:16 | within normal limits |
| | | 10 | 7:13 | within normal limits |
| | | | 8:25 | within normal limits |
| | | | 15:28 | within normal limits |
| | | 11 | 7:15 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:41 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 70 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 5 | 87 | 12 | 7:23 | within normal limits |
| | | | 8:44 | within normal limits |
| | | | 14:12 | within normal limits |
| | | 13 | 7:19 | within normal limits |
| | | | 8:28 | within normal limits |
| | | | 14:26 | within normal limits |
| | | 14 | 7:31 | within normal limits |
| | | | 8:46 | within normal limits |
| | | | 15:18 | within normal limits |
| | | 15 | 8:01 | within normal limits |
| | | | 8:01 | scheduled sacrifice |
| | 89 | 1 | 7:14 | within normal limits |
| | | | 8:42 | within normal limits |
| | | 2 | 7:47 | within normal limits |
| | | | 9:12 | within normal limits |
| | | 3 | 7:42 | within normal limits |
| | | | 8:50 | within normal limits |
| | | 4 | 7:27 | within normal limits |
| | | | 8:31 | within normal limits |
| | | | 15:01 | within normal limits |
| | | 5 | 7:27 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:44 | within normal limits |
| | | 6 | 7:19 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 14:48 | within normal limits |
| | | 7 | 7:36 | within normal limits |
| | | | 8:41 | within normal limits |
| | | | 14:20 | within normal limits |
| | | 8 | 7:48 | within normal limits |
| | | | 9:20 | within normal limits |
| | | | 14:59 | within normal limits |
| | | 9 | 7:18 | within normal limits |
| | | | 8:33 | within normal limits |
| | | | 14:16 | within normal limits |
| | | 10 | 7:14 | within normal limits |
| | | | 8:25 | within normal limits |
| | | | 15:29 | within normal limits |
| | | 11 | 7:16 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:41 | within normal limits |
| | | 12 | 7:23 | efflux of the dosing compound |
| | | | 8:44 | within normal limits |
| | | | 14:12 | within normal limits |
| | | 13 | 7:20 | within normal limits |
| | | | 8:28 | within normal limits |
| | | | 14:26 | within normal limits |
| | | 14 | 7:32 | within normal limits |
| | | | 8:46 | within normal limits |
| | | | 15:18 | nose: chromodacryorrhea |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 71 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 5 | 89 | 15 | 8:19 | within normal limits |
| | | | 8:19 | scheduled sacrifice |
| | 181 | 1 | 7:49 | within normal limits |
| | | | 9:12 | within normal limits |
| | | 2 | 7:43 | within normal limits |
| | | | 8:55 | within normal limits |
| | | 3 | 7:28 | within normal limits |
| | | | 8:39 | within normal limits |
| | | | 15:01 | within normal limits |
| | | 4 | 7:28 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:44 | within normal limits |
| | | 5 | 7:20 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 14:48 | within normal limits |
| | | 6 | 7:37 | within normal limits |
| | | | 8:42 | within normal limits |
| | | | 14:20 | within normal limits |
| | | 7 | 7:53 | within normal limits |
| | | | 9:20 | within normal limits |
| | | | 14:59 | within normal limits |
| | | 8 | 7:19 | within normal limits |
| | | | 8:33 | within normal limits |
| | | | 14:17 | within normal limits |
| | | 9 | 7:14 | within normal limits |
| | | | 8:25 | within normal limits |
| | | | 15:29 | within normal limits |
| | | 10 | 7:17 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:42 | within normal limits |
| | | 11 | 7:24 | within normal limits |
| | | | 8:44 | within normal limits |
| | | | 14:12 | within normal limits |
| | | 12 | 7:21 | within normal limits |
| | | | 8:28 | within normal limits |
| | | | 14:26 | within normal limits |
| | | 13 | 7:33 | within normal limits |
| | | | 8:46 | within normal limits |
| | | | 15:18 | within normal limits |
| | | 14 | 7:11 | within normal limits |
| | | | 8:40 | within normal limits |
| | | | 15:27 | within normal limits |
| | | 15 | 6:15 | within normal limits |
| | | | 6:15 | scheduled sacrifice |
| | 183 | 1 | 7:51 | within normal limits |
| | | | 9:12 | within normal limits |
| | | 2 | 7:44 | within normal limits |
| | | | 8:55 | within normal limits |
| | | 3 | 7:28 | within normal limits |
| | | | 8:39 | within normal limits |
| | | | 15:01 | Piloerection |

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 5 | 183 | 4 | 7:29 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:45 | within normal limits |
| | | 5 | 7:20 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 14:48 | within normal limits |
| | | 6 | 7:39 | within normal limits |
| | | | 8:42 | within normal limits |
| | | | 14:20 | within normal limits |
| | | 7 | 7:54 | within normal limits |
| | | | 9:20 | within normal limits |
| | | | 14:59 | within normal limits |
| | | 8 | 7:20 | within normal limits |
| | | | 8:33 | within normal limits |
| | | | 14:17 | within normal limits |
| | | 9 | 7:15 | within normal limits |
| | | | 8:25 | within normal limits |
| | | | 15:29 | within normal limits |
| | | 10 | 7:17 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:42 | within normal limits |
| | | 11 | 7:25 | within normal limits |
| | | | 8:44 | within normal limits |
| | | | 14:13 | Piloerection |
| | | 12 | 7:21 | within normal limits |
| | | | 8:28 | within normal limits |
| | | | 14:26 | within normal limits |
| | | 13 | 7:34 | within normal limits |
| | | | 8:46 | within normal limits |
| | | | 15:18 | within normal limits |
| | | 14 | 7:13 | within normal limits |
| | | | 8:40 | within normal limits |
| | | | 15:27 | within normal limits |
| | | 15 | 6:30 | within normal limits |
| | | | 6:30 | scheduled sacrifice |
| | 185 | 1 | 7:53 | within normal limits |
| | | | 9:12 | within normal limits |
| | | 2 | 7:46 | within normal limits |
| | | | 8:55 | nose: chromodacryorrhea |
| | | 3 | 7:29 | within normal limits |
| | | | 8:39 | within normal limits |
| | | | 15:01 | nose: chromodacryorrhea |
| | | 4 | 7:30 | within normal limits |
| | | | 8:36 | nose: chromodacryorrhea |
| | | | 14:45 | within normal limits |
| | | 5 | 7:21 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 14:48 | within normal limits |
| | | 6 | 7:40 | within normal limits |
| | | | 8:42 | within normal limits |
| | | | 14:20 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 73 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 5 | 185 | 7 | 7:54 | within normal limits |
| | | | 9:20 | within normal limits |
| | | | 14:59 | within normal limits |
| | | 8 | 7:21 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 14:17 | within normal limits |
| | | 9 | 7:16 | within normal limits |
| | | | 8:25 | within normal limits |
| | | | 15:29 | within normal limits |
| | | 10 | 7:18 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:42 | within normal limits |
| | | 11 | 7:26 | within normal limits |
| | | | 8:44 | within normal limits |
| | | | 14:13 | within normal limits |
| | | 12 | 7:22 | within normal limits |
| | | | 8:28 | within normal limits |
| | | | 14:26 | within normal limits |
| | | 13 | 7:35 | within normal limits |
| | | | 8:46 | within normal limits |
| | | | 15:18 | within normal limits |
| | | 14 | 7:14 | within normal limits |
| | | | 8:41 | within normal limits |
| | | | 15:27 | nose: chromodacryorrhea |
| | | 15 | 6:46 | within normal limits |
| | | | 6:47 | scheduled sacrifice |
| | 187 | 1 | 7:54 | within normal limits |
| | | | 9:12 | within normal limits |
| | | 2 | 7:46 | within normal limits |
| | | | 8:59 | nose: chromodacryorrhea |
| | | 3 | 7:29 | within normal limits |
| | | | 8:39 | within normal limits |
| | | | 15:02 | within normal limits |
| | | 4 | 7:31 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:46 | within normal limits |
| | | 5 | 7:22 | within normal limits |
| | | | 8:27 | within normal limits |
| | | | 14:49 | within normal limits |
| | | 6 | 7:40 | within normal limits |
| | | | 8:42 | within normal limits |
| | | | 14:21 | within normal limits |
| | | 7 | 7:55 | within normal limits |
| | | | 9:20 | within normal limits |
| | | | 14:59 | within normal limits |
| | | 8 | 7:21 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 14:17 | within normal limits |
| | | 9 | 7:17 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 15:29 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 74 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 5 | 187 | 10 | 7:20 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:42 | within normal limits |
| | | 11 | 7:27 | within normal limits |
| | | | 8:44 | within normal limits |
| | | | 14:13 | within normal limits |
| | | 12 | 7:22 | within normal limits |
| | | | 8:29 | within normal limits |
| | | | 14:26 | within normal limits |
| | | 13 | 7:36 | within normal limits |
| | | | 8:46 | within normal limits |
| | | | 15:18 | within normal limits |
| | | 14 | 7:16 | within normal limits |
| | | | 8:41 | within normal limits |
| | | | 15:27 | within normal limits |
| | | 15 | 7:05 | within normal limits |
| | | | 7:05 | scheduled sacrifice |
| | 189 | 1 | 7:55 | within normal limits |
| | | | 9:12 | within normal limits |
| | | 2 | 7:47 | within normal limits |
| | | | 8:59 | nose: chromodacryorrhea |
| | | 3 | 7:30 | within normal limits |
| | | | 8:39 | within normal limits |
| | | | 15:02 | within normal limits |
| | | 4 | 7:32 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:46 | within normal limits |
| | | 5 | 7:23 | within normal limits |
| | | | 8:27 | within normal limits |
| | | | 14:49 | within normal limits |
| | | 6 | 7:41 | within normal limits |
| | | | 8:42 | within normal limits |
| | | | 14:21 | within normal limits |
| | | 7 | 7:56 | within normal limits |
| | | | 9:20 | within normal limits |
| | | | 14:59 | within normal limits |
| | | 8 | 7:22 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 14:17 | within normal limits |
| | | 9 | 7:17 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 15:29 | within normal limits |
| | | 10 | 7:21 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:42 | within normal limits |
| | | 11 | 7:27 | within normal limits |
| | | | 8:44 | within normal limits |
| | | | 14:14 | within normal limits |
| | | 12 | 7:23 | within normal limits |
| | | | 8:29 | within normal limits |
| | | | 14:26 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 75 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 5 | 189 | 13 | 7:37 | within normal limits |
| | | | 8:46 | within normal limits |
| | | | 15:18 | within normal limits |
| | | 14 | 7:17 | within normal limits |
| | | | 8:41 | within normal limits |
| | | | 15:27 | within normal limits |
| | | 15 | 7:20 | within normal limits |
| | | | 7:20 | scheduled sacrifice |
| | 191 | 1 | 7:56 | within normal limits |
| | | | 9:12 | within normal limits |
| | | 2 | 7:48 | within normal limits |
| | | | 8:59 | nose: chromodacryorrhea |
| | | 3 | 7:31 | within normal limits |
| | | | 8:39 | within normal limits |
| | | | 15:02 | within normal limits |
| | | 4 | 7:32 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:47 | within normal limits |
| | | 5 | 7:23 | within normal limits |
| | | | 8:27 | within normal limits |
| | | | 14:49 | within normal limits |
| | | 6 | 7:42 | within normal limits |
| | | | 8:42 | within normal limits |
| | | | 14:21 | within normal limits |
| | | 7 | 7:56 | within normal limits |
| | | | 9:20 | within normal limits |
| | | | 14:59 | within normal limits |
| | | 8 | 7:22 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 14:17 | within normal limits |
| | | 9 | 7:18 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 15:29 | within normal limits |
| | | 10 | 7:21 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:43 | within normal limits |
| | | 11 | 7:28 | within normal limits |
| | | | 8:44 | within normal limits |
| | | | 14:14 | within normal limits |
| | | 12 | 7:24 | within normal limits |
| | | | 8:29 | within normal limits |
| | | | 14:26 | within normal limits |
| | | 13 | 7:39 | within normal limits |
| | | | 8:46 | within normal limits |
| | | | 15:18 | within normal limits |
| | | 14 | 7:19 | within normal limits |
| | | | 8:41 | within normal limits |
| | | | 15:27 | within normal limits |
| | | 15 | 7:38 | within normal limits |
| | | | 7:38 | scheduled sacrifice |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 76 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 5 | 193 | 1 | 7:57 | within normal limits |
| | | | 9:13 | within normal limits |
| | | 2 | 7:49 | within normal limits |
| | | | 8:59 | within normal limits |
| | | 3 | 7:31 | within normal limits |
| | | | 8:39 | within normal limits |
| | | | 15:02 | nose: chromodacryorrhea |
| | | 4 | 7:33 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:47 | within normal limits |
| | | 5 | 7:24 | within normal limits |
| | | | 8:27 | within normal limits |
| | | | 14:49 | within normal limits |
| | | 6 | 7:43 | within normal limits |
| | | | 8:46 | within normal limits |
| | | | 14:21 | within normal limits |
| | | 7 | 7:57 | within normal limits |
| | | | 9:20 | within normal limits |
| | | | 14:59 | within normal limits |
| | | 8 | 7:24 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 14:17 | within normal limits |
| | | 9 | 7:19 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 15:29 | within normal limits |
| | | 10 | 7:22 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:43 | within normal limits |
| | | 11 | 7:30 | within normal limits |
| | | | 8:44 | within normal limits |
| | | | 14:14 | nose: chromodacryorrhea |
| | | 12 | 7:25 | within normal limits |
| | | | 8:29 | within normal limits |
| | | | 14:27 | within normal limits |
| | | 13 | 7:40 | within normal limits |
| | | | 8:46 | within normal limits |
| | | | 15:18 | within normal limits |
| | | 14 | 7:20 | within normal limits |
| | | | 8:42 | within normal limits |
| | | | 15:28 | within normal limits |
| | | 15 | 7:59 | within normal limits |
| | | | 7:59 | scheduled sacrifice |
| | 195 | 1 | 7:58 | within normal limits |
| | | | 9:13 | within normal limits |
| | | 2 | 7:49 | within normal limits |
| | | | 8:59 | within normal limits |
| | | 3 | 7:32 | within normal limits |
| | | | 8:39 | within normal limits |
| | | | 15:02 | nose: chromodacryorrhea |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 77 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 5 | 195 | 4 | 7:34 | within normal limits |
| | | | 8:36 | nose: chromodacryorrhea |
| | | | 14:47 | within normal limits |
| | | 5 | 7:25 | within normal limits |
| | | | 8:27 | within normal limits |
| | | | 14:49 | within normal limits |
| | | 6 | 7:44 | within normal limits |
| | | | 8:46 | within normal limits |
| | | | 14:21 | within normal limits |
| | | 7 | 7:58 | within normal limits |
| | | | 9:20 | within normal limits |
| | | | 14:59 | within normal limits |
| | | 8 | 7:24 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 14:17 | within normal limits |
| | | 9 | 7:19 | within normal limits |
| | | | 8:26 | within normal limits |
| | | | 15:29 | within normal limits |
| | | 10 | 7:23 | within normal limits |
| | | | 8:24 | within normal limits |
| | | | 14:43 | within normal limits |
| | | 11 | 7:30 | within normal limits |
| | | | 8:44 | within normal limits |
| | | | 14:14 | within normal limits |
| | | 12 | 7:25 | within normal limits |
| | | | 8:29 | within normal limits |
| | | | 14:27 | within normal limits |
| | | 13 | 7:40 | within normal limits |
| | | | 8:46 | within normal limits |
| | | | 15:18 | within normal limits |
| | | 14 | 7:22 | within normal limits |
| | | | 8:42 | within normal limits |
| | | | 15:28 | within normal limits |
| | | 15 | 8:03 | within normal limits |
| | | | 8:03 | scheduled sacrifice |
| 6 | 91 | 1 | 7:18 | within normal limits |
| | | | 8:42 | within normal limits |
| | | 2 | 8:09 | within normal limits |
| | | | 9:13 | within normal limits |
| | | 3 | 7:51 | within normal limits |
| | | | 9:01 | within normal limits |
| | | 4 | 7:33 | within normal limits |
| | | | 8:41 | within normal limits |
| | | | 15:03 | within normal limits |
| | | 5 | 7:36 | within normal limits |
| | | | 8:47 | within normal limits |
| | | | 14:48 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 78 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 6 | 91 | 6 | 7:27 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:49 | within normal limits |
| | | 7 | 7:45 | within normal limits |
| | | | 8:58 | within normal limits |
| | | | 14:21 | within normal limits |
| | | 8 | 8:08 | within normal limits |
| | | | 9:20 | within normal limits |
| | | | 15:00 | within normal limits |
| | | 9 | 7:26 | within normal limits |
| | | | 8:35 | within normal limits |
| | | | 14:19 | nose: chromodacryorrhea |
| | | 10 | 7:22 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 15:30 | within normal limits |
| | | 11 | 7:24 | within normal limits |
| | | | 8:35 | within normal limits |
| | | | 14:43 | within normal limits |
| | | 12 | 7:31 | within normal limits |
| | | | 8:45 | within normal limits |
| | | | 14:14 | Lethargic |
| | | 13 | 7:27 | within normal limits |
| | | | 8:32 | within normal limits |
| | | | 14:27 | within normal limits |
| | | 14 | 7:41 | within normal limits |
| | | | 8:47 | within normal limits |
| | | | 15:20 | within normal limits |
| | | 15 | 6:18 | within normal limits |
| | | | 6:18 | scheduled sacrifice |
| | 93 | 1 | 7:19 | within normal limits |
| | | | 8:42 | within normal limits |
| | | 2 | 8:10 | within normal limits |
| | | | 9:13 | within normal limits |
| | | 3 | 7:52 | within normal limits |
| | | | 9:01 | within normal limits |
| | | 4 | 7:34 | within normal limits |
| | | | 8:41 | within normal limits |
| | | | 15:03 | within normal limits |
| | | 5 | 7:36 | within normal limits |
| | | | 8:48 | within normal limits |
| | | | 14:48 | within normal limits |
| | | 6 | 7:27 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:49 | within normal limits |
| | | 7 | 7:45 | within normal limits |
| | | | 8:58 | within normal limits |
| | | | 14:21 | within normal limits |
| | | 8 | 8:09 | within normal limits |
| | | | 9:21 | within normal limits |
| | | | 15:00 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 79 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 6 | 93 | 9 | 7:27 | within normal limits |
| | | | 8:35 | within normal limits |
| | | | 14:19 | within normal limits |
| | | 10 | 7:23 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 15:30 | within normal limits |
| | | 11 | 7:24 | within normal limits |
| | | | 8:35 | within normal limits |
| | | | 14:43 | within normal limits |
| | | 12 | 7:32 | within normal limits |
| | | | 8:45 | within normal limits |
| | | | 14:15 | Lethargic |
| | | 13 | 7:28 | within normal limits |
| | | | 8:32 | within normal limits |
| | | | 14:27 | within normal limits |
| | | 14 | 7:42 | within normal limits |
| | | | 8:47 | within normal limits |
| | | | 15:20 | within normal limits |
| | | 15 | 6:40 | within normal limits |
| | | | 6:40 | scheduled sacrifice |
| | 95 | 1 | 7:19 | within normal limits |
| | | | 8:42 | within normal limits |
| | | 2 | 8:10 | within normal limits |
| | | | 9:13 | within normal limits |
| | | 3 | 7:53 | within normal limits |
| | | | 9:01 | within normal limits |
| | | 4 | 7:35 | within normal limits |
| | | | 8:41 | within normal limits |
| | | | 15:03 | within normal limits |
| | | 5 | 7:37 | within normal limits |
| | | | 8:48 | within normal limits |
| | | | 14:49 | within normal limits |
| | | 6 | 7:28 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:49 | within normal limits |
| | | 7 | 7:46 | within normal limits |
| | | | 8:58 | within normal limits |
| | | | 14:21 | within normal limits |
| | | 8 | 8:10 | within normal limits |
| | | | 9:21 | within normal limits |
| | | | 15:00 | within normal limits |
| | | 9 | 7:28 | within normal limits |
| | | | 8:35 | within normal limits |
| | | | 14:19 | within normal limits |
| | | 10 | 7:23 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 15:30 | within normal limits |
| | | 11 | 7:25 | within normal limits |
| | | | 8:35 | within normal limits |
| | | | 14:43 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 80 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 6 | 95 | 12 | 7:33 | within normal limits |
| | | | 8:45 | within normal limits |
| | | | 14:15 | within normal limits |
| | | 13 | 7:29 | within normal limits |
| | | | 8:32 | within normal limits |
| | | | 14:27 | within normal limits |
| | | 14 | 7:43 | within normal limits |
| | | | 8:47 | within normal limits |
| | | | 15:20 | within normal limits |
| | | 15 | 6:58 | within normal limits |
| | | | 6:58 | scheduled sacrifice |
| | 97 | 1 | 7:20 | within normal limits |
| | | | 8:42 | within normal limits |
| | | 2 | 8:11 | within normal limits |
| | | | 9:13 | within normal limits |
| | | 3 | 7:54 | within normal limits |
| | | | 9:01 | within normal limits |
| | | 4 | 7:35 | within normal limits |
| | | | 8:41 | within normal limits |
| | | | 15:03 | within normal limits |
| | | 5 | 7:38 | within normal limits |
| | | | 8:48 | within normal limits |
| | | | 14:49 | within normal limits |
| | | 6 | 7:29 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:50 | within normal limits |
| | | 7 | 7:47 | within normal limits |
| | | | 8:58 | within normal limits |
| | | | 14:21 | within normal limits |
| | | 8 | 8:10 | within normal limits |
| | | | 9:21 | within normal limits |
| | | | 15:00 | within normal limits |
| | | 9 | 7:28 | within normal limits |
| | | | 8:35 | within normal limits |
| | | | 14:19 | within normal limits |
| | | 10 | 7:24 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 15:30 | within normal limits |
| | | 11 | 7:26 | within normal limits |
| | | | 8:35 | within normal limits |
| | | | 14:43 | within normal limits |
| | | 12 | 7:33 | efflux of the dosing compound |
| | | | 8:45 | within normal limits |
| | | | 14:15 | nose: chromodacryorrhea |
| | | 13 | 7:29 | within normal limits |
| | | | 8:32 | within normal limits |
| | | | 14:27 | within normal limits |
| | | 14 | 7:44 | within normal limits |
| | | | 8:47 | within normal limits |
| | | | 15:20 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 81 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 6 | 97 | 15 | 7:18 | within normal limits |
| | | | 7:18 | scheduled sacrifice |
| | 99 | 1 | 7:21 | within normal limits |
| | | | 8:42 | within normal limits |
| | | 2 | 8:12 | within normal limits |
| | | | 9:13 | within normal limits |
| | | 3 | 7:54 | within normal limits |
| | | | 9:01 | nose: chromodacryorrhea |
| | | 4 | 7:36 | within normal limits |
| | | | 8:41 | within normal limits |
| | | | 15:03 | nose: chromodacryorrhea |
| | | 5 | 7:39 | within normal limits |
| | | | 8:48 | within normal limits |
| | | | 14:49 | within normal limits |
| | | 6 | 7:30 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:50 | within normal limits |
| | | 7 | 7:47 | within normal limits |
| | | | 8:58 | within normal limits |
| | | | 14:22 | within normal limits |
| | | 8 | 8:11 | within normal limits |
| | | | 9:21 | within normal limits |
| | | | 15:00 | within normal limits |
| | | 9 | 7:29 | within normal limits |
| | | | 8:35 | within normal limits |
| | | | 14:20 | nose: chromodacryorrhea |
| | | 10 | 7:25 | within normal limits |
| | | | 8:34 | nose: chromodacryorrhea |
| | | | 15:30 | nose: chromodacryorrhea |
| | | 11 | 7:26 | nose: chromodacryorrhea |
| | | | 8:35 | nose: chromodacryorrhea |
| | | | 14:44 | nose: chromodacryorrhea |
| | | 12 | 7:34 | within normal limits |
| | | | 8:45 | within normal limits |
| | | | 14:15 | nose: chromodacryorrhea |
| | | | 14:15 | Ataxia |
| | | 13 | 7:30 | within normal limits |
| | | | 8:33 | within normal limits |
| | | | 14:28 | within normal limits |
| | | 14 | 7:44 | within normal limits |
| | | | 8:47 | within normal limits |
| | | | 15:21 | within normal limits |
| | | 15 | 7:39 | within normal limits |
| | | | 7:39 | scheduled sacrifice |
| | 101 | 1 | 7:23 | struggling during dosing |
| | | | 7:24 | gasping: post dosing |
| | | | 8:42 | within normal limits |
| | | 2 | 8:12 | within normal limits |
| | | | 9:14 | within normal limits |
| | | 3 | 7:55 | within normal limits |
| | | | 9:02 | nose: chromodacryorrhea |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 82 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 6 | 101 | 4 | 7:37 | within normal limits |
| | | | 8:41 | within normal limits |
| | | | 15:03 | Ataxia |
| | | 5 | 7:39 | within normal limits |
| | | | 8:48 | nose: chromodacryorrhea |
| | | | 14:50 | nose: chromodacryorrhea |
| | | 6 | 7:30 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:50 | within normal limits |
| | | 7 | 7:48 | within normal limits |
| | | | 8:58 | within normal limits |
| | | | 14:22 | within normal limits |
| | | 8 | 8:11 | within normal limits |
| | | | 9:21 | within normal limits |
| | | | 15:00 | within normal limits |
| | | 9 | 7:30 | within normal limits |
| | | | 8:35 | within normal limits |
| | | | 14:20 | within normal limits |
| | | 10 | 7:26 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 15:30 | within normal limits |
| | | 11 | 7:27 | within normal limits |
| | | | 8:35 | within normal limits |
| | | | 14:44 | within normal limits |
| | | 12 | 0:00 | Ataxia |
| | | | 7:35 | within normal limits |
| | | | 8:46 | within normal limits |
| | | 13 | 7:31 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 14:28 | within normal limits |
| | | 14 | 7:45 | within normal limits |
| | | | 8:47 | within normal limits |
| | | | 15:21 | within normal limits |
| | | 15 | 8:02 | efflux of the dosing compound |
| | | | 8:02 | scheduled sacrifice |
| | 103 | 1 | 7:24 | within normal limits |
| | | | 8:42 | within normal limits |
| | | 2 | 8:13 | within normal limits |
| | | | 9:14 | within normal limits |
| | | 3 | 7:55 | within normal limits |
| | | | 9:02 | within normal limits |
| | | 4 | 7:37 | within normal limits |
| | | | 8:41 | within normal limits |
| | | | 15:04 | within normal limits |
| | | 5 | 7:40 | within normal limits |
| | | | 8:48 | nose: chromodacryorrhea |
| | | | 14:50 | within normal limits |
| | | 6 | 7:31 | within normal limits |
| | | | 8:37 | within normal limits |
| | | | 14:50 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 83 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 6 | 103 | 7 | 7:48 | within normal limits |
| | | | 8:58 | within normal limits |
| | | | 14:22 | within normal limits |
| | | 8 | 8:12 | within normal limits |
| | | | 9:21 | within normal limits |
| | | | 15:00 | within normal limits |
| | | 9 | 7:30 | within normal limits |
| | | | 8:35 | nose: chromodacryorrhea |
| | | | 14:20 | nose: chromodacryorrhea |
| | | 10 | 7:27 | efflux of the dosing compound |
| | | | 7:27 | received partial dose: half |
| | | | 8:34 | nose: chromodacryorrhea |
| | | | 15:31 | nose: chromodacryorrhea |
| | | 11 | 7:28 | within normal limits |
| | | | 8:35 | within normal limits |
| | | | 14:44 | within normal limits |
| | | 12 | 7:35 | within normal limits |
| | | | 8:46 | within normal limits |
| | | | 14:17 | nose: chromodacryorrhea |
| | | | 14:17 | Piloerection |
| | | 13 | 7:32 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 14:28 | Piloerection |
| | | 14 | 7:46 | within normal limits |
| | | | 8:48 | within normal limits |
| | | | 15:21 | Piloerection |
| | | 15 | 8:21 | within normal limits |
| | | | 8:21 | scheduled sacrifice |
| | 105 | 1 | 7:25 | within normal limits |
| | | | 8:42 | within normal limits |
| | | 2 | 8:14 | within normal limits |
| | | | 9:14 | within normal limits |
| | | 3 | 7:56 | within normal limits |
| | | | 9:02 | within normal limits |
| | | 4 | 7:38 | within normal limits |
| | | | 8:41 | within normal limits |
| | | | 15:04 | within normal limits |
| | | 5 | 7:41 | within normal limits |
| | | | 8:49 | nose: chromodacryorrhea |
| | | | 14:51 | within normal limits |
| | | 6 | 7:32 | within normal limits |
| | | | 8:37 | within normal limits |
| | | | 14:50 | within normal limits |
| | | 7 | 7:49 | within normal limits |
| | | | 8:58 | within normal limits |
| | | | 14:22 | within normal limits |
| | | 8 | 8:12 | within normal limits |
| | | | 9:21 | within normal limits |
| | | | 15:00 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 84 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 6 | 105 | 9 | 7:31 | within normal limits |
| | | | 8:35 | within normal limits |
| | | | 14:20 | within normal limits |
| | | 10 | 7:28 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 15:31 | within normal limits |
| | | 11 | 7:29 | within normal limits |
| | | | 8:35 | within normal limits |
| | | | 14:44 | within normal limits |
| | | 12 | 7:36 | within normal limits |
| | | | 8:46 | within normal limits |
| | | | 14:17 | within normal limits |
| | | 13 | 7:32 | within normal limits |
| | | | 8:34 | within normal limits |
| | | | 14:28 | within normal limits |
| | | 14 | 7:46 | within normal limits |
| | | | 8:48 | within normal limits |
| | | | 15:21 | within normal limits |
| | | 15 | 8:32 | within normal limits |
| | | | 8:32 | scheduled sacrifice |
| | 197 | 1 | 8:15 | within normal limits |
| | | | 9:27 | within normal limits |
| | | 2 | 7:59 | within normal limits |
| | | | 9:02 | within normal limits |
| | | 3 | 7:38 | within normal limits |
| | | | 8:42 | within normal limits |
| | | | 15:05 | within normal limits |
| | | 4 | 7:43 | within normal limits |
| | | | 8:50 | within normal limits |
| | | | 14:51 | within normal limits |
| | | 5 | 7:33 | within normal limits |
| | | | 8:37 | within normal limits |
| | | | 14:50 | within normal limits |
| | | 6 | 7:50 | within normal limits |
| | | | 8:59 | within normal limits |
| | | | 14:22 | within normal limits |
| | | 7 | 8:13 | within normal limits |
| | | | 9:21 | within normal limits |
| | | | 15:00 | within normal limits |
| | | 8 | 7:32 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:21 | within normal limits |
| | | 9 | 7:28 | within normal limits |
| | | | 8:35 | within normal limits |
| | | | 15:31 | within normal limits |
| | | 10 | 7:29 | within normal limits |
| | | | 8:35 | within normal limits |
| | | | 14:44 | within normal limits |
| | | 11 | 7:37 | within normal limits |
| | | | 8:46 | within normal limits |
| | | | 14:20 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 85 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 6 | 197 | 12 | 7:33 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:29 | within normal limits |
| | | 13 | 7:47 | within normal limits |
| | | | 8:48 | within normal limits |
| | | | 15:22 | Lethargic |
| | | 14 | 7:23 | within normal limits |
| | | | 8:43 | within normal limits |
| | | | 15:28 | nose: chromodacryorrhea |
| | | 15 | 6:16 | within normal limits |
| | | | 6:16 | scheduled sacrifice |
| | 199 | 1 | 8:35 | within normal limits |
| | | | 9:36 | within normal limits |
| | | 2 | 7:59 | within normal limits |
| | | | 9:02 | within normal limits |
| | | 3 | 7:39 | within normal limits |
| | | | 8:42 | nose: chromodacryorrhea |
| | | | 15:05 | within normal limits |
| | | 4 | 7:43 | within normal limits |
| | | | 8:50 | within normal limits |
| | | | 14:51 | nose: chromodacryorrhea |
| | | 5 | 7:34 | within normal limits |
| | | | 8:37 | within normal limits |
| | | | 14:51 | within normal limits |
| | | 6 | 7:50 | within normal limits |
| | | | 8:59 | within normal limits |
| | | | 14:22 | within normal limits |
| | | 7 | 8:14 | within normal limits |
| | | | 9:21 | within normal limits |
| | | | 15:01 | within normal limits |
| | | 8 | 7:32 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:21 | within normal limits |
| | | 9 | 7:29 | within normal limits |
| | | | 8:35 | within normal limits |
| | | | 15:31 | within normal limits |
| | | 10 | 7:30 | within normal limits |
| | | | 8:35 | within normal limits |
| | | | 14:44 | within normal limits |
| | | 11 | 7:37 | within normal limits |
| | | | 8:46 | within normal limits |
| | | | 14:20 | within normal limits |
| | | 12 | 7:33 | alopecia: limb(s) |
| | | | 8:37 | alopecia: limb(s) |
| | | | 14:29 | alopecia: limb(s) |
| | | 13 | 7:48 | alopecia: limb(s) |
| | | | 8:49 | alopecia: limb(s) |
| | | | 15:22 | alopecia: limb(s) |
| | | | 15:22 | nose: chromodacryorrhea |

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 6 | 199 | 14 | 7:24 | alopecia: limb(s) |
| | | | 8:43 | alopecia: limb(s) |
| | | | 15:29 | alopecia: limb(s) |
| | | 15 | 6:33 | alopecia: limb(s) |
| | | | 6:33 | scheduled sacrifice |
| | 201 | 1 | 8:36 | within normal limits |
| | | | 9:37 | eye(s): chromodacryorrhea |
| | | 2 | 8:00 | within normal limits |
| | | | 9:03 | within normal limits |
| | | 3 | 7:40 | within normal limits |
| | | | 8:43 | within normal limits |
| | | | 15:05 | within normal limits |
| | | 4 | 7:44 | within normal limits |
| | | | 8:50 | within normal limits |
| | | | 14:52 | within normal limits |
| | | 5 | 7:34 | within normal limits |
| | | | 8:37 | within normal limits |
| | | | 14:51 | within normal limits |
| | | 6 | 7:51 | nose: chromodacryorrhea |
| | | | 8:59 | Lethargic |
| | | | 14:23 | eye(s): chromodacryorrhea |
| | | 7 | 8:14 | nose: chromodacryorrhea |
| | | | 8:15 | rust colored fur: limb(s) |
| | | | 9:22 | rust colored fur: limb(s) |
| | | | 9:22 | Lethargic |
| | | | 9:22 | nose: chromodacryorrhea |
| | | | 15:02 | rust colored fur: limb(s) |
| | | | 15:02 | Lethargic |
| | | | 15:02 | eye(s): chromodacryorrhea |
| | | | 15:02 | nose: chromodacryorrhea |
| | | 8 | 7:34 | rust colored fur: limb(s) |
| | | | 7:34 | nose: chromodacryorrhea |
| | | | 8:37 | rust colored fur: limb(s) |
| | | | 8:37 | Lethargic |
| | | | 8:37 | nose: chromodacryorrhea |
| | | | 14:21 | nose: chromodacryorrhea |
| | | | 14:21 | Ataxia |
| | | 9 | 7:30 | nose: chromodacryorrhea |
| | | | 8:36 | nose: chromodacryorrhea |
| | | | 15:32 | nose: chromodacryorrhea |
| | | 10 | 7:30 | nose: chromodacryorrhea |
| | | | 8:36 | nose: chromodacryorrhea |
| | | | 14:45 | nose: chromodacryorrhea |
| | | 11 | 7:39 | nose: chromodacryorrhea |
| | | | 8:46 | nose: chromodacryorrhea |
| | | | 14:20 | Ataxia |
| | | | 14:20 | nose: chromodacryorrhea |
| | | 12 | 7:34 | nose: chromodacryorrhea |
| | | | 8:37 | nose: chromodacryorrhea |
| | | | 14:29 | nose: chromodacryorrhea |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 87 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 6 | 201 | 13 | 7:49 | nose: chromodacryorrhea |
| | | | 8:49 | nose: chromodacryorrhea |
| | | | 15:22 | nose: chromodacryorrhea |
| | | 14 | 7:27 | nose: chromodacryorrhea |
| | | | 8:43 | nose: chromodacryorrhea |
| | | | 15:29 | nose: chromodacryorrhea |
| | | 15 | 6:50 | nose: chromodacryorrhea |
| | | | 6:50 | scheduled sacrifice |
| | 203 | 1 | 8:37 | eye(s): chromodacryorrhea |
| | | | 9:37 | eye(s): chromodacryorrhea |
| | | 2 | 8:01 | within normal limits |
| | | | 9:03 | within normal limits |
| | | 3 | 7:40 | within normal limits |
| | | | 8:43 | nose: chromodacryorrhea |
| | | | 15:05 | within normal limits |
| | | 4 | 7:45 | within normal limits |
| | | | 8:50 | within normal limits |
| | | | 14:52 | within normal limits |
| | | 5 | 7:35 | within normal limits |
| | | | 8:37 | within normal limits |
| | | | 14:51 | within normal limits |
| | | 6 | 7:52 | within normal limits |
| | | | 8:59 | nose: chromodacryorrhea |
| | | | 14:23 | within normal limits |
| | | 7 | 8:18 | nose: chromodacryorrhea |
| | | | 8:18 | hypothermia, cold to touch |
| | | | 8:19 | Lethargic |
| | | | 8:20 | urine: abnormal color, brown |
| | | | 9:22 | hypothermia, cold to touch |
| | | | 9:22 | nose: chromodacryorrhea |
| | | | 9:22 | Prone |
| | | | 15:02 | hypothermia, cold to touch |
| | | | 15:02 | Prone |
| | | | 15:02 | euthanized moribund |
| | 205 | 1 | 8:38 | within normal limits |
| | | | 9:38 | within normal limits |
| | | 2 | 8:01 | within normal limits |
| | | | 9:04 | within normal limits |
| | | 3 | 7:41 | within normal limits |
| | | | 8:44 | within normal limits |
| | | | 15:05 | within normal limits |
| | | 4 | 7:46 | within normal limits |
| | | | 8:50 | within normal limits |
| | | | 14:52 | within normal limits |
| | | 5 | 7:36 | within normal limits |
| | | | 8:37 | within normal limits |
| | | | 14:51 | within normal limits |
| | | 6 | 7:52 | within normal limits |
| | | | 9:00 | within normal limits |
| | | | 14:23 | within normal limits |

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 6 | 205 | 7 | 8:22 | within normal limits |
| | | | 9:23 | within normal limits |
| | | | 15:03 | within normal limits |
| | | 8 | 7:35 | within normal limits |
| | | | 8:38 | within normal limits |
| | | | 14:22 | within normal limits |
| | | 9 | 7:31 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 15:32 | within normal limits |
| | | 10 | 7:31 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:45 | within normal limits |
| | | 11 | 7:40 | within normal limits |
| | | | 8:47 | within normal limits |
| | | | 14:20 | within normal limits |
| | | 12 | 7:35 | within normal limits |
| | | | 8:38 | within normal limits |
| | | | 14:29 | within normal limits |
| | | 13 | 7:50 | within normal limits |
| | | | 8:51 | within normal limits |
| | | | 15:22 | within normal limits |
| | | 14 | 7:29 | within normal limits |
| | | | 8:44 | within normal limits |
| | | | 15:29 | within normal limits |
| | | 15 | 7:22 | within normal limits |
| | | | 7:22 | scheduled sacrifice |
| | 207 | 1 | 0:00 | within normal limits |
| | | | 8:38 | efflux of the dosing compound |
| | | 2 | 8:02 | within normal limits |
| | | | 9:04 | within normal limits |
| | | 3 | 7:41 | within normal limits |
| | | | 8:44 | within normal limits |
| | | | 15:05 | within normal limits |
| | | 4 | 7:47 | within normal limits |
| | | | 8:50 | nose: chromodacryorrhea |
| | | | 14:52 | within normal limits |
| | | 5 | 7:37 | within normal limits |
| | | | 8:38 | within normal limits |
| | | | 14:51 | within normal limits |
| | | 6 | 7:53 | within normal limits |
| | | | 9:00 | within normal limits |
| | | | 14:23 | within normal limits |
| | | 7 | 8:22 | within normal limits |
| | | | 9:23 | within normal limits |
| | | | 15:03 | within normal limits |
| | | 8 | 7:35 | within normal limits |
| | | | 8:38 | within normal limits |
| | | | 14:22 | within normal limits |
| | | 9 | 7:31 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 15:32 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 89 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 6 | 207 | 10 | 7:32 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:45 | within normal limits |
| | | 11 | 7:40 | within normal limits |
| | | | 8:47 | within normal limits |
| | | | 14:20 | within normal limits |
| | | 12 | 7:36 | within normal limits |
| | | | 8:38 | within normal limits |
| | | | 14:29 | within normal limits |
| | | 13 | 7:50 | within normal limits |
| | | | 8:51 | within normal limits |
| | | | 15:23 | within normal limits |
| | | 14 | 7:29 | within normal limits |
| | | | 8:44 | within normal limits |
| | | | 15:30 | within normal limits |
| | | 15 | 7:40 | within normal limits |
| | | | 7:40 | scheduled sacrifice |
| | 209 | 1 | 8:39 | within normal limits |
| | | | 9:39 | within normal limits |
| | | 2 | 8:02 | within normal limits |
| | | | 9:04 | within normal limits |
| | | 3 | 7:42 | within normal limits |
| | | | 8:44 | within normal limits |
| | | | 15:05 | within normal limits |
| | | 4 | 7:47 | within normal limits |
| | | | 8:50 | nose: chromodacryorrhea |
| | | | 14:53 | within normal limits |
| | | 5 | 7:37 | within normal limits |
| | | | 8:38 | within normal limits |
| | | | 14:51 | within normal limits |
| | | 6 | 7:54 | within normal limits |
| | | | 9:00 | within normal limits |
| | | | 14:24 | within normal limits |
| | | 7 | 8:23 | within normal limits |
| | | | 9:23 | within normal limits |
| | | | 15:03 | within normal limits |
| | | 8 | 7:36 | within normal limits |
| | | | 8:38 | within normal limits |
| | | | 14:22 | within normal limits |
| | | 9 | 7:32 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 15:32 | within normal limits |
| | | 10 | 7:32 | within normal limits |
| | | | 8:36 | within normal limits |
| | | | 14:45 | within normal limits |
| | | 11 | 7:41 | within normal limits |
| | | | 8:47 | within normal limits |
| | | | 14:20 | within normal limits |

Table I-3. Individual Male Clinical Observations During the Exposure Period (page 90 of 90)

| Group[a] | Animal ID | Test Day | Time[b] | Clinical Observation |
|---|---|---|---|---|
| 6 | 209 | 12 | 7:36 | within normal limits |
| | | | 8:38 | within normal limits |
| | | | 14:30 | within normal limits |
| | | 13 | 7:51 | within normal limits |
| | | | 8:51 | within normal limits |
| | | | 15:23 | within normal limits |
| | | 14 | 7:31 | within normal limits |
| | | | 8:44 | within normal limits |
| | | | 15:30 | within normal limits |
| | | 15 | 8:01 | within normal limits |
| | | | 8:01 | scheduled sacrifice |

[a]Groups are 0 is control; 1 is 25 mg/kg/day of Phenobarbital; 2 is 50 mg/kg/day of Phenobarbital; 3 is 100 mg/kg/day of Phenobarbital; 4 is 50 mg/kg/day of Linuron; 5 is 100 mg/kg/day of Linuron; and 6 is 150 mg/kg/day of Linuron.

[b]Time at which clinical observation was seen. A time of “0:00” indicates that the observation was edited.

Table I-4. Individual Male Necropsy Weights (g) (page 1 of 4)

| Group[a] | Animal ID | Thyroid | Liver | Left Testis | Right Testis | Paired Testis | Paired Epididymis | Prostate | Seminal Vesicles with Fluid and Coagulating Gland | Accessory Sex Gland[b] |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 1 | 0.0196 | 15.1968 | 1.8748 | 1.9250 | 3.7998 | 1.0392 | 0.7887 | 0.9118 | 1.7005 |
| | 3 | 0.0202 | 15.0023 | 1.7717 | 1.6773 | 3.4490 | 1.2369 | 1.0671 | 1.2978 | 2.3649 |
| | 5 | 0.0166 | 14.1308 | 1.4759 | 1.5162 | 2.9921 | 0.9541 | 1.0147 | 0.9946 | 2.0093 |
| | 7 | 0.0185 | 13.6104 | 1.5280 | 1.5248 | 3.0528 | 1.0543 | 0.9148 | 1.1453 | 2.0601 |
| | 9 | 0.0145 | 15.5187 | 1.9363 | 1.8931 | 3.8294 | 1.2192 | 1.2103 | 1.5181 | 2.7284 |
| | 11 | 0.0153 | 14.3236 | 1.5827 | 1.5914 | 3.1741 | 1.1007 | 0.7586 | 1.3501 | 2.1087 |
| | 13 | 0.0161 | 16.4537 | 1.8380 | 1.7986 | 3.6366 | 1.2656 | 0.9465 | 1.2791 | 2.2256 |
| | 15 | 0.0153 | 16.1255 | 1.8256 | 1.8012 | 3.6268 | 1.1856 | 1.0842 | 1.1391 | 2.2233 |
| | 107 | 0.0185 | 18.1688 | 1.5389 | 1.4684 | 3.0073 | 1.1762 | 0.7495 | 1.3140 | 2.0635 |
| | 109 | 0.0157 | 15.7131 | 1.6498 | 1.6486 | 3.2984 | 1.2073 | 1.1736 | 1.3941 | 2.5677 |
| | 111 | 0.0162 | 14.6785 | 1.5934 | 1.5504 | 3.1438 | 1.1301 | 0.8088 | 1.1516 | 1.9604 |
| | 113 | 0.0200 | 16.0578 | 1.5455 | 1.5548 | 3.1003 | 1.1144 | 1.0114 | 1.0266 | 2.0380 |
| | 115 | 0.0139 | 13.4479 | 1.6268 | 1.6530 | 3.2798 | 1.0314 | 0.9308 | 1.1289 | 2.0597 |
| | 117 | 0.0196 | 15.7874 | 1.5760 | 1.5766 | 3.1526 | 1.0849 | 1.0374 | 1.4025 | 2.4399 |
| | 119 | 0.0170 | 17.3374 | 1.3720 | 1.4721 | 2.8441 | 0.9007 | 1.0186 | 1.2127 | 2.2313 |
| 1 | 17 | 0.0165 | 17.6667 | 1.5148 | 1.4347 | 2.9495 | 1.1135 | 0.7375 | 1.0824 | 1.8199 |
| | 19 | 0.0161 | 16.0443 | 1.6965 | 1.6434 | 3.3399 | 1.2558 | 0.7860 | 1.2935 | 2.0795 |
| | 21 | 0.0208 | 17.4161 | 1.7025 | 1.6728 | 3.3753 | 1.1669 | 1.2186 | 1.5721 | 2.7907 |
| | 23 | 0.0203 | 15.1762 | 1.5724 | 1.5435 | 3.1159 | 1.0716 | 1.0107 | 1.3097 | 2.3204 |
| | 25 | 0.0193 | 15.0507 | 1.5497 | 1.5062 | 3.0559 | 1.1505 | 0.7428 | 1.1053 | 1.8481 |
| | 27 | 0.0241 | 20.2024 | 1.6269 | 1.6713 | 3.2982 | 1.1098 | 1.1310 | 1.3197 | 2.4507 |
| | 29 | 0.0321 | 21.0784 | 1.5386 | 1.5512 | 3.0898 | 1.0082 | 1.0692 | 1.0484 | 2.1176 |
| | 121 | 0.0149 | 18.1587 | 1.6364 | 1.6164 | 3.2528 | 1.1747 | 0.9845 | 1.5798 | 2.5643 |
| | 123 | 0.0251 | 15.4726 | 1.6110 | 1.6377 | 3.2487 | 1.1415 | 0.9441 | 1.1932 | 2.1373 |
| | 125 | 0.0192 | 17.6126 | 1.8049 | 1.8394 | 3.6443 | 1.1950 | 1.2494 | 1.3038 | 2.5532 |
| | 127 | 0.0227 | 20.3696 | 1.7443 | 1.7779 | 3.5222 | 1.1824 | 1.2466 | 1.4581 | 2.7047 |
| | 129 | 0.0106 | 18.2710 | 1.8216 | 1.7628 | 3.5844 | 1.0540 | 0.7517 | 1.0796 | 1.8313 |
| | 131 | 0.0212 | 18.5197 | 1.6099 | 1.5855 | 3.1954 | 1.2355 | 0.9207 | 1.7398 | 2.6605 |
| | 133 | 0.0194 | 16.4800 | 1.6551 | 1.6810 | 3.3361 | 1.0788 | 1.1059 | 1.5005 | 2.6064 |
| | 135 | 0.0248 | 21.0739 | 1.7375 | 1.7068 | 3.4443 | 1.1057 | 0.9825 | 1.3087 | 2.2912 |

Table I-4. Individual Male Necropsy Weights (g) (page 2 of 4)

| Group[a] | Animal ID | Thyroid | Liver | Left Testis | Right Testis | Paired Testis | Paired Epididymis | Prostate | Seminal Vesicles with Fluid and Coagulating Gland | Accessory Sex Gland[b] |
|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 31 | 0.0225 | 18.4116 | 1.4765 | 1.4629 | 2.9394 | 1.0838 | 1.0508 | 1.2799 | 2.3307 |
| | 33 | 0.0215 | 20.4432 | 1.7874 | 1.7816 | 3.5690 | 1.1548 | 1.3325 | 1.6305 | 2.9630 |
| | 35 | 0.0290 | 18.6340 | 1.8917 | 1.8266 | 3.7183 | 1.3647 | 1.0707 | 1.9626 | 3.0333 |
| | 37 | 0.0279 | 21.3350 | 1.8435 | 1.8649 | 3.7084 | 1.1397 | 0.9048 | 1.1069 | 2.0117 |
| | 39 | 0.0197 | 19.8723 | 1.6468 | 1.5949 | 3.2417 | 1.0139 | 0.7476 | 1.4750 | 2.2226 |
| | 41 | 0.0275 | 18.5491 | 1.6214 | 1.5970 | 3.2184 | 1.1720 | 1.1892 | 1.6336 | 2.8228 |
| | 43 | .[c] | 21.7569 | 1.5443 | 1.5698 | 3.1141 | 1.1589 | 1.0947 | 1.0648 | 2.1595 |
| | 45 | 0.0216 | 20.0548 | 1.5104 | 1.5658 | 3.0762 | 1.1594 | 1.3622 | 1.2262 | 2.5884 |
| | 137 | 0.0240 | 17.3282 | 1.6333 | 1.6214 | 3.2547 | 1.0941 | 0.9661 | 1.2394 | 2.2055 |
| | 139 | 0.0241 | 19.6790 | 1.7480 | 1.8171 | 3.5651 | 1.0961 | 0.8422 | 1.0568 | 1.8990 |
| | 141 | 0.0189 | 18.1850 | 1.4287 | 1.4492 | 2.8779 | 1.1173 | 1.1726 | 1.2412 | 2.4138 |
| | 143 | 0.0205 | 16.4504 | 1.6944 | 1.6895 | 3.3839 | 1.0822 | 0.8724 | 1.2717 | 2.1441 |
| | 145 | 0.0175 | 19.7689 | 1.4803 | 1.5390 | 3.0193 | 1.1100 | 0.8967 | 1.4065 | 2.3032 |
| | 147 | 0.0249 | 21.3187 | 1.8832 | 1.7351 | 3.6183 | 1.1204 | 0.9325 | 1.1710 | 2.1035 |
| | 149 | 0.0194 | 23.3644 | 1.7433 | 1.8133 | 3.5566 | 1.2431 | 0.8508 | .[d] | .[d] |
| 3 | 47 | 0.0266 | 18.1965 | 1.5542 | 1.4732 | 3.0274 | 1.2303 | 0.8619 | 1.4273 | 2.2892 |
| | 49 | 0.0263 | 17.1087 | 1.8537 | 1.8238 | 3.6775 | 1.3114 | 0.9474 | 2.3817 | 3.3291 |
| | 51[e] | | | | | | | | | |
| | 53 | 0.0257 | 19.4886 | 1.6974 | 1.7532 | 3.4506 | 1.0988 | 1.1925 | 0.9667 | 2.1592 |
| | 55 | 0.0222 | 15.2849 | 1.7516 | 1.7091 | 3.4607 | 1.1207 | 0.8424 | 0.6689 | 1.5113 |
| | 57 | .[c] | 22.4752 | 1.5845 | 1.5621 | 3.1466 | 1.0090 | 1.0615 | 1.7446 | 2.8061 |
| | 59 | 0.0169 | 17.0189 | 1.8680 | 1.8157 | 3.6837 | 1.2762 | 1.3328 | 2.0016 | 3.3344 |
| | 151 | 0.0253 | 17.7212 | 1.8176 | 1.8507 | 3.6683 | 1.2751 | 1.2414 | 1.4888 | 2.7302 |
| | 153 | 0.0271 | 18.9710 | 1.5872 | 1.6406 | 3.2278 | 1.0856 | 1.1896 | 1.3686 | 2.5582 |
| | 155 | 0.0211 | 14.3656 | 1.4572 | 1.4221 | 2.8793 | 1.0500 | 0.9449 | 1.7144 | 2.6593 |
| | 157 | 0.0119 | 17.2448 | 1.6065 | 1.6132 | 3.2197 | 1.0086 | 0.7563 | 1.2050 | 1.9613 |
| | 159 | 0.0205 | 17.4653 | 1.5819 | 1.6650 | 3.2469 | 1.0913 | 0.9603 | 0.9894 | 1.9497 |
| | 161 | 0.0251 | 20.3156 | 1.7884 | 1.7822 | 3.5706 | 1.0744 | 1.1022 | 1.6219 | 2.7241 |
| | 163 | 0.0261 | 21.2907 | 1.7513 | 1.6500 | 3.4013 | 1.1907 | 1.0588 | 1.3390 | 2.3978 |
| | 165[f] | | | | | | | | | |

Table I-4. Individual Male Necropsy Weights (g) (page 3 of 4)

| Group[a] | Animal ID | Thyroid | Liver | Left Testis | Right Testis | Paired Testis | Paired Epididymis | Prostate | Seminal Vesicles with Fluid and Coagulating Gland | Accessory Sex Gland[b] |
|---|---|---|---|---|---|---|---|---|---|---|
| 4 | 61 | 0.0168 | 13.7791 | 1.8240 | 1.7775 | 3.6015 | 1.0027 | 0.8962 | 1.0137 | 1.9099 |
| | 63 | 0.0214 | 14.3487 | 1.5590 | 1.5525 | 3.1115 | 1.0626 | 0.9428 | 1.2532 | 2.1960 |
| | 65 | 0.0192 | 14.0587 | 1.7417 | 1.8074 | 3.5491 | 1.1719 | 0.7297 | 1.1258 | 1.8555 |
| | 67 | 0.0227 | 18.4722 | 1.6870 | 1.6932 | 3.3802 | 1.1888 | 0.9875 | 1.3669 | 2.3544 |
| | 69 | 0.0166 | 14.3782 | 1.7259 | 1.6897 | 3.4156 | 1.1022 | 0.5708 | 1.7789 | 2.3497 |
| | 71 | 0.0170 | 14.9399 | 1.7487 | 1.7261 | 3.4748 | 1.1253 | 0.8636 | 1.4415 | 2.3051 |
| | 73 | 0.0177 | 13.8906 | 1.5359 | 1.4874 | 3.0233 | 1.0306 | 0.9823 | 1.0436 | 2.0259 |
| | 75 | 0.0210 | 15.2154 | 1.6733 | 1.7141 | 3.3874 | 0.9655 | 0.9026 | 0.8329 | 1.7355 |
| | 167 | 0.0154 | 12.7321 | 1.5659 | 1.5802 | 3.1461 | 1.1461 | 1.1970 | 1.0931 | 2.2901 |
| | 169 | 0.0149 | 13.2150 | 1.5639 | 1.4803 | 3.0442 | 1.0493 | 0.9241 | 0.9229 | 1.8470 |
| | 171 | 0.0206 | 15.9246 | 1.8077 | 1.7933 | 3.6010 | 1.2101 | 0.8159 | 2.0310 | 2.8469 |
| | 173 | 0.0168 | 15.3303 | 1.5963 | 1.6264 | 3.2227 | 1.0664 | 0.6992 | 1.5493 | 2.2485 |
| | 175 | 0.0137 | 14.3851 | 1.7324 | 1.7448 | 3.4772 | 1.2782 | 0.9341 | 0.9746 | 1.9087 |
| | 177 | 0.0177 | 16.0016 | 1.7304 | 1.7931 | 3.5235 | 1.1244 | 0.7519 | 1.0158 | 1.7677 |
| | 179 | 0.0203 | 17.6877 | 1.5986 | 1.5775 | 3.1761 | 1.0057 | 1.3280 | 1.4337 | 2.7617 |
| 5 | 77 | 0.0188 | 11.4769 | 1.5087 | 1.5108 | 3.0195 | 0.9708 | 0.7811 | 1.1731 | 1.9542 |
| | 79 | 0.0184 | 13.8915 | 1.8167 | 1.8538 | 3.6705 | 1.1413 | 1.1976 | 1.0394 | 2.2370 |
| | 81 | 0.0155 | 12.5978 | 1.5143 | 1.5543 | 3.0686 | 1.0578 | 0.7507 | 1.5121 | 2.2628 |
| | 83 | 0.0198 | 12.8665 | 1.6056 | 1.5616 | 3.1672 | 0.9798 | 0.7971 | 1.4985 | 2.2956 |
| | 85 | .[c] | 15.0316 | 1.7466 | 1.7163 | 3.4629 | 0.8701 | 0.8140 | 1.1440 | 1.9580 |
| | 87 | 0.0140 | 12.0765 | 1.4799 | 1.4680 | 2.9479 | 1.1730 | 0.7839 | 1.2156 | 1.9995 |
| | 89 | 0.0226 | 14.0068 | 1.6915 | 1.6850 | 3.3765 | 0.9853 | 0.6434 | 0.9108 | 1.5542 |
| | 181 | 0.0134 | 13.2393 | 1.6199 | 1.5868 | 3.2067 | 0.9853 | 0.9596 | 1.4248 | 2.3844 |
| | 183 | 0.0117 | 11.4020 | 1.5518 | 1.5513 | 3.1031 | 0.9359 | 0.7849 | 0.4322 | 1.2171 |
| | 185 | 0.0174 | 12.7445 | 1.6576 | 1.6458 | 3.3034 | 1.0677 | 0.7561 | .[d] | .[d] |
| | 187 | 0.0178 | 13.7520 | 1.6374 | 1.6812 | 3.3186 | 0.9611 | 0.7882 | 1.0058 | 1.7940 |
| | 189 | 0.0157 | 14.5664 | 1.8940 | 1.8695 | 3.7635 | 1.1971 | 0.8669 | 0.7464 | 1.6133 |
| | 191 | 0.0141 | 13.8589 | 1.6300 | 1.6816 | 3.3116 | 1.0633 | 0.9877 | 1.0670 | 2.0547 |
| | 193 | 0.0202 | 14.1288 | 1.5527 | 1.5809 | 3.1336 | 0.9540 | 0.7850 | 0.8352 | 1.6202 |
| | 195 | 0.0199 | 15.2459 | 1.5201 | 1.5537 | 3.0738 | 0.9215 | 0.7894 | 0.9959 | 1.7853 |

Table I-4. Individual Male Necropsy Weights (g) (page 4 of 4)

| Group[a] | Animal ID | Thyroid | Liver | Left Testis | Right Testis | Paired Testis | Paired Epididymis | Prostate | Seminal Vesicles with Fluid and Coagulating Gland | Accessory Sex Gland[b] |
|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 91 | 0.0197 | 14.2699 | 1.6419 | 1.6903 | 3.3322 | 1.0793 | 1.0284 | 1.2468 | 2.2752 |
| | 93 | 0.0166 | 12.1855 | 1.7391 | 1.7198 | 3.4589 | 1.0111 | 0.8996 | 1.1749 | 2.0745 |
| | 95 | 0.0145 | 11.3428 | 1.6328 | 1.6551 | 3.2879 | 1.0465 | 0.7244 | 0.9131 | 1.6375 |
| | 97 | 0.0175 | 10.7068 | 1.3807 | 1.3329 | 2.7136 | 0.9616 | 0.8777 | 0.9192 | 1.7969 |
| | 99 | 0.0172 | 11.3927 | 1.4938 | 1.4006 | 2.8944 | 0.8049 | 0.5241 | 0.6478 | 1.1719 |
| | 101 | 0.0178 | 15.9429 | 1.4739 | 1.4918 | 2.9657 | 0.8913 | 0.8829 | 1.1280 | 2.0109 |
| | 103 | 0.0187 | 13.1163 | 1.6735 | 1.6712 | 3.3447 | 1.0444 | 0.5583 | 0.5919 | 1.1502 |
| | 105 | 0.0189 | 14.0625 | 1.4820 | 1.4832 | 2.9652 | 0.9633 | 0.5539 | 1.0950 | 1.6489 |
| | 197 | 0.0154 | 13.0023 | 1.5192 | 1.5164 | 3.0356 | 0.9255 | 0.6440 | 0.6633 | 1.3073 |
| | 199 | 0.0151 | 11.5767 | 1.5692 | 1.5234 | 3.0926 | 0.8993 | 0.6045 | 0.6123 | 1.2168 |
| | 201 | 0.0169 | 11.7042 | 1.6070 | 1.5774 | 3.1844 | 1.1606 | 0.7158 | 1.0366 | 1.7524 |
| | 203[g] | | | | | | | | | |
| | 205 | 0.0156 | 13.5539 | 1.9129 | 2.0607 | 3.9736 | 1.2068 | 0.8992 | 1.0254 | 1.9246 |
| | 207 | 0.0176 | 12.7921 | 1.6469 | 1.6906 | 3.3375 | 1.0212 | 0.8041 | 0.8395 | 1.6436 |
| | 209 | 0.0252 | 16.2763 | 1.7598 | 1.6931 | 3.4529 | 1.1348 | 0.8167 | 1.2390 | 2.0557 |

[a]Groups are 0 is control; 1 is 25 mg/kg/day of Phenobarbital; 2 is 50 mg/kg/day of Phenobarbital; 3 is 100 mg/kg/day of Phenobarbital; 4 is 50 mg/kg/day of Linuron; 5 is 100 mg/kg/day of Linuron; and 6 is 150 mg/kg/day of Linuron.

[b]Accessory sex gland includes the prostate and the seminal vesicles with fluid and coagulating gland.

[c]Part or all of the tissue for this animal was not present in the tissue cup at the time of weighing the fixed tissue.

[d]The seminal vesicles were nicked causing fluid to leak, therefore an accurate weight could not be obtained.

[e]Male was euthanized moribund on test day 3.

[f]Male was euthanized moribund on test day 5.

[g]Male was euthanized moribund on test day 7.

Table I-5. Individual Male Macroscopic Necropsy Findings (page 1 of 3)

## A. Scheduled Sacrifice

| Group[a] | Animal ID | Test Day | Macroscopic Necropsy Finding |
|---|---|---|---|
| 0 | 1 | 15 | no remarkable observations |
| | 3 | 15 | no remarkable observations |
| | 5 | 15 | no remarkable observations |
| | 7 | 15 | no remarkable observations |
| | 9 | 15 | no remarkable observations |
| | 11 | 15 | no remarkable observations |
| | 13 | 15 | no remarkable observations |
| | 15 | 15 | kidney: hydronephrosis right |
| | 107 | 15 | no remarkable observations |
| | 109 | 15 | no remarkable observations |
| | 111 | 15 | no remarkable observations |
| | 113 | 15 | no remarkable observations |
| | 115 | 15 | no remarkable observations |
| | 117 | 15 | no remarkable observations |
| | 119 | 15 | no remarkable observations |
| 1 | 17 | 15 | no remarkable observations |
| | 19 | 15 | no remarkable observations |
| | 21 | 15 | no remarkable observations |
| | 23 | 15 | no remarkable observations |
| | 25 | 15 | no remarkable observations |
| | 27 | 15 | no remarkable observations |
| | 29 | 15 | skin: alopecia limb (s) |
| | 121 | 15 | no remarkable observations |
| | 123 | 15 | no remarkable observations |
| | 125 | 15 | no remarkable observations |
| | 127 | 15 | skin: alopecia limb (s) |
| | 129 | 15 | no remarkable observations |
| | 131 | 15 | no remarkable observations |
| | 133 | 15 | no remarkable observations |
| | 135 | 15 | no remarkable observations |
| 2 | 31 | 15 | no remarkable observations |
| | 33 | 15 | no remarkable observations |
| | 35 | 15 | no remarkable observations |
| | 37 | 15 | no remarkable observations |
| | 39 | 15 | no remarkable observations |
| | 41 | 15 | no remarkable observations |
| | 43 | 15 | no remarkable observations |
| | 45 | 15 | no remarkable observations |
| | 137 | 15 | no remarkable observations |
| | 139 | 15 | no remarkable observations |
| | 141 | 15 | no remarkable observations |
| | 143 | 15 | no remarkable observations |
| | 145 | 15 | no remarkable observations |
| | 147 | 15 | no remarkable observations |
| | 149 | 15 | seminal vesicles with coagulating glands: fluid leaked |

**A. Scheduled Sacrifice**

| Group[a] | Animal ID | Test Day | Macroscopic Necropsy Finding |
|---|---|---|---|
| 3 | 47 | 15 | no remarkable observations |
| | 49 | 15 | no remarkable observations |
| | 53 | 15 | no remarkable observations |
| | 55 | 15 | no remarkable observations |
| | 57 | 15 | no remarkable observations |
| | 59 | 15 | no remarkable observations |
| | 151 | 15 | no remarkable observations |
| | 153 | 15 | no remarkable observations |
| | 155 | 15 | prostate, ventral: missing left lobe |
| | 157 | 15 | no remarkable observations |
| | 159 | 15 | no remarkable observations |
| | 161 | 15 | no remarkable observations |
| | 163 | 15 | no remarkable observations |
| 4 | 61 | 15 | no remarkable observations |
| | 63 | 15 | no remarkable observations |
| | 65 | 15 | no remarkable observations |
| | 67 | 15 | no remarkable observations |
| | 69 | 15 | no remarkable observations |
| | 71 | 15 | no remarkable observations |
| | 73 | 15 | no remarkable observations |
| | 75 | 15 | no remarkable observations |
| | 167 | 15 | no remarkable observations |
| | 169 | 15 | no remarkable observations |
| | 171 | 15 | no remarkable observations |
| | 173 | 15 | skin: alopecia limb (s) |
| | 175 | 15 | no remarkable observations |
| | 177 | 15 | no remarkable observations |
| | 179 | 15 | no remarkable observations |
| 5 | 77 | 15 | no remarkable observations |
| | 79 | 15 | no remarkable observations |
| | 81 | 15 | no remarkable observations |
| | 83 | 15 | no remarkable observations |
| | 85 | 15 | no remarkable observations |
| | 87 | 15 | no remarkable observations |
| | 89 | 15 | no remarkable observations |
| | 181 | 15 | no remarkable observations |
| | 183 | 15 | seminal vesicles with coagulating glands: reduced bilateral |
| | 185 | 15 | seminal vesicles with coagulating glands: fluid leaked |
| | 187 | 15 | no remarkable observations |
| | 189 | 15 | no remarkable observations |
| | 191 | 15 | no remarkable observations |
| | 193 | 15 | no remarkable observations |
| | 195 | 15 | no remarkable observations |

**A. Scheduled Sacrifice**

| Group[a] | Animal ID | Test Day | Macroscopic Necropsy Finding |
|---|---|---|---|
| 6 | 91 | 15 | no remarkable observations |
| | 93 | 15 | no remarkable observations |
| | 95 | 15 | no remarkable observations |
| | 97 | 15 | no remarkable observations |
| | 99 | 15 | no remarkable observations |
| | 101 | 15 | no remarkable observations |
| | 103 | 15 | kidney: hydronephrosis right |
| | 105 | 15 | no remarkable observations |
| | 197 | 15 | no remarkable observations |
| | 199 | 15 | no remarkable observations |
| | 201 | 15 | no remarkable observations |
| | 205 | 15 | no remarkable observations |
| | 207 | 15 | no remarkable observations |
| | 209 | 15 | no remarkable observations |

**B. Unscheduled Sacrifice**

| Group[a] | Animal ID | Test Day | Macroscopic Necropsy Finding |
|---|---|---|---|
| 3 | 51 | 3 | urinary bladder: cyst |
| | 165 | 5 | kidney: hydronephrosis right |
| | | | stomach: no food present |
| 6 | 203 | 7 | intestines, small: no ingesta |
| | | | intestines, large: hard dry feces |
| | | | stomach: abnormally distended with food |
| | | | testes: flaccid left |
| | | | urinary bladder: thickened |
| | | | urinary bladder: calculi present |

[a]Groups are 0 is control; 1 is 25 mg/kg/day of Phenobarbital; 2 is 50 mg/kg/day of Phenobarbital; 3 is 100 mg/kg/day of Phenobarbital; 4 is 50 mg/kg/day of Linuron; 5 is 100 mg/kg/day of Linuron; and 6 is 150 mg/kg/day of Linuron.

Table I-6. Individual Male Hormone Data (page 1 of 4)

| Group[a] | Animal ID | Serum Testosterone (ng/ml) | Luteinizing Hormone (ng/ml) | Thyroid Stimulating Hormone (ng/ml) | Thyroxine (µg/dL) | Triiodothyronine (ng/dL) | Follicle Stimulating Hormone (ng/ml) | Estradiol (pg/ml) | Prolactin (ng/ml) | Dihydrotestosterone (pg/ml) |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 1 | 8.53 | 1.28 | 11.09 | 5.88 | 105.85 | 13.32 | 24.93 | 2.34 | 450.93 |
| | 3 | 1.95 | 1.39 | 15.30 | 4.96 | 71.35 | 15.63 | 22.03 | 7.12 | 159.35 |
| | 5 | 0.83 | .[b] | 59.41 | 5.67 | 86.81 | 16.94 | 22.49 | 12.31 | 120.24 |
| | 7 | 3.18 | .[b] | 18.65 | 5.10 | 96.04 | 15.17 | 24.93 | 66.99 | 226.73 |
| | 9 | 1.43 | 1.82 | 13.44 | 6.45 | 81.05 | 14.75 | 28.61 | 5.90 | 138.61 |
| | 11 | 3.48 | 1.28 | 13.63 | 5.91 | 108.03 | 15.72 | 29.36 | 4.70 | 195.28 |
| | 13 | 1.01 | 1.45 | 16.53 | 5.96 | 91.91 | 14.96 | 25.73 | 8.40 | 106.48 |
| | 15 | 0.23 | 1.22 | 10.60 | 7.74 | 108.66 | 18.50 | 30.19 | 16.33 | 82.14 |
| | 107 | 1.63 | 0.84 | 17.92 | 5.55 | 92.15 | 11.94 | 26.61 | 6.51 | 170.04 |
| | 109 | 1.88 | 0.82 | 12.44 | 5.18 | 87.89 | 15.81 | 18.16 | 1.73 | 131.85 |
| | 111 | 10.92 | 1.33 | 21.40 | 4.78 | 67.34 | 15.05 | 16.71 | 9.86 | 542.44 |
| | 113 | 2.81 | 1.17 | 13.04 | 6.09 | 80.70 | 12.03 | 19.86 | 7.71 | 197.50 |
| | 115 | 4.28 | 1.29 | 11.66 | 5.28 | 81.67 | 19.67 | 18.62 | 4.10 | 278.27 |
| | 117 | 4.62 | 1.28 | 29.64 | 4.33 | 81.53 | 15.54 | 18.23 | 3.42 | 245.08 |
| | 119 | 3.84 | 1.50 | 13.22 | 4.38 | 71.93 | 15.76 | 17.82 | 5.11 | 245.35 |
| 1 | 17 | 0.96 | 0.96 | 32.30 | 4.64 | 66.62 | 11.91 | 30.10 | 7.66 | 140.12 |
| | 19 | 5.45 | 1.30 | 16.31 | 5.31 | 91.12 | 12.73 | 28.97 | 11.78 | 278.43 |
| | 21 | 2.33 | 1.29 | 16.31 | 3.98 | 76.42 | 17.62 | 24.97 | 5.48 | 217.20 |
| | 23 | 2.48 | 1.08 | 32.99 | 3.99 | 77.43 | 12.98 | 37.84 | 3.85 | 261.08 |
| | 25 | 2.08 | 1.43 | 26.40 | 4.78 | 73.56 | 10.87 | 33.44 | 8.89 | 154.88 |
| | 27 | 0.82 | 1.86 | 15.49 | 5.12 | 108.15 | 9.62 | 28.95 | 3.72 | 104.07 |
| | 29 | 0.51 | 1.17 | 21.09 | 5.34 | 72.21 | 9.74 | 27.97 | 2.71 | 93.24 |
| | 121 | 2.75 | 1.06 | 18.64 | 4.18 | 76.70 | 10.69 | 33.85 | 8.86 | 161.55 |
| | 123 | 4.30 | 1.54 | 14.77 | 4.26 | 81.74 | 11.13 | 23.14 | 15.43 | 232.11 |
| | 125 | 4.27 | 1.11 | 25.41 | 4.24 | 48.41 | 10.04 | 25.29 | 2.47 | 290.73 |
| | 127 | 1.86 | 1.48 | 33.90 | 4.25 | 72.12 | 14.40 | 22.00 | 1.99 | 166.65 |
| | 129 | 4.58 | 0.96 | 18.16 | 5.54 | 90.01 | 11.95 | 19.63 | 9.69 | 272.28 |
| | 131 | 2.59 | .[b] | 16.89 | 5.02 | 87.69 | 12.71 | 18.93 | 6.18 | 193.82 |
| | 133 | 1.78 | 1.40 | 14.32 | 4.28 | 62.35 | 11.87 | 19.69 | 6.50 | 160.26 |
| | 135 | 2.57 | 1.28 | 22.39 | 4.91 | 89.00 | 11.83 | 20.21 | 1.51 | 145.52 |

Table I-6. Individual Male Hormone Data (page 2 of 4)

| Group[a] | Animal ID | Serum Testosterone (ng/ml) | Luteinizing Hormone (ng/ml) | Thyroid Stimulating Hormone (ng/ml) | Thyroxine (µg/dL) | Triiodothyronine (ng/dL) | Follicle Stimulating Hormone (ng/ml) | Estradiol (pg/ml) | Prolactin (ng/ml) | Dihydrotestosterone (pg/ml) |
|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 31 | 3.08 | 1.28 | 24.28 | 4.31 | 70.99 | 13.04 | 36.03 | 1.93 | 227.92 |
| | 33 | 3.24 | 1.31 | 16.50 | 4.78 | 72.40 | 13.45 | 29.14 | 3.63 | 228.22 |
| | 35 | 1.91 | 1.78 | 52.16 | 3.53 | 56.79 | 13.88 | 39.73 | 10.03 | 169.45 |
| | 37 | 1.09 | 1.18 | 37.19 | 3.56 | 61.03 | 10.74 | 34.27 | 6.74 | 140.81 |
| | 39 | 1.39 | 1.29 | 24.37 | 4.31 | 81.72 | 10.56 | 39.94 | 5.02 | 119.03 |
| | 41 | 1.53 | 1.05 | 26.10 | 4.44 | 79.18 | 11.15 | 35.34 | 4.08 | 138.95 |
| | 43 | 0.57 | 0.88 | 21.23 | 5.73 | 87.50 | 12.57 | 33.20 | 2.19 | 92.00 |
| | 45 | 1.78 | 1.41 | 14.11 | 3.57 | 68.57 | 14.04 | 43.14 | 8.04 | 153.84 |
| | 137 | 1.16 | 1.07 | 23.43 | 4.11 | 70.39 | 11.85 | 36.17 | 5.00 | 139.24 |
| | 139 | 9.61 | .[b] | 34.70 | 5.01 | 71.75 | 14.28 | 23.14 | 10.31 | 442.91 |
| | 141 | 4.87 | 1.32 | 18.73 | 4.45 | 66.94 | 16.61 | 21.80 | 4.56 | 256.02 |
| | 143 | 1.69 | .[b] | 29.69 | 4.02 | 80.97 | 10.44 | 19.66 | 1.12 | 166.28 |
| | 145 | 1.54 | 0.90 | 25.03 | 4.74 | 76.03 | 11.89 | 20.08 | 2.01 | 182.05 |
| | 147 | 3.97 | 1.41 | 28.82 | 4.11 | 79.71 | 12.01 | 20.98 | 1.85 | 325.07 |
| | 149 | 2.17 | 1.21 | 22.68 | 4.18 | 75.17 | 12.11 | 25.97 | 4.52 | 148.47 |
| 3 | 47 | 1.79 | 0.98 | 15.86 | 3.33 | 75.59 | 11.32 | 25.76 | 1.79 | 252.68 |
| | 49 | 2.72 | .[b] | 29.77 | 2.68 | 57.39 | 10.75 | 45.33 | 2.47 | 247.92 |
| | 51 | .[c] | | | | | | | | |
| | 53 | 0.66 | 1.20 | 20.77 | 3.50 | 63.13 | 12.01 | 57.45 | 2.25 | 109.25 |
| | 55 | 0.67 | 1.02 | 10.96 | 2.55 | 59.89 | 9.66 | 40.75 | 1.81 | 97.96 |
| | 57 | 1.26 | 1.22 | 19.86 | 3.00 | 64.11 | 12.39 | 28.84 | 2.99 | 138.44 |
| | 59 | 0.63 | 1.32 | 43.38 | 1.77 | 37.59 | 11.85 | 34.29 | 6.99 | 107.82 |
| | 151 | 1.78 | 1.21 | 26.18 | 3.45 | 56.57 | 10.76 | 38.19 | 16.32 | 168.16 |
| | 153 | 7.27 | 1.21 | 28.44 | 3.15 | 64.38 | 12.42 | 25.61 | 3.84 | 335.09 |
| | 155 | 1.94 | 1.00 | 58.20 | 2.20 | 45.01 | 13.77 | 23.94 | 2.29 | 221.62 |
| | 157 | 1.22 | 0.88 | 16.20 | 3.19 | 60.05 | 13.15 | 31.26 | 2.75 | 127.49 |
| | 159 | 3.01 | 1.24 | 22.31 | 4.26 | 61.25 | 12.34 | 23.35 | 6.45 | 176.40 |
| | 161 | 6.70 | 1.46 | 20.66 | 2.99 | 60.40 | 11.46 | 20.39 | 1.25 | 416.02 |
| | 163 | 4.90 | 1.46 | 30.39 | 2.56 | 50.46 | 11.55 | 29.89 | 5.99 | 281.78 |
| | 165 | .[d] | | | | | | | | |

Table I-6. Individual Male Hormone Data (page 3 of 4)

| Group[a] | Animal ID | Serum Testosterone (ng/ml) | Luteinizing Hormone (ng/ml) | Thyroid Stimulating Hormone (ng/ml) | Thyroxine (µg/dL) | Triiodothyronine (ng/dL) | Follicle Stimulating Hormone (ng/ml) | Estradiol (pg/ml) | Prolactin (ng/ml) | Dihydrotestosterone (pg/ml) |
|---|---|---|---|---|---|---|---|---|---|---|
| 4 | 61 | 6.50 | 0.89 | 19.16 | 4.27 | 109.66 | 18.58 | 35.09 | 5.64 | 528.79 |
| | 63 | 3.97 | 1.38 | 9.16 | 3.84 | 88.11 | 20.01 | 36.72 | 7.49 | 292.36 |
| | 65 | 3.07 | 1.84 | 25.87 | 3.53 | 73.83 | 13.86 | 34.75 | 9.05 | 262.40 |
| | 67 | 2.12 | 1.88 | 36.28 | 3.60 | 100.60 | 14.88 | 50.60 | 25.45 | 194.19 |
| | 69 | 3.06 | 1.45 | 11.39 | 4.03 | 80.58 | 19.50 | 42.17 | 4.07 | 207.21 |
| | 71 | 5.28 | 1.28 | 17.66 | 4.36 | 87.86 | 12.47 | 32.65 | 6.81 | 331.06 |
| | 73 | 1.92 | 1.25 | 19.74 | 4.17 | 82.43 | 14.61 | 33.59 | 2.00 | 203.50 |
| | 75 | 2.09 | 1.59 | 11.00 | 4.79 | 87.65 | 14.11 | 42.71 | 15.15 | 220.25 |
| | 167 | 6.45 | 1.23 | 16.99 | 2.77 | 71.48 | 17.16 | 24.44 | 2.65 | 292.64 |
| | 169 | 6.47 | 1.20 | 10.95 | 3.43 | 84.97 | 13.37 | 24.41 | 3.23 | 334.88 |
| | 171 | 3.25 | 0.83 | 18.37 | 3.65 | 78.16 | 15.36 | 23.39 | 1.24 | 207.65 |
| | 173 | 3.05 | 1.19 | 22.16 | 4.26 | 95.31 | 15.17 | 26.17 | 4.41 | 204.85 |
| | 175 | 4.00 | 0.94 | 14.59 | 4.32 | 87.96 | 14.58 | 19.40 | 9.59 | 205.09 |
| | 177 | 5.22 | 1.45 | 21.97 | 3.06 | 64.21 | 17.05 | 18.61 | 32.33 | 264.36 |
| | 179 | 4.54 | 1.55 | 22.34 | 3.98 | 92.13 | 20.37 | 21.15 | 3.37 | 227.12 |
| 5 | 77 | 2.33 | 1.30 | 8.49 | 2.23 | 85.13 | 23.54 | 38.94 | 4.74 | 181.66 |
| | 79 | 3.10 | 1.34 | 10.63 | 1.96 | 69.69 | 16.30 | 36.29 | 5.13 | 242.30 |
| | 81 | 2.43 | 2.03 | 14.66 | 1.53 | 63.25 | 20.45 | 40.73 | 3.00 | 240.26 |
| | 83 | 1.62 | 1.54 | 9.05 | 3.75 | 94.80 | 27.07 | 44.11 | 7.41 | 156.45 |
| | 85 | 2.38 | 0.91 | 13.10 | 3.53 | 85.13 | 20.68 | 33.44 | 4.11 | 148.44 |
| | 87 | 1.81 | .[b] | 16.12 | 1.67 | 69.24 | 13.07 | 32.97 | 2.24 | 143.77 |
| | 89 | 1.46 | 1.64 | 17.72 | 3.48 | 82.62 | 16.97 | 39.98 | 5.30 | 157.35 |
| | 181 | 1.05 | .[b] | 14.30 | 2.47 | 82.30 | 12.72 | 25.24 | 11.81 | 143.70 |
| | 183 | 1.85 | 1.02 | 7.84 | 2.03 | 73.89 | 13.07 | 28.49 | 3.92 | 185.09 |
| | 185 | 5.16 | 1.04 | 14.84 | 2.43 | 74.99 | 19.59 | 24.34 | 4.36 | 272.50 |
| | 187 | 5.12 | 1.16 | 7.77 | 2.80 | 74.93 | 20.39 | 25.33 | 14.53 | 263.03 |
| | 189 | 4.06 | 1.04 | 15.53 | 1.83 | 70.82 | 19.52 | 29.77 | 7.17 | 248.51 |
| | 191 | 0.65 | .[b] | 9.47 | 2.68 | 66.35 | 11.57 | 25.49 | 2.66 | 86.23 |
| | 193 | 2.57 | 1.00 | 8.62 | 3.78 | 74.89 | 12.92 | 26.08 | 15.50 | 169.68 |
| | 195 | 2.84 | 1.70 | 10.42 | 2.92 | 85.48 | 25.42 | 25.08 | 3.72 | 219.66 |

Table I-6. Individual Male Hormone Data (page 4 of 4)

| Group[a] | Animal ID | Serum Testosterone (ng/ml) | Luteinizing Hormone (ng/ml) | Thyroid Stimulating Hormone (ng/ml) | Thyroxine (µg/dL) | Triiodothyronine (ng/dL) | Follicle Stimulating Hormone (ng/ml) | Estradiol (pg/ml) | Prolactin (ng/ml) | Dihydrotestosterone (pg/ml) |
|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 91 | 7.36 | 1.66 | 16.58 | 1.78 | 84.18 | 24.29 | 55.53 | 16.51 | 443.17 |
| | 93 | 1.20 | .[b] | 7.45 | 1.75 | 84.64 | 18.30 | 55.29 | 4.86 | 158.80 |
| | 95 | 3.71 | 1.15 | 11.57 | 1.25 | 60.20 | 13.46 | 55.88 | 4.27 | 284.71 |
| | 97 | 0.76 | 2.19 | 9.35 | 2.35 | 80.96 | 22.69 | 44.27 | 5.55 | 130.50 |
| | 99 | 0.59 | 1.24 | 8.48 | 1.86 | 74.95 | 14.64 | 54.56 | 1.43 | 142.69 |
| | 101 | 1.25 | 0.94 | 14.76 | 1.59 | 80.07 | 19.22 | 53.50 | 1.92 | 121.93 |
| | 103 | 3.46 | 1.51 | 16.25 | 1.26 | 55.29 | 18.72 | 45.88 | 11.81 | 234.40 |
| | 105 | 3.08 | 1.48 | 10.54 | 1.92 | 105.00 | 11.82 | 45.27 | 20.85 | 210.31 |
| | 197 | 0.29 | 1.46 | 13.36 | 1.42 | 79.62 | 11.47 | 31.50 | 19.78 | 127.69 |
| | 199 | 2.73 | 1.18 | 30.39 | 1.30 | 75.74 | 16.80 | 35.86 | 4.08 | 212.77 |
| | 201 | 2.01 | .[b] | 10.08 | 1.85 | 82.69 | 19.64 | 32.02 | 28.42 | 179.19 |
| | 203 | .[e] | | | | | | | | |
| | 205 | 2.65 | 1.25 | 12.37 | 1.42 | 60.74 | 21.81 | 28.24 | 3.64 | 177.73 |
| | 207 | 4.30 | 1.18 | 15.20 | 1.62 | 81.12 | 17.50 | 29.51 | 24.52 | 291.36 |
| | 209 | 1.42 | 1.69 | 17.50 | 2.23 | 86.51 | 16.57 | 34.90 | 30.93 | 120.47 |

[a]Groups are 0 is control; 1 is 25 mg/kg/day of Phenobarbital; 2 is 50 mg/kg/day of Phenobarbital; 3 is 100 mg/kg/day of Phenobarbital; 4 is 50 mg/kg/day of Linuron; 5 is 100 mg/kg/day of Linuron; and 6 is 150 mg/kg/day of Linuron.
[b]Value was below the detection limit of 0.8 ng/ml.
[c]Male 51 was euthanized moribund on test day 3.
[d]Male 165 was euthanized moribund on test day 5.
[e]Male 203 was euthanized moribund on test day 7.

# Appendix II

# Summary Tables

Table II-1. Summary of the Phenobarbital Treated Male Body Weights During the Dosing Period
(page 1 of 2)

| | Phenobarbital (mg/kg/day, po) | | | |
|---|---|---|---|---|
| | 0 | 25 | 50 | 100 |
| Number of Males on Study | 15 | 15 | 15 | 15 |
| Body Weight (td 1) (g)[a] | 342.8 ± 2.7 N=15 | 340.9 ± 3.2 N=15 | 343.8 ± 3.1 N=15 | 342.3 ± 3.1 N=15 |
| Body Weight (td 2) (g)[a] | 347.3 ± 2.8 N=15 | 351.0 ± 3.7 N=15 | 350.6 ± 3.0 N=15 | 348.5 ± 3.9 N=15 |
| Body Weight (td 3) (g)[a] | 352.5 ± 3.1 N=15 | 358.5 ± 3.6 N=15 | 357.4 ± 3.5 N=15 | 337.0 ± 4.4 N=15 |
| Body Weight (td 4) (g)[a] | 357.8 ± 3.2 N=15 | 364.2 ± 3.9 N=15 | 362.1 ± 3.8 N=15 | 333.6 ± 4.3 N=14[b] |
| Body Weight (td 5) (g)[a] | 363.9 ± 3.4 N=15 | 368.8 ± 4.0 N=15 | 366.6 ± 4.1 N=15 | 329.7 ± 5.4 N=14 |
| Body Weight (td 6) (g)[a] | 368.5 ± 3.6 N=15 | 373.5 ± 4.2 N=15 | 369.0 ± 4.3 N=15 | 332.5 ± 5.8 N=13[c] |
| Body Weight (td 7) (g)[a] | 374.0 ± 4.0 N=15 | 376.1 ± 4.2 N=15 | 372.0 ± 4.6 N=15 | 335.2 ± 5.6 N=13 |
| Body Weight (td 8) (g)[a] | 378.4 ± 3.9 N=15 | 378.1 ± 4.9 N=15 | 375.8 ± 4.8 N=15 | 339.6 ± 5.6 N=13 |
| Body Weight (td 9) (g)[a] | 382.3 ± 3.8 N=15 | 383.7 ± 5.2 N=15 | 382.4 ± 5.4 N=15 | 346.9 ± 5.3 N=13 |
| Body Weight (td 10) (g)[a] | 387.8 ± 4.4 N=15 | 389.2 ± 5.5 N=15 | 386.3 ± 5.6 N=15 | 350.4 ± 5.3 N=13 |

Table II-1. Summary of the Phenobarbital Treated Male Body Weights During the Dosing Period
(page 2 of 2)

| | Phenobarbital (mg/kg/day, po) | | | |
|---|---|---|---|---|
| | 0 | 25 | 50 | 100 |
| Body Weight (td 11) (g)[a] | 391.0 ± 4.2 N=15 | 393.7 ± 5.7 N=15 | 389.9 ± 5.8 N=15 | 350.6 ± 6.0 N=13 |
| Body Weight (td 12) (g)[a] | 395.4 ± 4.3 N=15 | 396.2 ± 5.9 N=15 | 393.7 ± 6.0 N=15 | 353.9 ± 6.4 N=13 |
| Body Weight (td 13) (g)[a] | 398.5 ± 4.2 N=15 | 399.1 ± 5.9 N=15 | 397.0 ± 6.4 N=15 | 356.1 ± 6.3 N=13 |
| Body Weight (td 14) (g)[a] | 402.7 ± 4.6 N=15 | 404.4 ± 6.4 N=15 | 401.9 ± 6.5 N=15 | 359.3 ± 6.3 N=13 |
| Body Weight (td 15) (g)[a] | 403.9 ± 4.0 N=15 | 405.8 ± 6.2 N=15 | 403.6 ± 6.2 N=15 | 361.8 ± 6.5 N=13 |

[a]Reported as the mean ± S.E.M.; td = test day.
[b]Male 51 was euthanized moribund on test day 3.
[c]Male 165 was euthanized moribund on test day 5.

Table II-2. Summary of the Linuron Treated Male Body Weights During the Dosing Period (page 1 of 2)

| | Linuron (mg/kg/day, po) | | | |
|---|---|---|---|---|
| | 0 | 50 | 100 | 150 |
| Number of Males on Study | 15 | 15 | 15 | 15 |
| Body Weight (td 1) (g)[a] | 342.8 ± 2.7 N=15 | 341.2 ± 3.1 N=15 | 343.4 ± 2.8 N=15 | 339.0 ± 3.0 N=15 |
| Body Weight (td 2) (g)[a] | 347.3 ± 2.8 N=15 | 340.8 ± 3.3 N=15 | 328.0 ± 2.9 N=15 | 326.5 ± 2.7 N=15 |
| Body Weight (td 3) (g)[a] | 352.5 ± 3.1 N=15 | 337.2 ± 3.3 N=15 | 318.5 ± 3.4 N=15 | 318.3 ± 3.2 N=15 |
| Body Weight (td 4) (g)[a] | 357.8 ± 3.2 N=15 | 339.6 ± 3.8 N=15 | 322.3 ± 3.2 N=15 | 312.3 ± 3.9 N=15 |
| Body Weight (td 5) (g)[a] | 363.9 ± 3.4 N=15 | 343.9 ± 3.9 N=15 | 325.9 ± 3.5 N=15 | 310.7 ± 4.3 N=15 |
| Body Weight (td 6) (g)[a] | 368.5 ± 3.6 N=15 | 347.3 ± 4.3 N=15 | 329.9 ± 3.5 N=15 | 307.4 ± 5.7 N=15 |
| Body Weight (td 7) (g)[a] | 374.0 ± 4.0 N=15 | 349.1 ± 4.5 N=15 | 331.4 ± 4.3 N=15 | 305.9 ± 6.9 N=15 |
| Body Weight (td 8) (g)[a] | 378.4 ± 3.9 N=15 | 351.2 ± 4.8 N=15 | 333.7 ± 4.9 N=15 | 310.6 ± 5.8 N=14[b] |
| Body Weight (td 9) (g)[a] | 382.3 ± 3.8 N=15 | 356.6 ± 4.5 N=15 | 337.2 ± 5.0 N=15 | 313.0 ± 5.2 N=14 |
| Body Weight (td 10) (g)[a] | 387.8 ± 4.4 N=15 | 362.6 ± 4.6 N=15 | 338.4 ± 5.2 N=15 | 314.5 ± 5.4 N=14 |

Table II-2. Summary of the Linuron Treated Male Body Weights During the Dosing Period (page 2 of 2)

| | Linuron (mg/kg/day, po) | | | |
|---|---|---|---|---|
| | 0 | 50 | 100 | 150 |
| Body Weight (td 11) (g)[a] | 391.0 ± 4.2 N=15 | 366.8 ± 5.5 N=15 | 340.1 ± 5.7 N=15 | 316.2 ± 4.9 N=14 |
| Body Weight (td 12) (g)[a] | 395.4 ± 4.3 N=15 | 367.9 ± 5.6 N=15 | 343.1 ± 5.4 N=15 | 320.1 ± 5.1 N=14 |
| Body Weight (td 13) (g)[a] | 398.5 ± 4.2 N=15 | 371.7 ± 5.1 N=15 | 345.9 ± 5.8 N=15 | 322.7 ± 4.5 N=14 |
| Body Weight (td 14) (g)[a] | 402.7 ± 4.6 N=15 | 373.5 ± 5.2 N=15 | 349.1 ± 5.8 N=15 | 325.0 ± 5.3 N=14 |
| Body Weight (td 15) (g)[a] | 403.9 ± 4.0 N=15 | 378.2 ± 4.8 N=15 | 351.0 ± 5.4 N=15 | 327.1 ± 6.1 N=14 |

[a]Reported as the mean ± S.E.M.; td = test day.
[b]Male 203 was euthanized moribund on test day 7.

Table II-3. Summary of the Clinical Observations for the Phenobarbital Treated Males During the Exposure Period (page 1 of 3)

## A. Clinical Observations Summarized by Group

| | Phenobarbital (mg/kg/day, po) | | | |
|---|---|---|---|---|
| Observation | 0 | 25 | 50 | 100 |
| Alopecia | 1 | 2 | | |
| Ataxia | | | | 15 |
| Broken toenail | 2 | 1 | | |
| Ear(s): damaged | | | | 1 |
| Efflux of the dosing compound | 2 | 5 | 5 | 7 |
| Euthanized moribund | | | | 2 |
| Eye(s): chromodacryorrhea | | 1 | 3 | 13 |
| Eye(s): discharge | | | | 1 |
| Gasping: post dosing | | | 1 | 1 |
| Hindlimb: apparent paralysis, right | | | | 1 |
| Lethargic | | | 3 | 15 |
| Loss of righting reflex | | | | 2 |
| Minimal response to physical or auditory stimulus | | | | 2 |
| Nasal discharge: red | | | 1 | 2 |
| Nose: chromodacryorrhea | | | 1 | 6 |
| Not dosed: moribund | | | | 1 |
| Piloerection | | | | 1 |
| Prone | | | | 8 |
| Received partial dose: half | 1 | 3 | 1 | 2 |
| Rough coat | | | | 3 |
| Salivating: post dosing | | | | 1 |
| Scheduled sacrifice | 15 | 15 | 15 | 13 |
| Struggling during dosing | | 1 | 1 | 2 |

## B. Clinical Observations Summarized by Group and Day

| | | Phenobarbital (mg/kg/day, po) | | | |
|---|---|---|---|---|---|
| Test Day | Observation[a] | 0 | 25 | 50 | 100 |
| 1 | Broken toenail | 1 | 1 | | |
| | Efflux of the dosing compound | 1 | | | |
| | Gasping: post dosing | | | 1 | |
| | Lethargic | | | | 6 |
| | Received partial dose: half | 1 | | | |
| | Struggling during dosing | | 1 | 1 | 2 |
| 2 | Alopecia: limb(s) | 1 | | | |
| | Broken toenail | 1 | | | |
| | Efflux of the dosing compound | 1 | 1 | | 4 |
| | Eye(s): chromodacryorrhea | | | | 3 |
| | Lethargic | | | | 6 |
| | Nasal discharge: red | | | | 2 |
| | Nose: chromodacryorrhea | | | | 1 |
| | Received partial dose: half | | 1 | | |

Table II-3. Summary of the Clinical Observations for the Phenobarbital Treated Males During the Exposure Period (page 2 of 3)

## B. Clinical Observations Summarized by Group and Day

| Test Day | Observation[a] | Phenobarbital (mg/kg/day, po) 0 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| 3 | Alopecia: limb(s) | 1 | 1 | | |
| | Ataxia | | | | 10 |
| | Efflux of the dosing compound | 1 | | 2 | 3 |
| | Euthanized moribund | | | | 1 |
| | Eye(s): chromodacryorrhea | | | 1 | 9 |
| | Eye(s): discharge | | | | 1 |
| | Gasping: post dosing | | | | 1 |
| | Hindlimb: apparent paralysis, right; veterinary finding upon observation | | | | 1 |
| | Lethargic | | | | 12 |
| | Loss of righting reflex | | | | 1 |
| | Minimal response to physical or auditory stimulus; veterinary finding upon observation | | | | 1 |
| | Nose: chromodacryorrhea | | | | 2 |
| | Piloerection | | | | 1 |
| | Prone | | | | 2 |
| | Received partial dose: half | | | 1 | |
| | Rough coat | | | | 1 |
| 4 | Alopecia: limb(s) | 1 | 1 | | |
| | Ataxia | | | | 10 |
| | Eye(s): chromodacryorrhea | | 1 | 1 | 8 |
| | Lethargic | | | | 14 |
| | Loss of righting reflex | | | | 1 |
| | Nose: chromodacryorrhea | | | | 4 |
| | Prone | | | | 6 |
| 5 | Alopecia: limb(s) | 1 | 1 | | |
| | Ataxia | | | | 8 |
| | Broken toenail | 1 | | | |
| | Efflux of the dosing compound | | 1 | | |
| | Euthanized moribund | | | | 1 |
| | Eye(s): chromodacryorrhea | | | | 7 |
| | Lethargic | | | | 12 |
| | Minimal response to physical or auditory stimulus | | | | 1 |
| | Nose: chromodacryorrhea | | | | 2 |
| | Not dosed: moribund | | | | 1 |
| | Prone | | | | 4 |
| | Received partial dose: half | | 1 | | |
| | Rough coat | | | | 2 |
| 6 | Alopecia: limb(s) | 1 | 2 | | |
| | Ataxia | | | | 10 |
| | Efflux of the dosing compound | | 1 | | |
| | Eye(s): chromodacryorrhea | | | 1 | 4 |
| | Lethargic | | | 2 | 12 |
| | Nose: chromodacryorrhea | | | 1 | 2 |
| | Prone | | | | 2 |
| | Rough coat | | | | 1 |

Table II-3. Summary of the Clinical Observations for the Phenobarbital Treated Males During the Exposure Period (page 3 of 3)

## B. Clinical Observations Summarized by Group and Day

| Test Day | Observation[a] | Phenobarbital (mg/kg/day, po) 0 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| 7 | Alopecia: limb(s) | 1 | 2 | | |
| | Ataxia | | | | 5 |
| | Efflux of the dosing compound | | | | 2 |
| | Eye(s): chromodacryorrhea | | | | 2 |
| | Lethargic | | | | 10 |
| | Prone | | | | 2 |
| 8 | Alopecia: limb(s) | 1 | 2 | | |
| | Ataxia | | | | 1 |
| | Efflux of the dosing compound | | 1 | | 1 |
| | Lethargic | | | | 10 |
| | Prone | | | | 2 |
| 9 | Alopecia: limb(s) | 1 | 2 | | |
| | Ataxia | | | | 1 |
| | Efflux of the dosing compound | | 1 | 1 | |
| | Lethargic | | | | 13 |
| | Nasal discharge: red | | | 1 | |
| | Received partial dose: half | | 1 | | |
| 10 | Alopecia: limb(s) | 1 | 2 | | |
| | Ataxia | | | | 1 |
| | Eye(s): chromodacryorrhea | | | | 1 |
| | Lethargic | | | 1 | 12 |
| 11 | Alopecia: limb(s) | 1 | 2 | | |
| | Ataxia | | | | 2 |
| | Efflux of the dosing compound | | | 1 | 1 |
| | Lethargic | | | | 13 |
| | Received partial dose: half | | | | 1 |
| 12 | Alopecia: limb(s) | 1 | 2 | | |
| | Efflux of the dosing compound | | | | 1 |
| | Lethargic | | | | 13 |
| 13 | Alopecia: limb(s) | 1 | 2 | | |
| | Ataxia | | | | 1 |
| | Efflux of the dosing compound | | | | 1 |
| | Lethargic | | | 2 | 13 |
| | Salivating: post dosing | | | | 1 |
| 14 | Alopecia: limb(s) | 1 | 2 | | |
| | Efflux of the dosing compound | | | | 1 |
| | Lethargic | | | | 12 |
| | Received partial dose: half | | | | 1 |
| 15 | Alopecia: limb(s) | 1 | 2 | | |
| | Ear(s): damaged | | | | 1 |
| | Efflux of the dosing compound | | | 1 | 1 |
| | Received partial dose: half | | | | 1 |
| | Scheduled sacrifice | 15 | 15 | 15 | 13 |

[a]Clinical observations are tabulated once per day per animal.

Table II-4. Summary of the Clinical Observations for the Linuron Treated Males During the Exposure Period (page 1 of 3)

## A. Clinical Observations Summarized by Group

| | Linuron (mg/kg/day, po) | | | |
|---|---|---|---|---|
| Observation | 0 | 50 | 100 | 150 |
| Alopecia | 1 | 2 | 2 | 1 |
| Ataxia | | | | 3 |
| Broken toenail | 2 | | | |
| Efflux of the dosing compound | 2 | 4 | | 4 |
| Euthanized moribund | | | | 1 |
| Eye(s): chromodacryorrhea | | | | 2 |
| Gasping: post dosing | | | | 1 |
| Hypothermia, cold to touch | | | | 1 |
| Lethargic | | 1 | | 5 |
| Nose: chromodacryorrhea | | 1 | 8 | 12 |
| Piloerection | | | 1 | 1 |
| Prone | | | | 1 |
| Received partial dose: half | 1 | | | 1 |
| Rust colored fur: limb(s) | | | | 1 |
| Scheduled sacrifice | 15 | 15 | 15 | 14 |
| Sore(s) | | 1 | | |
| Struggling during dosing | | | 1 | 1 |
| Urine: abnormal color, brown | | | | 1 |

## B. Clinical Observations Summarized by Group and Day

| | | Linuron (mg/kg/day, po) | | | |
|---|---|---|---|---|---|
| Test Day | Observation[a] | 0 | 50 | 100 | 150 |
| 1 | Broken toenail | 1 | | | |
| | Efflux of the dosing compound | 1 | | 1 | 1 |
| | Eye(s): chromodacryorrhea | | | | 2 |
| | Gasping: post dosing | | | | 1 |
| | Received partial dose: half | 1 | | | |
| | Sore(s): head | | 1 | | |
| | Struggling during dosing | | | 1 | 1 |
| 2 | Alopecia: limb(s) | 1 | 1 | | |
| | Broken toenail | 1 | | | |
| | Efflux of the dosing compound | 1 | 1 | 1 | |
| | Nose: chromodacryorrhea | | | 4 | |
| | Sore(s): head | | 1 | | |
| 3 | Alopecia: limb(s) | 1 | 1 | | |
| | Efflux of the dosing compound | 1 | | | |
| | Nose: chromodacryorrhea | | | 3 | 4 |
| | Piloerection | | | 1 | |
| | Sore(s): head | | 1 | | |
| 4 | Alopecia: limb(s) | 1 | 1 | | |
| | Ataxia | | | | 1 |
| | Nose: chromodacryorrhea | | 1 | 2 | 4 |
| | Sore(s): head | | 1 | | |

Table II-4. Summary of the Clinical Observations for the Linuron Treated Males During the Exposure Period (page 2 of 3)

## B. Clinical Observations Summarized by Group and Day

| Test Day | Observation[a] | Linuron (mg/kg/day, po) 0 | 50 | 100 | 150 |
|---|---|---|---|---|---|
| 5 | Alopecia: limb(s) | 1 | 1 | | |
| | Broken toenail | 1 | | | |
| | Nose: chromodacryorrhea | | | | 3 |
| | Sore(s): head | | 1 | | |
| 6 | Alopecia: limb(s) | 1 | 1 | | |
| | Efflux of the dosing compound | | 1 | | |
| | Eye(s): chromodacryorrhea | | | | 1 |
| | Lethargic | | | | 1 |
| | Nose: chromodacryorrhea | | | | 2 |
| | Sore(s): head | | 1 | | |
| 7 | Alopecia: limb(s) | 1 | 1 | | |
| | Efflux of the dosing compound | | 1 | | |
| | Euthanized moribund | | | | 1 |
| | Eye(s): chromodacryorrhea | | | | 1 |
| | Hypothermia, cold to touch | | | | 1 |
| | Lethargic | | | | 2 |
| | Nose: chromodacryorrhea | | | | 2 |
| | Prone | | | | 1 |
| | Rust colored fur: limb(s) | | | | 1 |
| | Sore(s): head | | 1 | | |
| | Urine: abnormal color, brown | | | | 1 |
| 8 | Alopecia: head | | 1 | | |
| | Alopecia: limb(s) | 1 | 1 | | |
| | Ataxia | | | | 1 |
| | Lethargic | | | | 1 |
| | Nose: chromodacryorrhea | | | | 1 |
| | Rust colored fur: limb(s) | | | | 1 |
| 9 | Alopecia: head | | 1 | | |
| | Alopecia: limb(s) | 1 | 1 | | |
| | Efflux of the dosing compound | | | 1 | |
| | Lethargic | | 1 | | |
| | Nose: chromodacryorrhea | | | | 4 |
| 10 | Alopecia: head | | 1 | | |
| | Alopecia: limb(s) | 1 | 1 | | |
| | Efflux of the dosing compound | | 1 | | 1 |
| | Nose: chromodacryorrhea | | | | 3 |
| | Received partial dose: half | | | | 1 |
| 11 | Alopecia: head | | 1 | | |
| | Alopecia: limb(s) | 1 | 1 | | |
| | Ataxia | | | | 1 |
| | Nose: chromodacryorrhea | | | 1 | 2 |
| | Piloerection | | | 1 | |

Table II-4. Summary of the Clinical Observations for the Linuron Treated Males During the Exposure Period (page 3 of 3)

## B. Clinical Observations Summarized by Group and Day

| Test Day | Observation[a] | Linuron (mg/kg/day, po) 0 | 50 | 100 | 150 |
|---|---|---|---|---|---|
| 12 | Alopecia: head | | 1 | | |
| | Alopecia: limb(s) | 1 | 1 | | 1 |
| | Ataxia | | | | 2 |
| | Efflux of the dosing compound | | | 1 | 1 |
| | Lethargic | | | | 2 |
| | Nose: chromodacryorrhea | | | | 4 |
| | Piloerection | | | | 1 |
| 13 | Alopecia: head | | 1 | | |
| | Alopecia: limb(s) | 1 | 1 | | 1 |
| | Lethargic | | | | 1 |
| | Nose: chromodacryorrhea | | | 1 | 2 |
| | Piloerection | | | | 1 |
| 14 | Alopecia: head | | 1 | | |
| | Alopecia: limb(s) | 1 | 1 | | 1 |
| | Nose: chromodacryorrhea | | | 2 | 2 |
| | Piloerection | | | | 1 |
| 15 | Alopecia: head | | 1 | | |
| | Alopecia: limb(s) | 1 | 1 | | 1 |
| | Efflux of the dosing compound | | 1 | | 1 |
| | Nose: chromodacryorrhea | | | | 1 |
| | Scheduled sacrifice | 15 | 15 | 15 | 14 |

[a]Clinical observations are tabulated once per day per animal.

Table II-5. Summary of the Macroscopic and Microscopic Necropsy Findings for the Phenobarbital Treated Males (page 1 of 1)

## MACROSCOPIC FINDINGS

### A. Unscheduled Sacrifice on Testday 3

| | Phenobarbital (mg/kg/day, po) | | | |
|---|---|---|---|---|
| Finding | 0 | 25 | 50 | 100 |
| Urinary Bladder: cyst | | | | 1 |

### B. Unscheduled Sacrifice on Testday 5

| | Phenobarbital (mg/kg/day, po) | | | |
|---|---|---|---|---|
| Finding | 0 | 25 | 50 | 100 |
| Kidney: hydronephrosis, right | | | | 1 |
| Stomach: no food present | | | | 1 |

### C. Scheduled Sacrifice on Testday 15

| | Phenobarbital (mg/kg/day, po) | | | |
|---|---|---|---|---|
| Finding | 0 | 25 | 50 | 100 |
| Kidney: hydronephrosis, right | 1 | | | |
| Prostate, Ventral: missing left lobe | | | | 1 |
| Seminal Vesicles with Coagulating Glands: fluid leaked | | | 1 | |
| Skin: alopecia limb(s) | | 2 | | |

## MICROSCOPIC FINDINGS

| | Phenobarbital (mg/kg/day, po) | | | |
|---|---|---|---|---|
| Finding | 0 | 25 | 50 | 100 |
| **EPIDIDYMIS** | | | | |
| Number Examined | 15 | 0 | 0 | 13 |
| Infiltrative Cell, Mononuclear Cell, Bilateral | | | | 7 |
| **TESTIS** | | | | |
| Number Examined | 15 | 0 | 0 | 13 |
| Degeneration, Seminiferous Tubule, Unilateral | | | | 1 |
| **THYROID** | | | | |
| Number Examined | 15 | 0 | 0 | 13 |
| No Findings | | | | |

Table II-6. Summary of the Macroscopic and Microscopic Necropsy Findings for the Linuron Treated Males (page 1 of 1)

## MACROSCOPIC FINDINGS

### A. Unscheduled Sacrifice on Testday 7

| | Linuron (mg/kg/day, po) | | | |
|---|---|---|---|---|
| Finding | 0 | 50 | 100 | 150 |
| Intestines, Large: hard dry feces | | | | 1 |
| Intestines, Small: no ingesta | | | | 1 |
| Stomach: abnormally distended with food | | | | 1 |
| Testes: flaccid, left | | | | 1 |
| Urinary Bladder: calculi present | | | | 1 |
| Urinary Bladder: thickened | | | | 1 |

### B. Scheduled Sacrifice on Testday 15

| | Linuron (mg/kg/day, po) | | | |
|---|---|---|---|---|
| Finding | 0 | 50 | 100 | 150 |
| Kidney: hydronephrosis, right | 1 | | | 1 |
| Seminal Vesicles with Coagulating Glands: fluid leaked | | | 1 | |
| Seminal Vesicles with Coagulating Glands: reduced bilateral | | | 1 | |
| Skin: alopecia limb(s) | | 1 | | |

## MICROSCOPIC FINDINGS

| | Linuron (mg/kg/day, po) | | | |
|---|---|---|---|---|
| Finding | 0 | 50 | 100 | 150 |
| **EPIDIDYMIS** | | | | |
| Number Examined | 15 | 0 | 0 | 14 |
| No Findings | | | | |
| **TESTIS** | | | | |
| Number Examined | 15 | 0 | 0 | 14 |
| Degeneration, Seminiferous Tubule, Bilateral | | | | 2 |
| Degeneration, Seminiferous Tubule, Unilateral | | | | 1 |
| **THYROID** | | | | |
| Number Examined | 15 | 0 | 0 | 14 |
| No Findings | | | | |

Table 1. Summary and Statistical Analysis of the Final Body Weight, Body Weight Changes and Feed Consumptions for the Phenobarbital Treated Animals
(page 1 of 2)

| | Vehicle Control | | | 25 mg/kg/day Phenobarbital | | | | | 50 mg/kg/day Phenobarbital | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE |
| Final Body Weight on Test Day 15 (g)[a] | 15 | 403.9 ± 5.5 | 3.8 | 15 | 405.8 ± 5.5 | 5.9 | 1.8 ± 7.8 | 100.5 ± 1.9 | 15 | 403.6 ± 5.5 | 6.0 | -0.3 ± 7.8 | 99.9 ± 1.9 |
| Body Weight Change on Test Days 1 to 8 (g/day)[b] | 15 | 5.1 ± 0.4 | 30.5 | 15 | 5.3 ± 0.4 | 26.6 | 0.2 ± 0.6 | 104.3 ± 11.2 | 15 | 4.6 ± 0.4 | 28.4 | -0.5 ± 0.6 | 89.7 ± 10.4 |
| Body Weight Change on Test Days 8 to 15 (g/day)[c] | 15 | 3.6 ± 0.3 | 28.5 | 15 | 4.0 ± 0.4 | 37.6 | 0.3 ± 0.5 | 108.5 ± 13.2 | 15 | 4.0 ± 0.3 | 25.9 | 0.3 ± 0.4 | 109.3 ± 10.9 |
| Body Weight Change on Test Days 1 to 15 (g/day)[a] | 15 | 4.4 ± 0.3 | 22.0 | 15 | 4.6 ± 0.3 | 24.5 | 0.3 ± 0.4 | 106.1 ± 10.1 | 15 | 4.3 ± 0.3 | 24.5 | -0.1 ± 0.4 | 97.9 ± 9.6 |

| | 100 mg/kg/day Phenobarbital | | | | | Linear Trend |
|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | Linear Trend Slope ± SE |
| Final Body Weight on Test Day 15 (g)[a] | 13[g] | 361.8 ** ± 5.9 | 6.4 | -42.2 ± 8.1 | 89.6 ± 1.9 | -28.8 ** ± 5.7 |
| Body Weight Change on Test Days 1 to 8 (g/day)[b] | 13 | -0.2 ** ± 0.4 | -824.7 | -5.3 ± 0.6 | -4.4 ± 8.2 | -3.7 ** ± 0.4 |
| Body Weight Change on Test Days 8 to 15 (g/day)[c] | 13 | 3.2 ± 0.4 | 45.8 | -0.5 ± 0.5 | 87.1 ± 12.8 | -0.3 ± 0.3 |
| Body Weight Change on Test Days 1 to 15 (g/day)[a] | 13 | 1.5 ** ± 0.3 | 76.8 | -2.9 ± 0.4 | 33.8 ± 7.8 | -2.0 ** ± 0.3 |

Table 1. Summary and Statistical Analysis of the Final Body Weight, Body Weight Changes and Feed Consumptions for the Phenobarbital Treated Animals


| | Vehicle Control | | | 25 mg/kg/day Phenobarbital | | | | | 50 mg/kg/day Phenobarbital | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE |
| Feed Consumption on Test Days 1 to 8 (g/kg/day)[a] | 15 | 73.8 ± 1.9 | 8.6 | 9[d,e] | 73.6 ± 2.5 | 6.7 | -0.2 ± 3.2 | 99.7 ± 4.3 | 11[d,e] | 78.2 ± 2.3 | 5.2 | 4.4 ± 3.0 | 106.0 ± 4.1 |
| Feed Consumption on Test Days 8 to 15 (g/kg/day)[c] | 15 | 65.3 ± 1.5 | 8.8 | 15 | 64.9 ± 1.4 | 8.3 | -0.4 ± 2.0 | 99.3 ± 3.1 | 15 | 65.9 ± 2.6 | 15.5 | 0.6 ± 3.0 | 100.9 ± 4.6 |
| Feed Consumption on Test Days 1 to 15 (g/kg/day)[b] | 15 | 69.4 ± 1.4 | 7.9 | 9[f] | 68.3 ± 1.9 | 6.3 | -1.1 ± 2.4 | 98.4 ± 3.4 | 11[f] | 71.6 ± 1.7 | 9.7 | 2.2 ± 2.2 | 103.2 ± 3.3 |

| | 100 mg/kg/day Phenobarbital | | | | | Linear Trend |
|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | Linear Trend Slope ± SE |
| Feed Consumption on Test Days 1 to 8 (g/kg/day)[a] | 10[e] | 58.9 ** ± 2.4 | 16.8 | -14.9 ± 3.1 | 79.8 ± 3.8 | -9.0 ** ± 2.2 |
| Feed Consumption on Test Days 8 to 15 (g/kg/day)[c] | 13 | 71.9 * ± 2.4 | 11.8 | 6.5 ± 2.8 | 110.0 ± 4.4 | 4.6 * ± 2.0 |
| Feed Consumption on Test Days 1 to 15 (g/kg/day)[b] | 10[f] | 65.6 ± 1.8 | 8.1 | -3.8 ± 2.3 | 94.5 ± 3.2 | -1.8 ± 1.6 |

[a]Common variances across all groups model used for analysis.
[b]Separate variances for each chemical (or control) model used for analysis.
[c]Separate variances for each treatment group model used for analysis.
[d]Decrease in N is due to one animal placing large amounts of bedding in his feed jar and therefore an accurate feed weight could not be obtained.
[e]Decrease in N is due to one or more animals pulling the feed out of the feed jar and into the cage and therefore an accurate feed weight could not be obtained.
[f]Decrease in N is due to one or more interim feed consumption value(s) being missing and therefore the overall feed consumption value could not be calculated.
[g]Male 51 was euthanized moribund on test day 3 and male 165 was euthanized moribund on test day 5.
*p<0.05 for treatment group compared to control or linear trend compared to 0 trend.
**p<0.006 for treatment group compared to control or linear trend compared to 0 trend.

Table 2. Summary and Statistical Analysis of the Final Body Weight, Body Weight Changes and Feed Consumptions for the Linuron Treated Animals
(page 1 of 2)

| | Vehicle Control | | | 50 mg/kg/day Linuron | | | | | 100 mg/kg/day Linuron | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE |
| Final Body Weight on Test Day 15 (g)[a] | 15 | 403.9 ± 5.5 | 3.8 | 15 | 378.2 ** ± 5.5 | 4.9 | -25.8 ± 7.8 | 93.6 ± 1.9 | 15 | 351.0 ** ± 5.5 | 5.9 | -52.9 ± 7.8 | 86.9 ± 1.8 |
| Body Weight Change on Test Days 1 to 8 (g/day)[b] | 15 | 5.1 ± 0.4 | 30.5 | 15 | 1.4 ** ± 0.6 | 114.5 | -3.7 ± 0.7 | 28.1 ± 11.8 | 15 | -1.4 ** ± 0.6 | -175.5 | -6.5 ± 0.7 | -27.4 ± 11.8 |
| Body Weight Change on Test Days 8 to 15 (g/day)[c] | 15 | 3.6 ± 0.3 | 28.5 | 15 | 3.8 ± 0.2 | 21.0 | 0.2 ± 0.3 | 105.8 ± 9.7 | 15 | 2.5 * ± 0.4 | 58.1 | -1.2 ± 0.5 | 68.1 ± 11.4 |
| Body Weight Change on Test Days 1 to 15 (g/day)[a] | 15 | 4.4 ± 0.3 | 22.0 | 15 | 2.6 ** ± 0.3 | 34.2 | -1.7 ± 0.4 | 60.5 ± 8.1 | 15 | 0.5 ** ± 0.3 | 255.6 | -3.8 ± 0.4 | 12.4 ± 6.9 |

| | 150 mg/kg/day Linuron | | | | | Linear Trend |
|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | Linear Trend Slope ± SE |
| Final Body Weight on Test Day 15 (g)[a] | 14[f] | 327.1 ** ± 5.7 | 6.9 | -76.8 ± 8.0 | 81.0 ± 1.8 | -57.6 ** ± 5.6 |
| Body Weight Change on Test Days 1 to 8 (g/day)[b] | 14 | -4.1 ** ± 0.6 | -64.9 | -9.2 ± 0.7 | -81.2 ± 13.6 | -6.8 ** ± 0.5 |
| Body Weight Change on Test Days 8 to 15 (g/day)[c] | 14 | 2.4 ± 0.6 | 94.7 | -1.3 ± 0.7 | 65.1 ± 17.2 | -1.2 * ± 0.5 |
| Body Weight Change on Test Days 1 to 15 (g/day)[a] | 14 | -0.9 ** ± 0.3 | -170.1 | -5.2 ± 0.4 | -20.2 ± 7.3 | -4.0 ** ± 0.3 |

Table 2. Summary and Statistical Analysis of the Final Body Weight, Body Weight Changes and Feed Consumptions for the Linuron Treated Animals
(page 2 of 2)

| | Vehicle Control | | | 50 mg/kg/day Linuron | | | | | 100 mg/kg/day Linuron | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE |
| Feed Consumption on Test Days 1 to 8 (g/kg/day)[a] | 15 | 73.8 ± 1.9 | 8.6 | 13[d] | 62.8 ** ± 2.1 | 13.5 | -11.0 ± 2.8 | 85.1 ± 3.6 | 8[d] | 55.8 ** ± 2.6 | 18.2 | -18.0 ± 3.3 | 75.6 ± 4.1 |
| Feed Consumption on Test Days 8 to 15 (g/kg/day)[c] | 15 | 65.3 ± 1.5 | 8.8 | 15 | 61.9 ± 3.8 | 24.0 | -3.5 ± 4.1 | 94.7 ± 6.2 | 15 | 65.7 ± 3.4 | 20.3 | 0.4 ± 3.8 | 100.6 ± 5.7 |
| Feed Consumption on Test Days 1 to 15 (g/kg/day)[b] | 15 | 69.4 ± 1.4 | 7.9 | 13[e] | 61.4 * ± 2.6 | 16.9 | -8.0 ± 2.9 | 88.4 ± 4.1 | 8[e] | 60.5 * ± 3.3 | 15.8 | -8.9 ± 3.6 | 87.2 ± 5.1 |

| | 150 mg/kg/day Linuron | | | | | Linear Trend |
|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | Linear Trend Slope ± SE |
| Feed Consumption on Test Days 1 to 8 (g/kg/day)[a] | 6[d] | 46.7 ** ± 3.1 | 15.4 | -27.1 ± 3.6 | 63.3 ± 4.5 | -19.7 ** ± 2.5 |
| Feed Consumption on Test Days 8 to 15 (g/kg/day)[c] | 14 | 58.7 ± 3.4 | 21.4 | -6.6 ± 3.7 | 89.9 ± 5.5 | -3.6 ± 2.7 |
| Feed Consumption on Test Days 1 to 15 (g/kg/day)[b] | 6[e] | 50.1 ** ± 3.8 | 10.6 | -19.3 ± 4.0 | 72.2 ± 5.7 | -13.1 ** ± 2.9 |

[a]Common variances across all groups model used for analysis.
[b]Separate variances for each chemical (or control) model used for analysis.
[c]Separate variances for each treatment group model used for analysis.
[d]Decrease in N is due to one or more animals pulling the feed out of the feed jar and into the cage and therefore an accurate feed weight could not be obtained.
[e]Decrease in N is due to one or more interim feed consumption value(s) being missing and therefore the overall feed consumption value could not be calculated.
[f]Male 203 was euthanized moribund on test day 7.
*p<0.05 for treatment group compared to control or linear trend compared to 0 trend.
**p<0.006 for treatment group compared to control or linear trend compared to 0 trend.

Table 3. Summary and Statistical Analysis of the Absolute Organ Weights for the Phenobarbital Treated Animals (page 1 of 2)

| | Vehicle Control | | | 25 mg/kg/day Phenobarbital | | | | | 50 mg/kg/day Phenobarbital | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE |
| Thyroid Weight (g)[a] | 15 | 0.0171 ± 0.0005 | 12.2760 | 15 | 0.0205 * ± 0.0012 | 24.8265 | 0.0033 ± 0.0013 | 119.49 ± 7.76 | 14[b] | 0.0228 ** ± 0.0012 | 15.7325 | 0.0057 ± 0.0013 | 132.99 ± 8.18 |
| Liver Weight (g)[a] | 15 | 15.4368 ± 0.3397 | 8.5229 | 15 | 17.9062 ** ± 0.5287 | 11.5058 | 2.4693 ± 0.6284 | 116.00 ± 4.27 | 15 | 19.6768 ** ± 0.5287 | 9.2380 | 4.2399 ± 0.6284 | 127.47 ± 4.43 |
| Left Testis Weight (g)[c] | 15 | 1.6490 ± 0.0336 | 9.8979 | 15 | 1.6548 ± 0.0336 | 5.7148 | 0.0058 ± 0.0475 | 100.35 ± 2.88 | 15 | 1.6622 ± 0.0336 | 9.1917 | 0.0132 ± 0.0475 | 100.80 ± 2.89 |
| Right Testis Weight (g)[c] | 15 | 1.6434 ± 0.0348 | 8.9927 | 15 | 1.6420 ± 0.0348 | 6.5669 | -0.0014 ± 0.0491 | 99.92 ± 2.99 | 15 | 1.6619 ± 0.0348 | 8.2599 | 0.0184 ± 0.0491 | 101.12 ± 3.01 |
| Paired Testis Weight (g)[c] | 15 | 3.2925 ± 0.0671 | 9.3375 | 15 | 3.2968 ± 0.0671 | 6.0357 | 0.0044 ± 0.0950 | 100.13 ± 2.89 | 15 | 3.3241 ± 0.0671 | 8.5606 | 0.0316 ± 0.0950 | 100.96 ± 2.90 |
| Paired Epididymis Weight (g)[c] | 15 | 1.1134 ± 0.0243 | 9.4561 | 15 | 1.1363 ± 0.0243 | 6.0043 | 0.0229 ± 0.0344 | 102.06 ± 3.12 | 15 | 1.1407 ± 0.0243 | 7.0804 | 0.0273 ± 0.0344 | 102.45 ± 3.13 |

| | 100 mg/kg/day Phenobarbital | | | | | Linear Trend |
|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | Linear Trend Slope ± SE |
| Thyroid Weight (g)[a] | 12[b,d] | 0.0229 ** ± 0.0013 | 20.3147 | 0.0058 ± 0.0014 | 133.66 ± 8.67 | 0.0044 ** ± 0.0010 |
| Liver Weight (g)[a] | 13 | 18.2267 ** ± 0.5679 | 12.4728 | 2.7898 ± 0.6617 | 118.07 ± 4.50 | 2.2674 ** ± 0.4744 |
| Left Testis Weight (g)[c] | 13 | 1.6846 ± 0.0360 | 7.7525 | 0.0356 ± 0.0492 | 102.16 ± 3.02 | 0.0255 ± 0.0347 |
| Right Testis Weight (g)[c] | 13 | 1.6739 ± 0.0373 | 7.9912 | 0.0305 ± 0.0510 | 101.86 ± 3.13 | 0.0253 ± 0.0359 |
| Paired Testis Weight (g)[c] | 13 | 3.3585 ± 0.0721 | 7.6985 | 0.0660 ± 0.0985 | 102.01 ± 3.02 | 0.0504 ± 0.0694 |
| Paired Epididymis Weight (g)[c] | 13 | 1.1402 ± 0.0261 | 9.1686 | 0.0268 ± 0.0357 | 102.41 ± 3.24 | 0.0190 ± 0.0251 |

Table 3. Summary and Statistical Analysis of the Absolute Organ Weights for the Phenobarbital Treated Animals (page 2 of 2)

| | Vehicle Control | | | 25 mg/kg/day Phenobarbital | | | | | 50 mg/kg/day Phenobarbital | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE |
| Prostate Weight (g)[c] | 15 | 0.9677 ± 0.0429 | 14.8606 | 15 | 0.9921 ± 0.0429 | 18.1837 | 0.0244 ± 0.0607 | 102.52 ± 6.35 | 15 | 1.0191 ± 0.0429 | 17.9995 | 0.0514 ± 0.0607 | 105.31 ± 6.44 |
| Seminal Vesicles with Fluid and Coagulating Gland Weight (g)[e] | 15 | 1.2178 ± 0.0434 | 13.8121 | 15 | 1.3263 ± 0.0539 | 15.7347 | 0.1086 ± 0.0692 | 108.91 ± 5.89 | 14[f] | 1.3404 ± 0.0688 | 19.1971 | 0.1227 ± 0.0813 | 110.07 ± 6.88 |
| Accessory Sex Gland Weight (g)[c] | 15 | 2.1854 ± 0.0937 | 11.8181 | 15 | 2.3184 ± 0.0937 | 14.3209 | 0.1330 ± 0.1325 | 106.08 ± 6.25 | 14[f] | 2.3715 ± 0.0970 | 14.8744 | 0.1861 ± 0.1349 | 108.52 ± 6.43 |

| | 100 mg/kg/day Phenobarbital | | | | | Linear Trend |
|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | Linear Trend Slope ± SE |
| Prostate Weight (g)[c] | 13[d] | 1.0378 ± 0.0461 | 16.4972 | 0.0702 ± 0.0630 | 107.25 ± 6.74 | 0.0531 ± 0.0444 |
| Seminal Vesicles with Fluid and Coagulating Gland Weight (g)[e] | 13 | 1.4552 ± 0.1265 | 31.3343 | 0.2375 ± 0.1337 | 119.50 ± 11.23 | 0.1625 ± 0.0918 |
| Accessory Sex Gland Weight (g)[c] | 13 | 2.4931 * ± 0.1007 | 21.1941 | 0.3076 ± 0.1375 | 114.08 ± 6.72 | 0.2183 * ± 0.0970 |

[a]Separate variances for each chemical (or control) model used for analysis.
[b]Decrease in N is due to part or all of the tissue for one animal not being present in the tissue cup at the time of weighing the fixed tissue.
[c]Common variances across all groups model used for analysis.
[d]Male 51 was euthanized moribund on test day 3 and male 165 was euthanized moribund on test day 5.
[e]Separate variances for each treatment group model used for analysis.
[f]Decrease in N is due to the seminal vesicles being nicked causing fluid to leak for one animal and therefore an accurate weight could not be obtained.
*p<0.05 for treatment group compared to control or linear trend compared to 0 trend.
**p<0.006 for treatment group compared to control or linear trend compared to 0 trend.

Table 4. Summary and Statistical Analysis of the Relative Organ Weights for the Phenobarbital Treated Animals (page 1 of 2)

| | Vehicle Control | | | 25 mg/kg/day Phenobarbital | | | | | 50 mg/kg/day Phenobarbital | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE |
| Relative Thyroid Weight (% of final body wt.)[a] | 15 | 0.0043 ± 0.0002 | 13.8569 | 15 | 0.0050 * ± 0.0003 | 22.4274 | 0.0008 ± 0.0003 | 118.31 ± 8.21 | 14[b] | 0.0057 ** ± 0.0003 | 17.4909 | 0.0015 ± 0.0003 | 134.26 ± 8.72 |
| Relative Liver Weight (% of final body wt.)[a] | 15 | 3.8202 ± 0.0856 | 7.1029 | 15 | 4.4070 ** ± 0.0856 | 8.1296 | 0.5868 ± 0.1210 | 115.36 ± 3.42 | 15 | 4.8697 ** ± 0.0856 | 5.0735 | 1.0495 ± 0.1210 | 127.47 ± 3.63 |
| Relative Left Testis Weight (% of final body wt.)[c] | 15 | 0.4084 ± 0.0101 | 9.5176 | 15 | 0.4090 ± 0.0101 | 7.7445 | 0.0006 ± 0.0143 | 100.14 ± 3.50 | 15 | 0.4131 ± 0.0101 | 10.7198 | 0.0047 ± 0.0143 | 101.14 ± 3.52 |
| Relative Right Testis Weight (% of final body wt.)[c] | 15 | 0.4070 ± 0.0104 | 8.6426 | 15 | 0.4057 ± 0.0104 | 8.0087 | -0.0013 ± 0.0147 | 99.67 ± 3.59 | 15 | 0.4129 ± 0.0104 | 9.5704 | 0.0058 ± 0.0147 | 101.43 ± 3.63 |
| Relative Paired Testis Weight (% of final body wt.)[c] | 15 | 0.8155 ± 0.0202 | 8.9678 | 15 | 0.8148 ± 0.0202 | 7.7817 | -0.0007 ± 0.0286 | 99.91 ± 3.50 | 15 | 0.8260 ± 0.0202 | 10.0160 | 0.0105 ± 0.0286 | 101.28 ± 3.53 |
| Relative Paired Epididymis Weight (% of final body wt.)[c] | 15 | 0.2758 ± 0.0080 | 9.4144 | 15 | 0.2813 ± 0.0080 | 9.7716 | 0.0055 ± 0.0113 | 101.98 ± 4.13 | 15 | 0.2833 ± 0.0080 | 8.2599 | 0.0074 ± 0.0113 | 102.70 ± 4.15 |

| | 100 mg/kg/day Phenobarbital | | | | | Linear Trend |
|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | Linear Trend Slope ± SE |
| Relative Thyroid Weight (% of final body wt.)[a] | 12[b,d] | 0.0064 ** ± 0.0003 | 21.1597 | 0.0022 ± 0.0004 | 150.73 ± 9.54 | 0.0016 ** ± 0.0003 |
| Relative Liver Weight (% of final body wt.)[a] | 13 | 5.0292 ** ± 0.0919 | 8.6360 | 1.2090 ± 0.1256 | 131.65 ± 3.81 | 0.9145 ** ± 0.0885 |
| Relative Left Testis Weight (% of final body wt.)[c] | 13 | 0.4671 ** ± 0.0109 | 9.3766 | 0.0587 ± 0.0148 | 114.36 ± 3.89 | 0.0402 ** ± 0.0105 |
| Relative Right Testis Weight (% of final body wt.)[c] | 13 | 0.4642 ** ± 0.0111 | 9.8036 | 0.0572 ± 0.0152 | 114.05 ± 3.99 | 0.0400 ** ± 0.0107 |
| Relative Paired Testis Weight (% of final body wt.)[c] | 13 | 0.9313 ** ± 0.0217 | 9.4512 | 0.1158 ± 0.0296 | 114.21 ± 3.88 | 0.0802 ** ± 0.0209 |
| Relative Paired Epididymis Weight (% of final body wt.)[c] | 13 | 0.3169 ** ± 0.0086 | 12.5465 | 0.0410 ± 0.0117 | 114.87 ± 4.55 | 0.0280 ** ± 0.0082 |

Table 4. Summary and Statistical Analysis of the Relative Organ Weights for the Phenobarbital Treated Animals (page 2 of 2)

| | Vehicle Control | | | 25 mg/kg/day Phenobarbital | | | | | 50 mg/kg/day Phenobarbital | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE |
| Relative Prostate Weight (% of final body wt.)[c] | 15 | 0.2393 ± 0.0115 | 13.7151 | 15 | 0.2445 ± 0.0115 | 17.5207 | 0.0051 ± 0.0162 | 102.15 ± 6.85 | 15 | 0.2532 ± 0.0115 | 18.4622 | 0.0139 ± 0.0162 | 105.80 ± 6.98 |
| Relative Seminal Vesicles with Fluid and Coagulating Gland Weight (% of final body wt.)[e] | 15 | 0.3012 ± 0.0099 | 12.6694 | 15 | 0.3276 ± 0.0134 | 15.7938 | 0.0263 ± 0.0166 | 108.73 ± 5.68 | 14[f] | 0.3363 ± 0.0188 | 20.8721 | 0.0351 ± 0.0212 | 111.64 ± 7.22 |
| Relative Accessory Sex Gland Weight (% of final body wt.)[c] | 15 | 0.5406 ± 0.0144 | 10.3270 | 15 | 0.5720 ± 0.0293 | 14.0759 | 0.0315 ± 0.0326 | 105.82 ± 6.11 | 14[f] | 0.5941 ± 0.0303 | 16.0370 | 0.0535 ± 0.0336 | 109.90 ± 6.33 |

| | 100 mg/kg/day Phenobarbital | | | | | Linear Trend |
|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | Linear Trend Slope ± SE |
| Relative Prostate Weight (% of final body wt.)[c] | 13[d] | 0.2877 ** ± 0.0123 | 17.4540 | 0.0483 ± 0.0168 | 120.18 ± 7.73 | 0.0344 ** ± 0.0119 |
| Relative Seminal Vesicles with Fluid and Coagulating Gland Weight (% of final body wt.)[e] | 13 | 0.4041 * ± 0.0368 | 32.8528 | 0.1029 ± 0.0381 | 134.15 ± 12.99 | 0.0710 ** ± 0.0261 |
| Relative Accessory Sex Gland Weight (% of final body wt.)[c] | 13 | 0.6918 ** ± 0.0315 | 22.5908 | 0.1512 ± 0.0346 | 127.97 ± 6.75 | 0.1064 ** ± 0.0251 |

[a]Separate variances for each chemical (or control) model used for analysis.
[b]Decrease in N is due to part or all of the tissue for one animal not being present in the tissue cup at the time of weighing the fixed tissue.
[c]Common variances across all groups model used for analysis.
[d]Male 51 was euthanized moribund on test day 3 and male 165 was euthanized moribund on test day 5.
[e]Separate variances for each treatment group model used for analysis.
[f]Decrease in N is due to the seminal vesicles being nicked causing fluid to leak for one animal and therefore an accurate weight could not be obtained.
*p<0.05 for treatment group compared to control or linear trend compared to 0 trend.
**p<0.006 for treatment group compared to control or linear trend compared to 0 trend.

Table 5. Summary and Statistical Analysis of the Absolute Organ Weights for the Linuron Treated Animals (page 1 of 2)

| | Vehicle Control | | | 50 mg/kg/day Linuron | | | | | 100 mg/kg/day Linuron | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE |
| Thyroid Weight (g)[a] | 15 | 0.0171 ± 0.0005 | 12.2760 | 15 | 0.0181 ± 0.0007 | 14.5119 | 0.0010 ± 0.0009 | 105.76 ± 5.39 | 14[b] | 0.0171 ± 0.0007 | 18.2231 | -0.00004 ± 0.00093 | 99.76 ± 5.40 |
| Liver Weight (g)[a] | 15 | 15.4368 ± 0.3397 | 8.5229 | 15 | 14.9573 ± 0.3859 | 10.4794 | -0.4796 ± 0.5141 | 96.89 ± 3.29 | 15 | 13.3924 ** ± 0.3859 | 8.8643 | -2.0445 ± 0.5141 | 86.76 ± 3.15 |
| Left Testis Weight (g)[c] | 15 | 1.6490 ± 0.0336 | 9.8979 | 15 | 1.6727 ± 0.0336 | 5.7118 | 0.0237 ± 0.0475 | 101.44 ± 2.90 | 15 | 1.6285 ± 0.0336 | 7.3067 | -0.0206 ± 0.0475 | 98.75 ± 2.86 |
| Right Testis Weight (g)[c] | 15 | 1.6434 ± 0.0348 | 8.9927 | 15 | 1.6696 ± 0.0348 | 6.6785 | 0.0261 ± 0.0491 | 101.59 ± 3.01 | 15 | 1.6334 ± 0.0348 | 7.1404 | -0.0101 ± 0.0491 | 99.39 ± 2.98 |
| Paired Testis Weight (g)[c] | 15 | 3.2925 ± 0.0671 | 9.3375 | 15 | 3.3423 ± 0.0671 | 6.0826 | 0.0498 ± 0.0950 | 101.51 ± 2.91 | 15 | 3.2618 ± 0.0671 | 7.1585 | -0.0306 ± 0.0950 | 99.07 ± 2.87 |
| Paired Epididymis Weight (g)[c] | 15 | 1.1134 ± 0.0243 | 9.4561 | 15 | 1.1020 ± 0.0243 | 7.9185 | -0.0114 ± 0.0344 | 98.98 ± 3.07 | 15 | 1.0176 * ± 0.0243 | 9.4146 | -0.0958 ± 0.0344 | 91.40 ± 2.96 |

| | 150 mg/kg/day Linuron | | | | | Linear Trend |
|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | Linear Trend Slope ± SE |
| Thyroid Weight (g)[a] | 14[d] | 0.0176 ± 0.0007 | 15.0526 | 0.0005 ± 0.0009 | 102.85 ± 5.45 | 0.0001 ± 0.0007 |
| Liver Weight (g)[a] | 14 | 12.9946 ** ± 0.3994 | 13.0625 | -2.4422 ± 0.5244 | 84.18 ± 3.18 | -1.9882 ** ± 0.3723 |
| Left Testis Weight (g)[c] | 14 | 1.6095 ± 0.0347 | 8.5935 | -0.0396 ± 0.0483 | 97.60 ± 2.89 | -0.0364 ± 0.0341 |
| Right Testis Weight (g)[c] | 14 | 1.6076 ± 0.0360 | 11.0299 | -0.0358 ± 0.0500 | 97.82 ± 3.01 | -0.0321 ± 0.0353 |
| Paired Testis Weight (g)[c] | 14 | 3.2171 ± 0.0695 | 9.7125 | -0.0754 ± 0.0966 | 97.71 ± 2.90 | -0.0686 ± 0.0682 |
| Paired Epididymis Weight (g)[c] | 14 | 1.0108 ** ± 0.0252 | 11.1291 | -0.1026 ± 0.0350 | 90.78 ± 3.01 | -0.0877 ** ± 0.0247 |

Table 5. Summary and Statistical Analysis of the Absolute Organ Weights for the Linuron Treated Animals (page 2 of 2)

| | Vehicle Control | | | 50 mg/kg/day Linuron | | | | | 100 mg/kg/day Linuron | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE |
| Prostate Weight (g)[c] | 15 | 0.9677 ± 0.0429 | 14.8606 | 15 | 0.9017 ± 0.0429 | 20.9036 | -0.0660 ± 0.0607 | 93.18 ± 6.07 | 15 | 0.8324 * ± 0.0429 | 15.6625 | -0.1353 ± 0.0607 | 86.02 ± 5.85 |
| Seminal Vesicles with Fluid and Coagulating Gland Weight (g)[e] | 15 | 1.2178 ± 0.0434 | 13.8121 | 15 | 1.2585 ± 0.0876 | 26.9674 | 0.0407 ± 0.0978 | 103.34 ± 8.08 | 14[f] | 1.0715 ± 0.0793 | 27.6949 | -0.1463 ± 0.0904 | 87.99 ± 7.23 |
| Accessory Sex Gland Weight (g)[c] | 15 | 2.1854 ± 0.0937 | 11.8181 | 15 | 2.1602 ± 0.0937 | 15.7630 | -0.0253 ± 0.1325 | 98.85 ± 6.03 | 14[f] | 1.9093 * ± 0.0970 | 17.4681 | -0.2761 ± 0.1349 | 87.37 ± 5.81 |

| | 150 mg/kg/day Linuron | | | | | Linear Trend |
|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | Linear Trend Slope ± SE |
| Prostate Weight (g)[c] | 14[d] | 0.7524 ** ± 0.0444 | 20.9783 | -0.2153 ± 0.0618 | 77.75 ± 5.74 | -0.1599 ** ± 0.0436 |
| Seminal Vesicles with Fluid and Coagulating Gland Weight (g)[e] | 14 | 0.9381 ** ± 0.0627 | 25.0253 | -0.2797 ± 0.0763 | 77.03 ± 5.84 | -0.2294 ** ± 0.0576 |
| Accessory Sex Gland Weight (g)[c] | 14 | 1.6905 ** ± 0.0970 | 21.6057 | -0.4950 ± 0.1349 | 77.35 ± 5.54 | -0.3881 ** ± 0.0954 |

[a]Separate variances for each chemical (or control) model used for analysis.
[b]Decrease in N is due to part or all of the tissue for one animal not being present in the tissue cup at the time of weighing the fixed tissue.
[c]Common variances across all groups model used for analysis.
[d]Male 203 was euthanized moribund on test day 7.
[e]Separate variances for each treatment group model used for analysis.
[f]Decrease in N is due to the seminal vesicles being nicked causing fluid to leak for one animal and therefore an accurate weight could not be obtained.
*p<0.05 for treatment group compared to control or linear trend compared to 0 trend.
**p<0.006 for treatment group compared to control or linear trend compared to 0 trend.

Table 6. Summary and Statistical Analysis of the Relative Organ Weights for the Linuron Treated Animals (page 1 of 2)

| | Vehicle Control | | | 50 mg/kg/day Linuron | | | | | 100 mg/kg/day Linuron | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE |
| Relative Thyroid Weight (% of final body wt.)[a] | 15 | 0.0043 ± 0.0002 | 13.8569 | 15 | 0.0048 * ± 0.0002 | 13.2781 | 0.0005 ± 0.0002 | 112.59 ± 6.07 | 14[b] | 0.0049 * ± 0.0002 | 18.0714 | 0.0006 ± 0.0003 | 115.11 ± 6.25 |
| Relative Liver Weight (% of final body wt.)[a] | 15 | 3.8202 ± 0.0856 | 7.1059 | 15 | 3.9511 ± 0.0856 | 7.8077 | 0.1309 ± 0.1210 | 103.43 ± 3.22 | 15 | 3.8180 ± 0.0856 | 7.8573 | -0.0022 ± 0.1210 | 99.94 ± 3.17 |
| Relative Left Testis Weight (% of final body wt.)[c] | 15 | 0.4084 ± 0.0101 | 9.5176 | 15 | 0.4430 * ± 0.0101 | 6.4812 | 0.0346 ± 0.0143 | 108.47 ± 3.65 | 15 | 0.4649 ** ± 0.0101 | 7.6770 | 0.0564 ± 0.0143 | 113.81 ± 3.75 |
| Relative Right Testis Weight (% of final body wt.)[c] | 15 | 0.4070 ± 0.0104 | 8.6426 | 15 | 0.4422 * ± 0.0104 | 7.2505 | 0.0351 ± 0.0147 | 108.63 ± 3.76 | 15 | 0.4662 ** ± 0.0104 | 7.3325 | 0.0592 ± 0.0147 | 114.53 ± 3.87 |
| Relative Paired Testis Weight (% of final body wt.)[c] | 15 | 0.8155 ± 0.0202 | 8.9678 | 15 | 0.8852 * ± 0.0202 | 6.7588 | 0.0697 ± 0.0286 | 108.55 ± 3.66 | 15 | 0.9311 ** ± 0.0202 | 7.4453 | 0.1156 ± 0.0286 | 114.17 ± 3.76 |
| Relative Paired Epididymis Weight (% of final body wt.)[c] | 15 | 0.2758 ± 0.0080 | 9.4144 | 15 | 0.2923 ± 0.0080 | 10.4258 | 0.0165 ± 0.0113 | 105.98 ± 4.21 | 15 | 0.2905 ± 0.0080 | 9.5764 | 0.0147 ± 0.0113 | 105.31 ± 4.20 |

| | 150 mg/kg/day Linuron | | | | | Linear Trend |
|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | Linear Trend Slope ± SE |
| Relative Thyroid Weight (% of final body wt.)[a] | 14[d] | 0.0054 ** ± 0.0002 | 13.2024 | 0.0011 ± 0.0003 | 126.71 ± 6.53 | 0.0008 ** ± 0.0002 |
| Relative Liver Weight (% of final body wt.)[a] | 14 | 3.9677 ± 0.0886 | 9.6263 | 0.1475 ± 0.1232 | 103.86 ± 3.28 | 0.0692 ± 0.0869 |
| Relative Left Testis Weight (% of final body wt.)[c] | 14 | 0.4936 ** ± 0.0105 | 9.8515 | 0.0852 ± 0.0146 | 120.86 ± 3.94 | 0.0620 ** ± 0.0103 |
| Relative Right Testis Weight (% of final body wt.)[c] | 14 | 0.4929 ** ± 0.0107 | 11.7268 | 0.0858 ± 0.0149 | 121.08 ± 4.06 | 0.0629 ** ± 0.0105 |
| Relative Paired Testis Weight (% of final body wt.)[c] | 14 | 0.9865 ** ± 0.0209 | 10.6723 | 0.1710 ± 0.0291 | 120.97 ± 3.94 | 0.1250 ** ± 0.0205 |
| Relative Paired Epididymis Weight (% of final body wt.)[c] | 14 | 0.3102 ** ± 0.0083 | 12.6990 | 0.0344 ± 0.0115 | 112.46 ± 4.42 | 0.0227 ** ± 0.0081 |

Table 6. Summary and Statistical Analysis of the Relative Organ Weights for the Linuron Treated Animals (page 2 of 2)

| | Vehicle Control | | | 50 mg/kg/day Linuron | | | | | 100 mg/kg/day Linuron | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE |
| Relative Prostate Weight (% of final body wt.)[c] | 15 | 0.2393 ± 0.0115 | 13.7151 | 15 | 0.2395 ± 0.0115 | 22.5283 | 0.0002 ± 0.0162 | 100.07 ± 6.78 | 15 | 0.2380 ± 0.0115 | 16.8394 | -0.0014 ± 0.0162 | 99.43 ± 6.76 |
| Relative Seminal Vesicles with Fluid and Coagulating Gland Weight (% of final body wt.)[e] | 15 | 0.3012 ± 0.0099 | 12.6694 | 15 | 0.3320 ± 0.0215 | 25.1102 | 0.0307 ± 0.0237 | 110.20 ± 8.00 | 14[f] | 0.3049 ± 0.0224 | 27.5304 | 0.0037 ± 0.0245 | 101.23 ± 8.15 |
| Relative Accessory Sex Gland Weight (% of final body wt.)[c] | 15 | 0.5406 ± 0.0144 | 10.3270 | 15 | 0.5715 ± 0.0237 | 14.9430 | 0.0309 ± 0.0277 | 105.72 ± 5.21 | 14[f] | 0.5446 ± 0.0245 | 17.4637 | 0.0040 ± 0.0284 | 100.75 ± 5.27 |

| | 150 mg/kg/day Linuron | | | | | Linear Trend |
|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | Linear Trend Slope ± SE |
| Relative Prostate Weight (% of final body wt.)[c] | 14[d] | 0.2293 ± 0.0119 | 18.2876 | -0.0101 ± 0.0165 | 95.79 ± 6.76 | -0.0071 ± 0.0117 |
| Relative Seminal Vesicles with Fluid and Coagulating Gland Weight (% of final body wt.)[e] | 14 | 0.2853 ± 0.0168 | 22.0008 | -0.0159 ± 0.0195 | 94.71 ± 6.37 | -0.0167 ± 0.0149 |
| Relative Accessory Sex Gland Weight (% of final body wt.)[c] | 14 | 0.5146 ± 0.0245 | 18.4094 | -0.0260 ± 0.0284 | 95.19 ± 5.20 | -0.0235 ± 0.0205 |

[a]Separate variances for each chemical (or control) model used for analysis.
[b]Decrease in N is due to part or all of the tissue for one animal not being present in the tissue cup at the time of weighing the fixed tissue.
[c]Common variances across all groups model used for analysis.
[d]Male 203 was euthanized moribund on test day 7.
[e]Separate variances for each treatment group model used for analysis.
[f]Decrease in N is due to the seminal vesicles being nicked causing fluid to leak for one animal and therefore an accurate weight could not be obtained.
*p<0.05 for treatment group compared to control or linear trend compared to 0 trend.
**p<0.006 for treatment group compared to control or linear trend compared to 0 trend.

Table 7. Summary and Statistical Analysis of the Hormone Data for the Phenobarbital Treated Animals (page 1 of 2)

| | Vehicle Control | | | 25 mg/kg/day Phenobarbital | | | | | 50 mg/kg/day Phenobarbital | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE |
| Serum Testosterone (ng/ml)[a] | 15 | 3.37 ± 0.75 | 86.58 | 15 | 2.62 ± 0.52 | 55.40 | -0.75 ± 0.92 | 77.70 ± 23.20 | 15 | 2.64 ± 0.52 | 85.40 | -0.73 ± 0.92 | 78.23 ± 23.29 |
| Luteinizing Hormone (ng/ml)[b] | 13[c] | 1.28 ± 0.08 | 20.19 | 14[c] | 1.28 ± 0.07 | 19.57 | -0.002 ± 0.108 | 99.82 ± 8.40 | 13[c] | 1.24 ± 0.08 | 19.23 | -0.04 ± 0.11 | 96.52 ± 8.42 |
| Thyroid Stimulating Hormone (ng/ml)[d] | 15 | 18.53 ± 3.18 | 66.54 | 15 | 21.69 ± 1.79 | 31.88 | 3.16 ± 3.65 | 117.05 ± 22.30 | 15 | 26.60 * ± 2.42 | 35.28 | 8.07 ± 4.00 | 143.55 ± 27.91 |
| Thyroxine (µg/dL)[b] | 15 | 5.55 ± 0.16 | 15.62 | 15 | 4.66 ** ± 0.16 | 11.29 | -0.89 ± 0.23 | 83.88 ± 3.82 | 15 | 4.32 ** ± 0.16 | 13.61 | -1.23 ± 0.23 | 77.89 ± 3.71 |
| Triiodothyronine (ng/dL)[b] | 15 | 87.53 ± 2.92 | 14.92 | 15 | 78.24 * ± 2.92 | 18.06 | -9.29 ± 4.13 | 89.38 ± 4.48 | 15 | 73.28 ** ± 2.92 | 11.07 | -14.25 ± 4.13 | 83.72 ± 4.35 |

| | 100 mg/kg/day Phenobarbital | | | | | Linear Trend |
|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | Linear Trend Slope ± SE |
| Serum Testosterone (ng/ml)[a] | 13[e] | 2.66 ± 0.56 | 84.75 | -0.72 ± 0.94 | 78.75 ± 24.14 | -0.48 ± 0.65 |
| Luteinizing Hormone (ng/ml)[b] | 12[c] | 1.18 ± 0.08 | 15.53 | -0.10 ± 0.11 | 92.28 ± 8.43 | -0.08 ± 0.08 |
| Thyroid Stimulating Hormone (ng/ml)[d] | 13 | 26.38 ± 3.50 | 47.80 | 7.85 ± 4.73 | 142.37 ± 30.90 | 6.37 ± 3.24 |
| Thyroxine (µg/dL)[b] | 13 | 2.97 ** ± 0.17 | 21.39 | -2.58 ± 0.24 | 53.54 ± 3.52 | -1.80 ** ± 0.17 |
| Triiodothyronine (ng/dL)[b] | 13 | 58.14 ** ± 3.14 | 16.33 | -29.39 ± 4.29 | 66.43 ± 4.22 | -20.82 ** ± 3.02 |

Table 7. Summary and Statistical Analysis of the Hormone Data for the Phenobarbital Treated Animals (page 2 of 2)

| | Vehicle Control | | | 25 mg/kg/day Phenobarbital | | | | | 50 mg/kg/day Phenobarbital | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE |
| Follicle Stimulating Hormone (ng/ml)[a] | 15 | 15.39 ± 0.53 | 13.36 | 15 | 12.01 ** ± 0.43 | 16.92 | -3.38 ± 0.68 | 78.03 ± 3.88 | 15 | 12.57 ** ± 0.43 | 13.37 | -2.81 ± 0.68 | 81.73 ± 3.97 |
| Estradiol (pg/ml)[a] | 15 | 22.95 ± 1.18 | 19.98 | 15 | 26.33 ± 2.12 | 22.31 | 3.38 ± 2.43 | 114.73 ± 10.98 | 15 | 30.57 ** ± 2.12 | 26.38 | 7.62 ± 2.43 | 133.20 ± 11.52 |
| Prolactin (ng/ml)[d] | 15 | 10.84 ± 4.13 | 147.69 | 15 | 6.45 ± 1.03 | 61.77 | -4.39 ± 4.26 | 59.51 ± 24.60 | 15 | 4.74 ± 0.75 | 61.59 | -6.10 ± 4.20 | 43.70 ± 18.06 |
| Dihydrotestosterone (pg/ml)[a] | 15 | 219.35 ± 32.77 | 57.86 | 15 | 191.46 ± 21.86 | 33.32 | -27.89 ± 39.39 | 87.29 ± 16.41 | 15 | 195.35 ± 21.86 | 46.47 | -24.00 ± 39.39 | 89.06 ± 16.62 |

| | 100 mg/kg/day Phenobarbital | | | | | Linear Trend |
|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | Linear Trend Slope ± SE |
| Follicle Stimulating Hormone (ng/ml)[a] | 13 | 11.80 ** ± 0.46 | 9.13 | -3.58 ± 0.70 | 76.71 ± 4.00 | -2.28 ** ± 0.49 |
| Estradiol (pg/ml)[a] | 13 | 32.70 ** ± 2.28 | 31.94 | 9.74 ± 2.57 | 142.45 ± 12.36 | 7.48 ** ± 1.85 |
| Prolactin (ng/ml)[d] | 13 | 4.40 ± 1.12 | 92.05 | -6.44 ± 4.28 | 40.60 ± 18.63 | -4.70 ± 2.89 |
| Dihydrotestosterone (pg/ml)[a] | 13 | 206.20 ± 23.48 | 47.35 | -13.15 ± 40.32 | 94.01 ± 17.66 | -7.95 ± 27.92 |

[a]Separate variances for each chemical (or control) model used for analysis.
[b]Common variances across all groups model used for analysis.
[c]Decrease in N is due to one or more values being below the detection limit of 0.8 ng/ml.
[d]Separate variances for each treatment group model used for analysis.
[e]Male 51 was euthanized moribund on test day 3 and male 165 was euthanized moribund on test day 5.
*p<0.05 for treatment group compared to control or linear trend compared to 0 trend.
**p<0.006 for treatment group compared to control or linear trend compared to 0 trend.

Table 8. Summary and Statistical Analysis of the Hormone Data for the Linuron Treated Animals (page 1 of 2)

| | Vehicle Control | | | 50 mg/kg/day Linuron | | | | | 100 mg/kg/day Linuron | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE |
| Serum Testosterone (ng/ml)[a] | 15 | 3.37 ± 0.75 | 86.58 | 15 | 4.07 ± 0.42 | 39.67 | 0.69 ± 0.86 | 120.49 ± 29.65 | 15 | 2.56 ± 0.42 | 52.29 | -0.81 ± 0.86 | 75.92 ± 21.01 |
| Luteinizing Hormone (ng/ml)[b] | 13[c] | 1.28 ± 0.08 | 20.19 | 15 | 1.33 ± 0.07 | 23.44 | 0.05 ± 0.11 | 103.72 ± 8.44 | 12[c] | 1.31 ± 0.08 | 26.66 | 0.03 ± 0.11 | 102.16 ± 8.83 |
| Thyroid Stimulating Hormone (ng/ml)[d] | 15 | 18.53 ± 3.18 | 66.54 | 15 | 18.51 ± 1.80 | 37.71 | -0.02 ± 3.66 | 99.88 ± 19.72 | 15 | 11.90 * ± 0.88 | 28.58 | -6.63 ± 3.30 | 64.24 ± 12.01 |
| Thyroxine (µg/dL)[b] | 15 | 5.55 ± 0.16 | 15.62 | 15 | 3.87 ** ± 0.16 | 13.85 | -1.68 ± 0.23 | 69.73 ± 3.57 | 15 | 2.61 ** ± 0.16 | 29.05 | -2.94 ± 0.23 | 46.95 ± 3.24 |
| Triiodothyronine (ng/dL)[b] | 15 | 87.53 ± 2.92 | 14.92 | 15 | 85.66 ± 2.92 | 13.41 | -1.86 ± 4.13 | 97.87 ± 4.67 | 15 | 76.90 * ± 2.92 | 11.30 | -10.63 ± 4.13 | 87.86 ± 4.44 |

| | 150 mg/kg/day Linuron | | | | | Linear Trend |
|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | Linear Trend Slope ± SE |
| Serum Testosterone (ng/ml)[a] | 14[e] | 2.49 ± 0.43 | 75.55 | -0.89 ± 0.87 | 73.68 ± 20.87 | -0.93 ± 0.60 |
| Luteinizing Hormone (ng/ml)[b] | 12[c] | 1.41 ± 0.08 | 23.56 | 0.13 ± 0.11 | 110.02 ± 9.17 | 0.08 ± 0.08 |
| Thyroid Stimulating Hormone (ng/ml)[d] | 14 | 13.85 ± 1.53 | 41.32 | -4.68 ± 3.53 | 74.73 ± 15.26 | -4.62 ± 2.41 |
| Thyroxine (µg/dL)[b] | 14 | 1.69 ** ± 0.17 | 20.40 | -3.87 ± 0.23 | 30.37 ± 3.16 | -2.88 ** ± 0.17 |
| Triiodothyronine (ng/dL)[b] | 14 | 77.98 * ± 3.02 | 16.21 | -9.55 ± 4.21 | 89.09 ± 4.56 | -8.36 ** ± 2.97 |

Table 8. Summary and Statistical Analysis of the Hormone Data for the Linuron Treated Animals (page 2 of 2)

| | Vehicle Control | | | 50 mg/kg/day Linuron | | | | | 100 mg/kg/day Linuron | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE |
| Follicle Stimulating Hormone (ng/ml)[a] | 15 | 15.39 ± 0.53 | 13.36 | 15 | 16.07 ± 1.01 | 15.80 | 0.69 ± 1.14 | 104.46 ± 7.47 | 15 | 18.22 * ± 1.01 | 26.99 | 2.83 ± 1.14 | 118.41 ± 7.72 |
| Estradiol (pg/ml)[a] | 15 | 22.95 ± 1.18 | 19.98 | 15 | 31.06 ** ± 2.36 | 30.69 | 8.10 ± 2.64 | 135.31 ± 12.42 | 15 | 31.75 ** ± 2.36 | 21.40 | 8.80 ± 2.64 | 138.34 ± 12.51 |
| Prolactin (ng/ml)[d] | 15 | 10.84 ± 4.13 | 147.69 | 15 | 8.83 ± 2.32 | 101.76 | -2.00 ± 4.74 | 81.51 ± 37.75 | 15 | 6.37 ± 1.09 | 66.43 | -4.46 ± 4.27 | 58.82 ± 24.59 |
| Dihydrotestosterone (pg/ml)[a] | 15 | 219.35 ± 32.77 | 57.86 | 15 | 265.09 ± 20.24 | 32.89 | 45.74 ± 38.52 | 120.85 ± 20.28 | 15 | 190.58 ± 20.24 | 28.46 | -28.78 ± 38.52 | 86.88 ± 15.93 |

| | 150 mg/kg/day Linuron | | | | | Linear Trend |
|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | Linear Trend Slope ± SE |
| Follicle Stimulating Hormone (ng/ml)[a] | 14 | 17.64 ± 1.04 | 21.99 | 2.25 ± 1.17 | 114.64 ± 7.85 | 1.99 * ± 0.85 |
| Estradiol (pg/ml)[a] | 14 | 43.02 ** ± 2.44 | 24.99 | 20.06 ± 2.71 | 187.41 ± 14.37 | 13.61 ** ± 1.97 |
| Prolactin (ng/ml)[d] | 14 | 12.76 ± 2.81 | 82.35 | 1.92 ± 5.00 | 117.72 ± 51.83 | 0.74 ± 3.40 |
| Dihydrotestosterone (pg/ml)[a] | 14 | 202.55 ± 20.95 | 44.24 | -16.80 ± 38.90 | 92.34 ± 16.78 | -27.93 ± 26.87 |

[a]Separate variances for each chemical (or control) model used for analysis.
[b]Common variances across all groups model used for analysis.
[c]Decrease in N is due to one or more values being below the detection limit of 0.8 ng/ml.
[d]Separate variances for each treatment group model used for analysis.
[e]Male 203 was euthanized moribund on test day 7.
*p<0.05 for treatment group compared to control or linear trend compared to 0 trend.
**p<0.006 for treatment group compared to control or linear trend compared to 0 trend.

Table 9. Summary and Statistical Analysis of Selected Body Weights, Body Weight Changes and Feed Consumption for the Phenobarbital Treated Animals Minus the Potential Outlier Values (page 1 of 1)

| | Vehicle Control | | | 25 mg/kg/day Phenobarbital | | | | | 50 mg/kg/day Phenobarbital | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE |
| Body Weight Change on Test Days 8 to 15 (g/day)[a] | 15 | 3.6 ± 0.3 | 28.5 | 14[b] | 3.6 ± 0.3 | 26.2 | 0.01 ± 0.37 | 100.3 ± 10.2 | 15 | 4.0 ± 0.3 | 25.9 | 0.3 ± 0.4 | 109.3 ± 10.9 |
| Feed Consumption on Test Days 8 to 15 (g/kg/day)[a] | 15 | 65.3 ± 1.5 | 8.8 | 15 | 64.9 ± 1.4 | 8.3 | -0.4 ± 2.0 | 99.3 ± 3.1 | 14[c] | 68.2 ± 1.4 | 7.6 | 2.9 ± 2.0 | 104.4 ± 3.2 |

| | 100 mg/kg/day Phenobarbital | | | | | Linear Trend |
|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | Linear Trend Slope ± SE |
| Body Weight Change on Test Days 8 to 15 (g/day)[a] | 13[d] | 3.2 ± 0.4 | 45.8 | -0.5 ± 0.5 | 87.1 ± 12.8 | -0.02 ± 0.03 |
| Feed Consumption on Test Days 8 to 15 (g/kg/day)[a] | 13 | 71.9 * ± 2.4 | 11.8 | 6.5 ± 2.8 | 110.0 ± 4.4 | 5.1 ** ± 1.9 |

[a]Separate variances for each treatment group model used for analysis.
[b]One value for animal 29 was a potential outlier and was excluded from the analysis.
[c]One value for animal 45 was a potential outlier and was excluded from the analysis.
[d]Male 51 was euthanized moribund on test day 3 and male 165 was euthanized moribund on test day 5.
*p<0.05 for treatment group compared to control or linear trend compared to 0 trend.
**p<0.006 for treatment group compared to control or linear trend compared to 0 trend.

Table 10. Summary and Statistical Analysis of Selected Body Weights, Body Weight Changes and Feed Consumption for the Linuron Treated Animals Minus the Potential Outlier Values (page 1 of 1)

| | Vehicle Control | | | 50 mg/kg/day Linuron | | | | | 100 mg/kg/day Linuron | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE |
| Body Weight Change on Test Days 8 to 15 (g/day)[a] | 15 | 3.6 ± 0.3 | 28.5 | 15 | 3.8 ± 0.2 | 21.0 | 0.2 ± 0.3 | 105.8 ± 9.7 | 15 | 2.5 * ± 0.4 | 58.1 | -1.2 ± 0.5 | 68.1 ± 11.4 |
| Feed Consumption on Test Days 8 to 15 (g/kg/day)[a] | 15 | 65.3 ± 1.5 | 8.8 | 14[b] | 65.4 ± 1.5 | 8.6 | 0.1 ± 2.1 | 100.2 ± 3.2 | 15 | 65.7 ± 3.4 | 20.3 | 0.4 ± 3.8 | 100.6 ± 5.7 |

| | 150 mg/kg/day Linuron | | | | | Linear Trend |
|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | Linear Trend Slope ± SE |
| Body Weight Change on Test Days 8 to 15 (g/day)[a] | 14[c] | 2.4 ± 0.6 | 94.7 | -1.3 ± 0.7 | 65.1 ± 17.2 | -1.2 * ± 0.5 |
| Feed Consumption on Test Days 8 to 15 (g/kg/day)[a] | 14 | 58.7 ± 3.4 | 21.4 | -6.6 ± 3.7 | 89.9 ± 5.5 | -4.4 ± 2.6 |

[a]Separate variances for each treatment group model used for analysis.
[b]One value for animal 75 was a potential outlier and was excluded from the analysis.
[c]Male 203 was euthanized moribund on test day 7.
*p<0.05 for treatment group compared to control or linear trend compared to 0 trend.

Table 11. Summary and Statistical Analysis of Selected Absolute Organ Weights for the Phenobarbital Treated Animals Minus the Potential Outlier Values
(page 1 of 1)

| | Vehicle Control | | | 25 mg/kg/day Phenobarbital | | | | | 50 mg/kg/day Phenobarbital | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE |
| Thyroid Weight (g)[a] | 15 | 0.0171 ± 0.0005 | 12.2760 | 15 | 0.0205 * ± 0.0012 | 24.8265 | 0.0033 ± 0.0013 | 119.49 ± 7.76 | 14[b] | 0.0228 ** ± 0.0012 | 15.7325 | 0.0057 ± 0.0013 | 132.99 ± 8.18 |
| Paired Epididymis Weight (g)[c] | 15 | 1.1134 ± 0.0237 | 9.4561 | 15 | 1.1363 ± 0.0237 | 6.0043 | 0.0229 ± 0.0335 | 102.06 ± 3.04 | 14[d] | 1.1247 ± 0.0245 | 4.7792 | 0.0113 ± 0.0340 | 101.02 ± 3.07 |
| Prostate Weight (g)[c] | 15 | 0.9677 ± 0.0420 | 14.8606 | 15 | 0.9921 ± 0.0420 | 18.1837 | 0.0244 ± 0.0594 | 102.52 ± 6.21 | 15 | 1.0191 ± 0.0420 | 17.9995 | 0.0514 ± 0.0594 | 105.31 ± 6.30 |

| | 100 mg/kg/day Phenobarbital | | | | | Linear Trend |
|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | Linear Trend Slope ± SE |
| Thyroid Weight (g)[a] | 12[b,e] | 0.0229 ** ± 0.0013 | 20.3147 | 0.0058 ± 0.0014 | 133.66 ± 8.67 | 0.0044 ** ± 0.0010 |
| Paired Epididymis Weight (g)[c] | 13 | 1.1402 ± 0.0254 | 9.1686 | 0.0268 ± 0.0347 | 102.41 ± 3.15 | 0.0154 ± 0.0245 |
| Prostate Weight (g)[c] | 13 | 1.0378 ± 0.0451 | 16.4972 | 0.0702 ± 0.0616 | 107.25 ± 6.58 | 0.0531 ± 0.0434 |

[a]Separate variances for each chemical (or control) model used for analysis.
[b]Decrease in N is due to part or all of the tissue for one animal not being present in the tissue cup at the time of weighing the fixed tissue.
[c]Common variances across all groups model used for analysis.
[d]One value for animal 35 was a potential outlier and was excluded from the analysis.
[e]Male 51 was euthanized moribund on test day 3 and male 165 was euthanized moribund on test day 5.
*p<0.05 for treatment group compared to control or linear trend compared to 0 trend.
**p<0.006 for treatment group compared to control or linear trend compared to 0 trend.

Table 12. Summary and Statistical Analysis of Selected Relative Organ Weights for the Phenobarbital Treated Animals Minus the Potential Outlier Values
(page 1 of 1)

| | Vehicle Control | | | 25 mg/kg/day Phenobarbital | | | | | 50 mg/kg/day Phenobarbital | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE |
| Relative Thyroid Weight (% of final body wt.)[a] | 15 | 0.0043 ± 0.0002 | 13.8569 | 15 | 0.0050 * ± 0.0003 | 22.4274 | 0.0008 ± 0.0003 | 118.31 ± 8.21 | 14[b] | 0.0057 ** ± 0.0003 | 17.4909 | 0.0015 ± 0.0003 | 134.26 ± 8.72 |
| Relative Paired Epididymis Weight (% of final body wt.)[c] | 15 | 0.2758 ± 0.0078 | 9.4144 | 15 | 0.2813 ± 0.0078 | 9.7716 | 0.0055 ± 0.0110 | 101.98 ± 4.03 | 14[d] | 0.2782 ± 0.0081 | 4.7884 | 0.0024 ± 0.0112 | 100.87 ± 4.08 |
| Relative Prostate Weight (% of final body wt.)[c] | 15 | 0.2393 ± 0.0112 | 13.7151 | 15 | 0.2445 ± 0.0112 | 17.5207 | 0.0051 ± 0.0158 | 102.15 ± 6.67 | 15 | 0.2532 ± 0.0112 | 18.4622 | 0.0139 ± 0.0158 | 105.80 ± 6.79 |

| | 100 mg/kg/day Phenobarbital | | | | | Linear Trend |
|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | Linear Trend Slope ± SE |
| Relative Thyroid Weight (% of final body wt.)[a] | 12[b,e] | 0.0064 ** ± 0.0003 | 21.1597 | 0.0022 ± 0.0004 | 150.73 ± 9.54 | 0.0016 ** ± 0.0003 |
| Relative Paired Epididymis Weight (% of final body wt.)[c] | 13 | 0.3169 ** ± 0.0084 | 12.5465 | 0.0410 ± 0.0114 | 114.87 ± 4.43 | 0.0268 ** ± 0.0081 |
| Relative Prostate Weight (% of final body wt.)[c] | 13 | 0.2877 ** ± 0.0120 | 17.4540 | 0.0483 ± 0.0164 | 120.18 ± 7.52 | 0.0344 ** ± 0.0115 |

[a]Separate variances for each chemical (or control) model used for analysis.
[b]Decrease in N is due to part or all of the tissue for one animal not being present in the tissue cup at the time of weighing the fixed tissue.
[c]Common variances across all groups model used for analysis.
[d]One value for animal 35 was a potential outlier and was excluded from the analysis.
[e]Male 51 was euthanized moribund on test day 3 and male 165 was euthanized moribund on test day 5.
*p<0.05 for treatment group compared to control or linear trend compared to 0 trend.
**p<0.006 for treatment group compared to control or linear trend compared to 0 trend.

Table 13. Summary and Statistical Analysis of Select Absolute Organ Weights for the Linuron Treated Animals Minus the Potential Outlier Values (page 1 of 1)

| | Vehicle Control | | | 50 mg/kg/day Linuron | | | | | 100 mg/kg/day Linuron | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE |
| Thyroid Weight (g)[a] | 15 | 0.0171 ± 0.0005 | 12.2760 | 15 | 0.0181 ± 0.0007 | 14.5119 | 0.0010 ± 0.0009 | 105.76 ± 5.09 | 14[b] | 0.0171 ± 0.0007 | 18.2231 | -0.00004 ± 0.00087 | 99.76 ± 5.08 |
| Paired Epididymis Weight (g)[c] | 15 | 1.1134 ± 0.0237 | 9.4561 | 15 | 1.1020 ± 0.0237 | 7.9185 | -0.0114 ± 0.0335 | 98.98 ± 2.99 | 15 | 1.0176 ** ± 0.0237 | 9.4146 | -0.0958 ± 0.0335 | 91.40 ± 2.88 |
| Prostate Weight (g)[c] | 15 | 0.9677 ± 0.0420 | 14.8606 | 15 | 0.9017 ± 0.0420 | 20.9036 | -0.0660 ± 0.0594 | 93.18 ± 5.93 | 14[d] | 0.8063 * ± 0.0435 | 10.6041 | -0.1614 ± 0.0604 | 83.32 ± 5.76 |

| | 150 mg/kg/day Linuron | | | | | Linear Trend |
|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | Linear Trend Slope ± SE |
| Thyroid Weight (g)[a] | 13[e,f] | 0.0170 ± 0.0007 | 9.2194 | -0.0001 ± 0.0009 | 99.45 ± 5.19 | -0.0003 ± 0.0006 |
| Paired Epididymis Weight (g)[c] | 14 | 1.0108 ** ± 0.0245 | 11.1291 | -0.1026 ± 0.0340 | 90.78 ± 2.92 | -0.0877 ** ± 0.0240 |
| Prostate Weight (g)[c] | 14 | 0.7524 ** ± 0.0435 | 20.9783 | -0.2153 ± 0.0604 | 77.75 ± 5.62 | -0.1657 ** ± 0.0427 |

[a]Separate variances for each chemical (or control) model used for analysis.
[b]Decrease in N is due to part or all of the tissue for one animal not being present in the tissue cup at the time of weighing the fixed tissue.
[c]Common variances across all groups model used for analysis.
[d]One value for animal 79 was a potential outlier and was excluded from the analysis.
[e]Male 203 was euthanized moribund on test day 7.
[f]One value for animal 209 was a potential outlier and was excluded from the analysis.
*p<0.05 for treatment group compared to control or linear trend compared to 0 trend.
**p<0.006 for treatment group compared to control or linear trend compared to 0 trend.

Table 14. Summary and Statistical Analysis of Select Relative Organ Weights for the Linuron Treated Animals Minus the Potential Outlier Values (page 1 of 1)

| | Vehicle Control | | | 50 mg/kg/day Linuron | | | | | 100 mg/kg/day Linuron | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE |
| Relative Thyroid Weight (% of final body wt.)[a] | 15 | 0.0043 ± 0.0002 | 13.8569 | 15 | 0.0048 * ± 0.0002 | 13.2781 | 0.0005 ± 0.0002 | 112.59 ± 5.85 | 14[b] | 0.0049 * ± 0.0002 | 18.0714 | 0.0006 ± 0.0002 | 115.11 ± 6.02 |
| Relative Paired Epididymis Weight (% of final body wt.)[c] | 15 | 0.2758 ± 0.0078 | 9.4144 | 15 | 0.2923 ± 0.0078 | 10.4258 | 0.0165 ± 0.0110 | 105.98 ± 4.11 | 15 | 0.2905 ± 0.0078 | 9.5764 | 0.0147 ± 0.0110 | 105.31 ± 4.10 |
| Relative Prostate Weight (% of final body wt.)[c] | 15 | 0.2393 ± 0.0112 | 13.7151 | 15 | 0.2395 ± 0.0112 | 22.5283 | 0.0002 ± 0.0158 | 100.07 ± 6.60 | 14[d] | 0.2305 ± 0.0116 | 12.4271 | -0.0089 ± 0.0169 | 96.30 ± 6.60 |

| | 150 mg/kg/day Linuron | | | | | Linear Trend |
|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | Linear Trend Slope ± SE |
| Relative Thyroid Weight (% of final body wt.)[a] | 13[e,f] | 0.0053 ** ± 0.0002 | 9.7823 | 0.0010 ± 0.0002 | 123.49 ± 6.34 | 0.0007 ** ± 0.0002 |
| Relative Paired Epididymis Weight (% of final body wt.)[c] | 14 | 0.3102 ** ± 0.0081 | 12.6990 | 0.0344 ± 0.0112 | 112.46 ± 4.31 | 0.0227 ** ± 0.0079 |
| Relative Prostate Weight (% of final body wt.)[c] | 14 | 0.2293 ± 0.0116 | 18.2876 | -0.0101 ± 0.0161 | 95.79 ± 6.58 | -0.0088 ± 0.0114 |

[a]Separate variances for each chemical (or control) model used for analysis.
[b]Decrease in N is due to part or all of the tissue for one animal not being present in the tissue cup at the time of weighing the fixed tissue.
[c]Common variances across all groups model used for analysis.
[d]One value for animal 79 was a potential outlier and was excluded from the analysis.
[e]Male 203 was euthanized moribund on test day 7.
[f]One value for animal 209 was a potential outlier and was excluded from the analysis.
*p<0.05 for treatment group compared to control or linear trend compared to 0 trend.
**p<0.006 for treatment group compared to control or linear trend compared to 0 trend.

Table 15. Summary and Statistical Analysis of Select Hormone Data for the Phenobarbital Treated Animals Minus the Potential Outlier Values (page 1 of 2)

| | Vehicle Control | | | 25 mg/kg/day Phenobarbital | | | | | 50 mg/kg/day Phenobarbital | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE |
| Serum Testosterone (ng/ml)[a] | 15 | 3.37 ± 0.75 | 86.58 | 15 | 2.62 ± 0.52 | 55.40 | -0.75 ± 0.92 | 77.70 ± 21.59 | 14[b] | 2.64 ± 0.52 | 56.59 | -0.73 ± 0.92 | 63.48 ± 19.43 |
| Thyroid Stimulating Hormone (ng/ml)[c] | 14[d] | 18.53 ± 3.18 | 32.66 | 15 | 21.69 ± 1.79 | 31.88 | 3.16 ± 3.65 | 138.95 ± 16.67 | 15 | 26.60 * ± 2.42 | 35.28 | 8.07 ± 4.00 | 170.40 ± 21.50 |
| Follicle Stimulating Hormone (ng/ml)[a] | 15 | 15.39 ± 0.53 | 13.36 | 14[e] | 12.01 ** ± 0.43 | 11.71 | -3.38 ± 0.68 | 75.43 ± 3.57 | 15 | 12.57 ** ± 0.43 | 13.37 | -2.81 ± 0.68 | 81.73 ± 3.68 |
| Prolactin (ng/ml)[c] | 14[f] | 10.84 ± 4.13 | 58.40 | 15 | 6.45 ± 1.03 | 61.77 | -4.39 ± 4.26 | 94.49 ± 21.09 | 15 | 4.74 ± 0.75 | 61.59 | -6.10 ± 4.20 | 69.39 ± 15.46 |
| Dihydrotestosterone (pg/ml)[a] | 15 | 219.35 ± 32.77 | 57.86 | 15 | 191.46 ± 21.86 | 33.32 | -27.89 ± 39.39 | 87.29 ± 16.41 | 15 | 195.35 ± 21.86 | 46.47 | -24.00 ± 39.39 | 89.06 ± 16.62 |

| | 100 mg/kg/day Phenobarbital | | | | | Linear Trend |
|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | Linear Trend Slope ± SE |
| Serum Testosterone (ng/ml)[a] | 13[g] | 2.66 ± 0.56 | 84.75 | -0.72 ± 0.94 | 78.75 ± 22.35 | -0.48 ± 0.65 |
| Thyroid Stimulating Hormone (ng/ml)[c] | 13 | 26.38 ± 3.50 | 47.80 | 7.85 ± 4.73 | 169.00 ± 26.83 | 6.37 ± 3.24 |
| Follicle Stimulating Hormone (ng/ml)[a] | 13 | 11.80 ** ± 0.46 | 9.13 | -3.58 ± 0.70 | 76.71 ± 3.67 | -2.28 ** ± 0.49 |
| Prolactin (ng/ml)[c] | 12[h] | 4.40 ± 1.12 | 57.95 | -6.44 ± 4.28 | 49.91 ± 11.42 | -4.70 ± 2.89 |
| Dihydrotestosterone (pg/ml)[a] | 13 | 206.20 ± 23.48 | 47.35 | -13.15 ± 40.32 | 94.01 ± 17.66 | -7.95 ± 27.92 |

[a]Separate variances for each chemical (or control) model used for analysis.
[b]Value for animal 139 was a potential outlier and was excluded from the analysis.
[c]Separate variances for each treatment group model used for analysis.
[d]Value for animal 5 was a potential outlier and was excluded from the analysis.
[e]Value for animal 21 was a potential outlier and was excluded from the analysis.
[f]Value for animal 7 was a potential outlier and was excluded from the analysis.
[g]Male 51 was euthanized moribund on test day 3 and male 165 was euthanized moribund on test day 5.
[h]Value for animal 151 was a potential outlier and was excluded from the analysis.
*p<0.05 for treatment group compared to control or linear trend compared to 0 trend.
**p<0.006 for treatment group compared to control or linear trend compared to 0 trend.

Table 16. Summary and Statistical Analysis of Select Hormone Data for the Linuron Treated Animals Minus the Potential Outlier Values (page 1 of 2)

| | Vehicle Control | | | 50 mg/kg/day Linuron | | | | | 100 mg/kg/day Linuron | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE |
| Serum Testosterone (ng/ml)[a] | 15 | 3.37 ± 0.75 | 86.58 | 15 | 4.07 ± 0.42 | 39.67 | 0.69 ± 0.86 | 120.49 ± 29.65 | 15 | 2.56 ± 0.42 | 52.29 | -0.81 ± 0.86 | 75.92 ± 21.01 |
| Thyroid Stimulating Hormone (ng/ml)[b] | 14[c] | 18.53 ± 3.18 | 32.66 | 15 | 18.51 ± 1.80 | 37.71 | -0.02 ± 3.66 | 118.56 ± 15.50 | 15 | 11.90 * ± 0.88 | 28.58 | -6.63 ± 3.30 | 76.25 ± 8.72 |
| Follicle Stimulating Hormone (ng/ml)[a] | 15 | 15.39 ± 0.53 | 13.36 | 15 | 16.07 ± 1.01 | 15.80 | 0.69 ± 1.14 | 104.46 ± 7.47 | 15 | 18.22 * ± 1.01 | 26.99 | 2.83 ± 1.14 | 118.41 ± 7.72 |
| Prolactin (ng/ml)[b] | 14[d] | 10.84 ± 4.13 | 58.40 | 15 | 8.83 ± 2.32 | 101.76 | -2.00 ± 4.74 | 129.42 ± 39.55 | 15 | 6.37 ± 1.09 | 66.43 | -4.46 ± 4.27 | 93.39 ± 21.66 |
| Dihydrotestosterone (pg/ml)[a] | 15 | 219.35 ± 32.77 | 57.86 | 14[e] | 265.09 ± 20.24 | 20.12 | 45.74 ± 38.52 | 112.26 ± 18.63 | 15 | 190.58 ± 20.24 | 28.46 | -28.78 ± 38.52 | 86.88 ± 15.17 |

| | 150 mg/kg/day Linuron | | | | | Linear Trend |
|---|---|---|---|---|---|---|
| | N | LS Mean ± SE | CV% | Change Mean from Control ± SE | Mean as % of Control ± SE | Linear Trend Slope ± SE |
| Serum Testosterone (ng/ml)[a] | 14[f] | 2.49 ± 0.43 | 75.55 | -0.89 ± 0.87 | 73.68 ± 20.87 | -0.93 ± 0.60 |
| Thyroid Stimulating Hormone (ng/ml)[b] | 13[g] | 13.85 ± 1.53 | 26.27 | -4.68 ± 3.53 | 80.56 ± 9.16 | -4.62 ± 2.41 |
| Follicle Stimulating Hormone (ng/ml)[a] | 14 | 17.64 ± 1.04 | 21.99 | 2.25 ± 1.17 | 114.64 ± 7.85 | 1.99 * ± 0.85 |
| Prolactin (ng/ml)[b] | 14 | 12.76 ± 2.81 | 82.35 | 1.92 ± 5.00 | 186.91 ± 50.43 | 0.74 ± 3.40 |
| Dihydrotestosterone (pg/ml)[a] | 14 | 202.55 ± 20.95 | 44.24 | -16.80 ± 38.90 | 92.34 ± 16.01 | -27.93 ± 26.87 |

[a]Separate variances for each chemical (or control) model used for analysis.
[b]Separate variances for each treatment group model used for analysis.
[c]Value for animal 5 was a potential outlier and was excluded from the analysis.
[d]Value for animal 7 was a potential outlier and was excluded from the analysis.
[e]Value for animal 61 was a potential outlier and was excluded from the analysis.
[f]Male 203 was euthanized moribund on test day 7.
[g]Value for animal 199 was a potential outlier and was excluded from the analysis.
*p<0.05 for treatment group compared to control or linear trend compared to 0 trend.

# Appendix III

# Figures

Figure 1. Means (with ± 2 Standard Error Bars) for Body Weight (g) for Each Dose of Phenobarbital

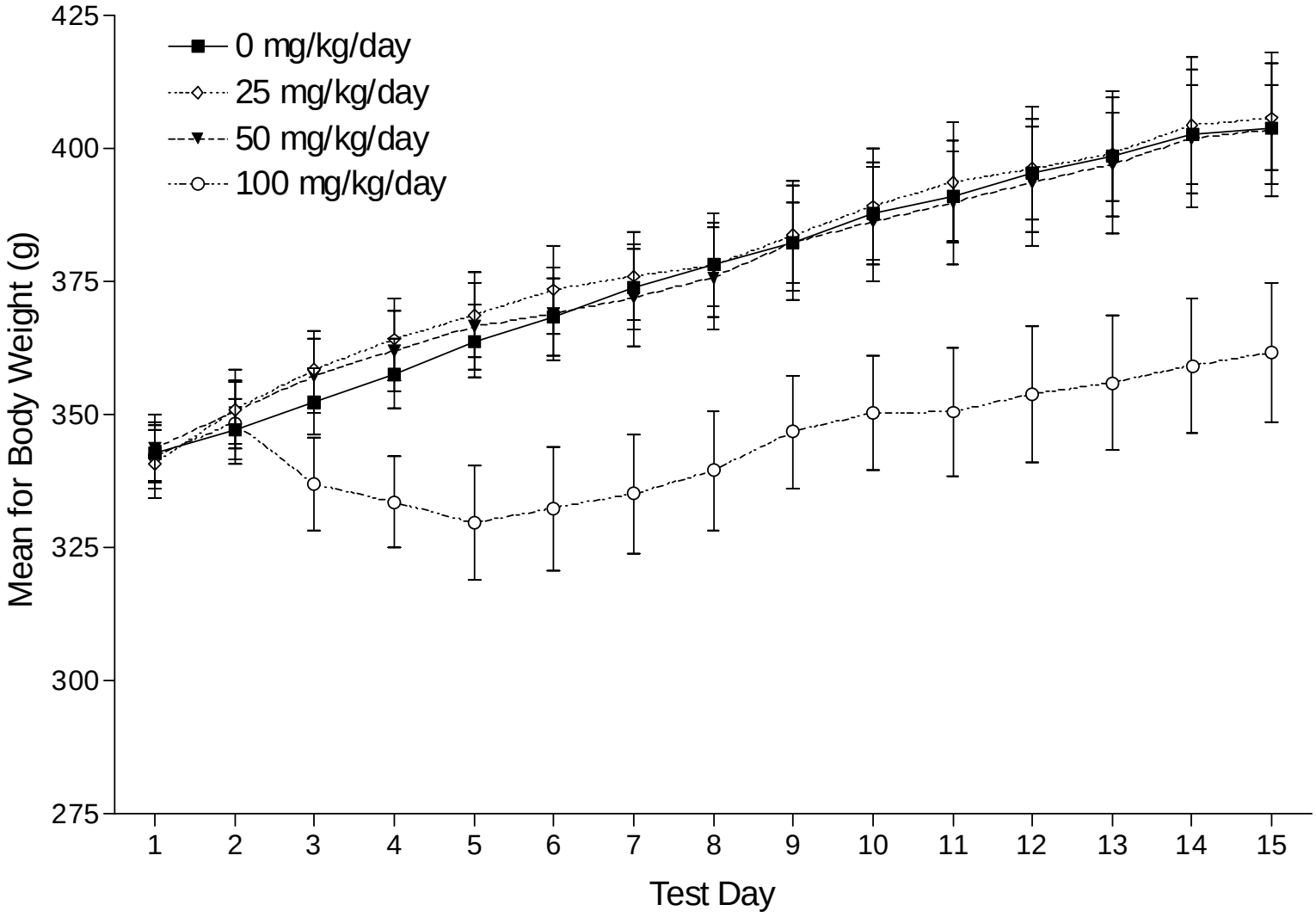


Figure 2. Means (with ± 2 Standard Error Bars) for Body Weights (g) for Each Dose Group of Linuron

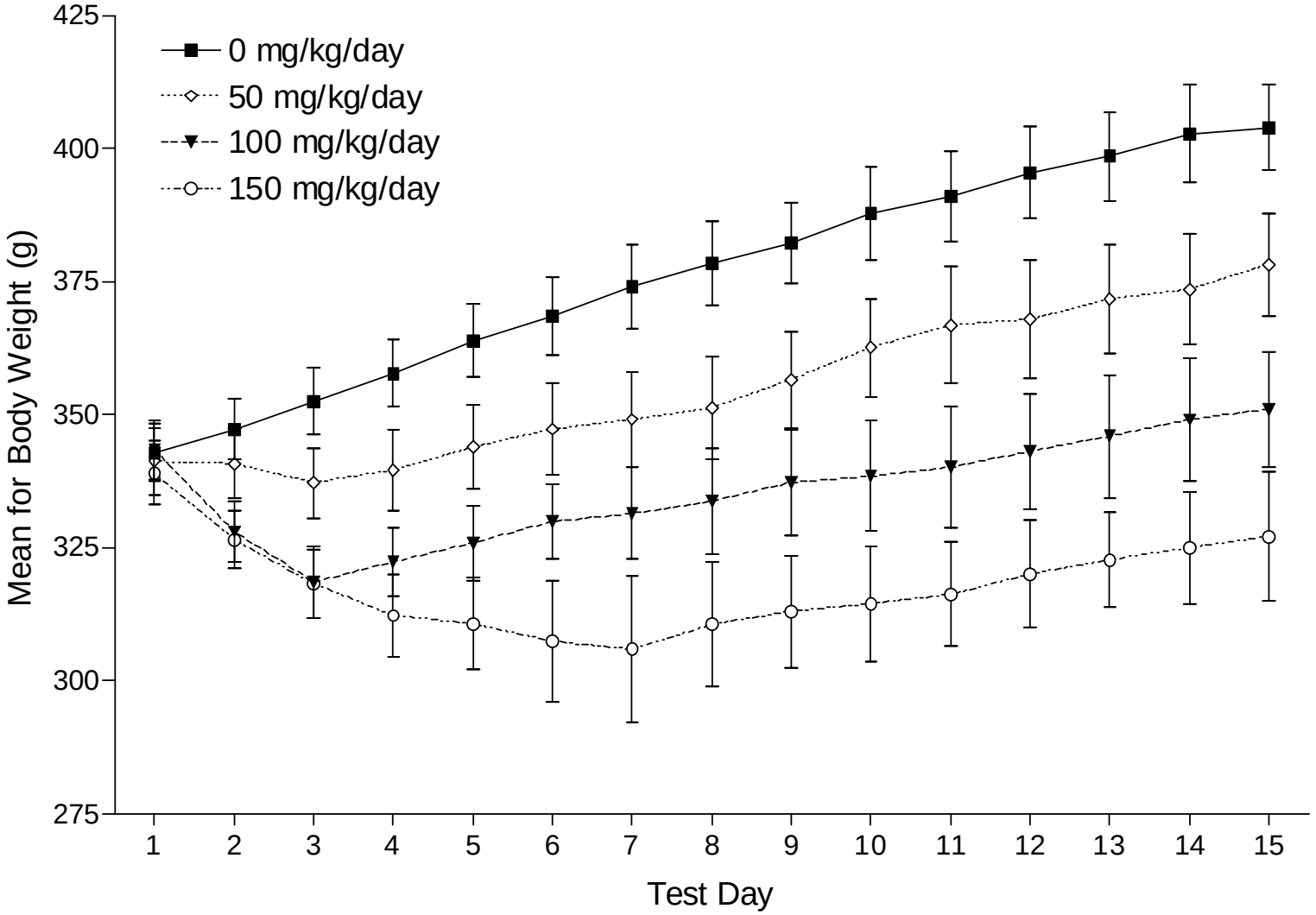


Figure 3. Least Squares Means (with ± 2 Standard Error Bars) for Final Body Weight (g) for Each Dose Group

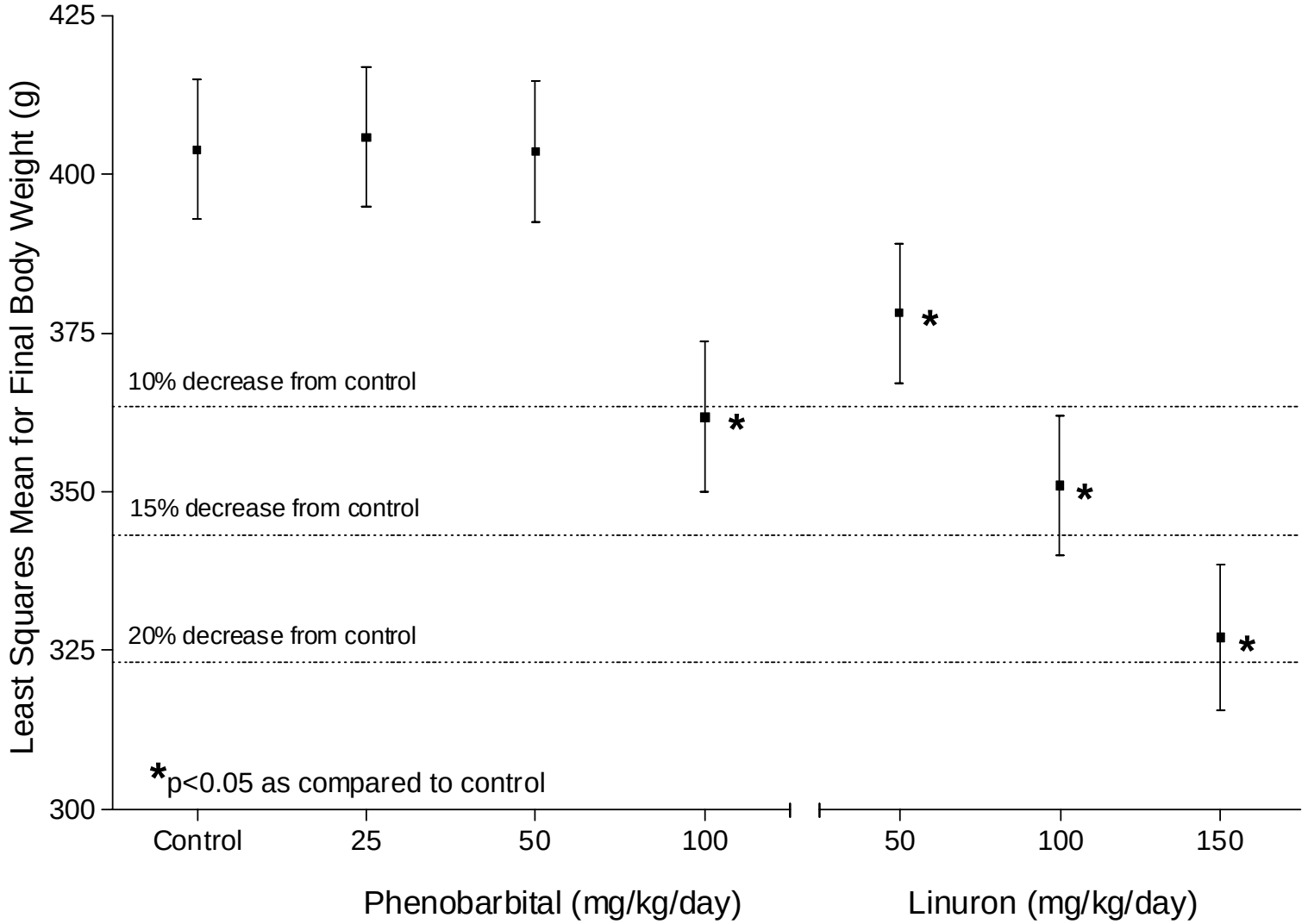


Figure 4. Least Squares Means (with ± 2 Standard Error Bars) for Body Weight Change (g/day) for Test Days 1 to 8 for Each Dose Group

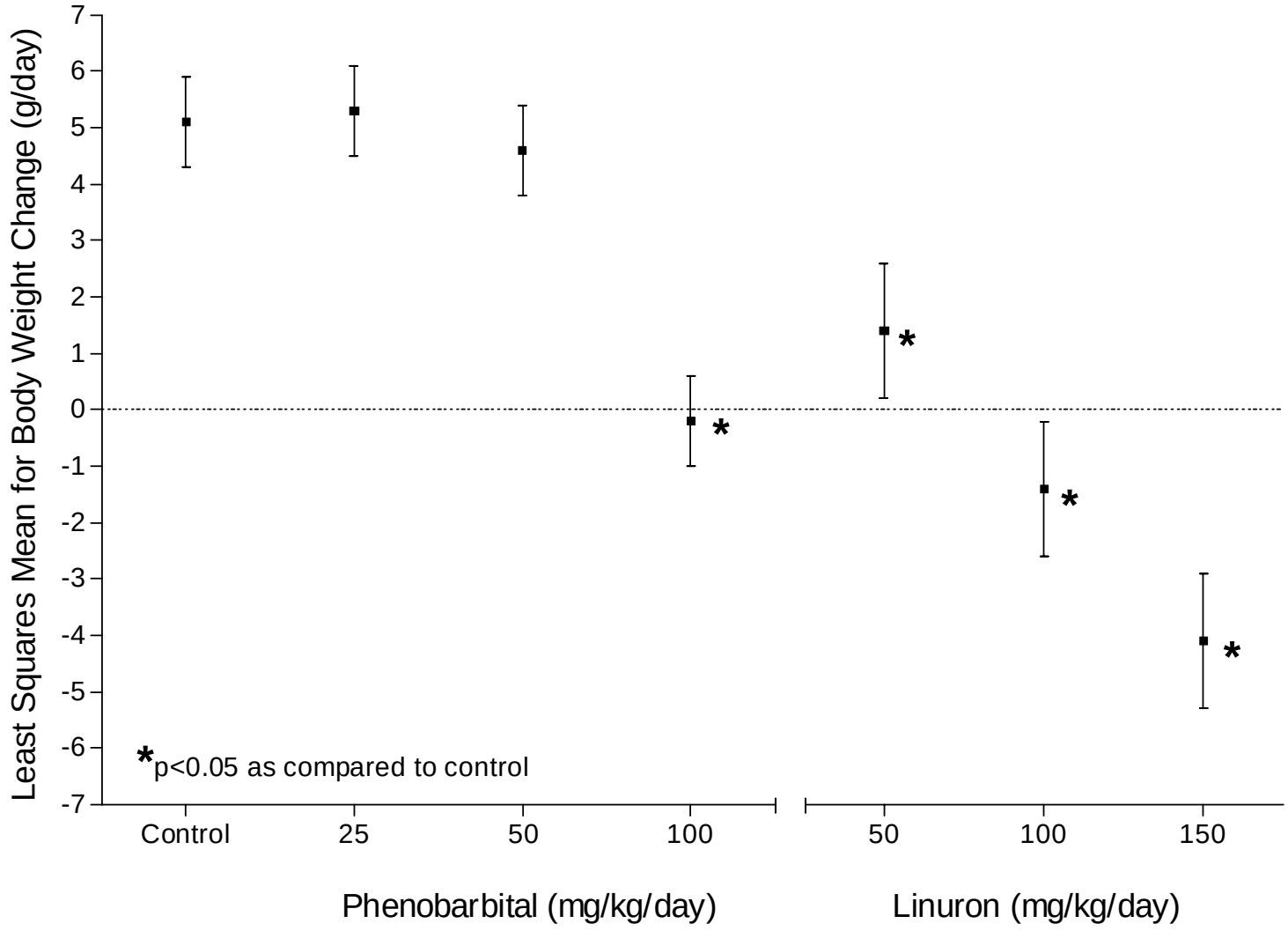


Figure 5. Least Squares Means (with ± 2 Standard Error Bars) for Body Weight Change (g/day) for Test Days 8 to 15 for Each Dose Group

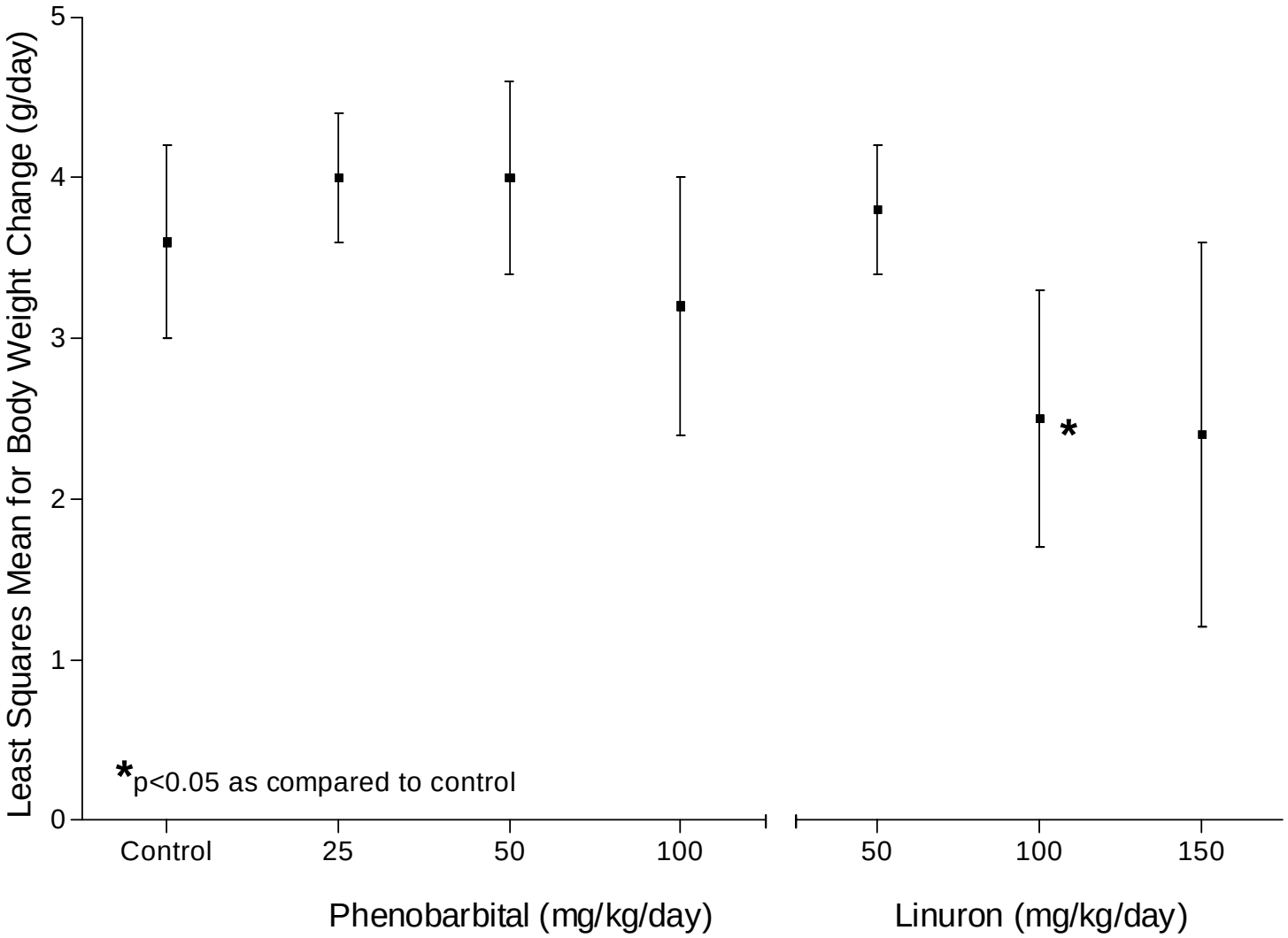


Figure 6. Least Squares Means (with ± 2 Standard Error Bars) for Body Weight Change (g/day) for Test Days 1 to 15 for Each Dose Group

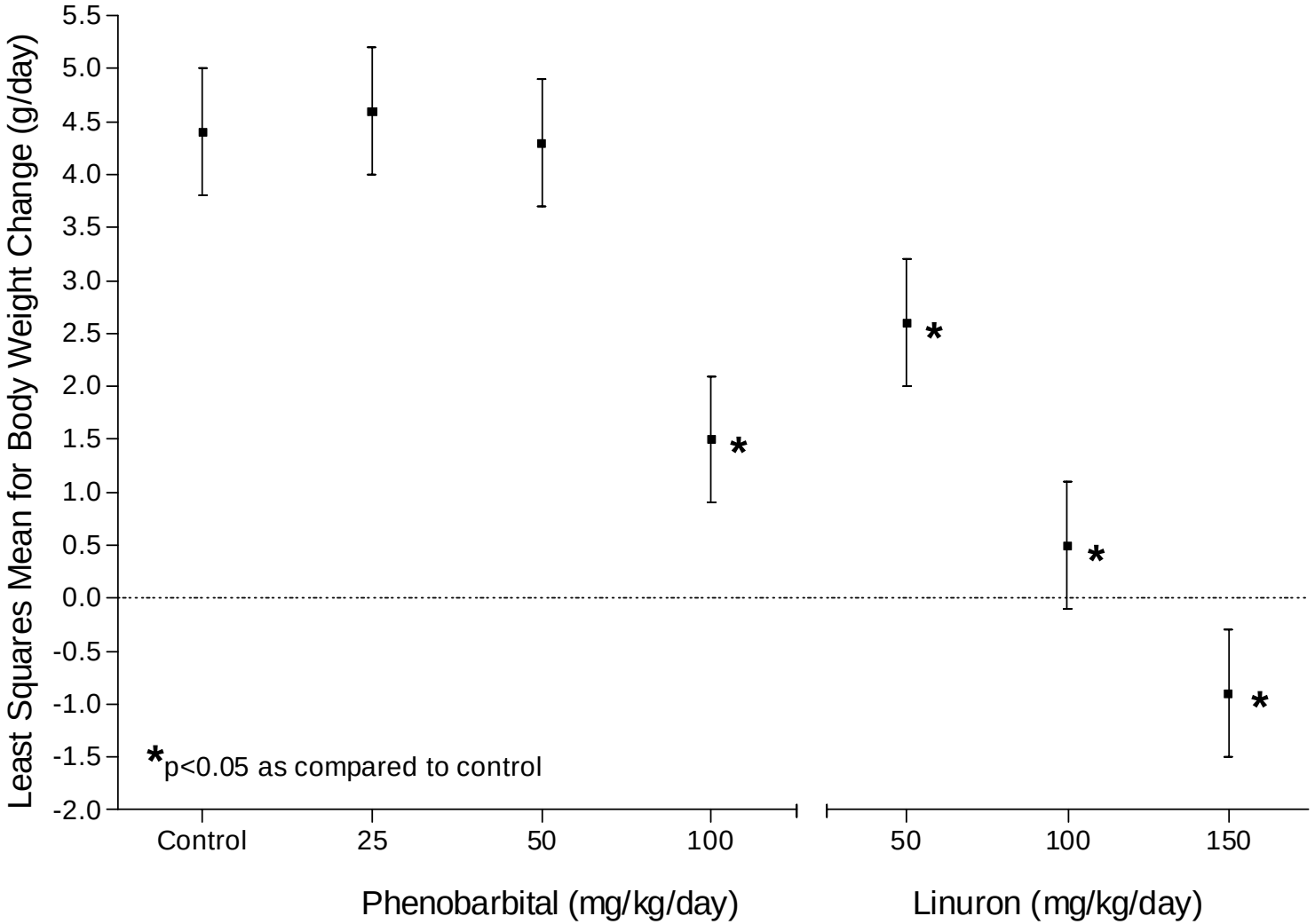


Figure 7. Least Squares Means (with ± 2 Standard Error Bars) for Feed Consumption (g/kg/day) for Test Days 1 to 8 for Each Dose Group

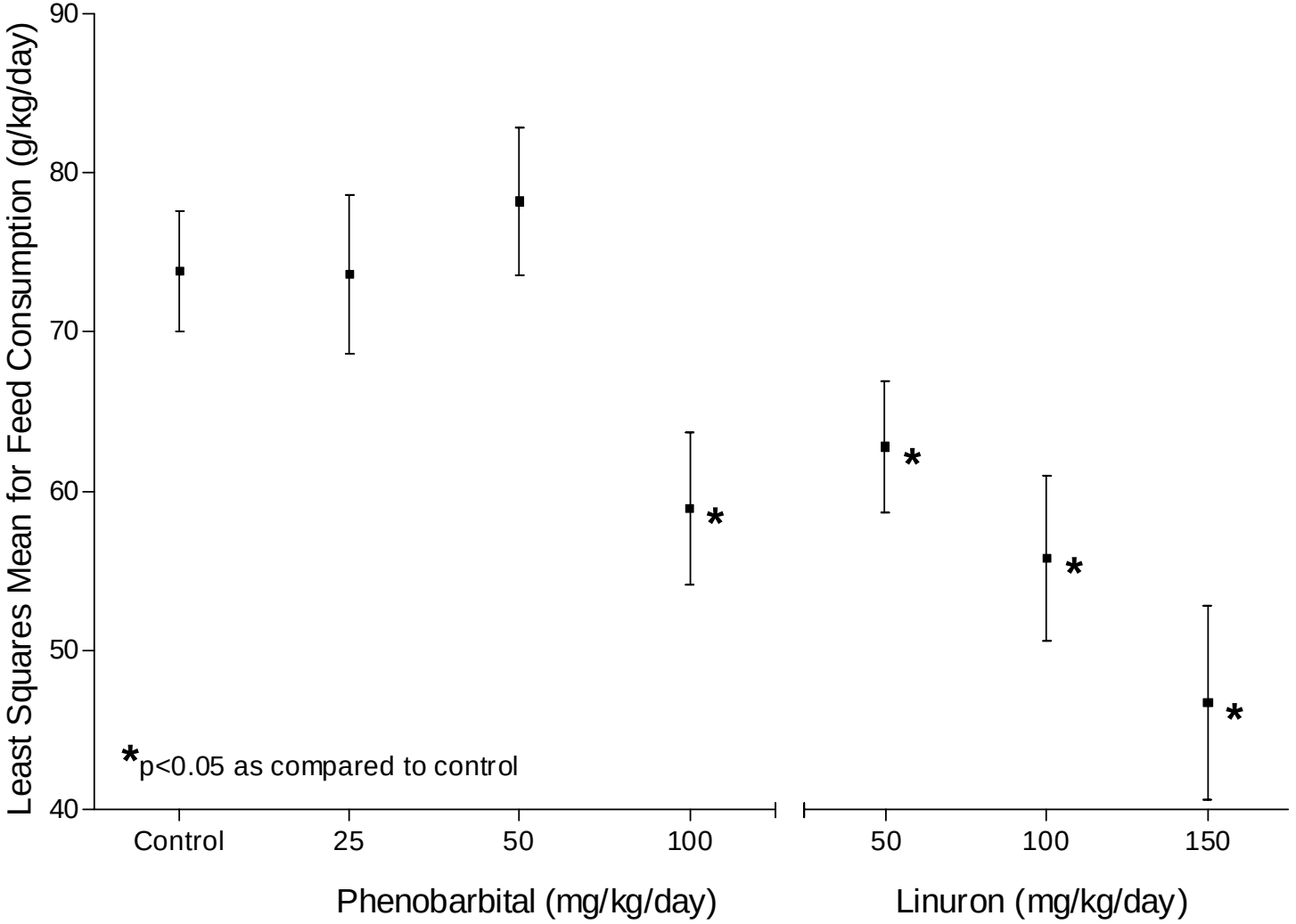


Figure 8. Least Squares Means (with ± 2 Standard Error Bars) for Feed Consumption (g/kg/day) for Test Days 8 to 15 for Each Dose Group

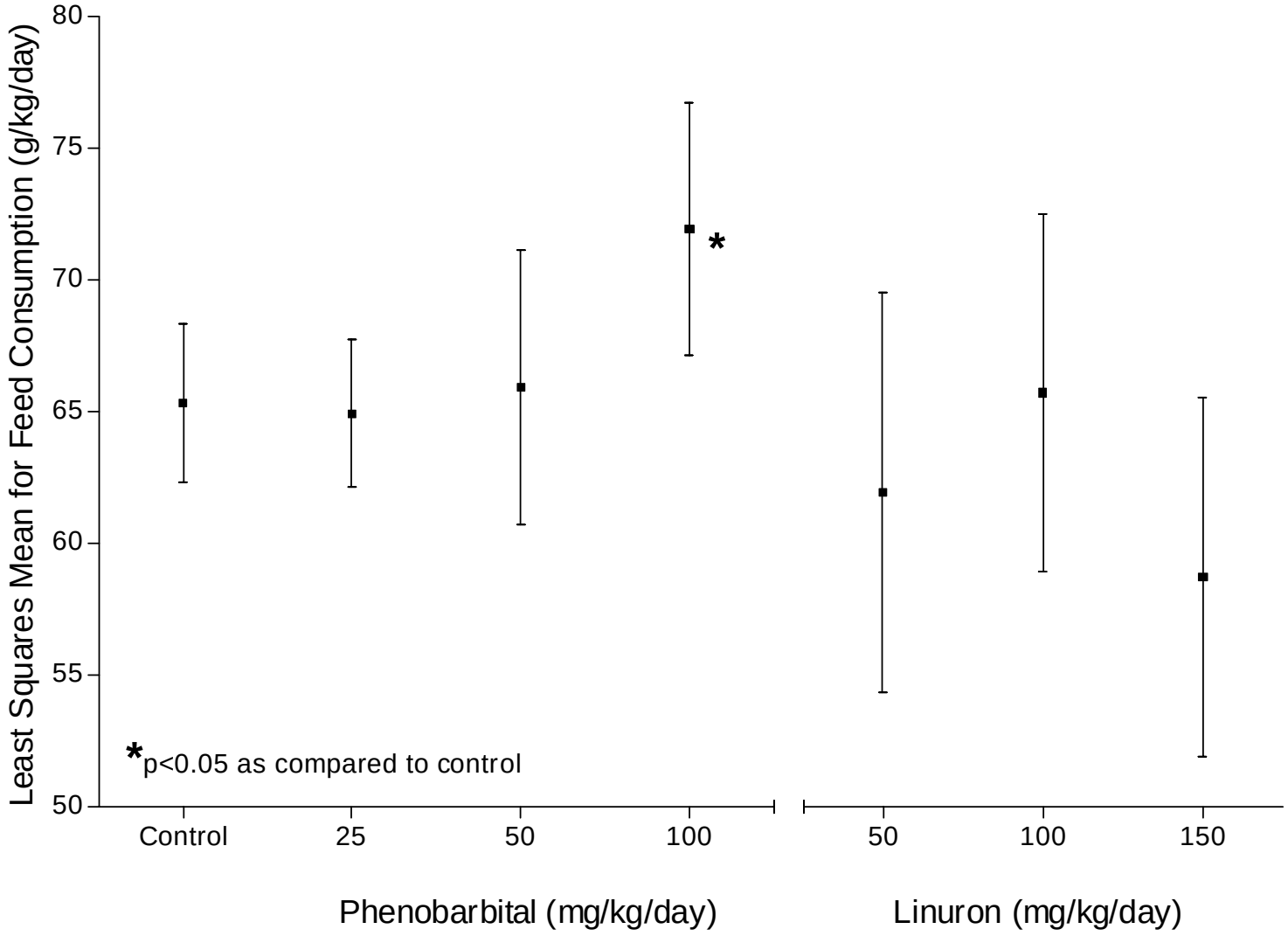


Figure 9. Least Squares Means (with ± 2 Standard Error Bars) for Feed Consumption (g/kg/day) for Test Days 1 to 15 for Each Dose Group

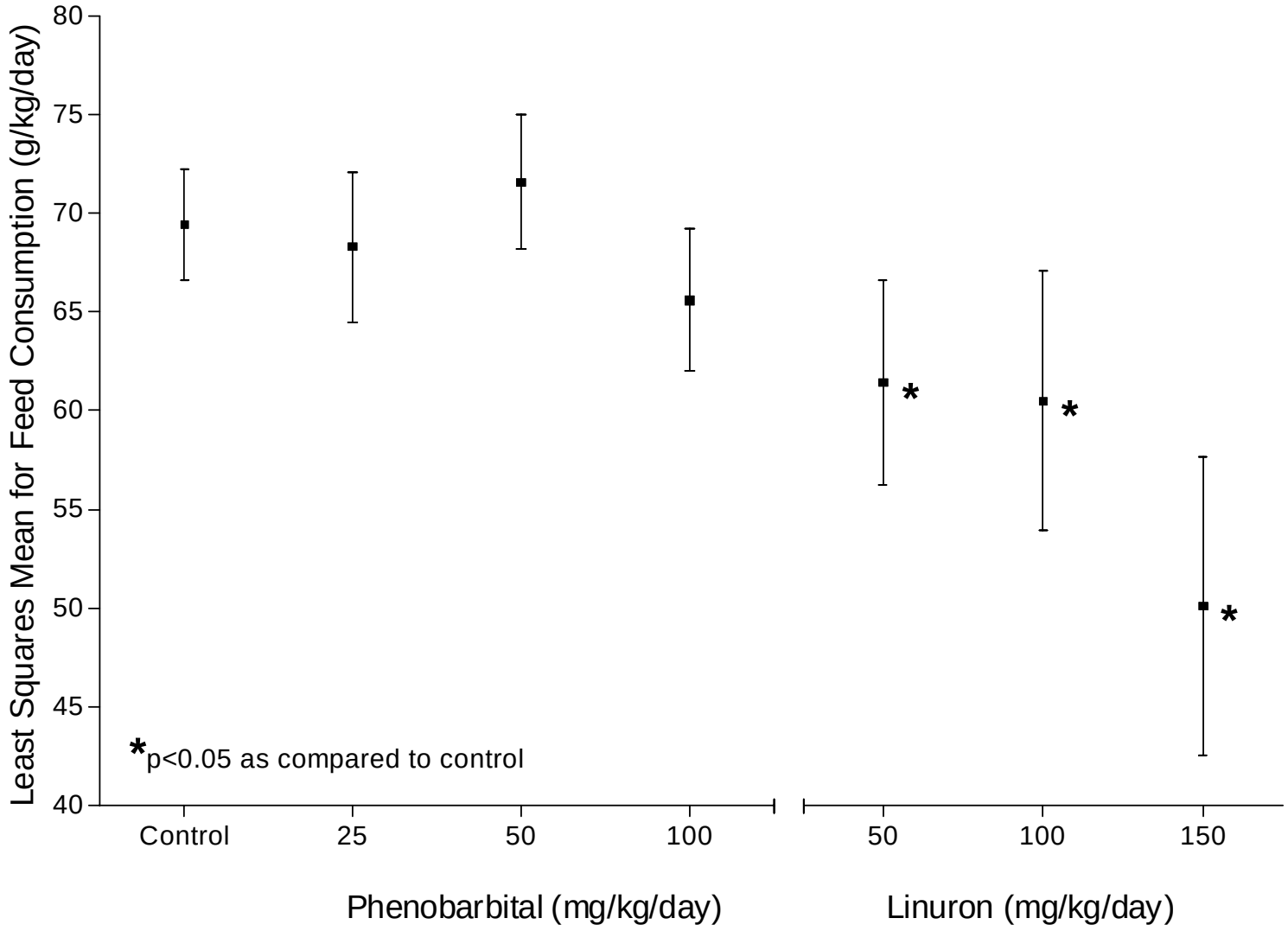


Figure 10. Least Squares Means (with ± 2 Standard Error Bars) for Thyroid Glands Weight (g) for Each Dose Group

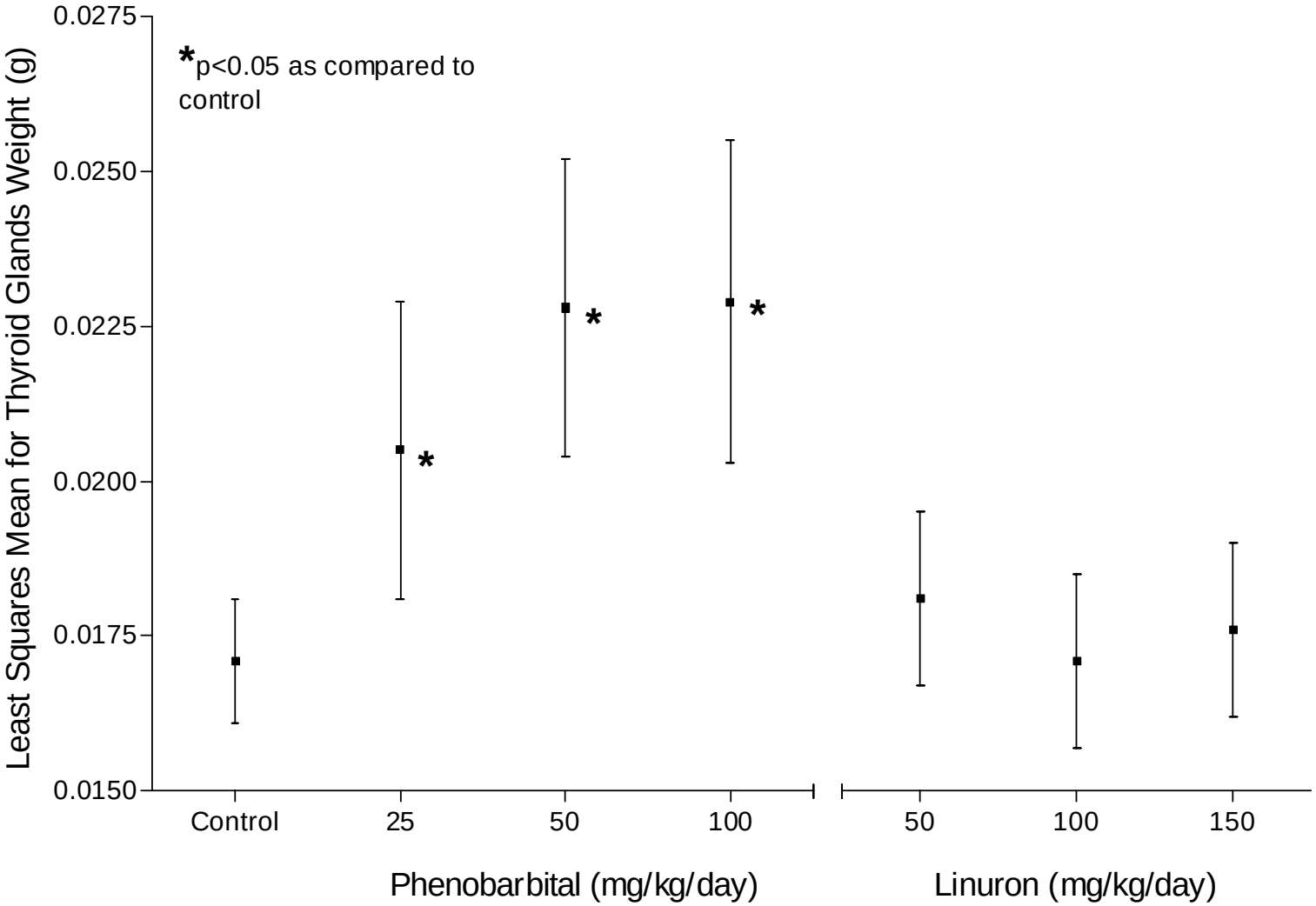


Figure 11. Least Squares Means (with ± 2 Standard Error Bars) for Liver Weight (g) for Each Dose Group

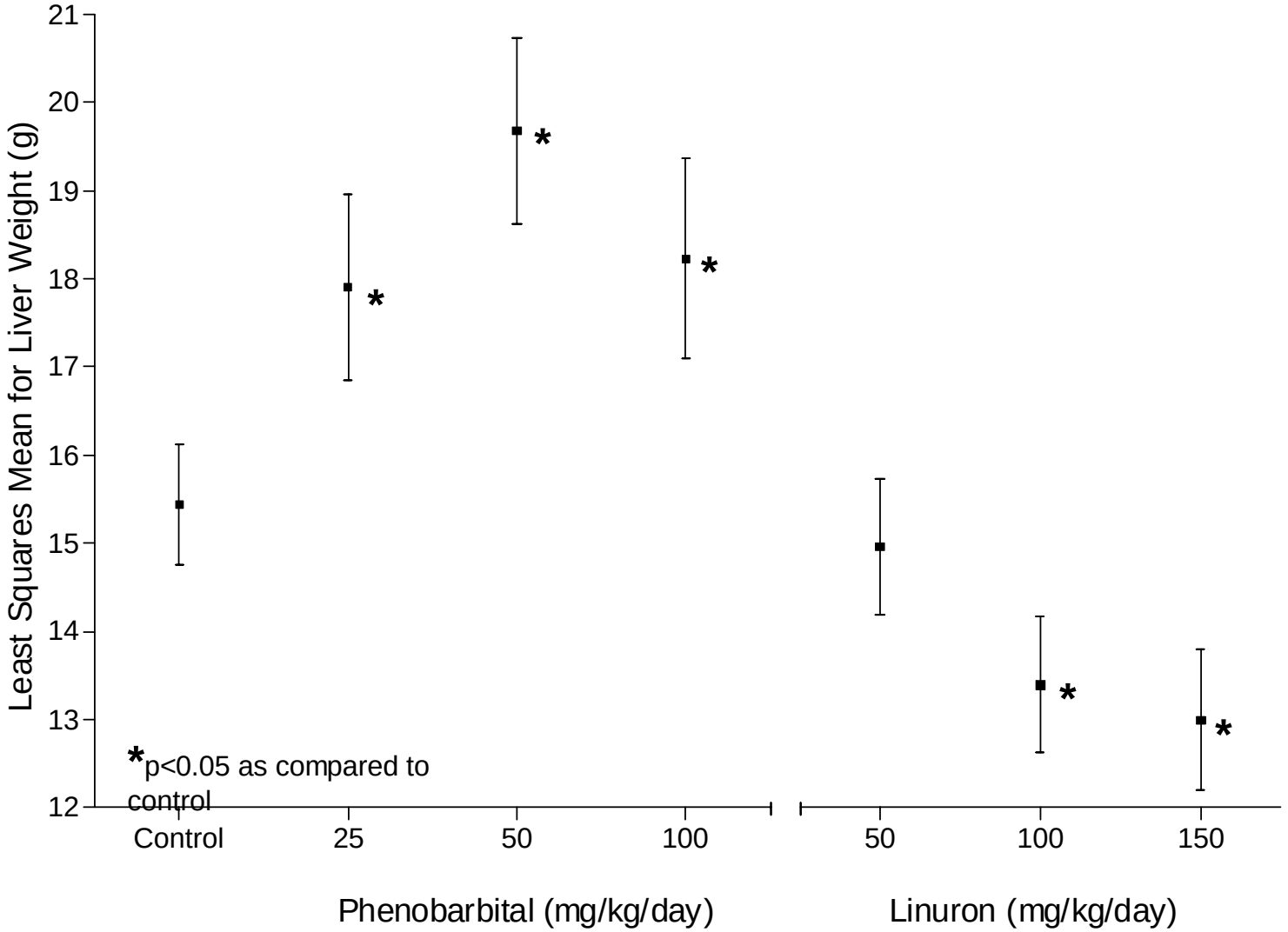


Figure 12. Least Squares Means (with ± 2 Standard Error Bars) for Left Testis Weight (g) for Each Dose Group

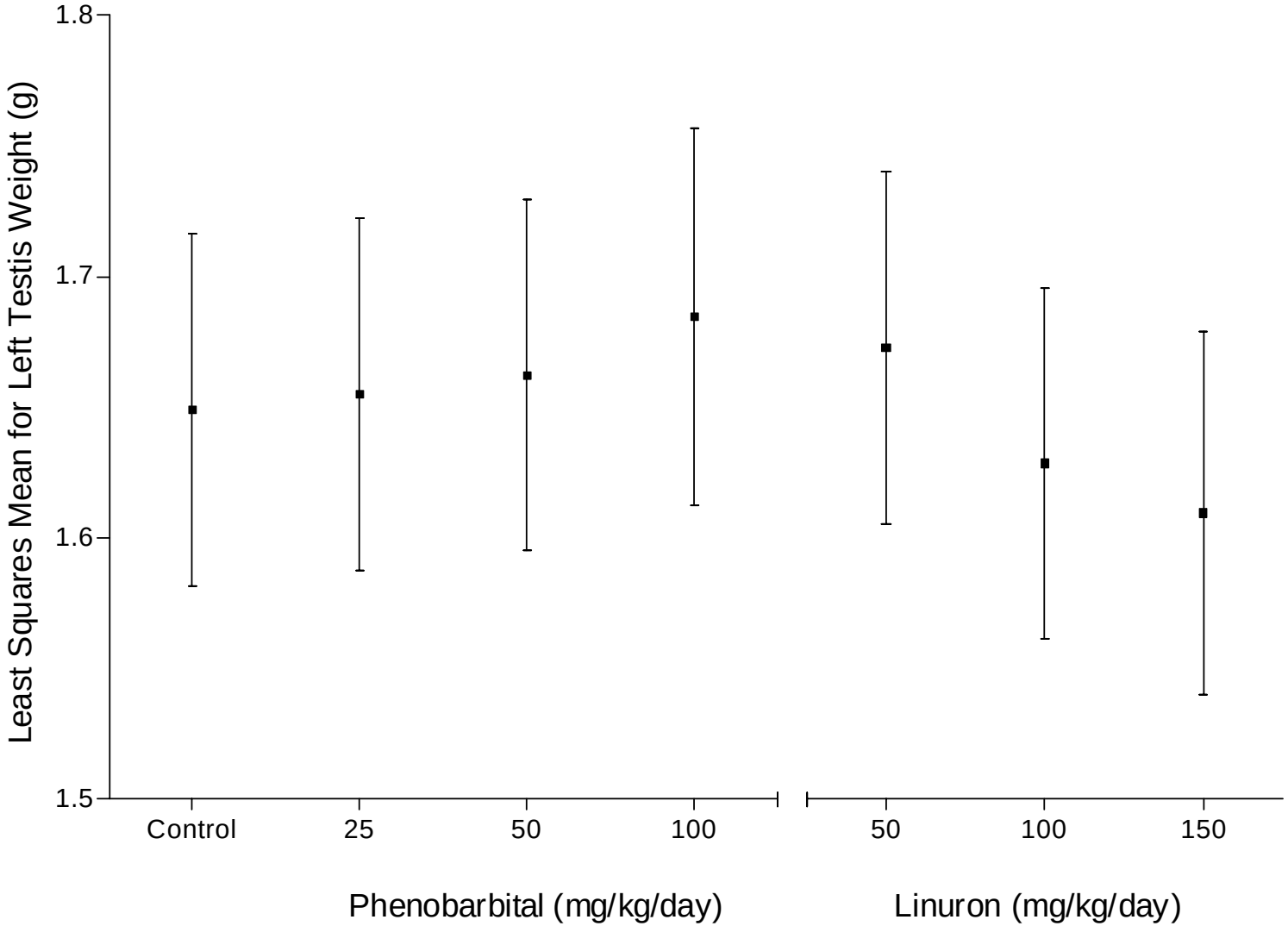


Figure 13. Least Squares Means (with ± 2 Standard Error Bars) for Right Testis Weight (g) for Each Dose Group

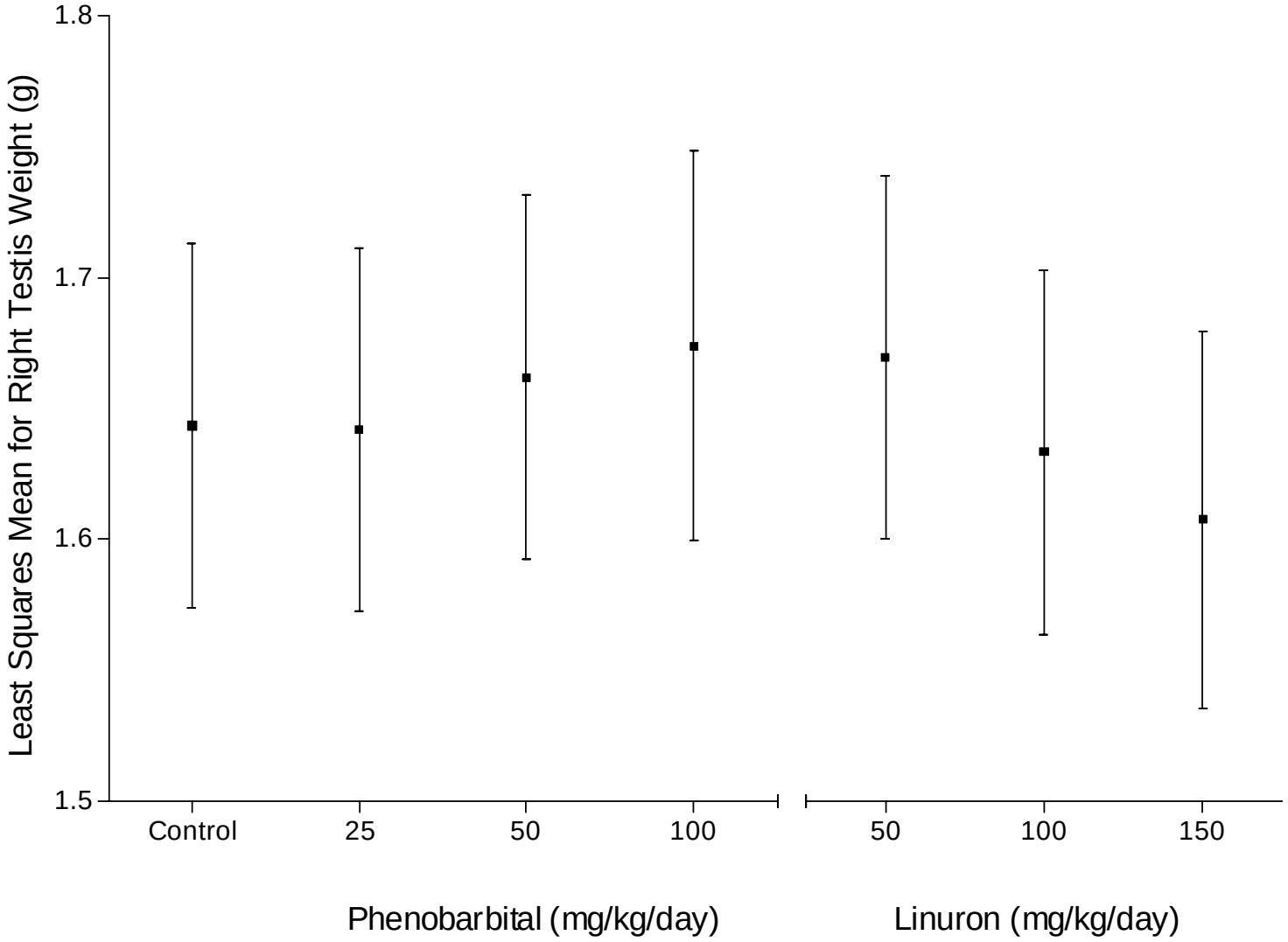


Figure 14. Least Squares Means (with ± 2 Standard Error Bars) for Paired Testis Weight (g) for Each Dose Group

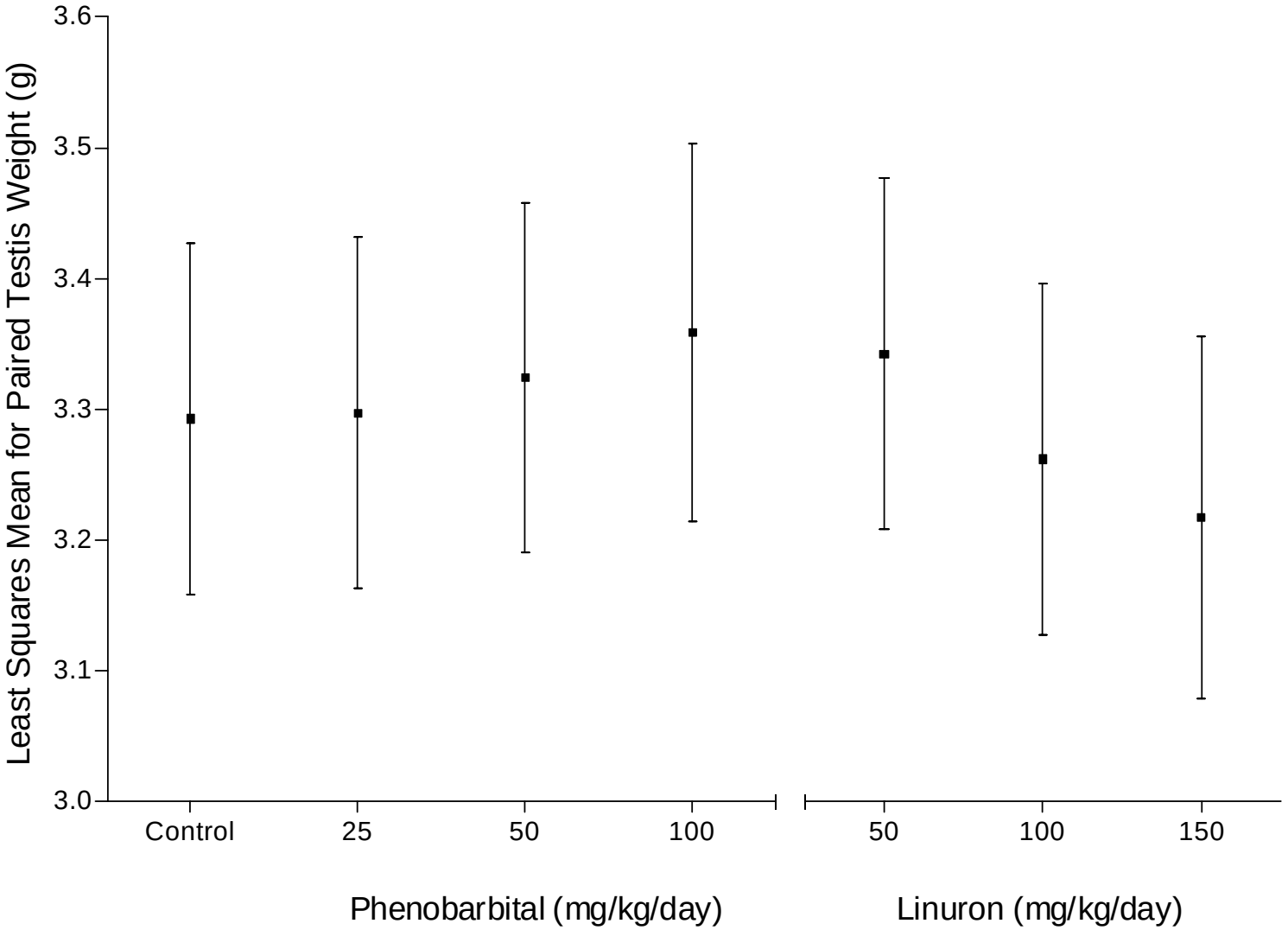


Figure 15. Least Squares Means (with ± 2 Standard Error Bars) for Paired Epididymis Weight (g) for Each Dose Group

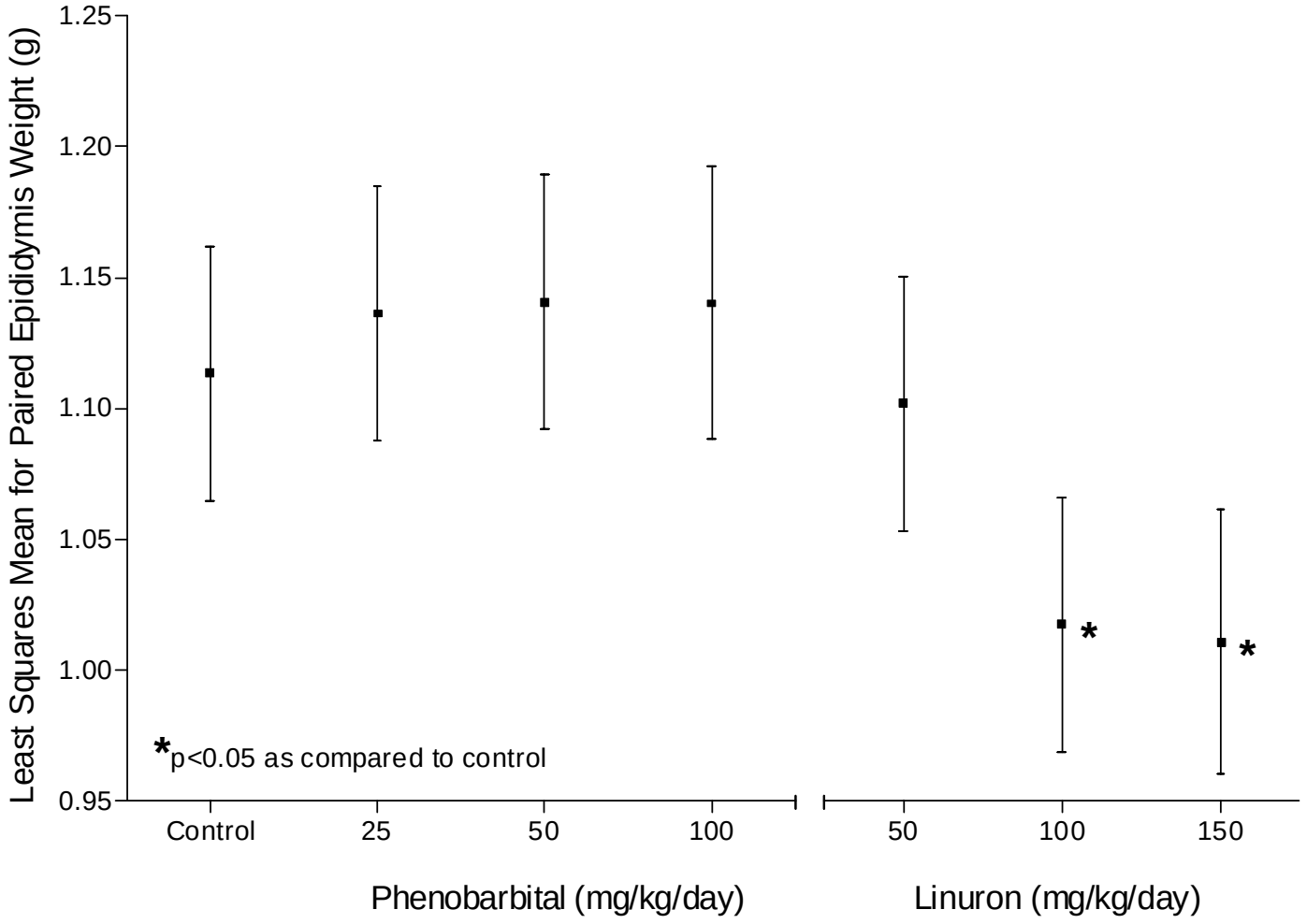


Figure 16. Least Squares Means (with ± 2 Standard Error Bars) for Prostate Weight (g) for Each Dose Group

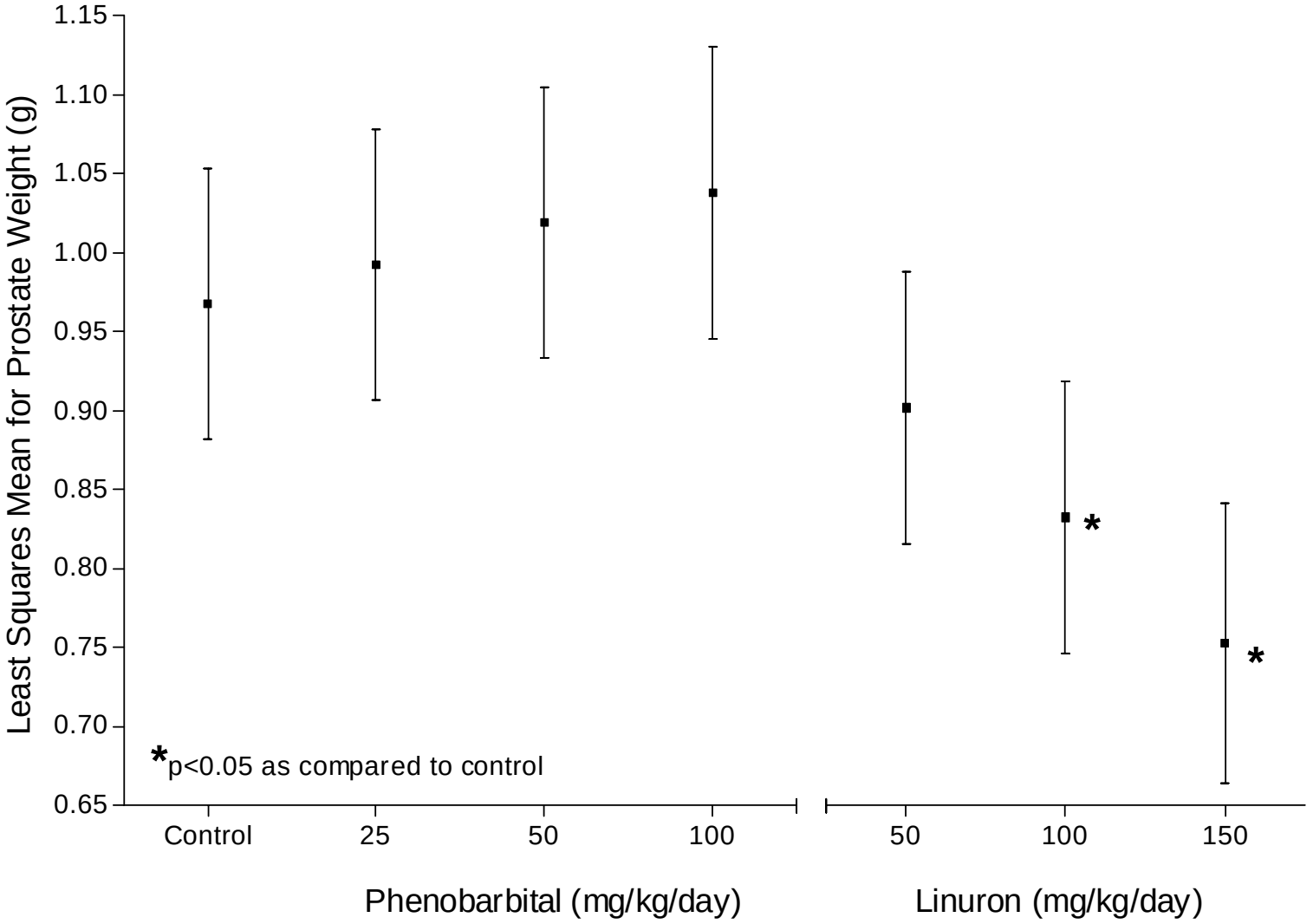


Figure 17. Least Squares Means (with ± 2 Standard Error Bars) for Seminal Vesicles with Fluid and Coagulating Gland Weight (g) for Each Dose Group

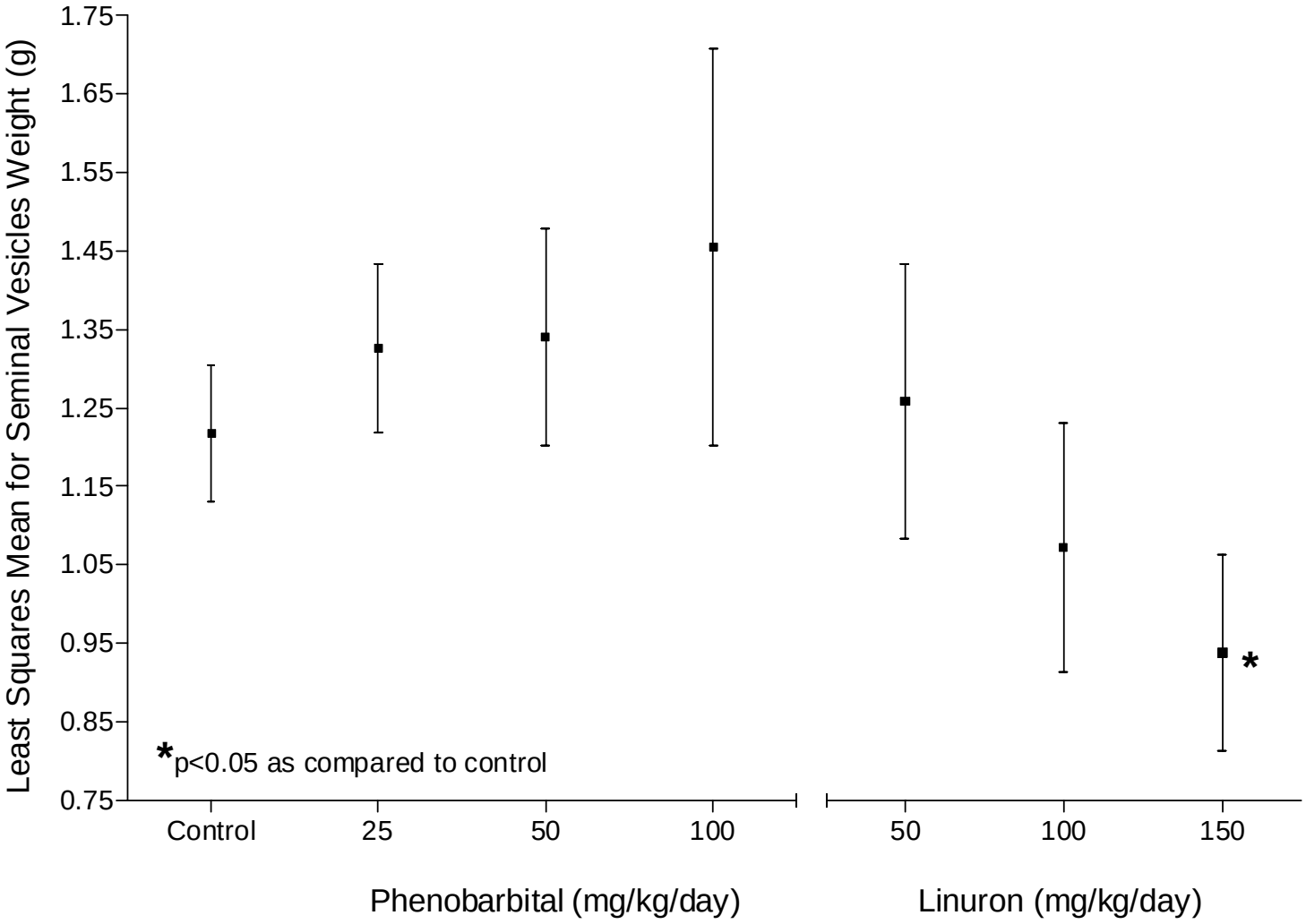


Figure 18. Least Squares Means (with ± 2 Standard Error Bars) for Accessory Sex Gland Weight (g) for Each Dose Group

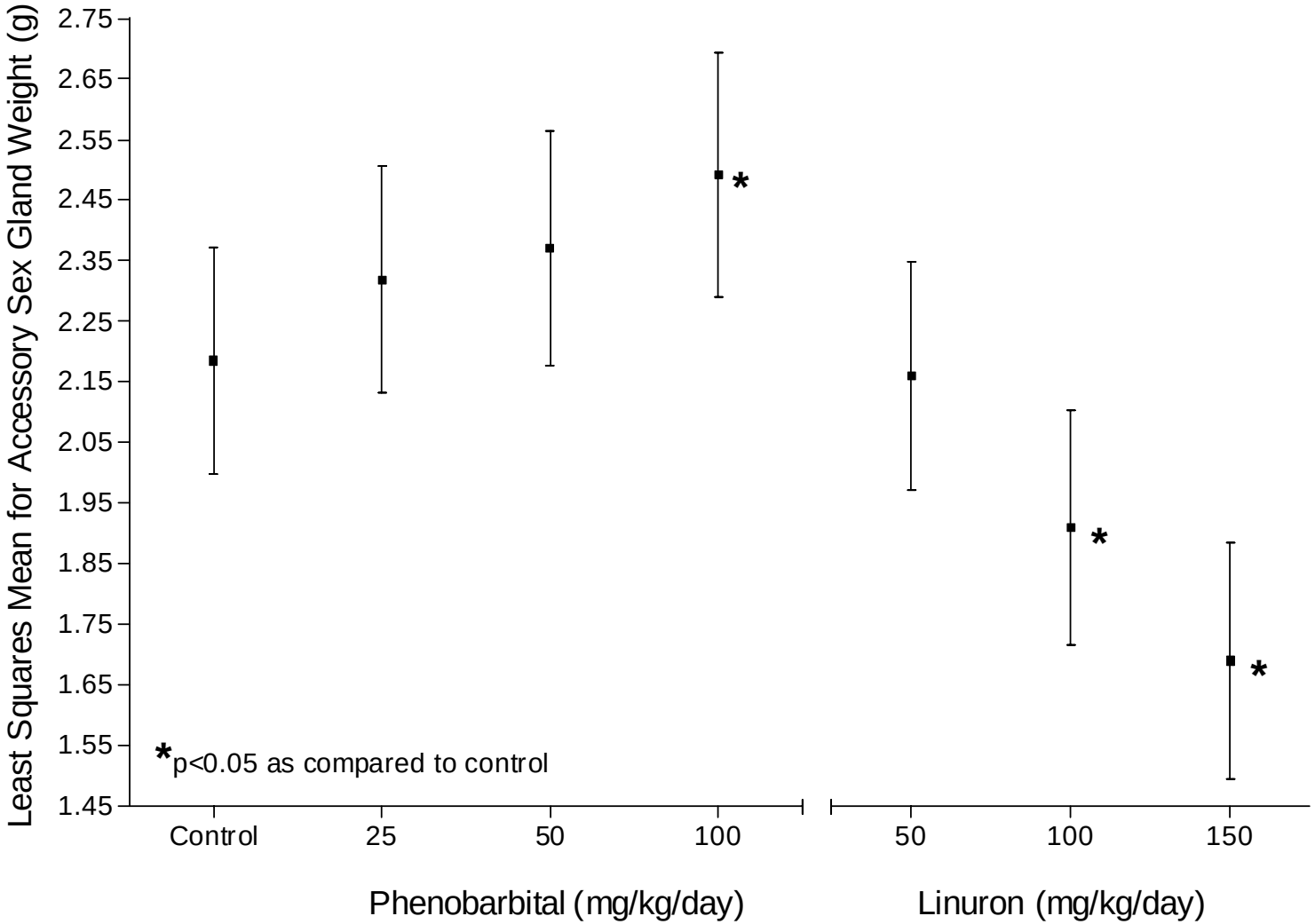


Figure 19. Least Squares Means (with ± 2 Standard Error Bars) for the Thyroid Glands to Body Weight Ratio (%) for Each Dose Group

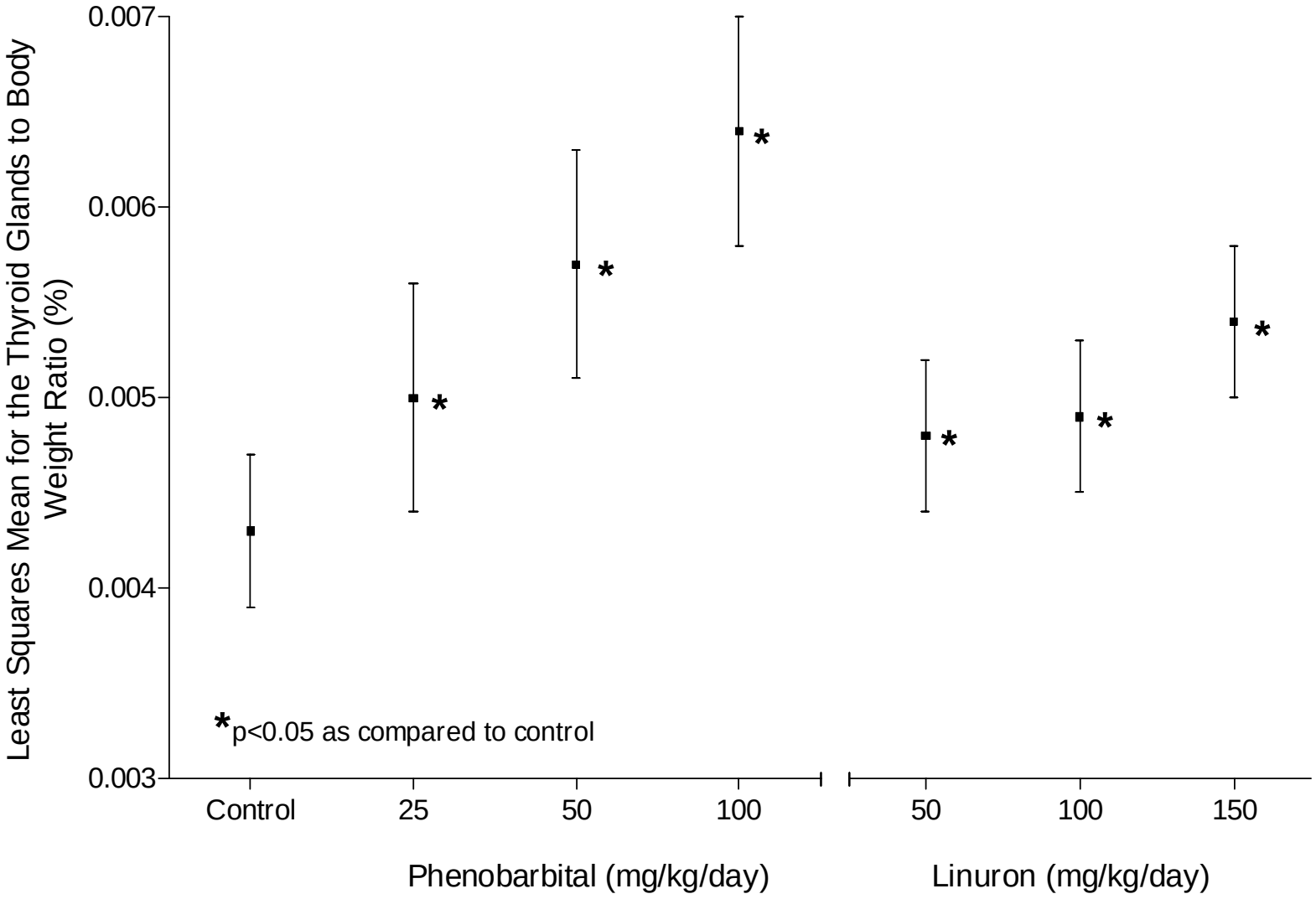


Figure 20. Least Squares Means (with ± 2 Standard Error Bars) for the Liver to Body Weight Ratio (%) for Each Dose Group

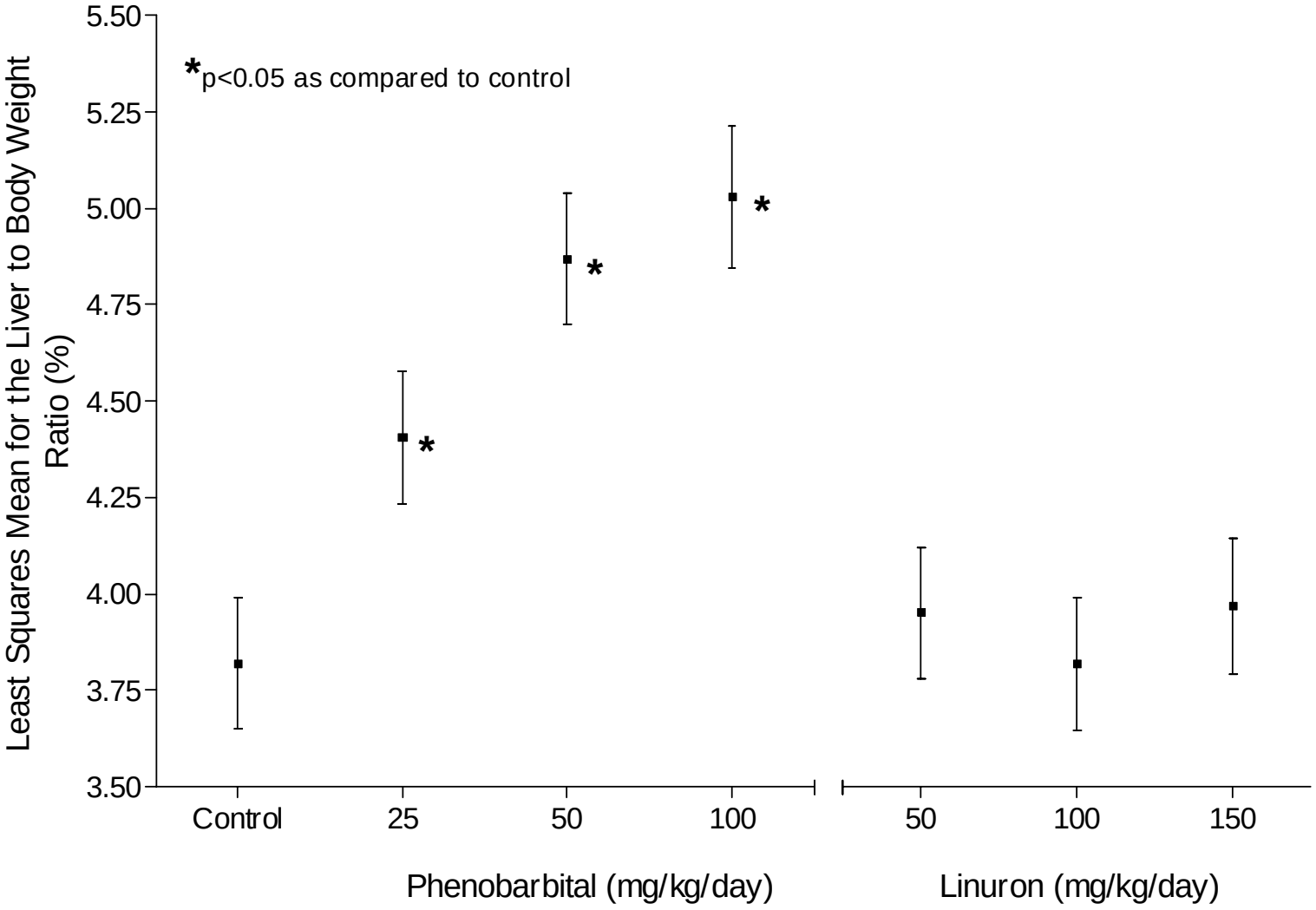


Figure 21. Least Squares Means (with ± 2 Standard Error Bars) for the Left Testis to Body Weight Ratio (%) for Each Dose Group

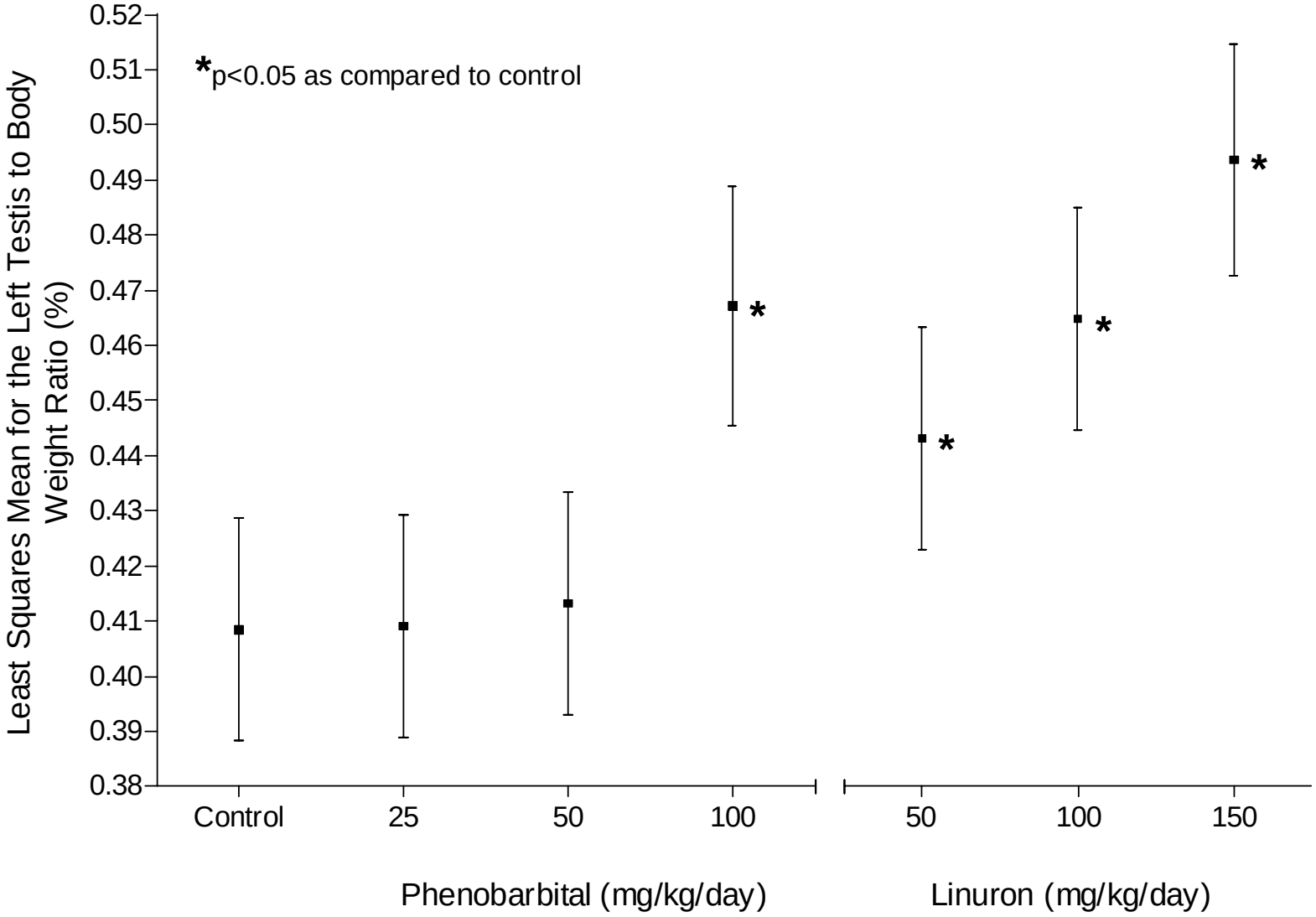


Figure 22. Least Squares Means (with ± 2 Standard Error Bars) for the Right Testis to Body Weight Ratio (%) for Each Dose Group

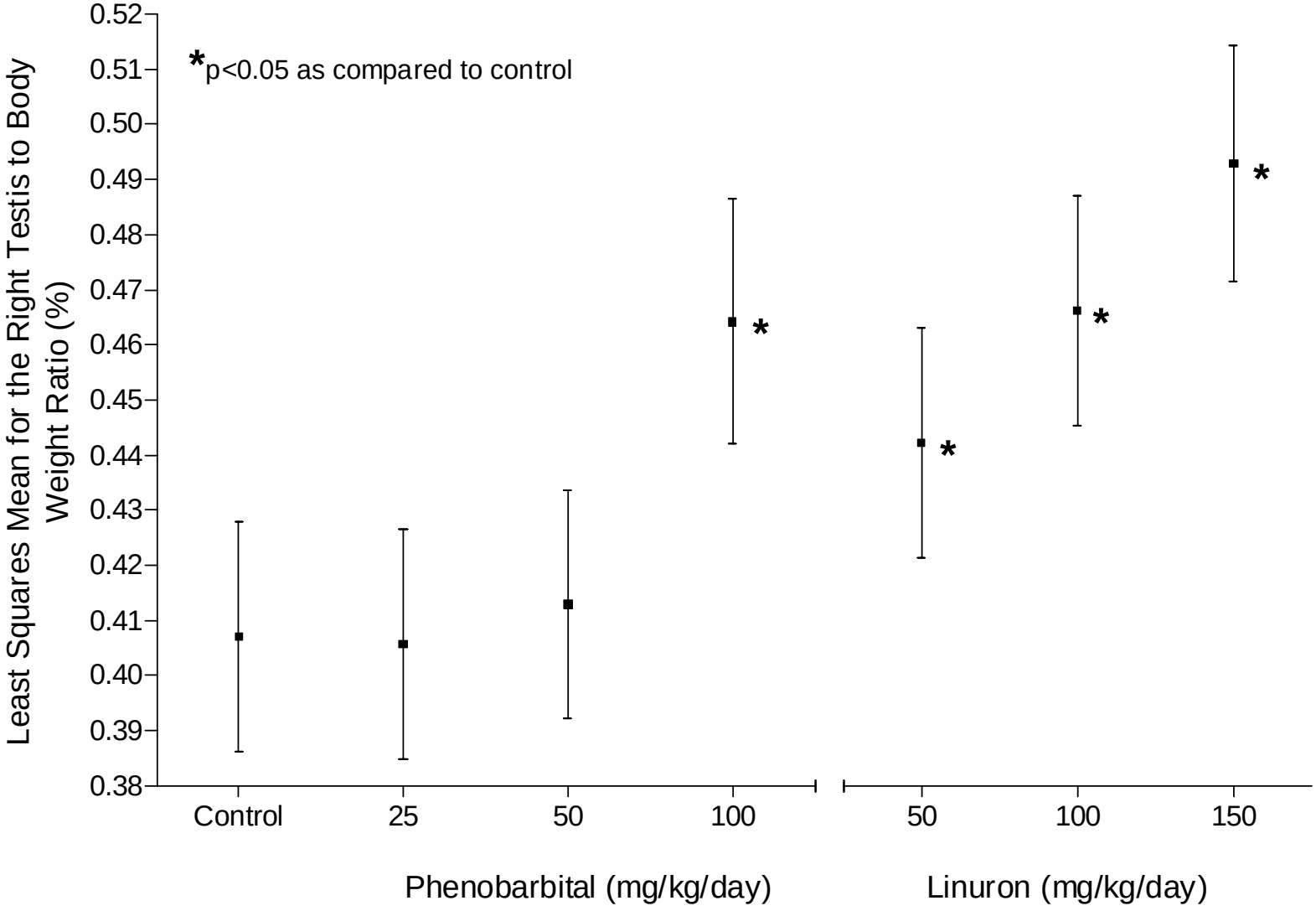


Figure 23. Least Squares Means (with ± 2 Standard Error Bars) for the Paired Testis to Body Weight Ratio (%) for Each Dose Group

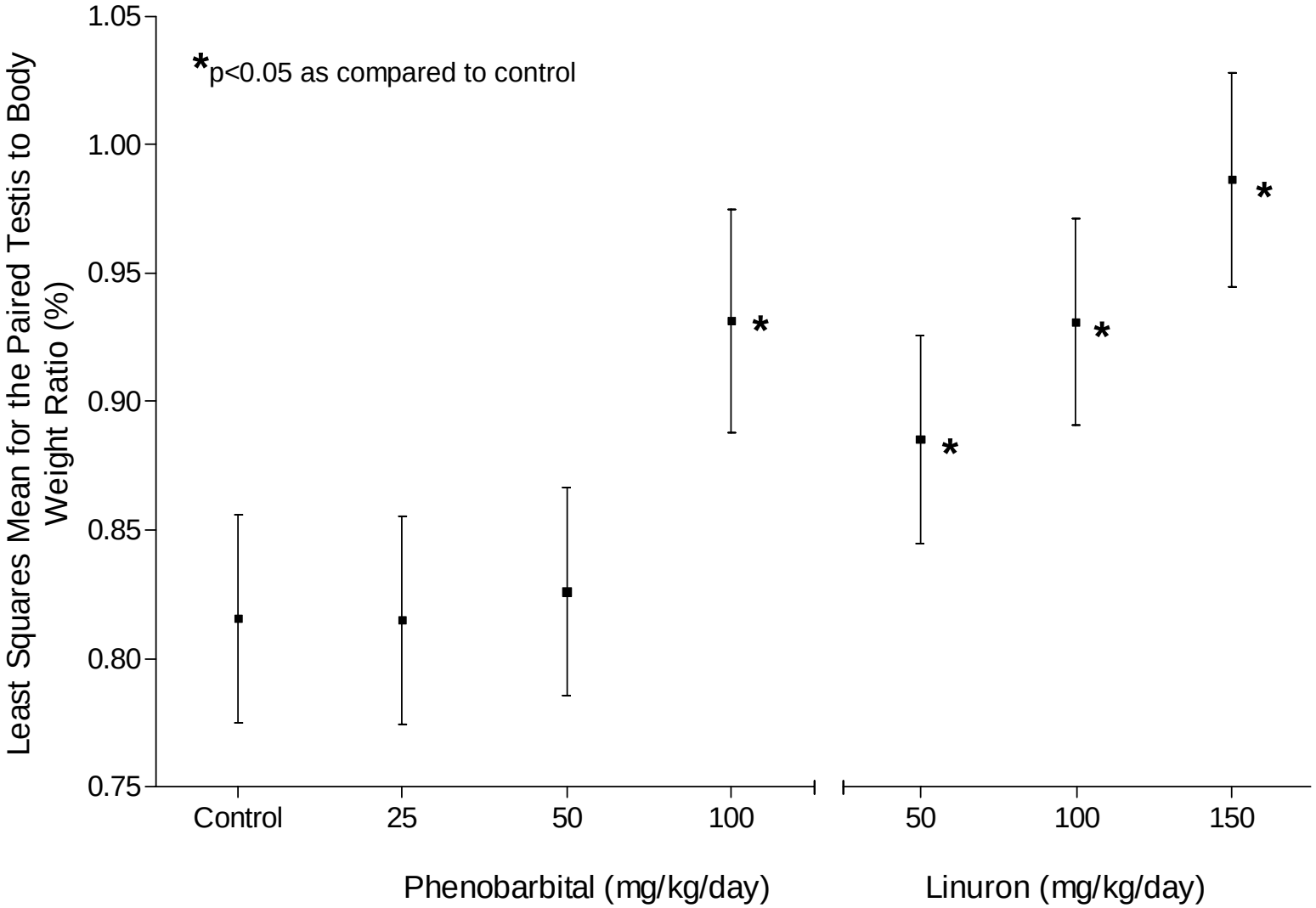


Figure 24. Leasts Squares Means (with ± 2 Standard Error Bars) for the Paired Epididymis to Body Weight Ratio (%) for Each Dose Group

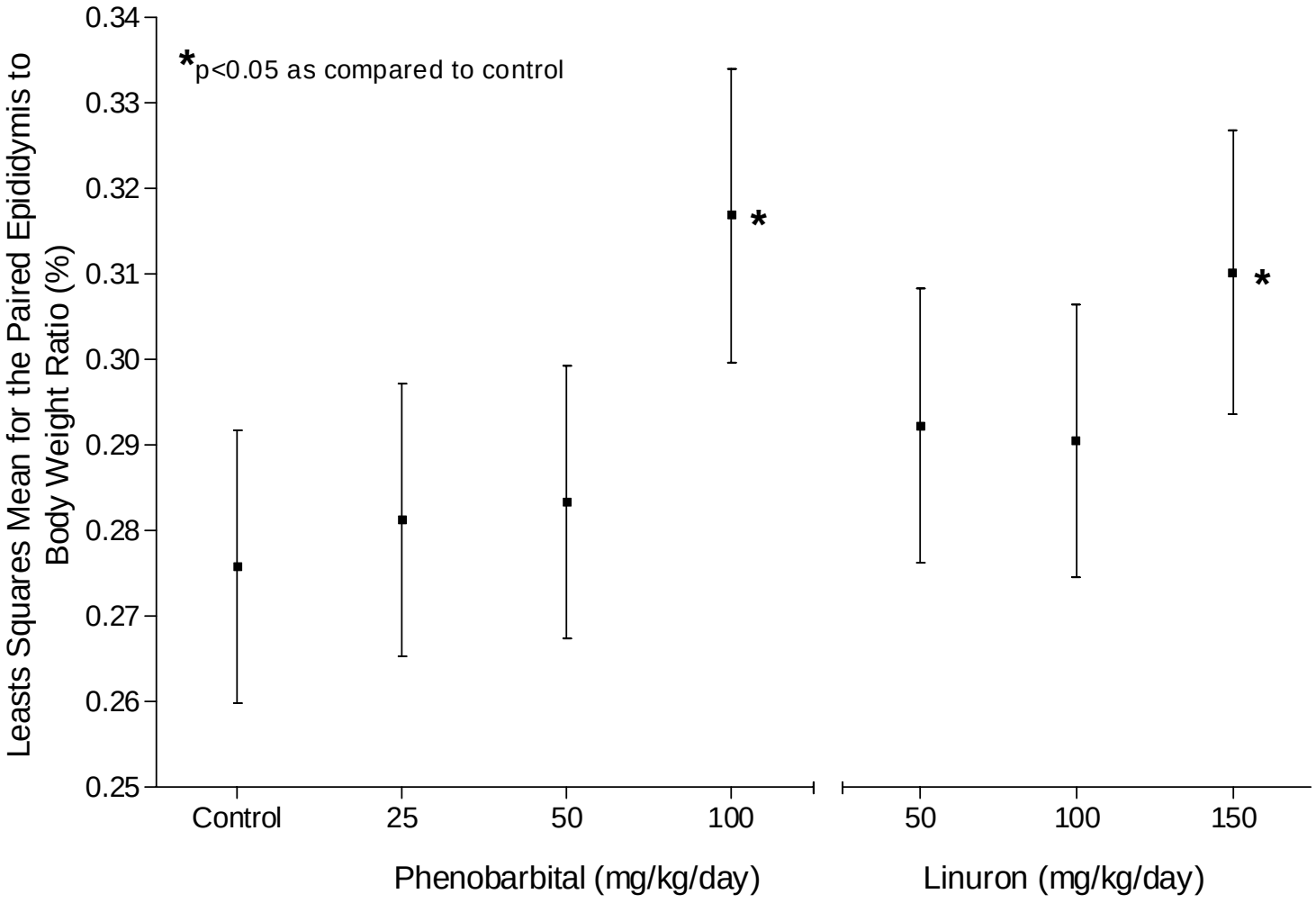


Figure 25. Least Squares Means (with ± 2 Standard Error Bars) for the Prostate to Body Weight Ratio (%) for Each Dose Group

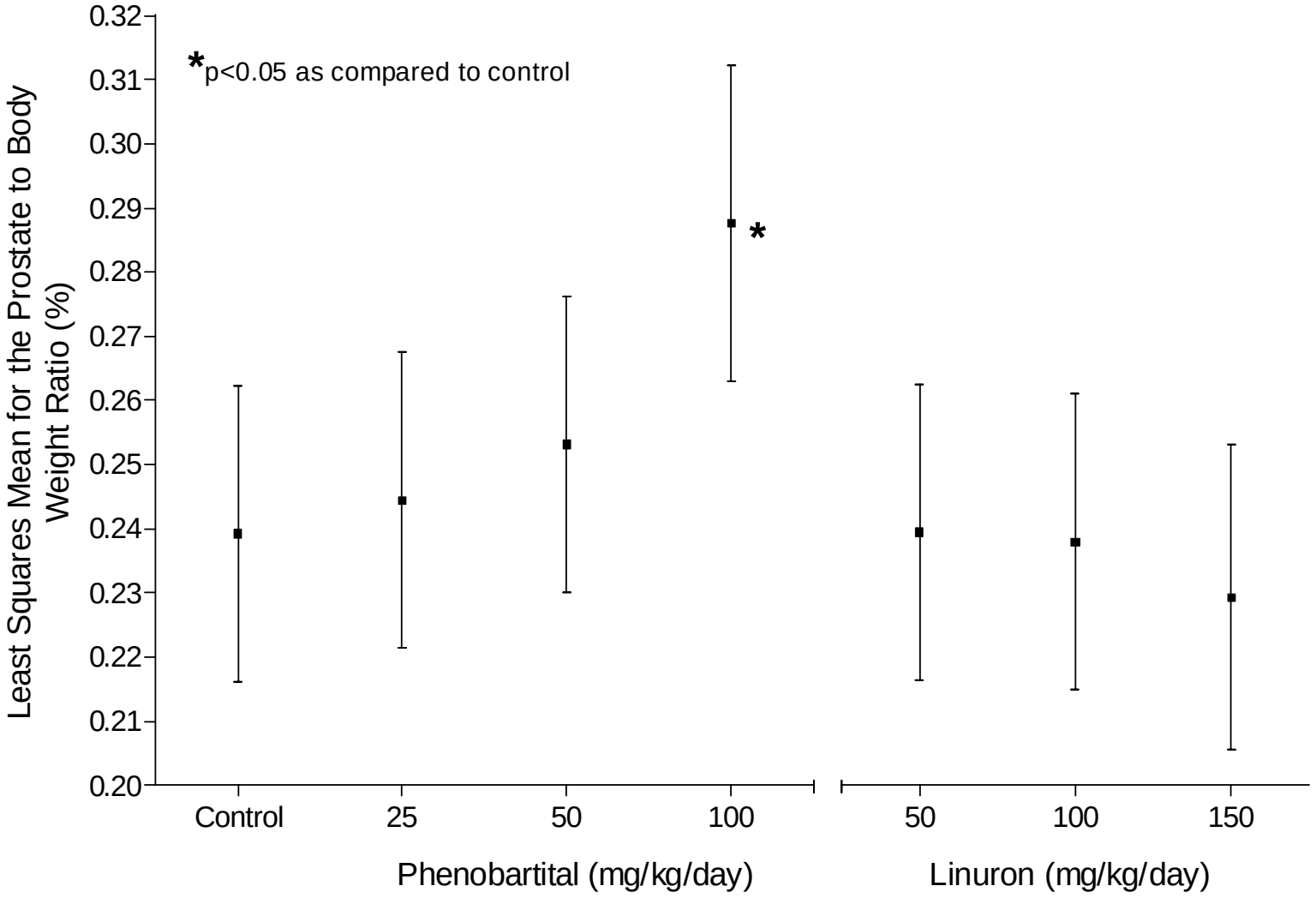


Figure 26. Leasts Squares Means (with ± 2 Standard Error Bars) for the Seminal Vesicles with Fluid and Coagulating Gland to Body Weight Ratio (%) for Each Dose Group

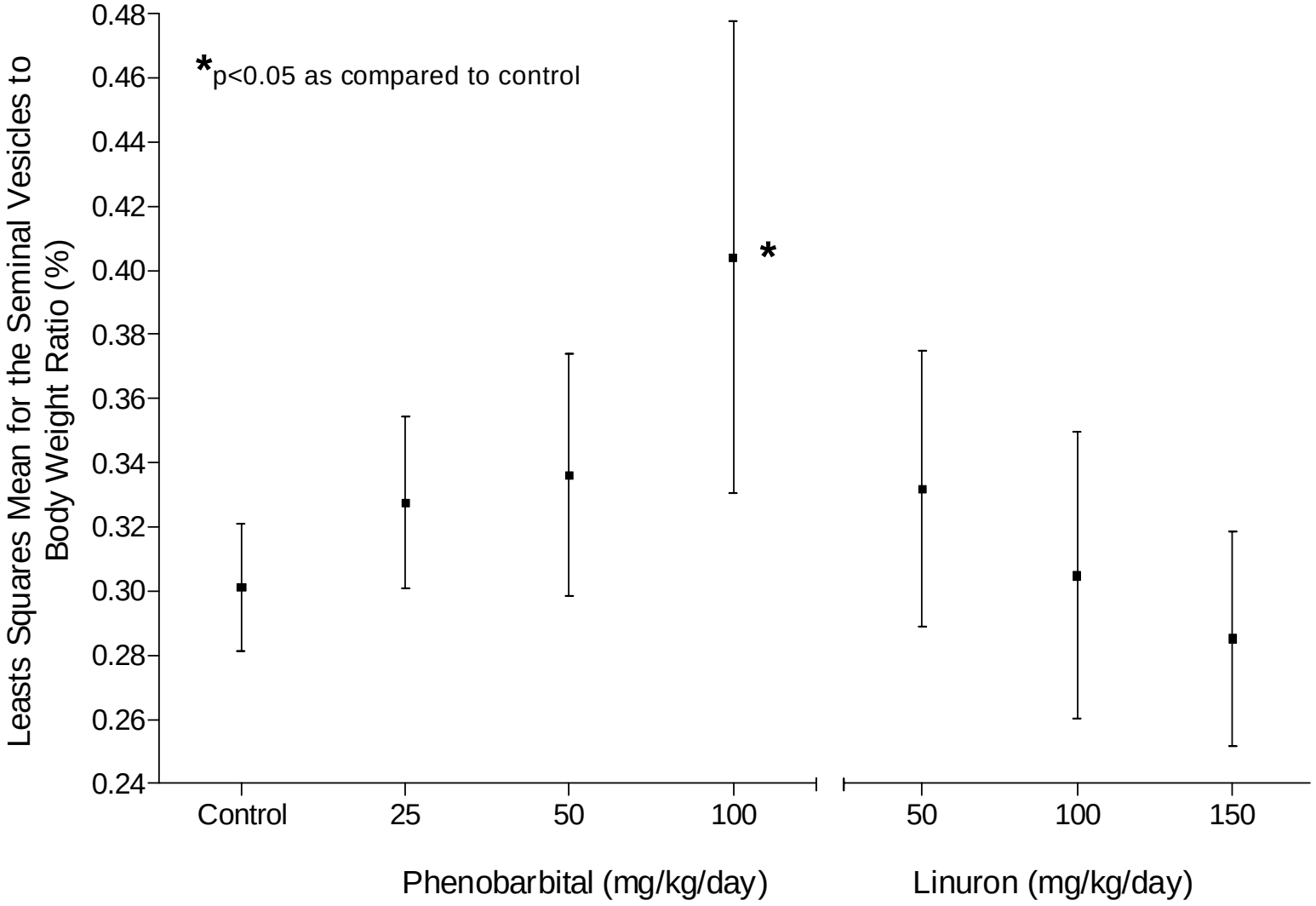


Figure 27. Least Squares Means (with ± 2 Standard Error Bars) for the Accessory Sex Gland to Body Weight Ratio (%) for Each Dose Group

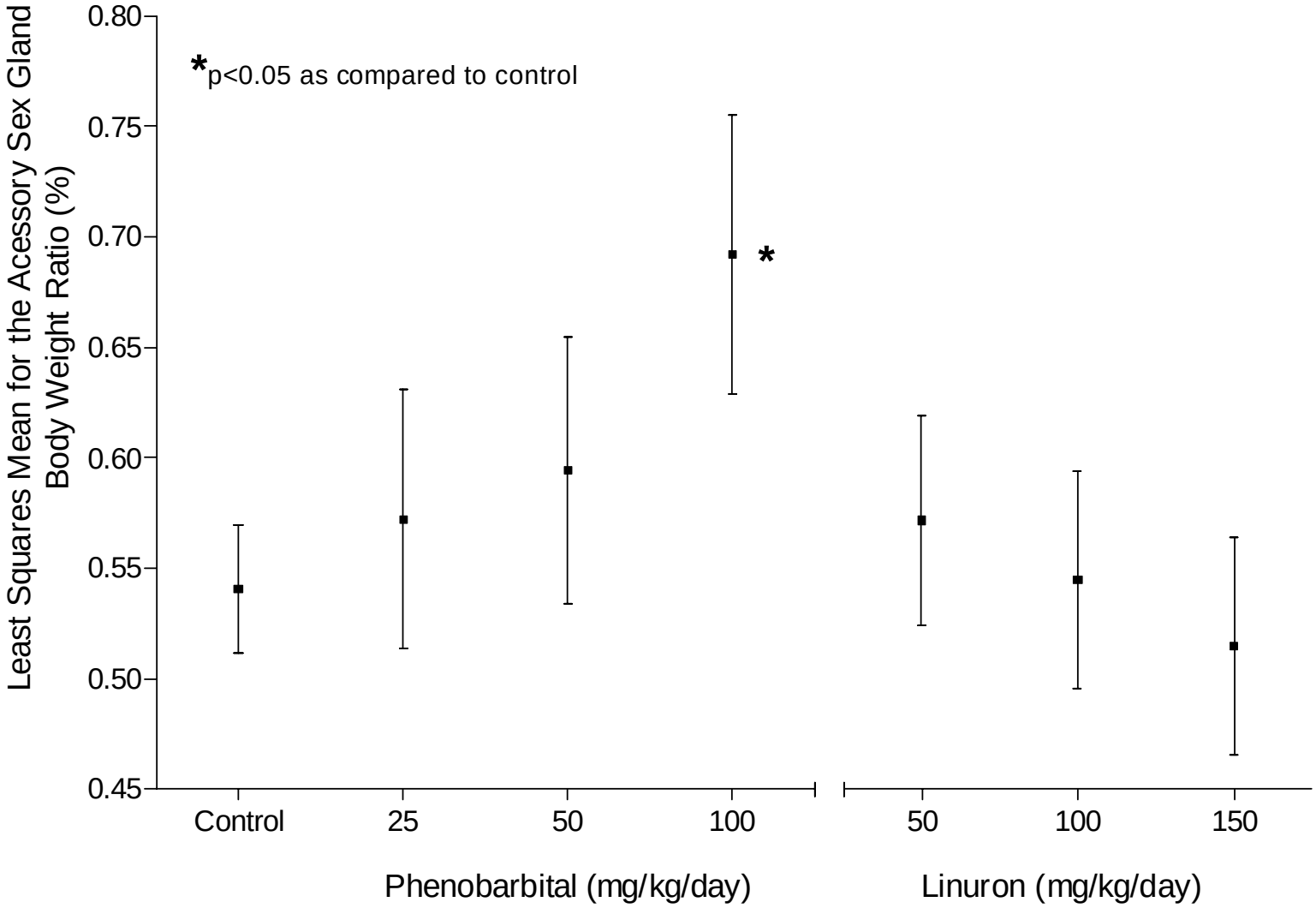


Figure 28. Least Squares Means (with ± 2 Standard Error Bars) for Serum Testosterone (ng/ml) for Each Dose Group

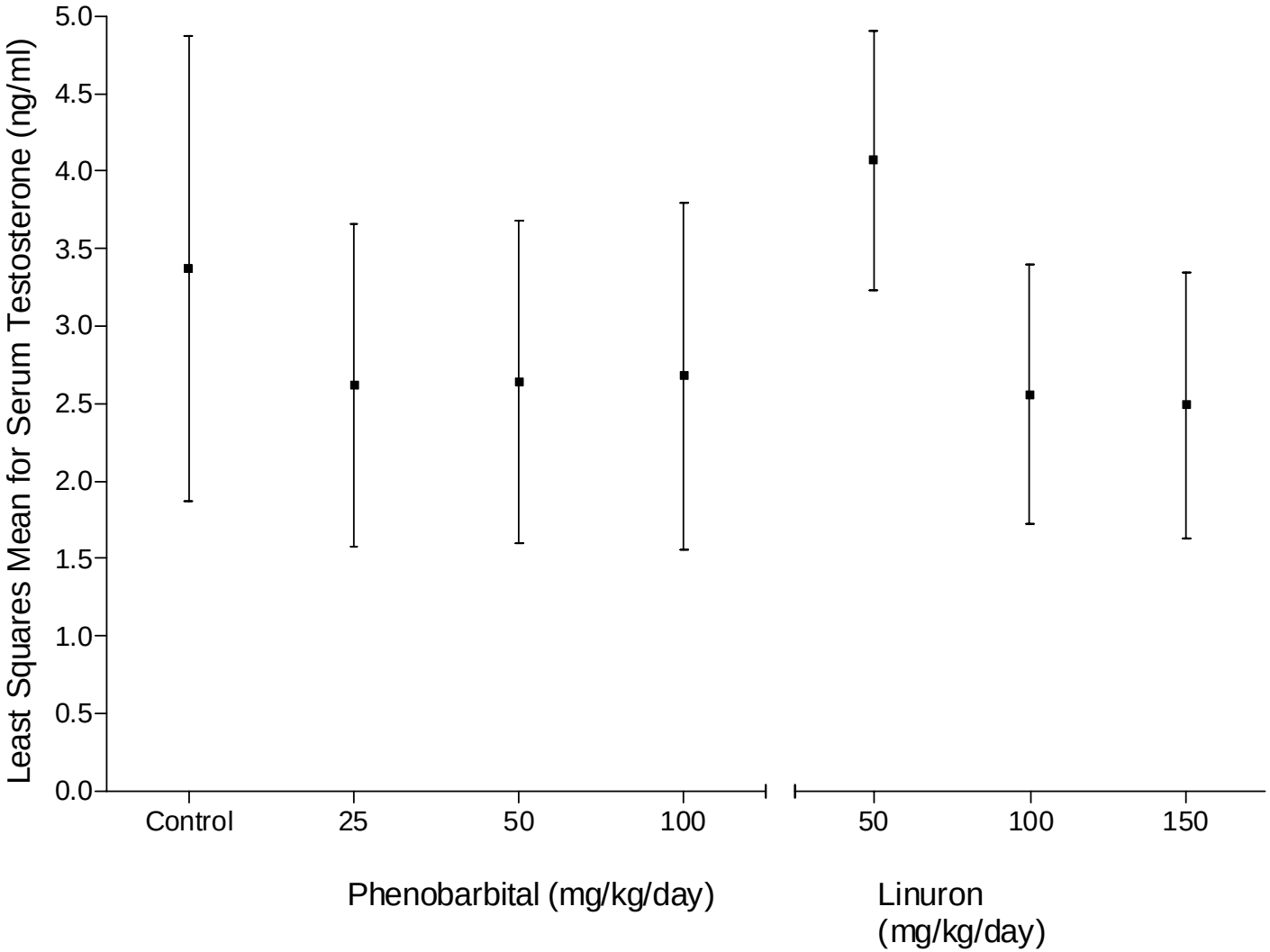


Figure 29. Least Squares Means (with ± 2 Standard Error Bars) for Luteinizing Hormone (ng/ml) for Each Dose Group

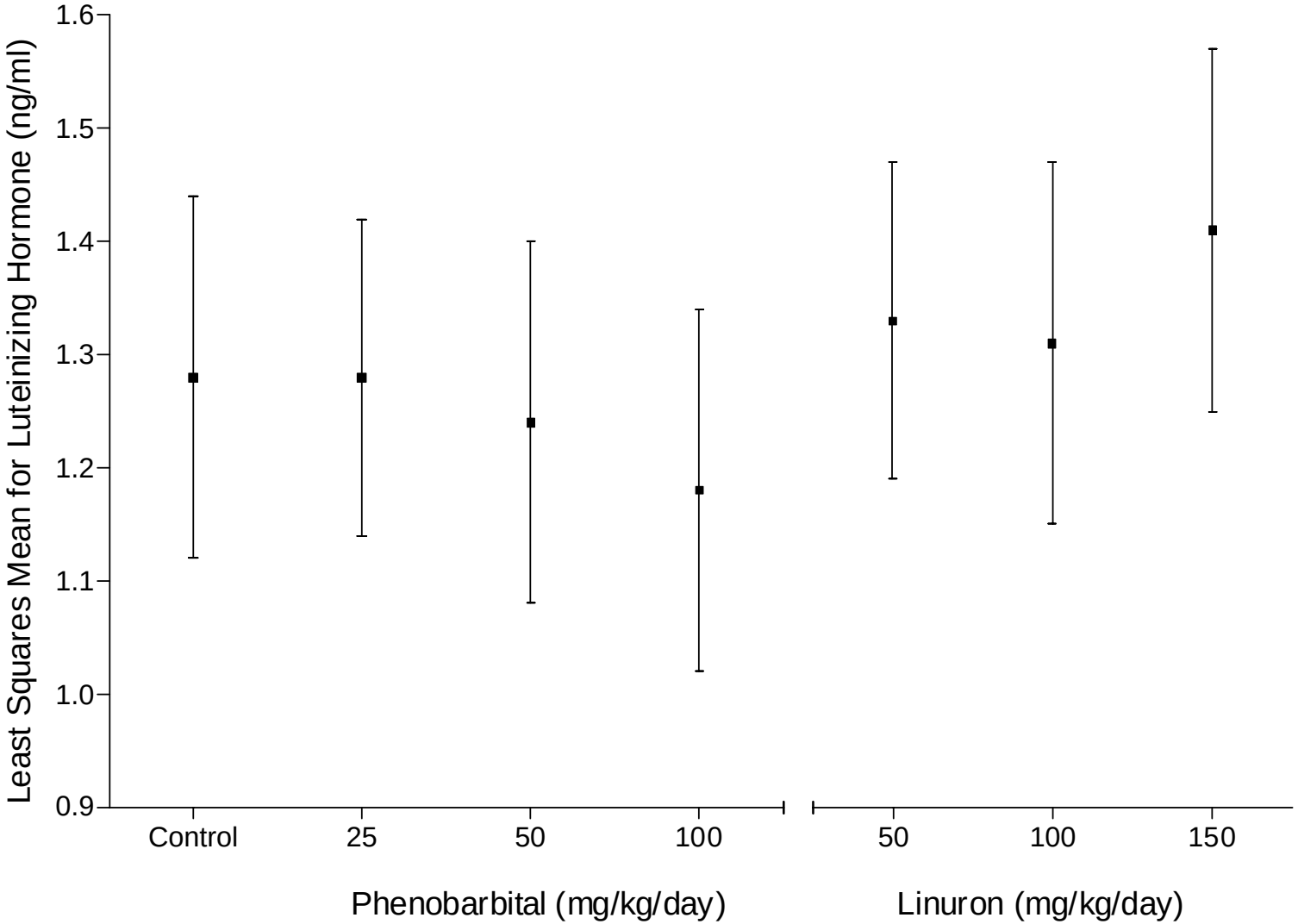


Figure 30. Least Squares Means (with ± 2 Standard Error Bars) for Thyroid Stimulating Hormone (ng/ml) for Each Dose Group

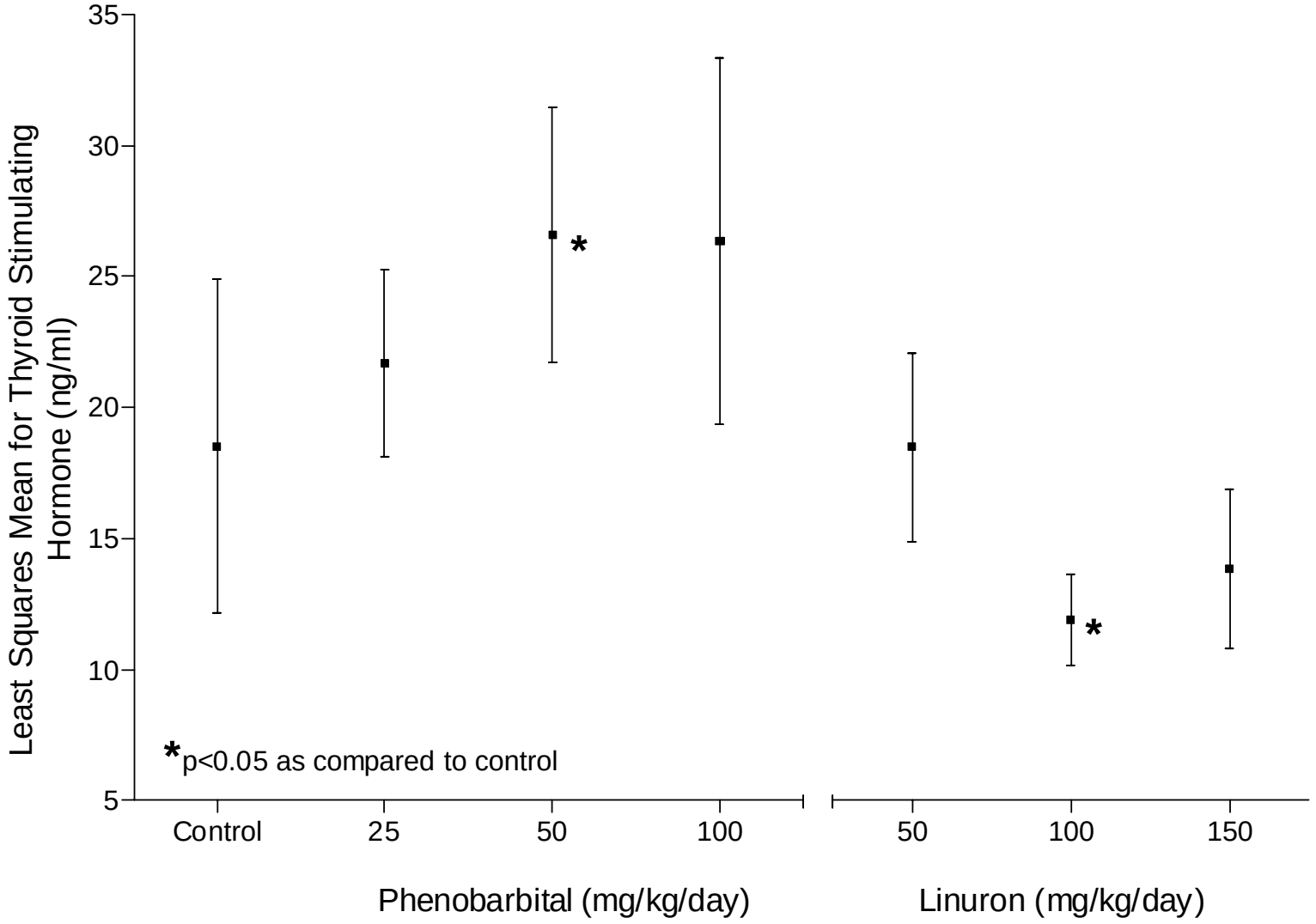


Figure 31. Least Squares Means (with ± 2 Standard Error Bars) for Thyroxine (μg/dL) for Each Dose Group

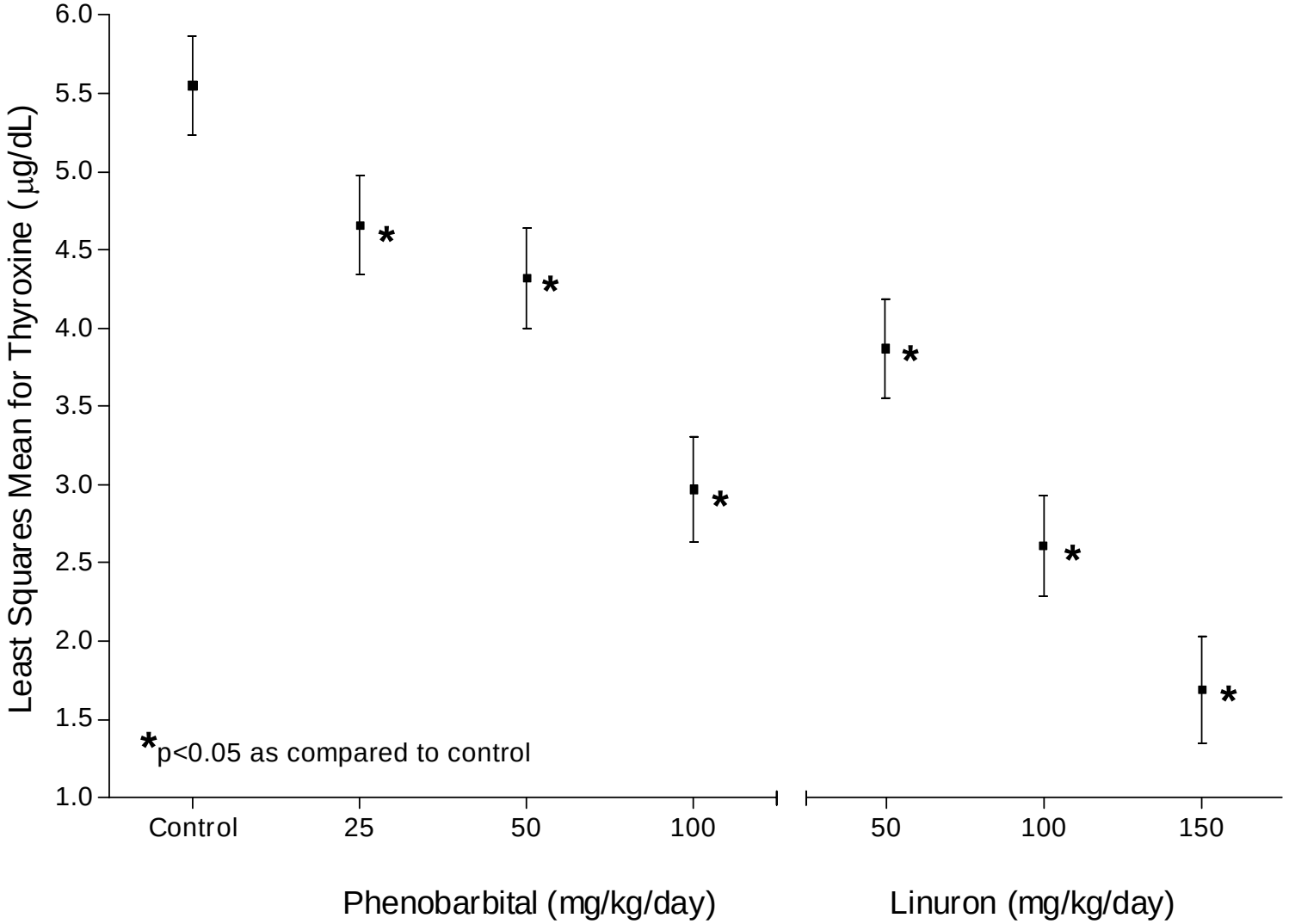


Figure 32. Least Squares Means (with ± 2 Standard Error Bars) for Triiodothyronine (ng/dL) for Each Dose Group

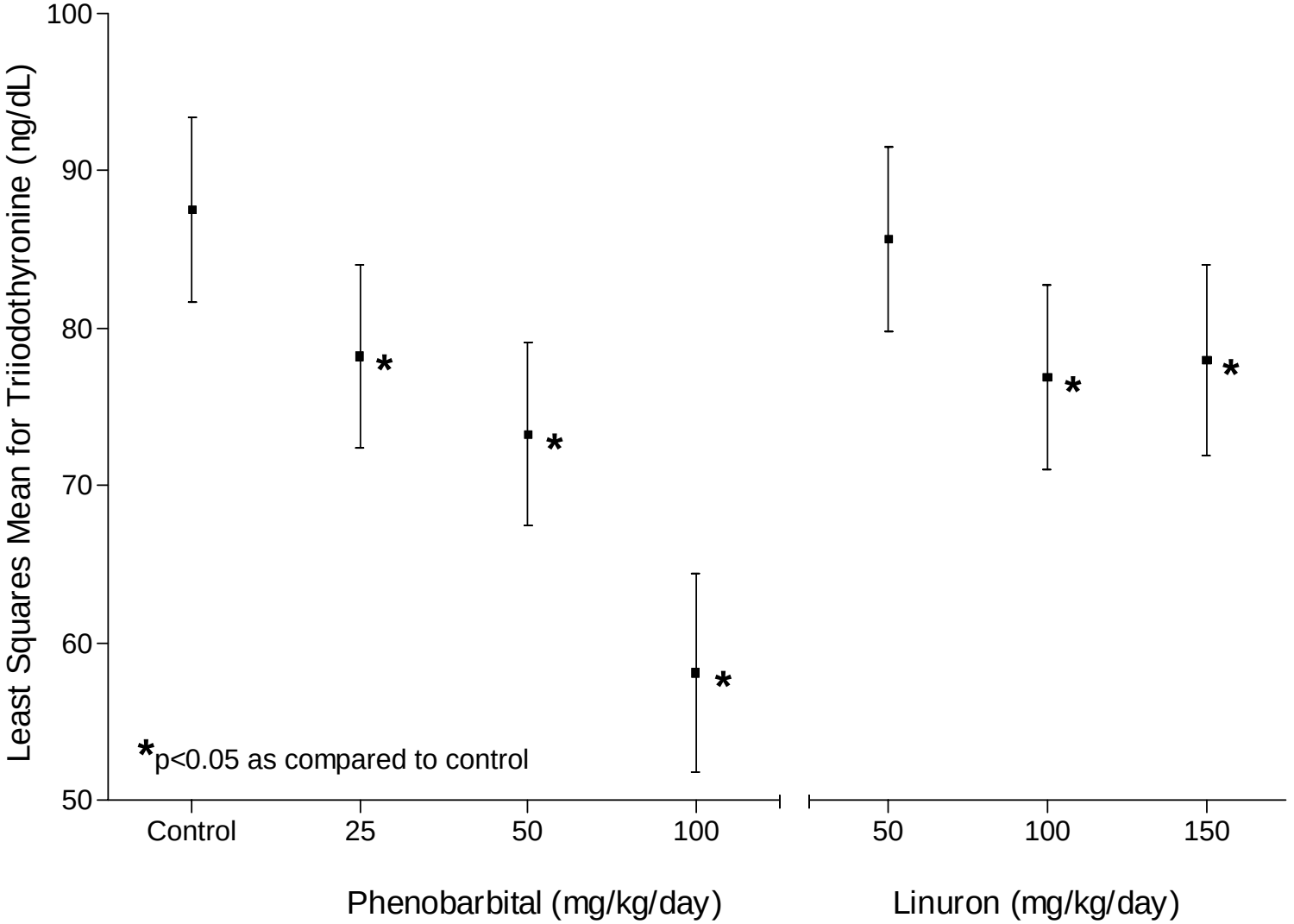


Figure 33. Least Squares Means (with ± 2 Standard Error Bars) for Follicle Stimulating Hormone (ng/ml) for Each Dose Group

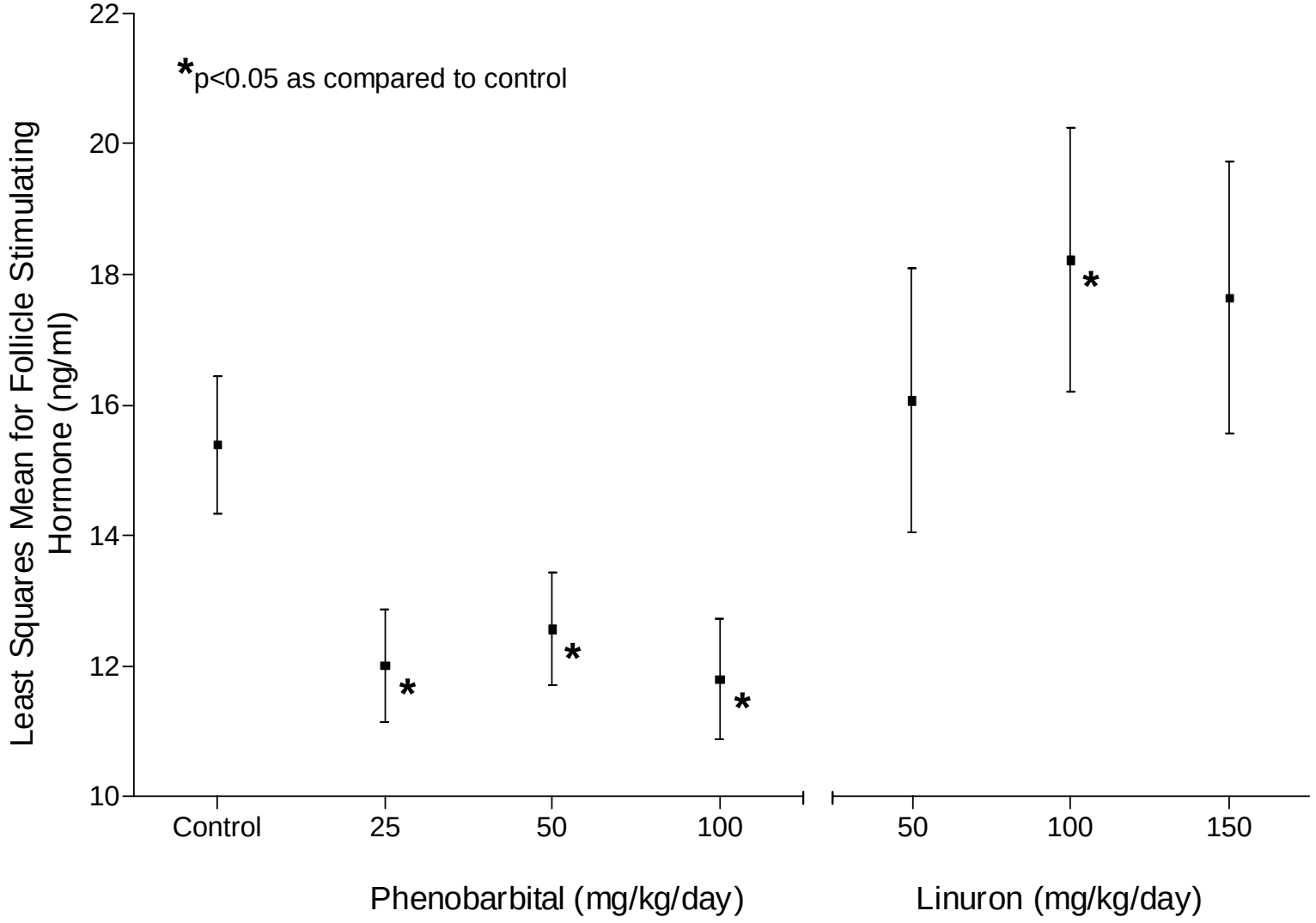


Figure 34. Least Squares Means (with ± 2 Standard Error Bars) for Estradiol (pg/ml) for Each Dose Group

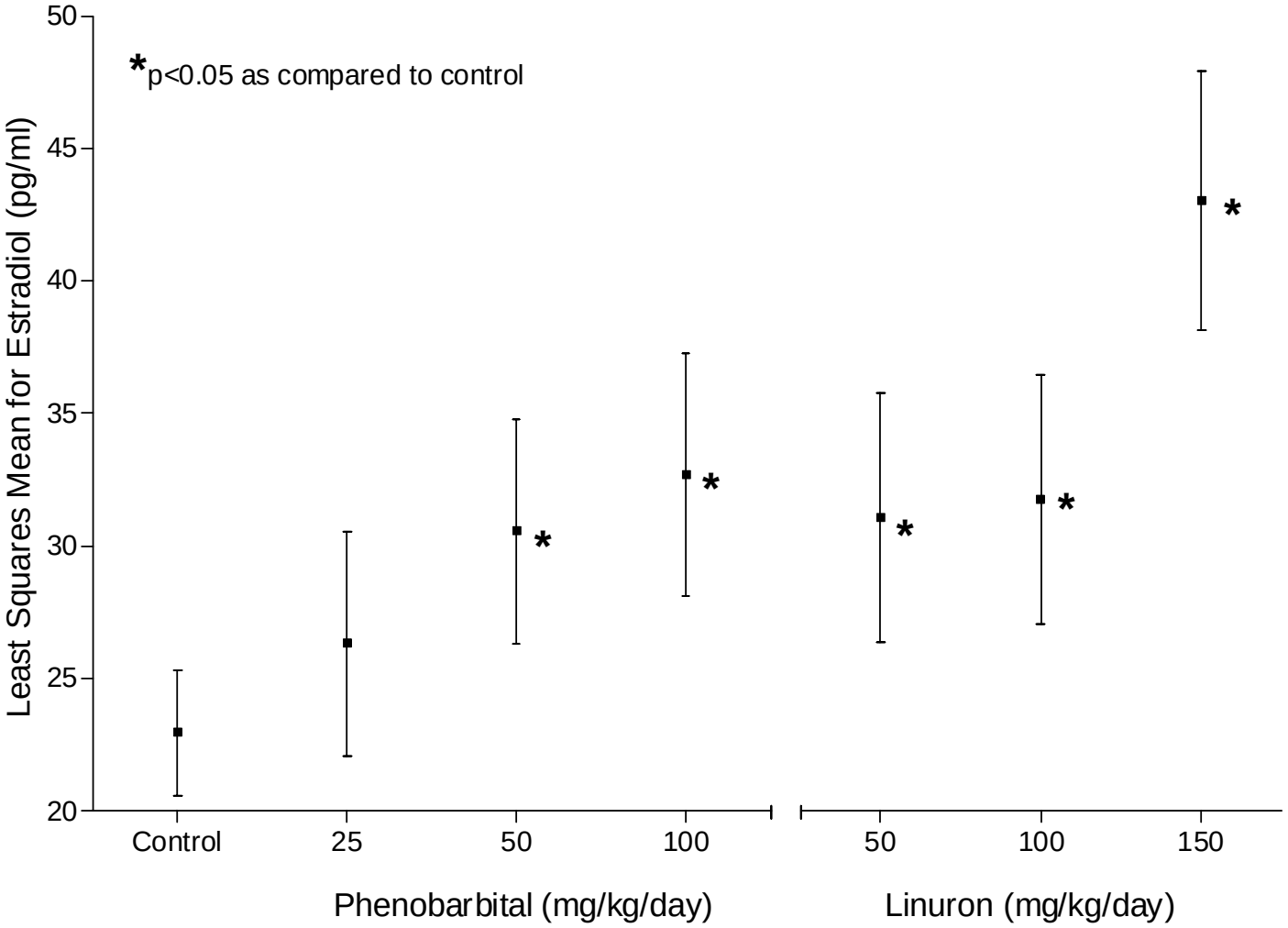


Figure 35. Least Squares Means (with ± 2 Standard Error Bars) for Prolactin (ng/ml) for Each Dose Group

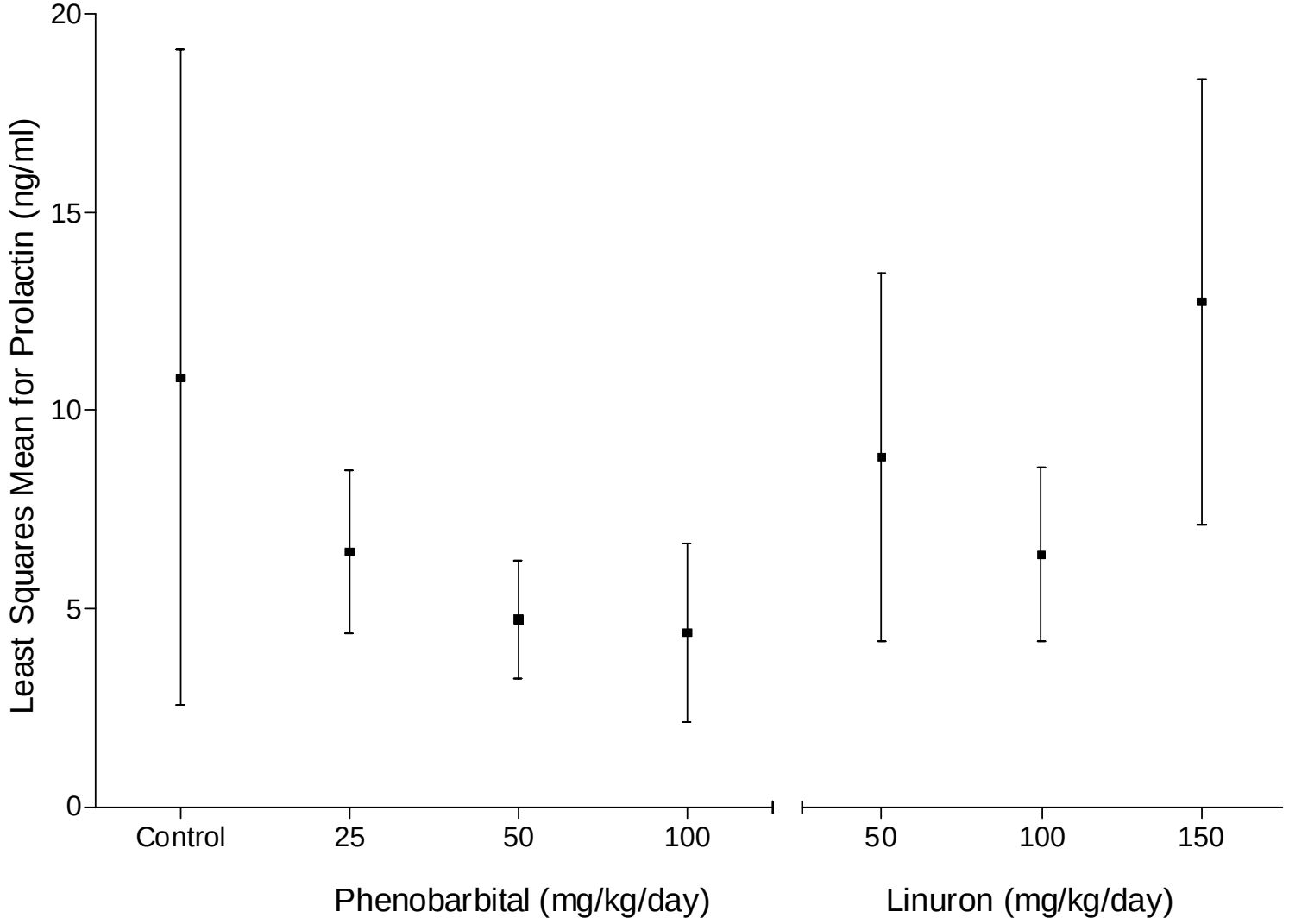
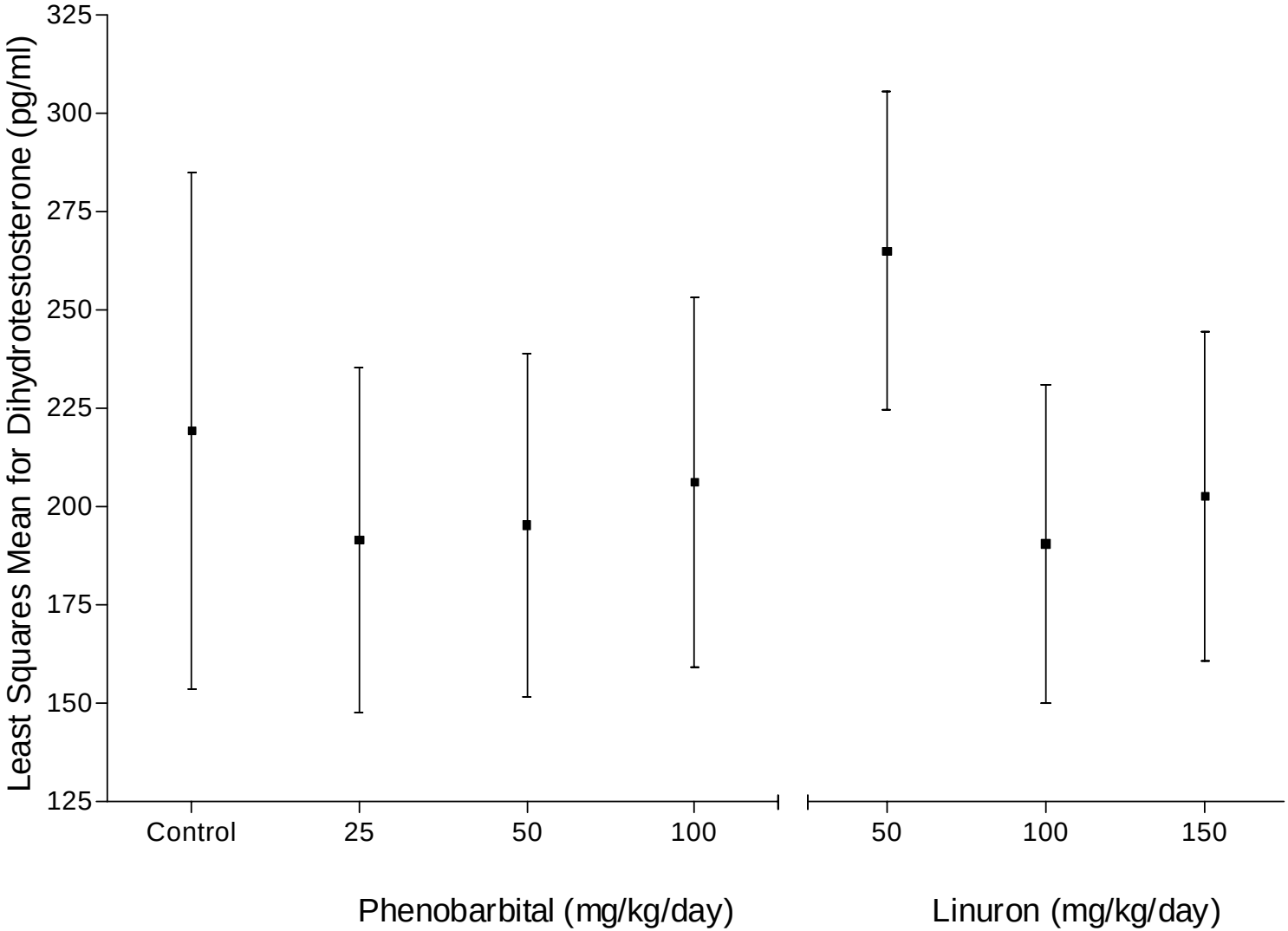


Figure 36. Least Squares Means (with ± 2 Standard Error Bars) for Dihydrotestosterone (pg/ml) for Each Dose Group



# Appendix IV

# Protocol and Amendments

EPA Contract No.: 68-W-01-023 (Battelle Prime Contractor)
EPA Work Assignment No.: WA-5-15
RTI Contract No.: 65U-08055.004.040
RTI Study Code: Rt05-ED09
RTI Master Protocol No.: RTI-956

TITLE: Inter-Laboratory Validation of the 15-Day Adult Intact Male Rat Assay with Linuron and Phenobarbital

SPONSOR: Battelle Memorial Institute
505 King Avenue
Columbus, OH 43201-2693

TESTING FACILITY: RTI International
Chemistry and Life Sciences
Center for Life Sciences and Toxicology
Post Office Box 12194, 3040 Cornwallis Road
Research Triangle Park, NC 27709

PROPOSED EXPERIMENTAL START DATE: September 19, 2005 (Staggered start)
PROPOSED EXPERIMENTAL TERMINATION DATE: October 12, 2005 (Staggered end)

AMENDMENTS:

| Number | Date | Section(s) | Page(s) |
|---|---|---|---|
| 1 | | | |
| 2 | | | |
| 3 | | | |
| 4 | | | |
| 5 | | | |

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9-19-05
Julia D. George, Ph.D. Date
Program Director
Endocrine Disruptor Screening Program
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David P. Houchens, Ph.D. Date
Program Manager
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## 1.0 OBJECTIVES AND BACKGROUND

The objectives of this study are:

1) To determine if similar results for each endpoint can be obtained among three different laboratories using a similar protocol, compounds, and dose levels.

2) To determine if the observed results in the more current studies are comparable to the expected results established in earlier studies using the same protocol.

3) 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.

The Food Quality Protection Act of 1996 requires the EPA to develop and implement a screening program using valid tests for determining the potential in humans for estrogenic effects from pesticides. This program has been expanded on the advice of the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) to include androgenic (and anti-androgenic) effects and effects from thyroid-hormone (TH)-like (and anti-TH) substances (EDSTAC, 1998). EPA proposed a two-tiered screening and testing program in a Federal Register notice in 1998 (63 FR 71542-71568, Dec. 28, 1998) that covered not only pesticides but also commercial chemicals subject to regulation under the Toxic Substances Control Act (TSCA; 15 USC 2601) and environmental and drinking water contaminants. One of the assays recommended as an alternative for potential inclusion in the Tier 1 screening battery is a short term 15-day-adult intact male rat assay. The adult male assay was developed by Dupont to identify compounds that have the potential to act as agonists or antagonists to the estrogen, androgen, progesterone, or dopamine receptor; 5α-reductase inhibitor; steroid biosynthesis inhibitors; and compounds that alter thyroid function. Results from this assay and/or with the use of intraperitoneal (ip) injection as the route of administration, and other assays with a similar purpose, have been reported (O'Connor et al., 1996, 1999, 2002a,b).

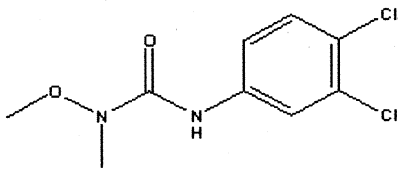
## 2.0 MATERIALS AND METHODS

### 2.1 Test Substances

#### 2.1.1 Linuron

CAS Number 330-55-2
Synonyms: Linuron ; N-(3,4-Dichlorophenyl)-N'-methoxy-N'-methylurea; Linuron; Lorox; Afalon; Linurex; N'-(3,4-Dichlorophenyl)-N-methoxy-N-methylurea; methoxydiuron; du Pont Herbicide 326; Hoe 2810; Linorox; Sarclex; Aflon; Linex 4L; Lorox 4L; Lorox 50W; Lorox DF; Lorox L; Lorox Plus; Alafon; Linex; Lorax; 1-Methoxy-1-methyl-3-(3,4-dichlorophenyl)urea; 3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea; Urea, 1-(3,4-dichlorophenyl)-3-methoxy-3-methyl-; Garnitan; Methoxy-1-methyl-3-(3,4-dichlorophenyl)urea; Premalin; Cephalon; 3-(3,4-Dichlorophenyl)-1-methoxy(methyl)urea;

Structure:

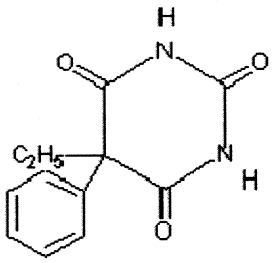



| | |
|---|---|
| Supplier: | Chem Services |
| Lot Number: | 348-8A |
| Purity: | 99.5% |
| Appearance: | Crystalline solid |
| Suspension/Solution: | Suspension |
| Molecular Formula: | $C_9H_{10}Cl_2N_2O_2$ |
| Molecular Weight: | 249.1 g/mole |
| Storage, Bulk Chemical: | Room Temperature |
| Storage, Test Suspension: | 4° C |

### 2.1.2 Phenobarbital

CAS Number 50-06-6

Structure:




| | |
|---|---|
| Supplier: | Sigma-Aldrich |
| Lot Number: | 104K2600 |
| Purity: | 99.1% |
| Appearance: | To be determined |
| Molecular Formula: | $C_{12}H_{12}N_2O_3$ |
| Molecular Weight: | 232.2 g/mole |
| Solution/Suspension: | Suspension |
| Storage, Bulk Chemical: | Room Temperature |
| Storage, Test Solution: | 4° C |

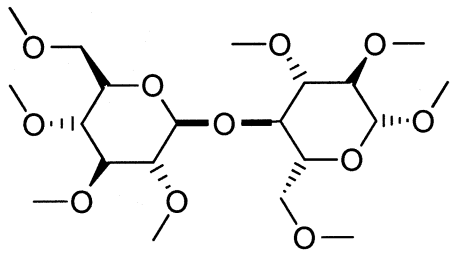
### 2.1.3 Vehicle: Methylcellulose

CAS number: 9004-67-5

Synonyms: Cologel; Celevac; tylose mh20; tylose mh50; tylose mh300; tylose mh1000; tylose mh2000; tylose mh4000; tylose mh300p; tylose sap; tylose sl; tylose sl 100; tylose sl 400; tylose sl 600; tylose twa; methylcellulose; viscol; viscontran l52; viscosol; walsroder mc 20000s; methyl ether cellulose; adulsin; bagolax; bufapto methalose; bulkaloid; celacol m; celacol m20; celacol m450; celacol mm; celacol mm 10p; celacol m 20p; cellapret; cellogran; cellothyl; cellulose methyl; cellulose methylate; cellumeth;

cethylose; cethytin; culminal k 42; edisol m; hydrolose; mapolose m25; mapolose 60sh50; mco 8000; mc 4000 cp; mc 20000s; mellose; methocel 10; methocel 15; methocel 181; methocel 400; methocel 4000; methocel a; methocel chg; methocel 400cps; methocel 4000cps; methocel mc; methocel mc 25; methocel mc4000; methocel mc 8000; methocel sm 100; methulose; methyl cellulose-a; methyl cellulose ether; metolose mc 8000; metolose 60sh; metolose 60sh400; metolose sm 15; metolose sm 100; metolose sm 4000; mmts-btr; napolone; Nicel; rhomellose; syncelose; tylose 444; tylose A4S; tylose mf; tylose mh; Cellulose, methyl ether; Citrucel; Methyl cellulose (viscosity: ca 15 cP (2% solution in water)); Methylcel MC;

Structure:



| | |
|---|---|
| Supplier: | Sigma-Aldrich |
| Lot Number: | 14601TC |
| Purity: | (to be added by sponsor) |
| Appearance: | (to be added by sponsor) |
| Molecular Formula: | $(C_7 H_{14} O_5)_x$ (polymer) |
| Molecular Weight: | 40,000 to 180,000 (polymer) |
| Storage, Bulk Chemical: | Room Temperature |
| Storage, Vehicle Solution: | 4°C |

Note: Chemical information not currently available will be added by amendment.

## 2.2 Chemical Safety and Handling

MSDSs of all chemicals in will be added in an amendment.

## 2.3 Dose Formulation and Analysis

The dosing formulations will be prepared at a frequency determined by stability tests initiated prior to the start of the study. Linuron stability, in 0.25% methylcellulose, has been established by Battelle Memorial Institute to be ≥90% of the target concentration for up to an estimated 6.5 weeks. The phenobarbital stability, in 0.25% methylcellulose, is currently being

determined and will be added by amendment. Stock formulations for the chemicals will be prepared by Battelle and supplied to each laboratory with instructions on how to prepare the various dose groups.

The dosing bottles will be identified at RTI by a five-digit random number Rx code, and a color code. Personnel, other than the safety personnel, analytical chemistry, and dose formulation personnel will not be informed of the test chemicals or formulation concentrations until all laboratory work is completed (i.e., the study technicians will be "blind" for chemical and dose). The dosing formulations will be stored at refrigerated temperatures. Prior to dosing each day, the formulations will be removed from the refrigerator. All dose levels, including the control, will be brought to room temperature while being stirred for about one hour before dosing. Each dose formulation will be stirred continuously throughout dosing. The dose volume will be 5 mL/kg. The vehicle control will be 0.25% methylcellulose and the route of administration will be oral gavage. The same volume of vehicle will be given to the control group.

Samples of each dose formulation will be collected in duplicate (one for dose analysis and one for back-up) on Test Days 1 and 15 and stored according to Battelle's instructions until dose analysis (i.e., homogeneity and concentration verification). RTI will analyze the concentration and homogeneity of these samples according to instructions from Battelle.

## 2.4 Animals

### 2.4.1 Species and Supplier

The proposed test animals will be the Sprague Dawley Derived Outbred Albino Rat Crl:CD®(SD) supplied by Charles River Laboratories, Inc., Raleigh, NC.

### 2.4.2 Live Animals and Species Justification

The use of live animals has been requested by the Sponsor. Alternative test systems are not available for the assessment of effects of chemicals on reproduction and development in intact mammals for determining the potential risk for humans from endocrine-mediated effects of pesticides and other chemicals. The Charles River CD® rat has been the subject of choice on reproductive and developmental toxicology contracts at RTI since 1976, and has been used for other toxicology studies with these test materials. Large historical data bases for growth, food, and water consumption are available from the supplier. The Crl:CD®(SD) rat has been selected on the basis of extensive experience with this strain and its suitability with respect to sensitivity to endocrine modulators. This study does not unnecessarily duplicate any previous study.

### 2.4.3 Total Number, Age, and Weight

Adult male Crl:CD®(SD) rats, approximately 9 weeks of age will be purchased specifically for this study with the expectation to be 225-350 grams at randomization and approximately 10 weeks of age at the start of dose administration. A total of 115 rats will be procured for this study.

### 2.4.4 Quality Control

The shipment of males will be quarantined on arrival, and quality control evaluation will be initiated that day. Animal Health testing records from Charles River Laboratories, Inc., Raleigh, will be kept with the study records. Animals will be observed twice daily and entries will be made on the Quarantine Evaluation Form. Animals will be housed under the conditions of the study with deionized water and low phytoestrogen feed.

### 2.4.5 Sentinels

If the experimental period is a month, after the selection of the study males, five of the remaining male rats will be randomly selected and designated as sentinels. Animals will be singly housed in the study room with feed and water available *ad libitum* (as described below). They will be examined once daily by cage side observation for morbidity or mortality at the same time as the clinical observations or morbidity/mortality checks for the study animals. The clinical condition of sentinel animals will be recorded only in the event that an animal is moribund or found dead. If a sentinel animal is terminated moribund, blood will be collected at termination and serum samples frozen. During the male necropsies, the surviving sentinel males will be terminated, blood samples collected, and serum samples prepared. All sentinel serum samples will be submitted to BioReliance (Rockville, MD) for serological evaluation (see above section on Quality Control).

### 2.4.6 Quarantine

The male rats will be quarantined for approximately one week, with the prior concurrence of the RTI Animal Research Facility (ARF) veterinarian. They will be observed daily for general health status and ability to adapt to the ARF husbandry conditions. The animals will be weighed three times (Test Day -5, -3 and 0) during this period and observed with respect to weight gain and any gross signs of disease or injury.

They will be released from quarantine, if suitable for use (based on QC results, such as body weight and absence of clinical signs), by the attending ARF veterinarian or her designee.

## 2.5 Animal Husbandry

### 2.5.1 Housing, Feed, and Water

During the quarantine period, animals will be assigned to cages. Males will be singly housed in solid-bottom, polycarbonate cages (8"x19"x10.5") fitted with stainless steel wire lids (Laboratory Products, Rochelle Park, NJ). Sani-Chip® cage bedding (P.J. Murphy, Forest Products, Inc., Montville, NJ) will be used in all cages. Powdered feed, Teklad 2018 CM diet (low phytoestrogen, lot # 082605 MA, analyzed August 26, 2005) and deionized water, produced at RTI from tap water from the Durham, NC water system, will be available *ad libitum* in plastic bottles with stainless steel sipper tubes throughout quarantine and study periods. The analysis of the rodent chow for chemical composition and possible chemical contamination, and analysis of Durham City water will be provided by the suppliers and maintained in the study records. Water samples will be analyzed for total bacterial counts, and the presence of coliforms. Feed samples will be analyzed for the presence of bacteria and fungi. Samples from freshly washed cages and cage racks will be analyzed to ensure adequate sanitation by the cage washers. It is anticipated that contaminant levels will be below certified levels for both feed and water and will not affect the design, conduct, or conclusions of this study. In addition, the lot number of Teklad 2018 diet used will be analyzed by the supplier for concentrations of the phytoestrogens genistein, daidzein, and glycitein. The "metabolizable energy content" of the feed (label value) will also be recorded and reported. The diet will be stored at approximately 60-70°F, and the period of use will not exceed six months from the milling date. At all times, animals will be housed, handled, and used according to the NRC Guide (NRC, 1996).

### 2.5.2 Environmental Conditions

Environmental conditions in the ARF will be continuously monitored, recorded, and controlled during the course of the study by an automated system (Siebe/Barber-Colman Network 8000 System) with Version 4.4.1 Signal® software (Siebe Environmental Controls (SEC)/Barber-Colman Company, Loves Park, IL). Animal rooms used for this study will be maintained on a 12:12 hour light:dark cycle. Target conditions for temperature and relative humidity in the animal rooms will be between 64-79°F (18-26°C) and 30-70%, respectively, with 10-15 air changes per hour (NRC, 1996). Temperature and/or relative humidity excursions will be documented in the study records and the final report.

### 2.5.3 Animal Identification

All male rats will be individually identified by ear tag after arrival at RTI on Test Day -6. In addition, all males assigned to the study will be given an animal study number. All data generated during the course of this study will be tracked by these numbers.

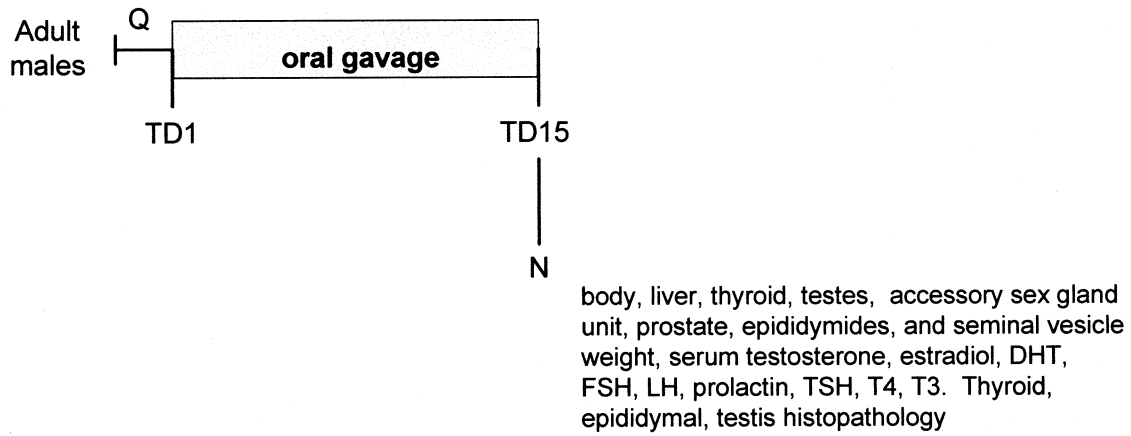
### 2.5.4 Limitation of Discomfort

Some toxicity may be caused by exposure at the high doses of each test material. Discomfort or injury to animals will be limited, in that if any animal becomes severely debilitated or moribund, it will be humanely terminated by carbon dioxide ($CO_2$) inhalation.

## 3.0 EXPERIMENTAL DESIGN

### 3.1 Study Design, Test Chemicals, and Dose Selection

The study will be conducted in two components with staggered start dates, and will consist of three dose groups each of two chemicals, and a vehicle control group (total of 7 groups). Part of each group, including controls, will start on each start day. Each group will be comprised of 15 males which have been randomized across treatment groups, based on body weight taken on TD 0 (the day before experimental start). The study males will be dosed by gavage once daily for 15 consecutive days. Table 1 presents the study design and target doses of the test chemicals. A graphical representation of the study design is presented in Figure 1 below.




Key:
Q = quarantine
TD = test day
N = necropsy

**Figure 1. Study Design for the 15-day Adult Male Assay**

The U.S. EPA selected two test chemicals for evaluation. The two test chemicals and their target/mechanism of action are as follows: (1) linuron, anti-androgen; competitive binding to androgen receptor, and (2) phenobarbital alters thyroid function.

**Tentative Study Dates** :

Males arrive at RTI: 9-19-05

Release of males from quarantine: September 26, 2005

Proposed Experimental Start Dates: September 27, 28, 2005

Proposed Experimental Termination Dates: October 11, 12, 2005

Submission of nonaudited draft final report: To be determined

Submission of audited draft final report: To be determined

**Table 1. Study Design, Test Chemicals, and Target Doses**

| Group No. | No. Males | Chemical | Dose (mg/kg/day) [b] | Concentration (mg/mL) | Dose Volume (mL/kg) |
|---|---|---|---|---|---|
| 1 | 15 | Vehicle Control[a] | 0 | 0.0 | 5 |
| 2 | 15 | Phenobarbital | 25 | 5.0 | 5 |
| 3 | 15 | Phenobarbital | 50 | 10.0 | 5 |
| 4 | 15 | Phenobarbital | 100 | 20.0 | 5 |
| 5 | 15 | Linuron | 50 | 10.0 | 5 |
| 6 | 15 | Linuron | 100 | 20.0 | 5 |
| 7 | 15 | Linuron | 150 | 30.0 | 5 |

[a] 0.25% aqueous methylcellulose, vehicle only

[b] Test compounds administered once daily by gavage on Test Days 1 through 15

## 3.2 Treatment of Adult Males

Beginning on Test Day 1, each male will be dosed with one of the test materials at one of the dose levels or the vehicle control (0.25 % aqueous methylcellulose). Each animal will be weighed daily on Test Days 1 to 14 prior to treatment and, on Test Day 15 after treatment, and the body weight recorded. Vehicle or dose formulations will be administered daily by oral gavage at a dosing volume of 5 ml/kg body weight from Test Days 1 – 14. Gavage dosing will

be used since it is part of the established protocol for this assay. Oral Administration is the usual route for phenobarbitol and is a possible exposure route for linuron. Gavage dosing will use a 16-gauge, two-inch curved dosing needle fitted with a glass or plastic (disposable) tuberculin syringe of the appropriate volume for each treatment group. The daily dosage for each compound will be administered at approximately 0600 hr daily so that animals can have blood collected and be necropsied between 0800 and 1100 hr on Test Day 15 (2-3 hr after the final dose). The treatments will be administered on an mg/kg body weight basis, adjusted based on the most recent body weight, with the exception of Test Day 15, when the dose will be based on the body weight taken on Test Day 14. On Test Days 1-14, the body weights will be taken and recorded, feed weights recorded on appropriate days, then dosing performed, and clinical observations recorded. On Test Day 15, the animals will be dosed, body weights taken, feed measured, and clinical observations recorded. The volume of the dose administered will be recorded each day.

## 3.3 Observation of Adult Males

### 3.3.1 Clinical Observations

Clinical observations of male study animals will be documented at least once daily during quarantine, and at least twice daily, at dosing and one to two hours postdosing on TD 1-14. On TD 15, clinical observations will be made at dosing. The examining technicians will be unaware of the test materials or of dosage levels. Observations will be made for (but not limited to):

A. Any response with respect to body position, activity, coordination, or gait
B. Any unusual behavior such as head flicking, compulsive biting or licking, circling, etc.
C. The presence of:

1. Convulsions, tremors, or fasciculations
2. Increased salivation
3. Increased lacrimation or red-colored tears (chromodacryorrhea)
4. Increased or decreased urination or defecation (including diarrhea)
5. Piloerection
6. Mydriasis or miosis (enlarged or constricted pupils)
7. Unusual respirations (fast, slow, labored, audible, gasping, or retching)
8. Vocalization.

Cage-side examinations to detect moribund or dead rats will be conducted at least once daily throughout the study by the Animal Research Facility staff. Moribund rats will be sacrificed. Moribund and dead rats will be given a gross pathological evaluation. At every

weighing, each rat will be individually handled and examined for abnormal behavior and appearance as part of the clinical observations.

### 3.3.2 Male Body Weights and Feed Consumption

All study males will be weighed in the morning of Test Day 0 (prior to assignment to treatment groups), and every day in the morning on Test Day 1-14, for adjustment of dosing volume based on the most recent body weight. They will also be weighed on Test Day 15 for calculation of food consumption. Male body weight and weight change will be calculated and analyzed for TD 1-8, 8-15 and 1-15. Feed weights for the individually-housed males will be recorded on TD 1, 8, and 15, and feed consumption will be reported as g/kg body weight/day.

## 3.4 Necropsy of Males

### 3.4.1 Gross Necropsy, Blood Collection, and Organ Weights

All rats found dead or sacrificed moribund prior to experimental start will be necropsied but tissues will not be saved or examined microscopically. After experimental start, all rats found dead or those euthanized by decapitation with prior anesthesia using carbon dioxide ($CO_2$) anesthesia for no more than 60 seconds after being found moribund will be necropsied. Gross lesions and target organs (as described below) will be saved for optional histopathology. Blood will be taken from the trunk of the animal at the time of necropsy. Rats sacrificed by design on Test Day 15 will be euthanized by decapitation with prior anesthesia using $CO_2$ for no more than 60 seconds within 2-3 hours after final dosing.

Animals will be dosed on Test Day 15, based on the body weight of Test Day 14, in the animal room and then moved to the necropsy area approximately 1 hour before necropsy to minimize stress-induced changes in hormone levels related to cage transport. Animals will not be fasted prior to necropsy. Rats will be euthanized by decapitation with prior anesthesia using CO2 for no more than 60 seconds and exsanguinated via the site of decapitation. Rapid euthanasia is necessary because of the likelihood that undue stress associated with the administration of anesthesia alone will interfere with the accurate measurement of the various hormones that are essential endpoints of this assay. Necropsies will take place between the hours of 0800 – 1100, within 2-3 hours of the last administered dose, on Test Day 15. This is necessary to minimize variability associated with serum hormone measurement. Time of death will be recorded for all animals.

All necropsies will be performed after animals are euthanized by decapitation with prior exposure to $CO_2$ for no more than 60 seconds. Animals will be evaluated for gross observations of toxicity, organ weights, and serum hormone concentrations. The testes, thyroid gland, and epididymides will be evaluated microscopically. The order in which animals will be necropsied for blood and tissue collection will be stratified across all groups.

Final body, liver, thyroid gland, left and right testis, entire prostate, epididymides, and seminal vesicles and coagulating glands (with fluid) weights will be taken. Total weight of the testes will be the combined weight of the left and right testis and, accessory sex gland unit weight, will be the combined weight of the entire prostate and seminal vesicles and coagulating gland with fluid weights. Care must be taken to remove mesenteric fat from these tissues such that the fluid in the accessory sex glands is retained. All organs will be weighed to four decimal places (0.0001g). Relative organ weights (% of live weight on Test Day 15) will be calculated. Blood will be collected into a serum separator tube and placed on ice until serum is prepared.

### 3.4.2 Histology and Pathology

The liver and epididymides from each rat will be placed in formalin fixative, and then embedded in paraffin. The thyroid glands and surrounding tissue will be removed and placed into formalin fixative for at least 48 hrs prior to trimming, weighing, and embedding in paraffin. Following fixation, final dissection of the thyroid will be performed, under a microscope, by one individual in order to reduce the variability of the dissection procedure and hence, reduce the variability of the thyroid weights. Testes will be placed in Bouin's fixative for 24 hours after which they will be rinsed and stored in 70% alcohol until embedded in paraffin. The testes(left and right), epididymides (left and right), and thyroid will be evaluated microscopically. The embedded tissues will be sectioned at 3-5 microns and stained with hematoxylin and eosin (H and E). Microscopic evaluations will be performed on control and high dose animals for all compounds. Compounds which show effects in the high dose group will have the remaining groups evaluated and this will be added by amendment. Stained sections of the control and high dose groups will be identified and evaluated by a Board Certified veterinary pathologist for pathologic abnormalities and potential treatment-related effects. Thyroids should be evaluated for morphologic changes such as altered follicular epithelial height, the relative number and staining characteristics of colloid, the extent of thyroid vascular supply, and the density, size, and shape of the thyroid follicles. The testes and epididymides will be evaluated for spermatogenesis, spermiogenesis, status of seminiferous tubules in the testis, and sperm in the epididymis, as well as the structural integrity of these organs. Liver will be evaluated microscopically at the discretion of the pathologist, the sponsor and the Study Director.

### 3.4.3 Hormone Evaluation

Blood will be collected from the trunk of the animal at the time of sacrifice from all animals. The blood will be placed in a serum separator tube on ice until the serum is prepared. The blood will be allowed to clot and centrifuged under refrigeration at a setting of

approximately 1400 x g for approximately ten minutes. Aliquots of serum should be made based on the number of different assays that will be run in a day to minimize the potential freeze and thaw effect on hormone concentrations. Serum will be stored between -65 °C and -85 °C until analyzed. The sequence in which the hormones should be assayed by commercially available radioimmunoassay (RIA) kits is testosterone, luteinizing hormone, thyroid stimulating hormone, thyroxine, triiodothyronine, follicle simulating hormone, estradiol, and prolactin. Only if relative liver weights are significantly increased should dihydrotestosterone levels be measured. If serum is limiting, the Study Director should contact the Sponsor to establish a priority list of hormones to be measured. Each sample should be run in duplicate and include high and low quality control (QC) serum samples. Each assay should include all samples from the control group and each dose level for both chemicals. For additional QC samples, the kit-supplied zero standard 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 all QC samples will be used to assess within-and between-assay variability for each laboratory. Proteinaceous rat hormones will be obtained from the National Hormone and Pituitary Program and the steroids will be purchased from commercial suppliers. All three laboratories will use the same sources.

## 4.0 STATISTICAL ANALYSES

A common statistical plan will be used by the three laboratories. The current plan is attached to this protocol.

## 5.0 RETENTION OF SPECIMENS AND RECORDS

All specimens will be disposed of after they no longer afford evaluation. All records, which remain the responsibility of RTI, will be retained in the RTI archives for the life of the contract.

## 6.0 QUALITY CONTROL/QUALITY ASSURANCE PROCEDURES

QC and quality assurance (QA) procedures will follow those outlined in the Quality Assurance Project Plan (QAPP) prepared for this study. The study is under OECD, Japanese and EPA/OPPTS GLP Guidelines.

## 7.0 REPORTING

### 7.1 Status Reports

Status reports will be provided monthly to the Sponsor's Representative, with contents to be determined by the Sponsor's Representative and/or the Sponsor's Study Monitor.

## 7.2 Draft and Final Reports

A draft report will be submitted to the Sponsor's Representative within two months of the final necropsy date. The final report will include:

- Abstract
- Objectives
- Materials and Methods
- Results
- Discussion
- Conclusions
- References
- Summary in-life and necropsy data with statistical analyses
- Individual animal data: in-life and necropsy
- Protocol, any amendments, or any deviations from the protocol
- QAPP, any amendments, or any deviations from the QAPP
- Histopathology report
- Analytical chemistry report

***Individual Data Male Rats***

a. Identification number
b. Clinical signs
c. Body weights daily; feed weights on test days 1, 8, and 15
d. Age at death and manner of death
e. Weight at necropsy
f. Organ weights
g. Gross Necropsy Observations
h. Serum Hormone levels

***Summary of Data From Male Rats***

a. Mean periodic body weights and weight gains
b. Feed consumption
c. Clinical signs
d. Organ weights
e. Histopathological Data
f. Serum Hormone Levels

## 8.0 PERSONNEL

| | |
|---|---|
| Study Director,<br>Work Assignment Leader | Carol D. Sloan, M.S. |
| Program Director: | Julia D. George, Ph.D. |
| Project Manager: | Bonnie T. Hamby, B.S. |
| Principal Investigator: | Rochelle W. Tyl, Ph.D., DABT |
| ARF Attending Veterinarian: | Jem Scott-Emuakpor, D.V.M. |
| ARF Manager: | Richard S. Silverstein, A.S. |
| Study Coordinator: | Melissa C. Marr, B.A., RLATG |
| Technician Supervisor: | Natalie A. Ostin, B.A. |
| Data Analyst and Reproductive Toxicity Supervisor: | Christina B. Myers, M.S. |
| Statistical Advisors: | Gayle S. Bieler, M.S.<br>Rick L. Williams, Ph.D |
| Research Assistant, Data Entry: | Timothy W. Wiley, B.S. |
| Biologists: | Vickie I. Wilson<br>Kristie D. Vick, B.S. |
| Biological Laboratory Assistants: | Malcolm D. Crews, ALAT<br>Christopher G. Leach, B.S.<br>Leslie L. MacDonald, B.S. |
| Endocrinology: | Carol D. Sloan, M.S.<br>Susan W. Pearce, B.S. |
| Quality Assurance: | Debra A. Drissel, B.S.<br>Celia D. Keller, M.S.<br>Carrie A. Ingalls, B.S. |
| Analytical Chemistry: | Nora P. Castillo, M.E.<br>Daniel J. Watkins, M.S.<br>Jeanne B. Whitaker, A.A.S. |
| Materials Handling Facility: | M. Michael Veselica, M.S., Manager<br>Donald L. Hubbard, B.A.<br>Randy A. Price<br>Jon E. Larson |
| Shipping Specialist: | Victor L. Parker |
| Histology: | EPL, Inc. |
| Pathology: | John C. Seely, D.V.M., ACVP (EPL, Inc.) |

Additional study team members to be determined.

## 9.0 STUDY RECORDS TO BE MAINTAINED

Protocol and any Amendments
QAPP and any Amendments
List of any Protocol Deviations
List of QAPP Deviations
List of Standard Operating Procedures
Animal Requisition and Receipt Records
Quarantine Records
Temperature and Humidity Records for the Animal Room(s)
Animal Research Facility Room Log(s)
Durham City Water Analysis (analyzed monthly, reported annually)
Feed Type, Source, Lot Number, Dates Used, Certification, Analytical Results
Dosage Code Records Containing Five-Digit Rx Code, Color Code, and Concentration
Dose Formulation Receipt and Use Records
Male Distribution into Groups
Male Dosing Forms
Body Weights
Clinical Signs
Food Weights
Male Necropsy Records: Body weight, organ weights, gross observations, required (and optional, if done) organ histopathology
Statistical Analysis Records
Histopathology Report
Serum Hormone Analyses (Serum testosterone, estradiol, DHT, FSH, LH, prolactin, TSH, T4, and T3 levels)
Correspondence

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# ATTACHMENT

## Material Safety Data Sheets (MSDSs)

| | |
| --- | --- |
| Linuron | CAS No. 330-55-2 |
| Phenobarbital | CAS No. 50-06-6 |

**Endocrine Disruptor Screening Program**
**Work Assignment 5-15**

# Interlaboratory Validation of the 15-Day Adult Intact Male Rat Assay

## Intra-Laboratory Analysis

**September 14, 2005**

## Introduction

Three laboratories will conduct the 15-day adult intact male rat assay according to the test method provided by the EPA.

Within each laboratory two chemicals will be tested, each at three dose levels specified by the EPA. In addition a vehicle control group will be tested in each laboratory. The sample size will be n=15 adult male rats per group, for a total of seven groups and 105 animals per laboratory. This statistical analysis plan specifies the summaries, displays, and statistical analyses that will be used to summarize the results within each laboratory.

## Data

The test method specifies four categories of data:

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)
   - $T_4$ (µg/dl)
   - $T_3$ (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 and added by amendment.

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 – (4 - 5 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 and added by amendment.

Microscopic evaluations will be performed on control and high dose animals for all compounds. 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 Test Day (TD)15.

If animals 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 animals 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 (1969) 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 bodyweight and food consumption endpoints. These will be TD15 body weight, 3 body weight change variables as shown in the data section, 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 animals 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

[1] If X, Y denote the control group least squares mean and the dose group lest squares mean respectively, with variance-covariance matrix ($S_X^2$, $S_Y^2$, $S_{XY}$), an approximate standard error for $R \equiv (Y/X)\times 100\%$ is

$$Se[R(X, Y)] \approx |1/X| \left[(Y/X)^2 S_X^2 + S_Y^2 - 2(Y/X) S_{XY}\right]^{1/2} \times 100\%$$

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.

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**

---

[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

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

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 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 ($\approx 0.05/8$) 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.

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

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!
Can 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

SECTION 5 - FIRE AND EXPLOSION DATA CONTINUED

Extinguishing 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: | 0.051mPa@20 C |
| Vapor Density: | Not Available |

| | |
|---|---|
| Solubility in Water: | Very slightly soluble |
| Odor: | Not Available |
| Evaporation 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: 4000mg/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.

Cat No.: PS-372
Page: 4

## 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.

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<br>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.

**ENDOCRINE DISRUPTOR SCREENING PROGRAM**
**AMENDMENT REPORT**

STUDY NUMBER:08055.004.040

WAL/STUDY DIRECTOR: C. Sloan

AMENDMENT NUMBER: 0 0 1

NOTEBOOK NUMBER:

TITLE OF STUDY: Inter-Laboratory Validation of the 15-Day Adult Intact Male Rat Assay with Linuron and Phenobarbital

QAPP/PROTOCOL ID (e.g. number and/or date): RTI-956

AMENDMENT RELATING TO:

[ ] QAPP [ ] QMP [x] Protocol [ ] GLPs
[ ] SOP [ ] Method

ORIGINAL DOCUMENT SPECIFICATIONS:

1.0 Objectives and Background
2.0 Materials and Methods
3.0 Experimental Design
4.0 Statistical Analyses
5.0 Retention of Specimens and Records
6.0 Quality Control/Quality Assurance Procedures
7.0 Reporting
8.0 Personnel
9.0 Study Records to be Maintained
10.0 References

AMENDMENT:
Addition of Section 4.0 and renumbering of Sections:

1.0 Objectives and Background
2.0 Materials and Methods
3.0 Experimental Design
**4.0 Data Collection**
5.0 Statistical Analyses
6.0 Retention of Specimens and Records
7.0 Quality Control/Quality Assurance Procedures
8.0 Reporting
9.0 Personnel
10.0 Study Records to be Maintained
11.0 References

Text for this section:

**The TASC automated data collection system will be used for collection of all body weights (including quarantine), feed weights, clinical observations, organ weights and gross necropsy findings. TASC will also calculate the volume of dosing solution to be administered to each animal on each day based on the appropriate body weight and recorded when each animal is dosed. Therefore, the raw data for these measurements will be the electronic data collected in TASC unless otherwise noted in the study records. TASC will not be used for automated collection of the hormone measurements.**

REASON FOR CHANGE:

The recently validated Toxicology Analysis System Customized (TASC) data collection system will be used for this study. This had not been determined at the time the protocol was developed.

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APPROVAL:

WAL/Study Director [signature] Date 9-21-05

Program Management [signature] Date 11/22/05

EDSP Battelle QAM [signature] Date 11-22-05

**ENDOCRINE DISRUPTOR SCREENING PROGRAM**
**AMENDMENT REPORT**

STUDY NUMBER:08055.004.040 WAL/STUDY DIRECTOR: C. Sloan

AMENDMENT NUMBER: 0 0 2

NOTEBOOK NUMBER:

TITLE OF STUDY: Inter-Laboratory Validation of the 15-Day Adult Intact Male Rat Assay with Linuron and Phenobarbital

QAPP/PROTOCOL ID (e.g. number and/or date): RTI-956

AMENDMENT RELATING TO:

[ ] QAPP [ ] QMP [x] Protocol [ ] GLPs
[ ] SOP [ ] Method

ORIGINAL DOCUMENT SPECIFICATIONS:

Clinical observations of male study animals will be documented at least once daily during quarantine, and at least twice daily, at dosing and one to two hours postdosing on TD 1-14. On TD 15, clinical observations will be made at dosing. The examining technicians will be unaware of the test materials or of dosage levels.

AMENDMENT:

Clinical observations of male study animals will be documented at least once daily during quarantine, and at dosing and one to two hours postdosing on TD 1-14 **and an additional clinical observation after 2:00 PM on TD 4-14 for Group 1 and on TD 3-14 for group 2.** On TD 15, clinical observations will be made at dosing. The examining technicians will be unaware of the test materials or of dosage levels.

REASON FOR CHANGE:

Positive clinical observations were being noticed in the afternoon of Test Days 1-3 for Group 1. Therefore, the sponsor requested the addition of PM observations.

APPROVAL:

WAL/Study Director Carol Sloan Date 9-30-05

Program Management David P. Mannina Date 11/22/05

EDSP Battelle QAM Terri L Pollock Date 11-22-05

**ENDOCRINE DISRUPTOR SCREENING PROGRAM**
**AMENDMENT REPORT**

STUDY NUMBER:08055.004.040 WAL/STUDY DIRECTOR: C. Sloan

AMENDMENT NUMBER: 0 0 3

NOTEBOOK NUMBER:

TITLE OF STUDY: Inter-Laboratory Validation of the 15-Day Adult Intact Male Rat Assay with Linuron and Phenobarbital

QAPP/PROTOCOL ID (e.g. number and/or date): RTI-956

AMENDMENT RELATING TO:

[ ] QAPP [ ] QMP [x] Protocol [ ] GLPs
[ ] SOP [ ] Method

ORIGINAL DOCUMENT SPECIFICATIONS: **3.4.1 Gross Necropsy, Blood Collection, and Organ Weights, p. 16**

Blood will be collected into a serum separator tube and placed on ice until serum is prepared.

AMENDMENT:

Blood will be collected into a tube and placed at room temperature until serum is prepared.

REASON FOR CHANGE:

Blood did not yield enough serum when collected and placed on ice.

APPROVAL:

WAL/Study Director Carl Sloan Date 11-11-05

Program Management Dirk P. Hawkins Date 11/22/05

EDSP Battelle QAM [illegible] Date 11-22-05

RTI QAU Review by Carrie Regalbuto Date 10-12-2005

**ENDOCRINE DISRUPTOR SCREENING PROGRAM**
**AMENDMENT REPORT**

STUDY NUMBER:08055.004.040 WAL/STUDY DIRECTOR: C. Sloan

AMENDMENT NUMBER: 0 0 4

NOTEBOOK NUMBER:

TITLE OF STUDY: Inter-Laboratory Validation of the 15-Day Adult Intact Male Rat Assay with Linuron and Phenobarbital

QAPP/PROTOCOL ID (e.g. number and/or date): RTI-956

AMENDMENT RELATING TO:

[ ] QAPP [ ] QMP [x] Protocol [ ] GLPs
[ ] SOP [ ] Method

ORIGINAL DOCUMENT SPECIFICATIONS: **3.4.3 Hormone Evaluation**

The sequence in which the hormones should be assayed by commercially available radioimmunoassay (RIA) kits is testosterone, luteinizing hormone, thyroid stimulating hormone, thyroxine, triiodothyronine, follicle simulating hormone, estradiol, and prolactin..

AMENDMENT:

The sequence in which the hormones will be assayed by commercially available radioimmunoassay (RIA) kits is follicle stimulating hormone, prolactin, thyroid stimulating hormone, testosterone, thyroxine, luteinizing hormone, estradiol, and triiodothyronine.

REASON FOR CHANGE:

Although the kits were ordered so that the sequence would be as stated in the protocol, the expiration dates dictated that the assays be run in the modified sequence.

APPROVAL:

WAL/Study Director Carol Sloan Date 10-11-05

Program Management Date 11/22/05

EDSP Battelle QAM Date 11-22-05

**ENDOCRINE DISRUPTOR SCREENING PROGRAM**
**AMENDMENT REPORT**

| | |
|---|---|
| STUDY NUMBER:08055.004.040 | WAL/STUDY DIRECTOR: C. Sloan |
| | AMENDMENT NUMBER: 0 0 5 |

NOTEBOOK NUMBER:

TITLE OF STUDY: Inter-Laboratory Validation of the 15-Day Adult Intact Male Rat Assay with Linuron and Phenobarbital

QAPP/PROTOCOL ID (e.g. number and/or date): RTI-956

AMENDMENT RELATING TO:

[ ] QAPP [ ] QMP [x] Protocol [ ] GLPs
[ ] SOP [ ] Method

ORIGINAL DOCUMENT SPECIFICATIONS: **6.0 QUALITY CONTROL/QUALITY ASSURANCE PROCEDURES and 10.0 REFERENCES**

QC and quality assurance (QA) procedures will follow those outlined in the Quality Assurance Project Plan (QAPP) prepared for this study. The study is under OECD, Japanese and EPA/OPPTS GLP Guidelines.

AMENDMENT:

QC and quality assurance (QA) procedures will follow those outlined in the Quality Assurance Project Plan (QAPP) prepared for this study. The study is under OECD, Japanese (MAFF) and EPA/ FIFRA/TSCA GLP regulations.

## 10.0 REFERENCES
**Add the following:**

**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.

REASON FOR CHANGE:

These sections were amended for clarification.

APPROVAL:

WAL/Study Director Carol Stoner Date 11-4-05

Program Management [signature] Date 11/22/05

EDSP Battelle QAM [signature] Date 11-21-05

**ENDOCRINE DISRUPTOR SCREENING PROGRAM**
**AMENDMENT REPORT**

STUDY NUMBER:08055.004.040 WAL/STUDY DIRECTOR: C. Sloan

AMENDMENT NUMBER: 0 0 6

NOTEBOOK NUMBER:

TITLE OF STUDY: Inter-Laboratory Validation of the 15-Day Adult Intact Male Rat Assay with Linuron and Phenobarbital

QAPP/PROTOCOL ID (e.g. number and/or date): RTI-956

AMENDMENT RELATING TO:

[ ] QAPP [ ] QMP [x] Protocol [ ] GLPs
[ ] SOP [ ] Method

ORIGINAL DOCUMENT SPECIFICATIONS:

**3.4.3 Hormone Evaluation**

**Only if relative liver weights are significantly increased should dihydrotestosterone levels be measured.** If serum is limiting, the Study Director should contact the Sponsor to establish a priority list of hormones to be measured. Each sample should be run in duplicate and include high and low quality control (QC) serum samples. Each assay should include all samples from the control group and each dose level for both chemicals.

AMENDMENT:

Determination of serum DHT concentrations 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:

Further clarification on the need to determine the DHT concentrations of the serum samples.

APPROVAL:

WAL/Study Director [signature] Date 10-13-05

Program Management [signature] Date 11/22/05

EDSP Battelle QAM [signature] Date 11-22-05

**ENDOCRINE DISRUPTOR SCREENING PROGRAM**
**AMENDMENT REPORT**

STUDY NUMBER:08055.004.040

WAL/STUDY DIRECTOR: C. Sloan

AMENDMENT NUMBER: 0 0 7

NOTEBOOK NUMBER:

TITLE OF STUDY: Inter-Laboratory Validation of the 15-Day Adult Intact Male Rat Assay with Linuron and Phenobarbital

QAPP/PROTOCOL ID (e.g. number and/or date): RTI-956

AMENDMENT RELATING TO: Protocol Amendment # 6

[ ] QAPP [ ] QMP [ ] [ ] GLPs
[ ] SOP [ ] Method

ORIGINAL DOCUMENT SPECIFICATIONS:
**3.4.3 Hormone Evaluation**

AMENDMENT # 6:

Determination of serum DHT concentrations 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.

**Amendment: All of the above statement will be deleted and replaced with the following:**

Determination of serum DHT concentrations will be done in all serum samples.

REASON FOR CHANGE:

Further clarification on the need to determine the DHT concentrations of the serum samples by EPA.

APPROVAL:

WAL/Study Director Carol Sloan Date 11-15-05

Program Management David P. Hamilton Date 11/22/05

EDSP Battelle QAM Jeri L Pollock Date 11-22-05

**ENDOCRINE DISRUPTOR SCREENING PROGRAM**
**AMENDMENT REPORT**

STUDY NUMBER:08055.004.040 WAL/STUDY DIRECTOR: C. Sloan

AMENDMENT NUMBER: 0 0 8

NOTEBOOK NUMBER:

TITLE OF STUDY: Inter-Laboratory Validation of the 15-Day Adult Intact Male Rat Assay with Linuron and Phenobarbital

QAPP/PROTOCOL ID (e.g. number and/or date): RTI-956

AMENDMENT RELATING TO: ~~Protocol Amendment # 6~~ Not applicable TH 11-22-05

[ ] QAPP [ ] QMP [X] Protocol [ ] GLPs
[ ] SOP [ ] Method

ORIGINAL DOCUMENT SPECIFICATIONS: **2.1** **Test Substances**

Phenobarbital
Appearance: To be determined

Methylcellulose
Purity: (to be added by sponsor)
Appearance: (to be added by sponsor)

AMENDMENT:

Phenobarbital
Appearance: White Powder

Methylcellulose
Purity: Not Available
Appearance: Off-white Powder

REASON FOR CHANGE:

Further clarification of materials used for study.

APPROVAL:

WAL/Study Director [signature] Date 11-17-05

Program Management [signature] Date 11/22/05

EDSP Battelle QAM [signature] Date 11-22-05

**ENDOCRINE DISRUPTOR SCREENING PROGRAM**
**AMENDMENT REPORT**

STUDY NUMBER:08055.004.040 WAL/STUDY DIRECTOR: C. Sloan

AMENDMENT NUMBER: 0 0 9

NOTEBOOK NUMBER:

TITLE OF STUDY: Inter-Laboratory Validation of the 15-Day Adult Intact Male Rat Assay with Linuron and Phenobarbital

QAPP/PROTOCOL ID (e.g. number and/or date): RTI-956

AMENDMENT RELATING TO: ~~Protocol Amendment # 6~~ Not applicable TNF 11-22-05

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| [ ] | QAPP | [ ] | QMP | [X] | Protocol | [ ] | GLPs |
| [ ] | SOP | [ ] | Method | | | | |

ORIGINAL DOCUMENT SPECIFICATIONS: **2.5.1 Housing, Feed, and Water**

In addition, the lot number of Teklad 2018 diet used will be analyzed by the supplier for concentrations of the phytoestrogens genistein, daidzein, and glycitein. The "metabolizable energy content" of the feed (label value) will also be recorded and reported.

AMENDMENT: delete the following sentence

The "metabolizable energy content" of the feed (label value) will also be recorded and reported.

REASON FOR CHANGE:

The sponsor had not requested this information and it was inadvertently included in the protocol.

APPROVAL:

WAL/Study Director Carol Sloan Date 11-21-05

Program Management Dirk P. Hawkins Date 11/22/05

EDSP Battelle QAM Jeri L Pollock Date 11-22-05

**ENDOCRINE DISRUPTOR SCREENING PROGRAM**
**AMENDMENT REPORT**

STUDY NUMBER:08055.004.040

WAL/STUDY DIRECTOR: C. Sloan

AMENDMENT NUMBER: 0 0 10

NOTEBOOK NUMBER:

TITLE OF STUDY: Inter-Laboratory Validation of the 15-Day Adult Intact Male Rat Assay with Linuron and Phenobarbital

QAPP/PROTOCOL ID (e.g. number and/or date): RTI-956

AMENDMENT RELATING TO: Protocol Amendment # 6

[ ] QAPP [ ] QMP [X] Protocol [ ] GLPs
[ ] SOP [ ] Method

ORIGINAL DOCUMENT SPECIFICATIONS:

**9.0 STUDY RECORDS TO BE MAINTAINED**

Protocol and any Amendments
QAPP and any Amendments
List of any Protocol Deviations
List of QAPP Deviations
List of Standard Operating Procedures
Animal Requisition and Receipt Records
Quarantine Records
Temperature and Humidity Records for the Animal Room(s)
Animal Research Facility Room Log(s)
Durham City Water Analysis (analyzed monthly, reported annually)
Feed Type, Source, Lot Number, Dates Used, Certification, Analytical Results
Dosage Code Records Containing Five-Digit Rx Code, Color Code, and Concentration
**Dose Formulation Receipt and Use Records**

AMENDMENT: Delete **Dose Formulation Receipt and Use Records**

And substitute with :

**Dose Formulation and Analysis Records**

REASON FOR CHANGE:

To clarify the records that will be maintained.

APPROVAL:

WAL/Study Director Carol Shaw Date 11-21-05

Program Management [signature] Date 11/22/05

EDSP Battelle QAM [signature] Date 11-22-05

**ENDOCRINE DISRUPTOR SCREENING PROGRAM**
**DEVIATION REPORT**

| | | | |
|---|---|---|---|
| Study Number: | 08055.004.040 | WAL/Study Director | Carol Sloan |
| Notebook Number: | | Deviation Number | 1 |
| Title of Study | Inter-Laboratory Validation of the 15-Day Adult Intact Male Rat Assay with Linuron and Phenobarbital | | |
| Deviation Relating to: **Protocol** | | | |

**ORIGINAL DOCUMENT SPECIFICATIONS: 2.5.1 Housing, Feed, and Water, p. 11**

During the quarantine period, animals will be assigned to cages. Males will be singly housed in solid-bottom, polycarbonate cages (8"x19"x10.5") fitted with stainless steel wire lids (Laboratory Products, Rochelle Park, NJ). Sani-Chip® cage bedding (P.J. Murphy, Forest Products, Inc., Montville, NJ) will be used in all cages. Powdered feed, Teklad 2018 CM diet (low phytoestrogen, lot # 082605 MA, analyzed August 26, 2005) and deionized water, produced at RTI from tap water from the Durham, NC water system, will be available *ad libitum* in plastic bottles with stainless steel sipper tubes throughout quarantine and study periods.

**DEVIATION:** Since the powdered feed had not arrived when the animals arrived on 9-19-05, they were fed pelleted feed (Purina Certified Rodent Diet 5002) until the morning of 9-20-05 when they were switched to the Teklad 2018 CM powdered diet.

**REASON/IMPACT OF CHANGE:**
No adverse impact on the integrity of the study. The animals were not started on the study until 9-27-05, therefore they were on the diet for one week before the study started which was the planned amount of time.

***Approval:***

WAL/Study Director: Carol Sloan
Date: 10-5-05

Program Management: Richard P. Kemmerer 11/22/05
Date:

EDSP Battelle QAM: Jean F Pollock 11-22-05
Date:

RTI QAU: Michelle R
Date: 11/22/05

**ENDOCRINE DISRUPTOR SCREENING PROGRAM**
**DEVIATION REPORT**

| | | | |
|---|---|---|---|
| Study Number: | 08055.004.040 | WAL/Study Director | Carol Sloan |
| Notebook Number: | | Deviation Number | 2 |
| Title of Study | Inter-Laboratory Validation of the 15-Day Adult Intact Male Rat Assay with Linuron and Phenobarbital | | |
| Deviation Relating to: **Protocol** | | | |

**ORIGINAL DOCUMENT SPECIFICATIONS: 2.3 Dose Formulation and Analysis, p.9**

The dosing bottles will be identified at RTI by a five-digit random number Rx code, and a color code. Personnel, other than the safety personnel, analytical chemistry, and dose formulation personnel will not be informed of the test chemicals or formulation concentrations until all laboratory work is completed (i.e., the study technicians will be "blind" for chemical and dose).

**DEVIATION:** The Materials Handling Facility labeled the dose formulation bottles with the name of the chemical.

**REASON/IMPACT OF CHANGE:**
No adverse impact on the integrity of the study. The codes had to be broken when animals became mordibund and had to be sacrificed. Dosing personnel were still "blinded " as to the concentration of the chemical except for obvious clinical signs.

***Approval:***

WAL/Study Director: Carol Sloan
Date: 10-05-05

Program Management: Dirk P. Dumas 11/22/05
Date:

EDSP Battelle QAM: Terri L Pollock 11-22-05
Date:

RTI QAU: Michelle R
Date: 11/22/05

**ENDOCRINE DISRUPTOR SCREENING PROGRAM**
**DEVIATION REPORT**

| | | | |
|---|---|---|---|
| Study Number: | 08055.004.040 | WAL/Study Director | Carol Sloan |
| Notebook Number: | | Deviation Number | 3 |
| Title of Study | Inter-Laboratory Validation of the 15-Day Adult Intact Male Rat Assay with Linuron and Phenobarbital | | |
| Deviation Relating to: **Protocol** | | | |

**ORIGINAL DOCUMENT SPECIFICATIONS: 2.4.5 Sentinels, p.10**

If the experimental period is a month, after the selection of the study males, five of the remaining male rats will be randomly selected and designated as sentinels.

**DEVIATION:** No animals were designated as sentinels.

**REASON/IMPACT OF CHANGE:**
No adverse impact on the integrity of the study. Originally we had not received the chemicals when the animals were ordered and so enough animals were ordered so that sentinels could be designated in case the period of time for the animals to be at RTI became greater than one month. By the time that study animals were assigned we had the chemicals and knew that the animals would not be at RTI for the one month time period. Therefore, no sentinels were designated.

***Approval:***

WAL/Study Director: Carol Sloan
Date: 10-05-05

Program Management: David P. Rhomberg 11/22/05
Date:

EDSP Battelle QAM: Jennifer Pollock 11-22-05
Date:

RTI QAU: Michelle Ok
Date: 11/22/05

**ENDOCRINE DISRUPTOR SCREENING PROGRAM**
**DEVIATION REPORT**

| | | | |
|---|---|---|---|
| Study Number: | 08055.004.040 | WAL/Study Director | Carol Sloan |
| Notebook Number: | | Deviation Number | 4 |
| Title of Study | Inter-Laboratory Validation of the 15-Day Adult Intact Male Rat Assay with Linuron and Phenobarbital | | |
| Deviation Relating to: **Protocol** | | | |

**ORIGINAL DOCUMENT SPECIFICATIONS: 2.3 Dose Formulation and Analysis p.9**

The dose volume will be 5 mL/kg. The vehicle control will be 0.25% methylcellulose and the route of administration will be oral gavage. The same volume of vehicle will be given to the control group.

**DEVIATION:** One animal (#91) in the black dose group was dosed with the purple dosage. The animal was then dosed so that the dosage (mg/kg/day) would be correct for that day but the volume was incorrect since it was 1.5 times what it should have been.

**REASON/IMPACT OF CHANGE:**
No adverse impact on the integrity of the study. That animal was watched for any adverse signs and none were seen.

***Approval:***

WAL/Study Director: Carol Sloan
Date: 10-05-05

Program Management: Richard P. Hardisty 11/22/05
Date:

EDSP Battelle QAM: Terri L Pollock 11-22-05
Date:

RTI QAU: Michelle Ho
Date: 11/22/05

**ENDOCRINE DISRUPTOR SCREENING PROGRAM**
**DEVIATION REPORT**

| | | | |
|---|---|---|---|
| Study Number: | 08055.004.040 | WAL/Study Director | Carol Sloan |
| Notebook Number: | | Deviation Number | 5 |
| Title of Study | Inter-Laboratory Validation of the 15-Day Adult Intact Male Rat Assay with Linuron and Phenobarbital | | |
| Deviation Relating to: **Protocol** | | | |

**ORIGINAL DOCUMENT SPECIFICATIONS: 2.3** **Dose Formulation and Analysis**

The dosing formulations will be prepared at a frequency determined by stability tests initiated prior to the start of the study. Linuron stability, in 0.25% methylcellulose, has been established by Battelle Memorial Institute to be ≥90% of the target concentration for up to an estimated 6.5 weeks. The phenobarbital stability, in 0.25% methylcellulose, is currently being determined and will be added by amendment.

**DEVIATION:** Battelle determined that the stability for phenobarbital was two weeks. The first dose formulation was prepared on 9-21-05 and was used through 10-06-05. This means that the three dose groups for Phenobarbital were dosed one day beyond the stability determined by Battelle.

**REASON/IMPACT OF CHANGE:**
No adverse impact on the integrity of the study. The animals on these doses were not seen to show any differences for the previous days of dosing and they received a new dose formulation from 10-07-05 until the end of the study. We did not know the stability results for 21 days until 10-07-05.

***Approval:***

WAL/Study Director: Carol Sloan
Date: 10-15-05

Program Management: Dirk P. Hawkins 11/22/05
Date:

EDSP Battelle QAM: Terri L Pollock
Date: 11-22-05

RTI QAU: Michelle R
Date: 11/22/05

**ENDOCRINE DISRUPTOR SCREENING PROGRAM**
**DEVIATION REPORT**

| | | | |
|---|---|---|---|
| Study Number: | 08055.004.040 | WAL/Study Director | Carol Sloan |
| Notebook Number: | | Deviation Number | 6 |
| Title of Study | Inter-Laboratory Validation of the 15-Day Adult Intact Male Rat Assay with Linuron and Phenobarbital | | |
| Deviation Relating to: **Protocol** | | | |

**ORIGINAL DOCUMENT SPECIFICATIONS:**

### 3.4.1 Gross Necropsy, Blood Collection, and Organ Weights

Final body, liver, thyroid gland, left and right testis, entire prostate, epididymides, and seminal vesicles and coagulating glands (with fluid) weights will be taken. Total weight of the testes will be the combined weight of the left and right testis and, accessory sex gland unit weight, will be the combined weight of the entire prostate and seminal vesicles and coagulating gland with fluid weights.

**DEVIATIONS**: The body weights for animals # 203 and 165 were taken when they were dosed in the room. The testes were weighed together for animal # 203.

**REASON/IMPACT OF CHANGE:**
**This is the way all of the animals were weighed for body weights using the TASC system, we just didn't know that this would be the day of sacrifice for these animals at the time of weighing. This should not impact the study since these weights were not used for any statistics. The paired testis weight was not used for this animal either, although it was the paired testis weight that was used for the animals euthanized on TD 15.**

***Approval:***

WAL/Study Director: Carol Sloan
Date: 11-21-05

Program Management: David P. Rhodes 11/22/05
Date:

EDSP Battelle QAM: Terri L Pollock
Date: 11-22-05

RTI QAU: Michelle A
Date: 11/22/05

## ENDOCRINE DISRUPTOR SCREENING PROGRAM
## DEVIATION REPORT

| | | | |
|---|---|---|---|
| Study Number: | 08055.004.040 | WAL/Study Director | Carol Sloan |
| Notebook Number: | | Deviation Number | 7 |
| Title of Study | Inter-Laboratory Validation of the 15-Day Adult Intact Male Rat Assay with Linuron and Phenobarbital | | |
| Deviation Relating to: **Protocol** | | | |

**ORIGINAL DOCUMENT SPECIFICATIONS:**

**Table 1. Study Design, Test Chemicals, and Target Doses**

| Group No. | No. Males | Chemical | Dose (mg/kg/day) | Concentration (mg/mL) | Dose Volume (mL/kg) |
|---|---|---|---|---|---|
| 1 | 15 | Vehicle Control[a] | 0 | 0.0 | 5 |
| 2 | 15 | Phenobarbital | 25 | 5.0 | 5 |
| 3 | 15 | Phenobarbital | 50 | 10.0 | 5 |
| 4 | 15 | Phenobarbital | 100 | 20.0 | 5 |
| 5 | 15 | Linuron | 50 | 10.0 | 5 |
| 6 | 15 | Linuron | 100 | 20.0 | 5 |
| 7 | 15 | Linuron | 150 | 30.0 | 5 |

[a] 0.25% aqueous methylcellulose, vehicle only

**DEVIATIONS:** Animal # 193 did not receive the proper dose on TD 15. He received 1.8 instead of 1.9 ml after being confused with # 191.

**REASON/IMPACT OF CHANGE:**
**Technician error/ No impact on the study.**

***Approval:***

WAL/Study Director: Carol Sloan
Date: 11-21-05

Program Management: [signature] 11/22/05
Date:

EDSP Battelle QAM: [signature] 11-22-05
Date:

RTI QAU: [signature]
Date: 11/22/05

**ENDOCRINE DISRUPTOR SCREENING PROGRAM**
**DEVIATION REPORT**

| | | | |
|---|---|---|---|
| Study Number: | 08055.004.040 | WAL/Study Director | Carol Sloan |
| Notebook Number: | | Deviation Number | 9 |
| Title of Study | Inter-Laboratory Validation of the 15-Day Adult Intact Male Rat Assay with Linuron and Phenobarbital | | |
| Deviation Relating to: **Protocol** | | | |

**ORIGINAL DOCUMENT SPECIFICATIONS:**

### 2.1.3 Vehicle: Methylcellulose

CAS number: 9004-67-5
Supplier: Sigma-Aldrich
**Lot Number:** **14601TC**

**DEVIATIONS:** The methylcellulose lot number actually sent from Battelle and used was 062K0144.

**REASON/IMPACT OF CHANGE:**
**Battelle sent the wrong information for the methylcellulose lot shipped to participating laboratories. / No impact on the study.**

***Approval:***

WAL/Study Director: Carol Sloan
Date: 12-05-05

Program Management: Dirk P. Hanson 12/6/05
Date:

EDSP Battelle QAM: Jeri L Pollock
Date: 12-15-05

# Appendix V

# QAPP

# 1.0 TITLE AND APPROVAL SHEET

## Inter-Laboratory Validation of the 15-Day Adult Intact Male Rat Assay

## Work Assignment 5-15

**for**

## EPA Contract Number 68-W-01-023

**September 20, 2005**

## SIGNATURE PAGE

### Quality Assurance Project Plan for WA 5-15

### Inter-Laboratory Validation of the 15-Day Adult Intact Male Rat Assay

### EPA Contract Number 68-W-01-023

### Concurrences and Approvals

| | Signature | Date |
|---|---|---|
| Terri L. Pollock, B.A.<br>EDSP Quality Assurance Manager<br>Battelle<br>Columbus, OH | Terri L Pollock | 9-19-05 |
| David P. Houchens, Ph.D.<br>EDSP Program Manager/Battelle<br>Work Assignment Leader<br>Battelle<br>Columbus, OH | David P. Houchens | 9/19/05 |
| J. Thomas McClintock, Ph.D.<br>Quality Assurance Manager<br>U.S. EPA<br>Washington, DC | J. Thomas McClintock | 9/20/05 |
| Linda Phillips, Ph.D.<br>EPA Project Officer<br>U.S. EPA<br>Washington, DC | Linda Phillips | 9/20/05 |
| Don Bergfelt, Ph.D.<br>EPA Work Assignment Manager<br>U.S. EPA<br>Washington, D.C. | D. Bergfelt | 9/20/05 |

# 2.0 TABLE OF CONTENTS

**Page**

**LIST OF FIGURES**

## 3.0 DISTRIBUTION LIST

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919-541-6337

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215-443-8710

## 4.0 WORK ASSIGNMENT ORGANIZATION

The US Environmental Protection Agency (EPA) is implementing the Endocrine Disruptor Screening Program (EDSP). To support this program, the EPA has contracted with Battelle to provide comprehensive toxicological and ecotoxicological testing services, including chemical, analytical, statistical, and quality assurance (QA)/quality control (QC) support, to assist EPA in developing, standardizing, and validating a suite of *in vitro* and in vivo mammalian, and ecotoxicological screens and tests for identifying and characterizing endocrine effects through exposure to pesticides, industrial chemicals, and environmental contaminants. The studies conducted will be used to develop, standardize and validate methods, prepare appropriate guidance documents for peer review of the methods, and develop technical guidance and test guidelines in support of the Office of Prevention, Pesticides and Toxic Substances regulatory programs. The inter-laboratory validation studies will be conducted under the EDSP Quality Management Plan (QMP), study protocol, applicable Quality Assurance Project Plans (QAPPs), relevant program and facility Standard Operating Procedures (SOPs) and guidance documents.

One of the assays recommended for validation and consideration for inclusion in the screening program is the 15-Day Adult Intact Male Rat Assay.

According to the requirements of the work assignment for this assay the study is to be conducted by three laboratories (Research Triangle Institute [RTI], Research Triangle Park, NC, WIL Research Laboratories, Ashland, OH; and Charles River-ARGUS Division, Horsham, PA). This QAPP will address work to be conducted on this study. A summary of the Work Assignment Organization for the adult male assay is shown in Figure 4-1.

Portions of this work assignment will be managed at RTI, WIL, and ARGUS. At each of these laboratories, there will be a person responsible for preparing the protocol, assigning appropriate staff to complete specified tasks within the protocol, and monitoring the progress of both technical and fiscal milestones as outlined in the technical work plan. A study director from each laboratory will report on the progress of the work assignment to **Dr. David P. Houchens** at Battelle through a series of planned conference calls and through the use of written monthly reports.

General scientific direction and supervision of the work performed under this work assignment will be provided by **Dr. Houchens,** Battelle. All technical questions will be forwarded to EPA for clarification.

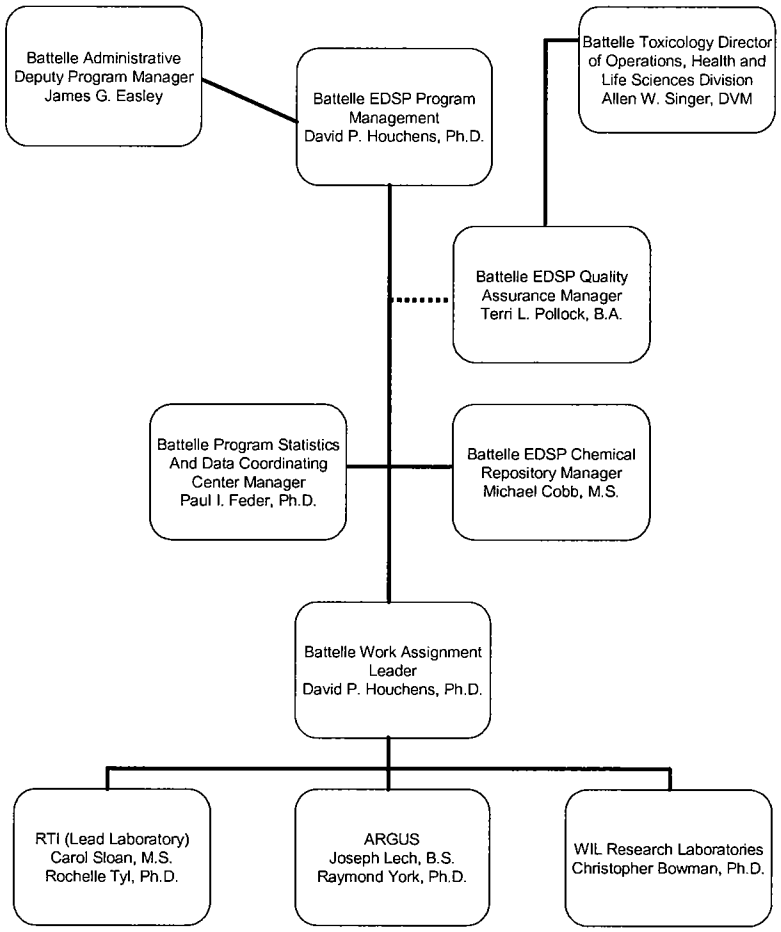


**Figure 4-1. WA 5-15 Work Assignment Organization Overview**

Each laboratory will have a study director in charge of overseeing the daily operation and conduct of the study. The individual laboratory teams will execute the necessary tasks required in the study protocols and ensure the data are collected and handled appropriately. All of these tasks are clearly defined in the study protocol.

The QA tasks are summarized as follows:

- Assist the Work Assignment Leader (WAL) in preparing the individual QAPP as required for the WA by defining appropriate QA requirements according to EPA/QA-R5, EPA Requirements for Quality Assurance Project Plans for Environmental Data Operations.

- Interact with the WAL to ensure that QA and QC procedures are understood by WA personnel.

- Conduct technical systems audits (TSAs) and audits of data quality (ADQs) to evaluate the implementation of the program WAs with respect to the EDSP QMP, the WA QAPP and protocol, and applicable program and facility SOPs.

- Prepare and track reports of deficiencies and submit them to both line and program management.

- Consult with the WAL and, as necessary, the EDSP Battelle QA Manager and Program Manager on actions required to correct deficiencies noted during the conduct of the WA.

- Ensure that all data produced as part of the EDSP WAs are maintained in secure, environmentally-protected archives.

- Ensure, during the conduct of TSAs, that all staff participating on the EDSP are adequately trained.

- Maintain complete facility-specific QA records related to the program.

- Submit copies of resolved audits to the EDSP Battelle QA Manager.

- Submit a QA Statement to the EDSP Battelle QA Manager and Program Manager with each written deliverable, which describes the audit and reviews activities completed.

- Maintain effective communication with the EDSP QA Managers.

- Act as the facility's EDSP SOP Custodian for all SOPs received from the SOP Administrator.

As EDSP program manager, **Dr. David Houchens** will have ultimate responsibility for quality, timeliness, and budget adherence for all activities on the contract. He also will serve as the principal interface with the EPA's project officer on all contract-level administrative and technical issues. Because of the high level of subcontracting and purchases required by the program, such as test laboratory subcontracts and purchases of chemical supplies, Dr. Houchens will be assisted by an administrative deputy manager, **Mr. James Easley**. Mr. Easley will manage the procurement of all subcontracts, consultants, and purchased materials and services, and will facilitate schedule and cost control. He has played a similar role on ten other large, multi-year, level-of-effort task-order contracts for EPA. Thus, he will be able to assure that all purchases are compliant with government regulations and that EPA is provided timely, accurate accounting of these substantial costs in our monthly progress reports.

**Ms. Terri Pollock**, the EDSP QA manager at Battelle, will direct a team of QA specialists, who will monitor the technical activities on the chemical repository program, and provide oversight to all associated QA functions. Ms. Pollock will be responsible for reporting

her findings and any quality concerns to Dr. Houchens. Ms. Pollock reports, for the purposes of this program, to **Dr. Allen Singer**, Vice President and General Manager of Battelle's Environmental Division. This reporting relationship assures that the QA function is independent of the technical activities on the program.

# 5.0 PROBLEM DEFINITION/BACKGROUND

## 5.1 Problem Definition

The Food Quality Protection Act of 1996 requires the EPA to develop and implement a screening program using valid tests for determining the potential for estrogenic, androgenic and thyrotropic-like effects from pesticides, industrial chemicals and environmental contaminants in humans. EPA proposed a two-tiered screening and testing program in a Federal Register notice in 1998 (63 FR 71542-71568, Dec. 28, 1998) that covered not only pesticides but also commercial chemicals subject to regulation under the Toxic Substances Control Act (TSCA; 15 USC 2601) and environmental and drinking water contaminants. One of the assays recommended by the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) as an alternate assay for inclusion in the screening program is a short term screen in an adult intact male rat. The adult male assay was developed to identify compounds that have the potential to act as agonists or antagonists to the estrogen, androgen, progesterone, or dopamine receptor; 5 $\alpha$-reductase inhibitors; steroid biosynthesis inhibitors; or compounds that alter thyroid function.

## 5.2 Background

This *in vivo* assay was developed to identify compounds that have the potential to act as agonists or antagonists to the estrogen, androgen, progesterone, or dopamine receptor; 5$\alpha$-reductase inhibitor; steroid biosynthesis inhibitors; and compounds that alter thyroid function. Results from this assay and/or with the use of ip injection as the route of administration, and other assays with a similar purpose, have been reported (O'Connor et al., 1999b, 2002a,b). The duration should be sufficient to detect effects on thyroid gland activity. The rats used are anatomically mature and have an intact hypothalamus-pituitary-gonadal (HPG) axis and therefore can be used for assessment of higher neuroendocrine control in male reproductive function and thyroid. It may: (1) be used as one of the protocols recommended by EDSTAC for the Tier 1 screening battery, and/or (2) serve as a follow-up test for certain substances for which additional data are required or desired.

The U.S. EPA selected two known test chemicals for evaluation. The two test chemicals and their target/mechanism of action are as follows: (1) linuron (anti-androgen; competitive binding to androgen receptor), and (2) phenobarbital (alters thyroid function).

### 5.2.1 Overview of Linuron

Linuron is an androgen receptor (AR) antagonist and competes with androgens for AR binding. It has been documented to inhibit androgen-induced gene expression *in vitro* and short-

term exposure to linuron reduces the size of androgen-dependent tissues *in vivo* . *In utero* linuron exposure appears to affect androgen-dependent development of the male reproductive system (McIntyre et al., 2000, 2002).

### 5.2.2 Overview of Phenobarbital

Animal studies show that exposure to phenobarbital in food or water harms the thyroid function in males and females. Phenobarbital induces P450 isoforms, predominantly in the liver, and accelerates the metabolism of hormones and exogenous xenobiotics.

## 6.0 WORK ASSIGNMENT/TASK DESCRIPTION

### 6.1 Work Assignment Overview

One of the alternate assays recommended for validation and consideration for inclusion in the screening program is the 15-day adult intact male rat assay. Briefly, adult male rats (15/group) are dosed daily for 15 days (test days 1 to 15) with the test compound and euthanized on the morning of Test Day 15, approximately 2-3 hours after the last administered dose. Blood samples are taken and selected organs are weighed and saved. Reproductive and thyroid hormone levels are measured and appropriate tissues are examined histologically.

### 6.2 Specific Study Objectives

The objectives of this study are to:

1) 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.

2) demonstrate that three contract laboratories can adopt this assay by analyzing the repeatability of the results for each endpoint across laboratories.

## 7.0 QUALITY OBJECTIVES AND CRITERIA FOR MEASUREMENT DATA

In the adult male assay, the primary endpoints will be body weights and weights and histology of the liver, reproductive organs and thyroid and the hormone concentration values for luteinizing hormone (LH), follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), triiodothyronine (T3), throxine (T4), dihydrotestosterone (DHT), testosterone, prolactin and estradiol. The level of sensitivity of the assays will be evaluated by examining effects of chemicals known to affect the endocrine system through various pathways and/or mechanisms of action.

Chemicals known to affect the endocrine system and/or the thyroid gland should show significant differences in hormone levels, organ weights and histological measurements between treated and control animals.

**Data Quality Indicators**

**1. Precision**

For the animals used on this project, the acceptable weight range for acceptance into the study will be the mean weight $\pm$ 20%. Animals with weights outside this range will not be used in the study.

For body weight, the weight of the animal on the day of necropsy but before sacrifice should be within approximately 10% of the control group. Animals that have body weights higher than that prescribed will be considered to have been overexposed to the chemical.

For organ weights, any value that shows up as a statistical outlier on data analysis will be flagged and eliminated from the summary tables and reported separately. The values for absolute and relative organs weights from the treated groups will be compared to those of the vehicle control group.

For hormone level analysis, duplicate samples will be used and the relative standard deviation (RSD) and coefficient of variance (CV) will be calculated. These values will be determined for each hormone assay individually. If values are not within the accepted value (normally 10-20% CV), they will be flagged and the samples will be repeated on another assay.

**2. Bias**

For the animal data, bias will be controlled by using stratified randomization to select animals for the study. Animals that do not fall within the acceptable weight range will not be used in the study.

For the hormone analysis, bias will be determined by use of blank and spiked matrix samples. If these values are incorrect, the assay is not used and all of the samples will be re-run in another assay. The reason for the incorrect values would be further explored and explained in the report.

**3. Accuracy**

For hormone assays, accuracy is determined by including samples called standards with known concentrations of the hormone to be measured. These standards must fall within the CV set for the assay or the assay is not used to report unknowns. The unknowns will then be run in another assay with acceptable values for the standards.

**4. Sensitivity**

For hormone assays, the standard curve will be run and will show the ranges of sensitivity for the individual assay. Calculation of assay sensitivity will be reported. This is usually the most linear portion of the curve.

## 8.0 SPECIAL TRAINING/CERTIFICATION

All personnel involved in handling radiolabeled materials will have completed a Radiation Safety Training course. Training documentation will be maintained in the individual training files. Each laboratory will be licensed to receive radiolabeled materials

## 9.0 DOCUMENTS AND RECORDS

### 9.1 Retention of Specimens and Records

Archiving procedures will be specified in the individual protocols.

### 9.2 Quality Assurance Project Plan

This QAPP will be distributed to the project participants initially, and whenever revised. Previous versions will be marked as "obsolete" when newer versions are distributed, or collected and destroyed so that there is no confusion regarding the version in effect. The right-justified document control header example shown is used to ensure that revision numbers and dates are obvious to document users. The QAPP will be reviewed annually and a determination made to either modify the document based on new or modified project requirements, or leave as is.

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Month, Year
Page 1 of 1

Copies of the QAPP are maintained, tracked, and managed by the laboratories' QAU through the use of a master distribution list.

### 9.3 Data Reporting Package

All data forms will include a title identifying the type of data to be recorded, a unique study code or protocol number and the initials and date of data recorder(s) to authenticate the records.

### 9.4 Environmental Conditions

Monitoring of environmental conditions in the Animal Resources Facility (ARF) will be described in the individual laboratory protocols.

## 9.5 Reports

### 9.5.1 Draft and Final Reports

A draft report will be submitted to the Sponsor's Representative within three months after the completion of the laboratory studies. The final report will include:

- Objectives
- Abstract
- Materials and Methods
- Results
- Discussion
- Conclusions
- References
- Summary in-life and necropsy data with statistical analyses when possible
- Individual animal data: in-life and necropsy
- Protocol, any amendments, or any deviations from the protocol and all values flagged as outliers
- QAPP, any amendments, or any deviations from the QAPP
- Histopathology Report
- Analytical Chemistry

***Individual Data Male Rats***

a. Identification number
b. Clinical signs
c. Daily body weight and weekly body weight change and feed consumption
d. Age at death and manner of death
e. Body weight on the day of necropsy but before sacrifice
f. Organ weights
g. Gross Necropsy Observations
h. Serum Hormone levels
i. Histology

***Summary of Data From Male Rats***

a. Mean weekly body weights and weight changes
b. Mean weekly feed consumption
c. Clinical signs
d. Mean body weight on Test Day 15
e. Mean organ weights (absolute and relative)
f. Histopathology Data
g. Mean Serum Hormone Levels

### 9.5.2 QA Assessment Reports

QA assessment reports (see section 20) will be maintained as confidential files in the QAU. When a WA report is finalized, the QA assessment report file will be removed to a separate file location designated for those to be transferred to the archives.

### 9.5.3 Status Reports

Status/progress reports will be submitted to the EPA Project Officer on a monthly basis as stipulated in the contract.

## 10.0 EXPERIMENTAL DESIGN

The individual testing laboratory protocols will describe the experimental design.

### 10.1 Number and Type of Samples to be Collected

As much blood as possible will be taken after decapitation following pre-exposure to carbon dioxide for preparation of serum for hormone concentration determination of the treated and control animals at the time of necropsy. The liver, thyroid gland, right and left testes, entire prostate, epididymides, and seminal vesicles and coagulating gland with fluid will be removed and weighed at necropsy. The liver, epididymides, thyroid, and testes will be saved for histology.

### 10.2 Frequency and Types of Measurement to be Made

The **body weight** (in grams) of the animals will be determined for randomization, on the day before the experimental start day, on the morning of each day of the study, Test Day (TD) 1-14 for determination of dosing volume, and on the day of necropsy. The dosing volume for TD 15 dosing will be determined by the body weight on TD 14.

The **feed weights** (in grams) will be determined on TD 1, 8, and 15 and will be reported as g/kg body weight/day.

The **organ weights** (in grams) will be determined at necropsy, expect that total testes weight (combined weights of left and right testis) and accessory sex gland weight (combined weights of entire prostate and seminal vesicles and coagulating gland with fluid) will be determined post-necropsy.

The **hormone concentrations** testosterone, LH, TSH, T4, T3, FSH, estradiol, and prolactin will determined in this sequence. Only if relative liver weights are significantly increased should DHT levels be measured. The blood samples for serum will be collected 2-3 hours after the final dose is administered.

### 10.3 Rationale for Experimental Design

Body weights of the animals will give an indication of exposure of the compounds being administered and are necessary for the volume of compound to be administered and for the relative organ weights to be determined at the conclusion of the experiment.

Food consumption is expected to support dramatic changes in body weights.

Absolute and relative weights of the organs will give a further indication of overexposure and the histology will reveal if there is any organ damage at the cellular level.

Hormone concentrations will be used to indicate effects on the endocrine system and glands that secrete or respond to the hormone.

All the above measurements are considered critical to the objectives of the work assignment since all can be used for evaluation of assay performance among laboratories.

## 11.0 SAMPLING METHODS

### 11.1 Blood Samples for Endocrine Assays

As much blood as possible will be collected from the trunk of the animal after decapitation following pre-exposure to carbon dioxide for preparation of serum as described in the protocols of respective laboratories. If serum is limited, priority of analysis will be determined by the study director and sponsor and documented. Any remaining serum will be discarded after the study is completed and with the concurrence of EPA.

## 12.0 SAMPLE HANDLING AND CUSTODY

### 12.1 Dosage Formulations

The dosing solutions will be prepared at a frequency determined by stability tests performed prior to the start of the study.

### 12.2 Sample Collection Documentation

The aliquots will be transferred to the Analytical Chemistry Laboratories at each site with a study specific transfer of material form. Aliquots of each treatment concentration will include the designated Rx Code and Color Code specific to that study. The samples will be analyzed according to the procedures received from Battelle.

Each aliquot of sera will be labeled with the designated Rx Code and Color Code specific to that study as well as the unique animal identification number. The samples will be stored until time for the hormone determinations.

The tissues saved will be labeled with the designated Rx Code and Color Code specific to that study as well as the unique animal identification number and sent for histopathology assessment.

## 13.0 ANALYTICAL METHODS

Analytical methods are described in each study protocol and appropriate SOPs.

## 14.0 QUALITY CONTROL

### 14.1 Methods

| Method | Acceptance Criteria | Corrective Action |
|---|---|---|
| Use of blanks | Blank values should be below 100 counts / minute (cpm), values are subtracted from sample values | Repeat assay with new blanks |
| Running duplicate samples | CV should be below 20% | Repeat samples in duplicate on a new assay |
| Running control samples | Should be within 20% CV of previous values | Rerun the entire assay |

### 14.2 Data Collection

Data collection documentation will be as described in applicable SOPs.

Assay data, including weights and/or volumes of chemicals, solvents or other materials used to prepare necessary solutions or samples, will be recorded manually on data sheets. All data sheets will include a title identifying the type of data to be recorded, the unique study code or protocol number, and the initials and date of the data recorder(s) to authenticate the records.

## 15.0 INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE

The following types of equipment are required for this WA: temperature controlled shaking water bath, pH meter, analytical balances, centrifuges, pipettors, spectrophotometer, and high performance liquid chromatography (HPLC) equipment (injector, pumps, detectors [radiochemical and ultraviolet {UV}], data collection system). The equipment will be tested, inspected and maintained according to schedules contained in the relevant SOPs.

## 16.0 INSTRUMENT/EQUIPMENT CALIBRATION AND FREQUENCY

Balances used to obtain weight measurements, as well as the check weights that are used to verify a balance's calibration status will be calibrated and maintained according to the schedule specified in relevant SOPs. Balances that do not meet the criteria specified in the SOP will not be used for this work assignment.

Radiation counters will be calibrated using procedures described in the relevant SOPs. Calibration of pH meters occurs as specified in relevant SOPs. The water bath, pipettes, spectrophotometer, and HPLC equipment are calibrated using the procedures and schedule in applicable SOPs. Any equipment or instrument that does not meet acceptance criteria as described in the relevant SOP will not be used for this work assignment.

## 17.0 INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES

Upon receipt, purchased items must be inspected for conformance to quality requirements prior to use. All use of the product must be prior to the expiration dates if applicable.

**Animals** must be inspected and weighed by Charles River Laboratories, Raleigh, NC before shipment. They will be checked on at the laboratories for general health and appropriate age. They will be quarantined for at least seven days after arrival and checked daily for health. They must be released by the laboratory veterinarian for use on a study.

**Food** will be analyzed and the results are available and will be included in the final report.

**Water** will be from the deionized water sources at the laboratories. The main water used is analyzed and the results are available and will be included in the final report.

**Hormone Assay Kits** will be shipped to each participating laboratory. They will be then checked for total radioactivity, spillage, breakage and expiration date before release. They will be labeled with the date received, opened and expiration dates on the kits upon receipt.

# 18.0 NON-DIRECT MEASUREMENTS

No collection of any samples or sample data will be obtained from non-direct measures such as computer data bases or programs.

# 19.0 DATA MANAGEMENT

## 19.1 Data Management Overview

Data will be maintained in notebooks and/or files according to applicable facility SOPs. The records will be kept in the appropriate rooms until there is a signed final report at which time they will be inventoried and placed in the facility archives according to applicable facility SOPs, unless the sponsor requests that they be transferred to another archive location.

## 19.2 Data Transfer

Information will be sent to the Data Coordination Center in electronic format as specified in SOP EDSP.D-003-01. Specifically all raw data, all tables, graphs summarizing results of statistical analyses as presented in study reports, statistical analysis data files, statistical analysis programs, and all study documents will be sent to the EDSP Data Coordination Center in electronic format.

# 20.0 ASSESSMENTS AND RESPONSE ACTIONS

EDSP QA team members will perform assessments on WA activities and operations affecting data quality and the raw data and final report. They will report any findings to the Study Director and management to ensure that the requirements in relevant SOPs, study protocols and WA QAPP, the QMP, and the FIFRA GLPs are met. The assessments for this study include TSAs and ADQs. Performance Evaluations do not apply to this QAPP.

## 20.1 Technical Systems Audits

A TSA is a process by which the quality of a study is assessed through evaluating a study activity's conformance with the protocols, applicable facility or program SOPs, QAPP, QMP, and GLPs. The acceptance criteria are that WA activities and operations must meet the requirements of these planning documents and the GLPs or be explained and evaluated in a deviation report. Deviations from the GLPs, QAPP, protocol, or SOPs will be properly documented and assessed by management and the study director as to their impact on the study.

## 20.2 Type, Scheduling, and Performance of Technical Systems Audits

The following paragraphs provide an example of how the laboratories may perform technical system audits.

Prior to the experimental start, the facility QA Team Member will convey a list of inspections targeted for the study to the study director. Whenever possible, TSAs should be done at the commencement of the WA critical phase to ensure WA integrity based on compliance with the protocol, QAPP, SOPs, and GLPs. Critical phases targeted for TSAs include, but are not limited to:

- Protocol review
- Dose formulation and analysis
- Dose administration
- Necropsy

During the TSA, EDSP QA team members will record observations to be used later in preparing the audit report. EDSP QA team members will observe the procedure, data recording, and any equipment maintenance and calibration procedures and/or documentation, noting whether or not the activities adhered to the study protocols and QAPP, applicable SOPs, QMP, and the GLPs. Any findings will be communicated to the technical personnel at the completion of the procedure unless an error could compromise the study (e.g., misdiluting the stock solution). EDSP QA team members will immediately notify the Study Director by telephone and/or e-mail of any adverse findings that could impact the conduct of the study. This direct communication will also be documented in the audit report.

## 20.3 Audits of Data Quality

An ADQ is a process by which the accuracy of data calculations and reporting will be assessed to ensure that the reported results are of high quality and accurately reflect the raw data and accurately describe the materials used in the study. The acceptance criteria for the ADQ are that data collection, analysis, and reporting must meet the requirements of the applicable facility and program SOPs, the WA protocols and QAPP, QMP, and the FIFRA GLPs, or be explained and evaluated in a deviation report, as previously described.

## 20.4 Scheduling and Performance of Audits of Data Quality

Direct and frequent communication between the WA Leader/Study Director, laboratory supervisor, and the QA Manager will provide for sufficient time to perform an ADQ so that the submission date of the draft final report meets that specified in the study protocol. The scheduling process should also allow for a reasonable amount of time for corrections and subsequent verification of the corrections by QA.

EDSP QA team members will audit the study records at a frequency adequate to ensure that approved protocol requirements are met. The frequency required is specified by the type of data in the QMP, Section 2.4.1. Findings will be reported and corrective actions undertaken as described earlier. EDSP QA team members review the final report using the audited data and corrected tables. The report text will be reviewed to ensure that every statement is supported by the data and any discussions or conclusions drawn from the study are supported by the data. Findings will then be reported and corrective actions undertaken as described earlier.

## 20.5 Audit Report Format

The following paragraphs provide an example of how the laboratories may format an audit report.

The audit report consists of a cover page for study information and additional page(s) with the audit findings. All pages have header information containing the study protocol number, audit report date, and audit type. The audit report date is the date on which the EDSP QA team member signs the audit report and sends it to the Study Director and management.

The cover page contains the study protocol title, number, and code; Sponsor; Study Director; audit type; audit date(s); EDSP QA team member; distribution list; the dated signature of the auditor; the date that the Study Director received the audit report; and the dated signatures of the Study Director and management. The distribution list may include additional names for individuals who have findings pertaining to their area of responsibility (e.g., the ARF Manager would address a finding pertaining to the ARF) and is used to ensure that the report is sent to all who need to respond. Subsequent page(s) contain the audit finding(s), any recommended remedial actions, and space for the Study Director to respond to the findings and document remedial actions taken or to be taken.

## 20.6 Response Actions and Resolution of Issues

The Study Director will respond to the TSA report within a specified number of working days of receipt of the report as required by the laboratory's SOPs. There is no deadline for the Study Director's response to an ADQ report except for the time constraint deriving from the submission date of the final WA report. The Study Director forwards the audit report to management for review. Management adds comments as necessary, signs and dates the report and returns it to the EDSP QA team member. The EDSP QA team member assesses the responses and verifies the corrective actions. If a disagreement between the Study Director and EDSP QA team member arises over a finding, it will be discussed among the other EDSP QA team members. The EDSP QA team member will then present the majority opinion to the Study Director for further consideration. If the disagreement remains, the issue will be reported to the Study Director's management. The action decided on by management will be documented in the QA files.

During an assessment, if the auditor determines that adverse health effects could result or WA objectives of acceptable quality cannot be achieved, the auditor follows the Stop Work Procedure specified in the EDSP QMP (Section 3.3).

## 20.7 Independent Assessments

The EDSP Battelle QA Manager (QAM), or designee, may conduct an independent TSA and ADQ during the conduct of this work assignment. Typically one independent audit may be conducted during the work assignment. If major deficiencies are uncovered, additional independent audits may be scheduled. The conduct and reporting of the audits will be consistent with the procedures described in the EDSP QMP (Section 3.3).

In addition, the EDSP EPA QAM, or designee, has the option of conducting external TSAs/ADQs.

## 21.0 REPORTS TO MANAGEMENT

The QA Manager will send periodic reports to the study director and management, which detail significant regulatory, protocol, and SOP issues. Also, the participating laboratories will report to the EDSP Program Manager and WAL.

## 22.0 DATA REVIEW, VERIFICATION, AND VALIDATION

The data produced under this work assignment will be reviewed by the technical personnel for the validation process and by EDSP QA team members for the verification process (see section 23). The criteria used for validation depend on the type of data. For dose solution sample data, information regarding the condition of the containers and whether or not samples were compromised will be recorded in the sample chain-of-custody records. Compromised samples will not be analyzed. The criteria for validating data are those found in Section 7 (Data Quality Objectives).

## 23.0 VERIFICATION AND VALIDATION METHODS

### 23.1 Chain of Custody for Data

Study data, records, and specimens will be maintained in a secure and designated location, e.g., in the respective laboratory offices until study completion. Chain-of-custody procedures will be implemented according to facility SOPs. Chain-of-custody information, including the date, study record(s) removed or returned, and the name of the person removing or returning the data will be documented. At study completion, the Study Director will follow the procedures specified in the facility SOP for archiving study materials.

### 23.2 Data Validation

Data validation is a process by which the WA Leader/Study Director and/or other technical personnel evaluate the data for conformance to the stated requirements for methodology and quality. These personnel are responsible for reviewing the data, evaluating any technical deviations or non-conformances, and then determining the degree to which the data meet the quality criteria stated in Section 7.

### 23.3 Data Verification

Data verification constitutes part of the ADQ process performed by EDSP QA team members and described earlier. Verification ensures that 1) the data are of high quality and were collected according to the planning documents' requirements, and 2) the reported results accurately reflect the raw data. Each data type will be evaluated against its collection and

reduction requirements specified in the planning documents. Errors discovered during the data evaluation will be corrected. The reported conclusions drawn from the data are verified by EDSP QA team members during the report audit to confirm that they are true and accurate. The procedure for resolving issues of data verification has been detailed in prior sections of this document.

## 24.0 RECONCILIATION AND USER REQUIREMENTS

Proposed methods for data analysis, including a test for statistical outliers, are specified in the Study Plan and/or protocols.

## 25.0 REFERENCES

The following references were used to prepare the QAPP. Not all references are cited in the text.

Battelle (2003). Endocrine Disruptor Screening Program Quality Management Plan, Version 2. May 12, 2003.

Battelle (2004). Technical Work Plan on Microsomal Aromatase Validation Study, EPA Contract Number 68-W-01-023, Work Assignment 4-16. September 8, 2004.

FQPA (1996). Food Quality Protection Act of 1996, U.S. Public Law 104-170, 21 U.S.C. 46a(p), Section 408(p), 110 STAT.1489. August 3, 1996.

McIntyre, B.S., Barlow, N.J., Wallace, D.G., Maness, S.C.,Gaido, K.W., Foster, P.M.(2000). Effects of in utero exposure to linuron on androgen-dependent reproductive development in the male Csl:CD(SD)BR rat. *Toxicol. Appl. Pharmacol.* 167(2):87-89.

McIntyre, B.S., Barlow, N.J., Foster, P.M.(2002). Male rats exposed to linuron exhibit permanent changes in anogenital distance, nipple retention, and epididymal malformations that result in subsequent testicular atrophy. Toxicol Sci 65:62-70.

O'Connor, J.C., Davis, L.G., Frame, S.R., and Cook, J.C. (2000a). Detection of dopaminergic modulators in a Tier I screening battery for identifying endocrine-active compounds (EACs). Reprod. Tox. In press.

O'Connor, J.C., Davis, L.G., Frame, S.R., and Cook, J.C. (2000b). Evaluation of a Tier I screening battery for detecting endocrine-active compounds (EACs) using the positive controls testosterone, coumestrol, progesterone, and RU486. Toxicological Sciences, 54: 338-354.

O'Connor, J.C., Cook, J.C., Frame, S.R., and Davis, L.G. (1999a). Detection of the environmental antiandrogen p,p'-DDE in Sprague-Dawley and Long-Evans rats using a Tier I screening battery and a Hershberger Assay. Toxicological Sciences, 51: 44-53.

O'Connor, J.C., Frame, S.R., and Cook, J.C. (1999b). Detection of thyroid toxicants in a Tier I screening battery and alterations in thyroid endpoints over 28 days of exposure. Toxicological Sciences, 51: 54-70.

O'Connor, J.C., Cook, J.C., Slone, T.W., Frame, S.R., and Davis, L.G. (1998). An ongoing validation of a Tier I screening battery for detecting endocrine-active compounds (EACs). Toxicological Sciences, 46: 45-60.

O'Connor, J.C., Frame, S.R., Biegel, L.B., Cook, J.C., and Davis, L.G. (1998). Sensitivity of a tier I screening battery compared to an in utero exposure for detecting the estrogen receptor agonist 17 -estradiol. Toxicological Sciences 44: 169-184.

# Appendix VI

# Statistical Plan/Intra-Laboratory Analysis

**Endocrine Disruptor Screening Program**
**Work Assignment 5-15**

# Interlaboratory Validation of the 15-Day Adult Intact Male Rat Assay

## Intra-Laboratory Analysis

**September 14, 2005**

## Introduction

Three laboratories will conduct the 15-day adult intact male rat assay according to the test method provided by the EPA.

Within each laboratory two chemicals will be tested, each at three dose levels specified by the EPA. In addition a vehicle control group will be tested in each laboratory. The sample size will be n=15 adult male rats per group, for a total of seven groups and 105 animals per laboratory. This statistical analysis plan specifies the summaries, displays, and statistical analyses that will be used to summarize the results within each laboratory.

## Data

The test method specifies four categories of data:

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)
    - $T_4$ (µg/dl)
    - $T_3$ (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 and added by amendment.

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 – (4 - 5 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 and added by amendment.

Microscopic evaluations will be performed on control and high dose animals for all compounds. 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 Test Day (TD)15.

If animals 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 animals 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 (1969) 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 bodyweight and food consumption endpoints. These will be TD15 body weight, 3 body weight change variables as shown in the data section, 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 animals 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

[1] If X, Y denote the control group least squares mean and the dose group lest squares mean respectively, with variance-covariance matrix ($S_X^2$, $S_Y^2$, $S_{XY}$), an approximate standard error for R ≡(Y/X)×100% is

$$Se[R(X, Y)] \approx |1/X| \left[(Y/X)^2 S_X^2 + S_Y^2 - 2(Y/X) S_{XY}\right]^{1/2} \times 100\%$$

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.

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

[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

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

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 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.

# Appendix VII

# Chemistry Reports

# DOSE FORMULATION AND ANALYSIS REPORT

RTI Project No.: 65U-08055.004.040
RTI Master Protocol No.: RTI-956
RTI Study Code.:Rt05-ED09

# LINURON IN 0.25% METHYLCELLULOSE

November 28, 2005

Prepared by:

Nora P. Castillo 11/28/05
Nora P. Castillo Date
Task Leader

Approved by:

11/28/05
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Prepared for:
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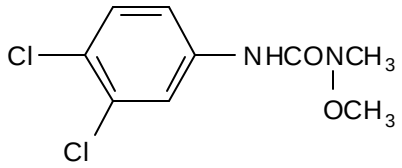
Columbus, OH 43201-2693

# LINURON IN 0.25 % METHYL CELLULOSE

| | |
|---|---|
| RTI Log Nos.:<br>10038-02-01 (Bulk Chemical)<br>10038-02-02 (Analytical aliquot) | Amount received:<br>100 g (Bulk chemical)<br>2 g (Analytical aliquot) |
| Cas No.: 330-55-2 | Received from: Battelle Marine Sciences. |
| RTI Receipt Date:<br>September 20, 2005 | Lot No: 348-8A |
| Appearance: Crystalline solid | Purity: 99.5% (ChemService COA) |
| Storage: Ambient | |

| STRUCTURE | MOL. WT. | FORMULA |
|---|---|---|
|  | 249.10 | $C_9H_{10}Cl_2N_2O_2$ |


**SUMMARY**

A neat sample of linuron (RTI Log No. 10038-02-01) was submitted for dose formulation studies in support of RTI Project No. 65U-08055.004.040. The dose formulation studies included mixing studies of the test chemical in vehicle (0.25% (w/v) methyl cellulose), concentration verification and homogeneity at day 1 and final day of the first dose formulations and concentration verification and homogeneity of the last day of administration for the second dose formulations.

The method validation and storage stability studies were conducted at Battelle.

The method: "Analysis of Linuron in Methylcellulose Using HPLC with UV/VIS Detection" assigned as EDSP.H4-033-01 was provided by Battelle. The method transfer consisted in the preparation of a solvent standard curve and the preparation of an initial calibration verification sample (ICV). Linearity was confirmed for the solvent standards data (correlation coefficient = 0.99988). Accuracy was confirmed with relative errors within ± 3.0%. The initial calibration verification was demonstrated with a relative error of 2.8%.

Analyses were performed to determine the linuron content of dose formulations mixed on September 21, 2005 and on October 05, 2005. Dose formulations of linuron prepared in 0.25% (w / v) methylcellulose at 10, 20, and 30 mg / mL and used in the animal study (Rt05-ED09) were found to contain 85.5 to 106% of the nominal concentrations of the test chemical. The relative standard deviations for duplicate or triplicate analyses were less than or equal to 15%. No test chemical was detected in the matrix blanks. Estimated limit of detection (LOD) was 0.40 µg / mL and the method detection limit (MDL) was 4.02 mg / mL.

Dose formulations used in animal studies were found to contain 68.2 to 108% of the nominal concentration of linuron from homogeneity samples. The relative standard deviations for six sample analyses were less than or equal to 17%.

**TABLE OF CONTENTS**

## FIGURES



## TABLES

# Linuron

# DOSE FORMULATION IN 0.25% (w/v) METHYL CELLULOSE

## 1.0 INTRODUCTION

The purpose of this work was to confirm the homogeneity of dose formulations of the test chemical in 0.25% (w/v) methylcellulose and to determine the test chemical content in dose formulations to be used in the animal studies for the RTI Study Code Rt05-ED09 entitled: "Inter-Laboratory Validation of the 15-Day Adult Intact Male Rat Assay with Linuron and Phenobarbital".

## 2.0 CHEMICAL HANDLING

RTI Notebook No.: 10038 pp.: 02

The bulk chemical was received from Battelle Marine Sciences on September 20, 2005, and was stored at ambient temperature at RTI. A 2 g aliquot of the bulk chemical was removed for use in the dose formulation studies.

## 3.0 METHOD TRANSFER

For WA 5-15 under project No. 08055.004.040 the following method was received from Battelle: Method # EDSP.H4-033-01 "Analysis of Linuron in Methylcellulose Using HPLC with UV/VIS Detection". The validation of this method and the stability in the vehicle were determined by Battelle. The following procedure was performed as method transfer.

RTI Notebook No.: 11595 pp.:13-25

### 3.1 Initial Calibration

Initial Calibration was performed preparing a calibration curve with 5 points from one stock solution, at a concentration range of 0.0505 µg/ml to 5.05 µg/mL.

#### 3.1.1 Preparation of Stock Solution

A stock calibration solution was prepared by directly weighing 50.5 mg of linuron into a 50-mL volumetric flask and dissolving the compound in acetonitrile. The solution was taken to the mark with acetonitrile and mixed by hand to yield a concentration of 1.01 mg of linuron/mL of acetonitrile.

### 3.1.2 Dilution Scheme

The following dilution scheme was prepared to obtain solution in the range of 0.0505 to 5.05 µg/mL

Table 1 Preparation of Solvent Solutions for Initial Calibration

| RTI ID | Sol. ID | Volume Stock Solution (mL) | Volume Solution A (mL) | Total Volume (mL) | Nominal Concentration (µg/mL) |
|---|---|---|---|---|---|
| 11595-17A | linuron A | 0.5 | - | 100 | 5.05 |
| 11595-17B | linuron B | - | 5 | 10 | 2.53 |
| 11595-17C | linuron C | - | 2 | 10 | 1.01 |
| 11595-17D | linuron D | - | 1 | 10 | 0.505 |
| 11595-17E | linuron E | - | 1 | 100 | 0.0505 |

All volumetric flasks were taken to volume with mobile phase (water/acetonitrile 40/60), mixed by hand and transferred to scintillation vials for storage under refrigeration conditions. An aliquot of each solvent standard solution was transferred into an autosampler vial and analyzed singly by HPLC as described in Section 3.4.

## 3.2 Initial Calibration Verification (ICV)

As part of the method transfer, an independent solution was prepared at a middle level range as ICV. This solution was prepared from the same neat material. A stock ICV solution was prepared by directly weighing 21.1 mg of linuron into a 20-mL volumetric flask and dissolving the compound in acetonitrile. The solution was taken to the mark with acetonitrile and mixed by hand to yield a concentration of 1.06 mg of linuron/mL of acetonitrile. A 0.1-mL aliquot of the stock ICV solution was transferred to a 100-mL volumetric flask and diluted to volume with mobile phase (water/acetonitrile (40/60). Concentration of the ICV solution was 1.06 µg/mL. An aliquot of the ICV solution was transferred into an autosampler vial and analyzed singly by HPLC as described in Section 3.4.

## 3.3 Method Detection Limit (MDL)

The Method Detection Limit (MDL) was determined by preparing 7 sample aliquots at the lowest concentration (10 mg/mL) as indicated in the method. The formulation used for this determination was prepared by RTI's material Handling Facilities (MHS) on September 21, 2005. The details of this preparation are presented in Section 4.1.

The formulation was stirred for approximately one hour before it was sampled. The

MDL samples were prepared by transferring a 1-mL aliquot of the formulation into a tared 100-mL volumetric flask using a 3 mL syringe with a 3.5 inch 16 gauge needle, and diluting to volume with acetonitrile. Ten microliters of this solution were combined with 0.99 mL of mobile phase in an autosampler vial and analyzed singly by HPLC as described in Section 3.4.

### 3.4 High Performance Liquid Chromatographic Analysis

Each blank, solvent standard, ICV solution and MDL sample was analyzed by single injection using HPLC as described in Table 2.

**Table 2 HPLC System**

| | |
|---|---|
| **Instrumentation** | |
| Pump : | Waters Alliance 2695 |
| Injector : | Waters Alliance 2695 |
| Column : | Synergy 4 µ Hydro-RP 80 Å (250 x 4.6 mm; 4 µm) (s/n 314628-13) |
| Detector : | Waters 2487 UV Detector |
| Data System : | Atlas 2000 (LabSystems Version R2) |
| **Conditions** | |
| Mobile Phase : | A: 100% water<br>B: 100% acetonitrile |
| Program : | Isocratic 40% water/60% acetonitrile |
| Flow Rate : | 1.0 mL/min |
| Detection : | 250 nm |
| Range : | 1.0 AUFS |
| Injection Volume : | 100 µL |
| Retention Times : | Analyte ~ 8.5 min |

### 3.5 Calculations

The peak area of linuron was noted for each injection. A linear regression equation (nominal concentration of the solvent standards versus peak area) (weighted 1/x) was computed for the calibration standards. The correlation coefficient (r) was determined to examine the linearity. The found concentration of the linuron in each solvent standard and sample expressed in µg/mL was determined from the linear regression equation and normalized to 1-g aliquots. For the MDL samples, the normalized found concentration in µg/mL was adjusted to mg/mL by applying a dilution factor of 10000/1000. The results were presented in terms of percent recovery based on the nominal concentration. The precision of the analysis was examined in terms of the relative standard deviation expressed as percent of the mean (%RSD). The value was obtained by averaging actual concentrations of the seven replicate dose formulations. To determine the MDL the standard deviation of the

7 aliquots expressed in mg/mL was multiplied by the student's T (3.143 for N = 7).

### 3.6 Results

Results for the initial calibration are listed in the Table 3, and for the MDL in Table 4.

Table 3 Initial Calibration Results

| Sample ID | Nominal Concentration (µg/mL) | Area Linuron | Found Concentration (µg/mL)[a] | % Recovery[b] | % Error |
|---|---|---|---|---|---|
| linuron A | 5.05 | 5420533 | 5.00 | 99.0 | -1.0 |
| linuron B | 2.53 | 2773454 | 2.56 | 101 | 1 |
| linuron C | 1.01 | 1128620 | 1.04 | 103 | 3 |
| linuron D | 0.505 | 540759 | 0.499 | 98.8 | -1.2 |
| linuron E | 0.0505 | 53015 | 0.0495 | 98.0 | -2.0 |
| ICV | 1.06 | 1112147 | 1.03 | 97.2 | -2.8 |

[a]Quantitation was based on the linear regression equation y = - 668.7 + 1084656 x ; r = 0.99988, expressed in µg/mL. The ICV sample was not included in the curve.
[b]Percent recovery = found concentration/nominal concentration * 100

Table 4. Method Detection Limit

| **Sample ID** | **Nominal Conc. (mg/mL)** | **Area** | **Amount Weighed (g)** | **Found Concencentration** | | | **% Recovery** |
|---|---|---|---|---|---|---|---|
| | | | | **(µg/mL)** | **Normalized (µg/mL)** | **Final (mg/mL)** | |
| MDL1 | 10 | 812686 | 1.1223 | 0.750 | 0.668 | 6.68 | 66.8 |
| MDL2 | 10 | 1000903 | 1.0372 | 0.923 | 0.890 | 8.90 | 89.0 |
| MDL3 | 10 | 1013505 | 0.977 | 0.935 | 0.957 | 9.57 | 95.7 |
| MDL4 | 10 | 1067152 | 0.9291 | 0.984 | 1.059 | 10.6 | 106 |
| MDL5 | 10 | 964995 | 1.0216 | 0.890 | 0.871 | 8.71 | 87.1 |
| MDL6 | 10 | 826695 | 1.0103 | 0.763 | 0.755 | 7.55 | 75.5 |
| MDL7 | 10 | 920161 | 0.9436 | 0.849 | 0.900 | 9.00 | 90.0 |
| | | | | | Mean | 8.72 | 87.2 |
| | | | | | SD | 1.28 | 12.8 |
| | | | | | % RSD | 15 | 15 |

### 3.7 Conclusions

The transfer of the method EDSP.H4-033-01 provided by Battelle was completed. Linearity was confirmed for the solvent standards data (correlation coefficient = 0.99988). Accuracy was confirmed with relative errors within ± 3.0%. The initial calibration verification was demonstrated with a relative error of 2.8%. The MDL was determined to be 4.02 mg/mL. See Figure 1 for chromatograms of solvent standards and Figure 2 for a plot of the solvent standards data for the calibration curve.

## 4.0 DOSE FORMULATION STUDIES

This section describes the procedures used in the mixing and storage of formulations of linuron in 0.25% (w/v) methylcellulose. Also included are the procedures for analyzing dose formulations of linuron in the 10 to 30 mg/mL concentration range for homogeneity and dose verification using the method EDSP.H4-033-01.

### 4.1 Mixing Procedure

The following procedure was used to prepare 500 mL of a 10 mg/mL formulation of linuron in 0.25% (w/v) methylcellulose. For formulations at other concentrations, the amount of test material was adjusted accordingly.

A glass beaker was calibrated to a 500-mL mark including a magnetic stir bar. An amount of the vehicle was added to the beaker. The chemical was sieved through an 80 mesh sieve. Then, it was weighed in a small beaker also color coded. The chemical was added to the large beaker and the weighing beaker was rinsed 3 times with the vehicle for a quantitative transfer. The mixture was taken to volume with 0.25% (w/v) methylcellulose and stirred for at least 30 minutes, and stirred an additional 10 minutes before sampling.

Duplicate 10-mL aliquots were taken from all dose formulations as an analytical and archive aliquot. The remaining formulation was transferred to 5 × 120 mL amber bottles as bulk formulations.

### 4.2 Storage Conditions

Formulations containing linuron in 0.25% (w/v) methylcellulose at concentrations of 10 to 30 mg/mL can be stored for a period of at least 30 days at refrigerator temperature ( ~ 2 - 8°C) as indicated in the RTI study protocol No. RTI-956.

### 4.3 Concentration Verification

| | | |
|---|---|---|
| RTI Notebook No.: | 11595 | pp.: 19-25, 28-29 |
| Vehicle: | 0.25% (w/v) methylcellulose | |
| Concentrations: | 10 mg/mL, 20 mg/mL, 30 mg/mL | |

Concentration verification was performed in dose formulations prepared in two

separate occasions, on September 21, 2005 and October 05, 2005. The lowest formulation from September 21, 2005 (10 mg/mL) was also used for the determination of the method detection limit as indicated in Section 3.3.

#### 4.3.1 Preparation of Calibration Standards

No calibration standards were prepared, solvent standards from the method transfer were used as continued calibration verification (CCV).

#### 4.3.2 Sample preparation

The formulations were removed from the refrigerator and stirred for approximately one hour prior to sample preparation. Duplicate 1-mL aliquots were transferred to tared 100-mL volumetric flasks using a 3-mL syringe equipped with a 3.5 inch needle of a wide bore (18 gauge). The weights were recorded and the formulations diluted with acetonitrile to the 100-mL mark. The samples were mixed by hand, and 0.01 mL were transferred to an autosampler vial and combined with 0.99 mL of mobile phase (40/60 water/acetonitrile). The samples were analyzed singly by HPLC as described in Section 4.3.3.

#### 4.3.3 High Performance Liquid Chromatographic Analysis

Each blank, continued calibration verification (CCV), and sample was analyzed by single injection using HPLC as described in Table 2.

#### 4.3.4 Calculations

The peak area of linuron was noted for each injection. The found concentration of the linuron in each CCV and sample expressed in µg/mL was determined from the linear regression equation from the method transfer. The amounts found were normalized to 1-g aliquots. The normalized found concentration in µg/mL was adjusted to mg/mL by applying a dilution factor of 10000/1000. The results were presented in terms of percent recovery based on the nominal concentration and an average of the two aliquots.

#### 4.3.5 Results

The results of these analyses are presented as interim reports in the attachment. See Figures 3 and 4 for typical chromatograms of concentration verification samples.

### 4.4 Homogeneity Analysis

| | | |
|---|---|---|
| RTI Notebook No.: | 11595 | pp.: 26-27, 30-33 |
| Vehicle: | 0.25% (w/v) methylcellulose | |
| Concentrations: | 10 mg/mL, 20 mg/mL, 30 mg/mL | |

Homogeneity samples were analyzed for the following dose formulations: a) during the first day of dosing (September 29, 2005) for the formulations prepared on September 21, 2005, b) for the last day of dosing (October 11, 2005) for the formulations prepared on

September 21, 2005 , and c) for the last day of dosing (October 13, 2005) for the formulations prepared on October 05, 2005.

### 4.4.1 Preparation of Calibration Standards

No calibration standards were prepared, solvent standards from the method transfer were used as continued calibration verification (CCV).

### 4.4.2 Sample preparation

The formulations were removed from the refrigerator and stirred for approximately one hour prior to sample preparation. Some of the formulations were prepared for analysis the same day they were received, but they were also stirred approximately one hour prior to sample preparation. Triplicate 1-mL aliquots were transferred to tared 100-mL volumetric flasks using a 3-mL syringe equipped with a 3.5 inch needle of a wide bore (16 gauge). The weights were recorded and the formulations diluted with acetonitrile to the 100-mL mark. The samples were mixed by hand, and 0.01 mL were transferred to an autosampler vial and combined with 0.99 mL of mobile phase (40/60 water/acetonitrile). The samples were analyzed singly by HPLC as described in Section 4.4.3.

### 4.4.3 High Performance Liquid Chromatographic Analysis

Each blank, continued calibration verification (CCV), and sample was analyzed by single injection using HPLC as described in Table 2.

### 4.4.4 Calculations

The peak area of linuron was noted for each injection. The found concentration of the linuron in each CCV and sample expressed in µg/mL was determined from the linear regression equation from the method transfer. The amounts found were normalized to 1-g aliquots. The percent error for the CCV samples was determined in µg/mL. The normalized found concentration in the samples, expressed in µg/mL was adjusted to mg/mL by applying a dilution factor of 10000/1000. The results were presented in terms of percent recovery based on the nominal concentration. The precision of the analysis was examined in terms of the relative standard deviation expressed as percent of the mean (%RSD). The value was obtained by averaging actual concentrations of the three replicate dose formulations at each level for each homogeneity sample. An overall mean for each dose formulation was determined from the six replicates (three top and 3 bottom aliquots).

### 4.4.5 Results

The results of these analyses are presented as interim reports in the attachment.

### 4.4.6 Conclusions

Dose formulations used in animal studies were found to contain 68.2 to 108% of the nominal concentration of linuron from homogeneity samples. The relative standard

deviations for six sample analyses were less than or equal to 17%.

## 5.0 CONTRIBUTORS

Personnel contributing to the formulation and analysis of linuron were Randy Price for the preparation of formulations and Nora Castillo for dose formulation analysis.

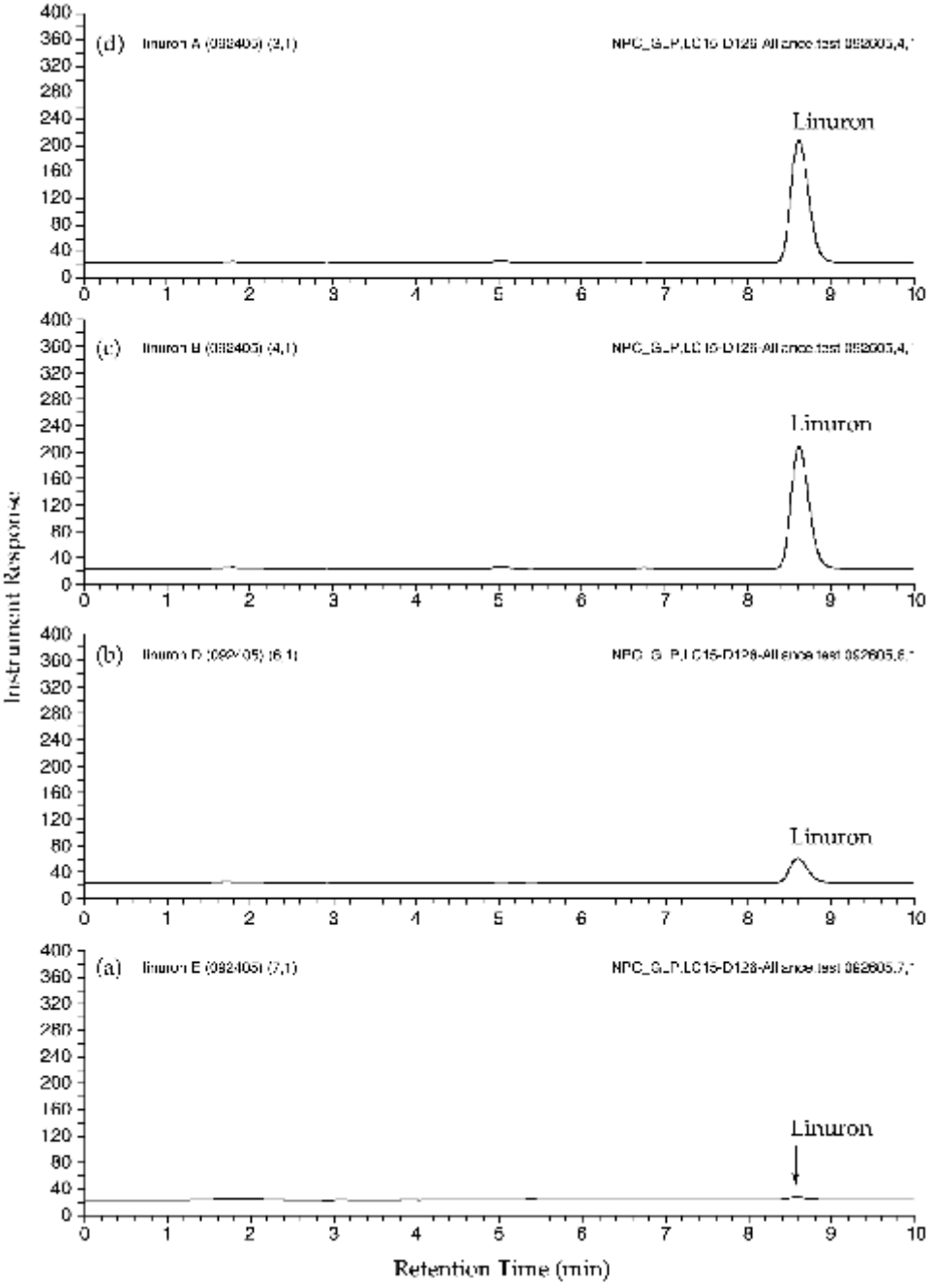



Figure 1. High Performance Liquid Chromatograms of Linuron in 0.25% (w / v) Methylcellulose from Initial Calibration Standards

(a) 0.0505 µg / mL; solvent standard
(b) 0.505 µg / mL; solvent standard
(c) 2.53 µg / mL; solvent standard
(d) 5.05 µg / mL; solvent standard

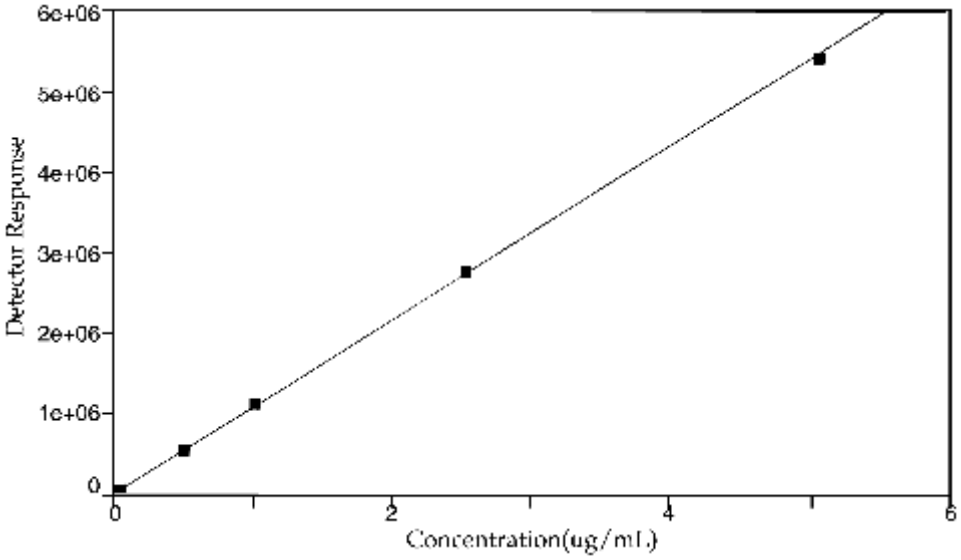



Figure 2. Plot of Initial Calibration Standards Data for Analysis of Dose Formulations of Linuron in 0.25% (w / v) Methylcellulose.

Linear regression Equation: y = -668.7 + 1084656 x
Correlation Coefficient (r) : r = 0.9999

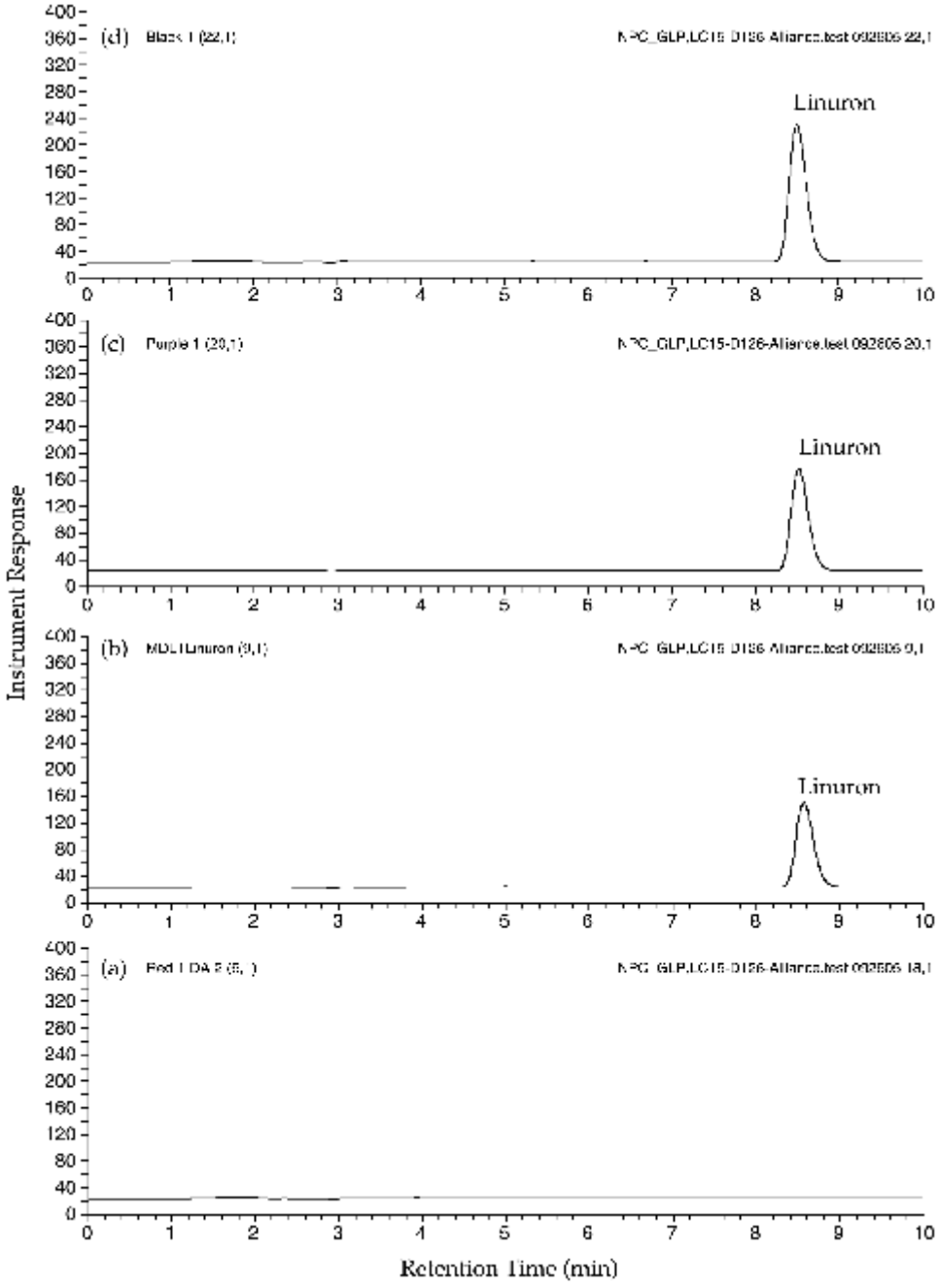



Figure 3. High Performance Liquid Chromatograms of Linuron Dose Formulations in 0.25% (w / v) Methylcellulose (Mix Date: September 21, 2005)

(a) 0 mg / mL; RTI Log No. 10038-14-09
(b) 10.0 mg / mL; RTI Log No. 10038-15-07
(c) 20.0 mg / mL; RTI Log No. 10038-15-08
(d) 30.0 mg / mL; RTI Log No. 10038-15-09

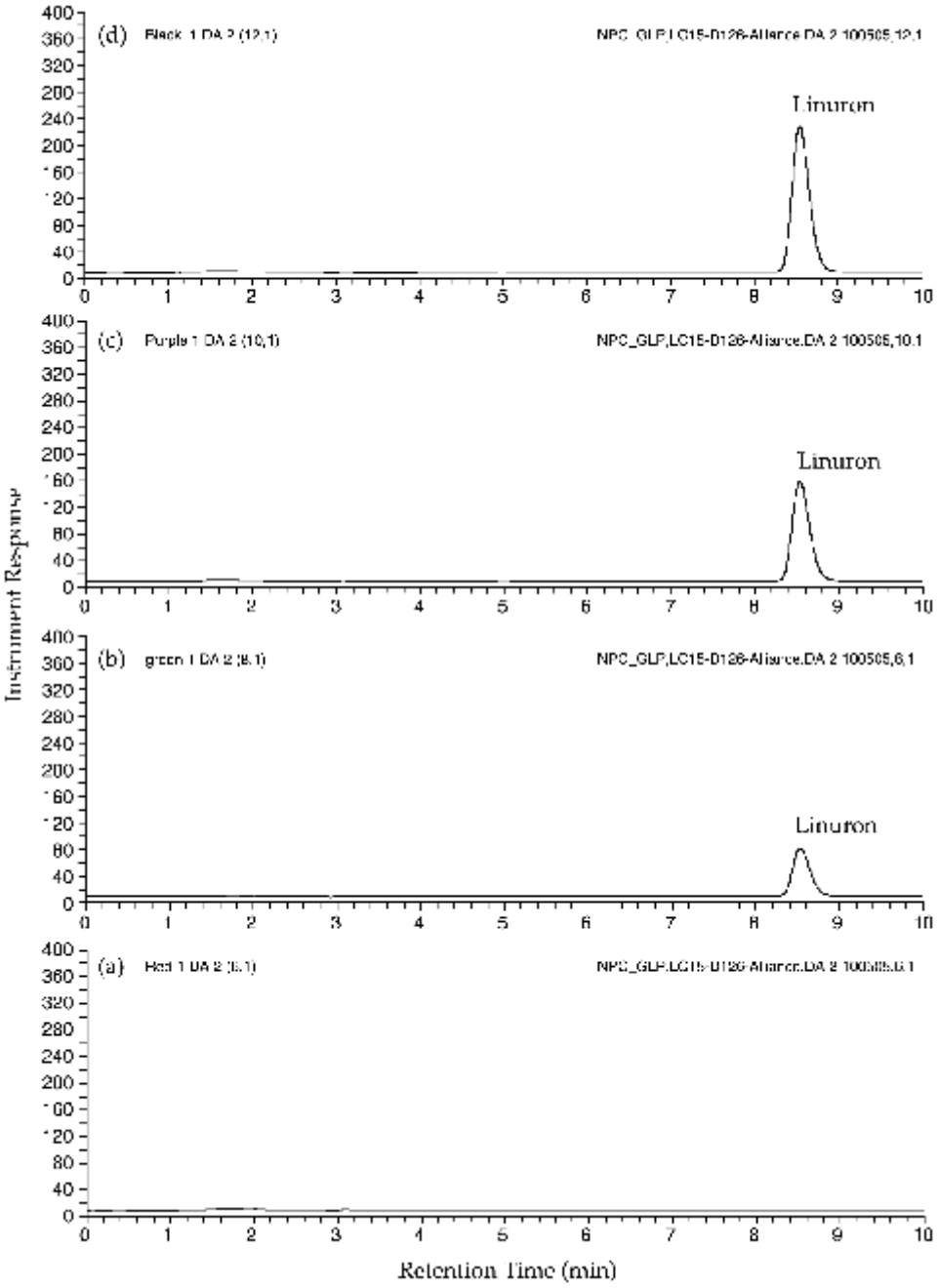



Figure 4. High Performance Liquid Chromatograms of Linuron Dose Formulations in 0.25% (w / v) Methylcellulose (Mix Date: October 05, 2005)

(a) 0 mg / mL; RTI Log No. 10038-20-09
(b) 10.0 mg / mL; RTI Log No. 10038-21-07
(c) 20.0 mg / mL; RTI Log No. 10038-21-08
(d) 30.0 mg / mL; RTI Log No. 10038-21-09

ATTACHMENT

**Dosage Analysis Results**

RTI INTERNATIONAL

Memorandum

**FORMULATION ANALYSIS INTERIM REPORT**
**DA1 Formulated September 21, 2005**

**Submission Date: November 18, 2005**

| | | | |
|---|---|---|---|
| Test Chemical (ID): | Linuron | Study Code: | Rt05-ED09 |
| Study Project No.: | 08055.004.040 | RTI ID No.: | 10038-15-07 to 10038-15-09<br>10038-14-09 |
| Lot No.(Vendor): | 348-8A (ChemServices) | Receipt Date: | 09 / 22 / 05 |
| Vehicle: | 0.25% Methyl Cellulose | Formulation Date: | 09 / 21 / 05 |
| No. of Samples: | 4 x 10 mL | Analysis Date: | 09 / 24 / 05 - 09 / 26 / 05 |

| Study Lab Sample ID | RTI Log Numbers | Nominal Concentration[a] | Found Concentration[a,b] | Percent of Nominal |
|---|---|---|---|---|
| Rx 75171 Red 1 | 10038-14-09 | 0 | ND[c] | NA[d] |
| Rx 75171 Red 2 | 10038-14-09 | 0 | ND | NA |
| Rx 80945 Green 1[e] | 10038-15-07 | 10 | 6.68 | 66.8 |
| Rx 80945 Green 2 | 10038-15-07 | 10 | 8.90 | 89.0 |
| Rx 80945 Green 3 | 10038-15-07 | 10 | 9.57 | 95.7 |
| Rx 80945 Green 4 | 10038-15-07 | 10 | 10.6 | 106 |
| Rx 80945 Green 5 | 10038-15-07 | 10 | 8.71 | 87.1 |
| Rx 80945 Green 6 | 10038-15-07 | 10 | 7.55 | 75.5 |
| Rx 80945 Green 7 | 10038-15-07 | 10 | 9.00 | 90.0 |
| | | Mean ± SD (%RPD) | 8.72 ± 1.28 (15%) | 87.2 ± 12.8 (15%) |
| Rx 21297 Purple 1 | 10038-15-08 | 20 | 19.8 | 99.0 |
| Rx 21297 Purple 2 | 10038-15-08 | 20 | 22.4 | 112 |
| | | Avg. (%RPD) | 21.1 (8.7%) | 106 (8.7%) |
| Rx 34208 Black 1 | 10038-15-09 | 30 | 26.6 | 88.7 |
| Rx 34208 Black 2 | 10038-15-09 | 30 | 24.7 | 82.3 |
| | | Avg. (%RPD) | 25.7 (5.2%) | 85.5 (5.3%) |

[a]Concentration units: mg / mL.
[b]Calibration range was 0.0505 ug / mL to 5.05 ug / mL; Linear regression equation ( weighted 1 / x):
y = -668.7 + 1084656 x; r = 0.99988.
[c]ND = Not detected. LOD = 0.40 µg/mL
[d]NA = Not applicable.
[e]This aliquot was repeated because the original one was not prepared correctly.

COMMENT: Rx 80945 Green was used to determine the MDL.= Student's T * SD = 3.143 * 1.28 = 4.02 mg / mL

RTI INTERNATIONAL

Memorandum

**FORMULATION ANALYSIS INTERIM REPORT**
**HOMOGENEITY DAY 1 for DA1**

**Submission Date: October 04, 2005**
**Revised Date: November 18, 2005**

| | | | |
|---|---|---|---|
| Test Chemical (ID): | Linuron | Study Code: | Rt05-ED09 |
| Study Project No.: | 08055.004.040 | RTI ID No.: | 10038-17-01 to 10038-17-02<br>10038-17-09 to 10038-17-14 |
| Lot No.(Vendor): | 348-8A (ChemServices) | Receipt Date: | 09 / 29 / 05 |
| Vehicle: | 0.25% Methyl Cellulose | Formulation Date: | 09 / 21 / 05 |
| No. of Samples: | 8 x 10 mL | Analysis Date: | 09 / 29 / 05 to 09 / 30 / 05 |

| Study Lab Sample ID | RTI Log Numbers | Nominal Concentration[a] | Found Concentration[a,b] | Percent of Nominal |
|---|---|---|---|---|
| Rx 75171 Red 1 T | 10038-17-01 | 0 | ND[c] | NA[d] |
| Rx 75171 Red 2 T | 10038-17-01 | 0 | ND | NA |
| Rx 75171 Red 3 T | 10038-17-01 | 0 | ND | NA |
| Rx 75171 Red 1 B | 10038-17-02 | 0 | ND | NA |
| Rx 75171 Red 2 B | 10038-17-02 | 0 | ND | NA |
| Rx 75171 Red 3 B | 10038-17-02 | 0 | ND | NA |
| Rx 80945 Green 1 T | 10038-17-09 | 10 | 5.45 | 54.5 |
| Rx 80945 Green 2 T | 10038-17-09 | 10 | 7.86 | 78.6 |
| Rx 80945 Green 3 T | 10038-17-09 | 10 | 8.20 | 82.0 |
| | | Mean ± SD (%RSD) | 7.17 ±1.50 (21%) | 71.7 ± 15.0 (21%) |
| Rx 80945 Green 1 B | 10038-17-10 | 10 | 6.82 | 68.2 |
| Rx 80945 Green 2 B | 10038-17-10 | 10 | 6.14 | 61.4 |
| Rx 80945 Green 3 B | 10038-17-10 | 10 | 8.35 | 83.5 |
| | | Mean ± SD (%RSD) | 7.10 ± 1.13 (16%) | 71.0 ± 11.3 (16%) |
| | **DOSE MEAN ± SD (%RSD) (N= 6)** | | **7.14 ± 1.19 (17%)** | **71.4 ± 11.9 (17%)** |

(Continued)

| Study Lab Sample ID | RTI Log Numbers | Nominal Concentration[a] | Found Concentration[a,b] | Percent of Nominal |
|---|---|---|---|---|
| Rx 21297 Purple 1 T[e] | 10038-17-11 | 20 | 15.9 | 79.5 |
| Rx 21297 Purple 2 T | 10038-17-11 | 20 | 18.3 | 91.5 |
| Rx 21297 Purple 3 T | 10038-17-11 | 20 | 18.9 | 94.5 |
| | Mean ± SD (%RSD) | | 17.7 ± 1.59 (9.0%) | 88.5 ± 7.94 (9.0%) |
| Rx 21297 Purple 1 B | 10038-17-12 | 20 | 1.79 | 89.5 |
| Rx 21297 Purple 2 B | 10038-17-12 | 20 | 17.7 | 88.5 |
| Rx 21297 Purple 3 B | 10038-17-12 | 20 | 17.3 | 86.5 |
| | Mean ± SD (%RSD) | | 17.6 ± 0.306 (1.7%) | 88.2 ± 1.53 (1.7%) |
| **DOSE MEAN ± SD (%RSD) (N=6)** | | | **17.7 ± 1.02 (5.8%)** | **88.3 ± 5.12 (5.8%)** |
| Rx 34208 Black 1 T[f] | 10038-17-13 | 30 | 23.9 | 79.7 |
| Rx 34208 Black 2 T | 10038-17-13 | 30 | 24.5 | 81.7 |
| Rx 34208 Black 3 T | 10038-17-13 | 30 | 23.8 | 79.3 |
| | Mean ± SD (%RSD) | | 24.1 ± 0.379 (1.6%) | 80.2 ± 1.29 (1.6%) |
| Rx 34208 Black 1 B | 10038-17-14 | 30 | 21.9 | 73.0 |
| Rx 34208 Black 2 B | 10038-17-14 | 30 | 22.0 | 73.3 |
| Rx 34208 Black 3 B | 10038-17-14 | 30 | 22.5 | 75.0 |
| | Mean ± SD (%RSD) | | 22.1 ± 0.321 (1.5%) | 73.8 ± 1.08 (1.5%) |
| **DOSE MEAN ± SD (%RSD) (N=6)** | | | **23.1 ± 1.10 (4.8%)** | **77.0 ± 3.70 (4.8%)** |

[a]Concentration units: mg / mL.
[b]Calibration range was 0.0505 ug / mL to 5.05 ug / mL; Linear regression equation ( weighted 1 / x):
y = -668.7 + 1084656 x; r = 0.99988.
[c]ND = Not detected. LOD = 0.40 µg / mL
[d]NA = Not applicable.
[e] Rx 21297 Purple homogeneity samples were received mislabeled as Rx 34208 Black.
[f] Rx 34208 Black homogeneity samples were received mislabeled as Rx 21297 Purple.

COMMENT: The error in the transfer of samples for homogeneity analysis was confirmed by repeating the test with new aliquots that gave similar results.

RTI INTERNATIONAL

Memorandum

**FORMULATION ANALYSIS INTERIM REPORT**
**HOMOGENEITY DAY 12 for DA1**

**Submission Date: October 18, 2005**
**Revised Date: November 18, 2005**

| | | | |
|---|---|---|---|
| Test Chemical (ID): | Linuron | Study Code: | Rt05-ED09 |
| Study Project No.: | 08055.004.040 | RTI ID No.: | 10038-22-01 to 10038-22-02<br>10038-22-09 to 10038-22-14 |
| Lot No.(Vendor): | 348-8A (ChemServices) | Receipt Date: | 10 / 11 / 05 |
| Vehicle: | 0.25% Methyl Cellulose | Formulation Date: | 09 / 21 / 05 |
| No. of Samples: | 8 x 10 mL | Analysis Date: | 10 / 11 / 05 to 10 / 12 / 05 |

| Study Lab Sample ID | RTI Log Numbers | Nominal Concentration[a] | Found Concentration[a,b] | Percent of Nominal |
|---|---|---|---|---|
| Rx 75171 Red 1 T | 10038-22-01 | 0 | ND[c] | NA[d] |
| Rx 75171 Red 2 T | 10038-22-01 | 0 | ND | NA |
| Rx 75171 Red 3 T | 10038-22-01 | 0 | ND | NA |
| Rx 75171 Red 1 B | 10038-22-02 | 0 | ND | NA |
| Rx 75171 Red 2 B | 10038-22-02 | 0 | ND | NA |
| Rx 75171 Red 3 B | 10038-22-02 | 0 | ND | NA |
| Rx 80945 Green 1 T | 10038-22-09 | 10 | 8.20 | 82.0 |
| Rx 80945 Green 2 T | 10038-22-09 | 10 | 7.23 | 72.3 |
| Rx 80945 Green 3 T | 10038-22-09 | 10 | 5.97 | 59.7 |
| | | Mean ± SD (%RSD) | 7.13 ±1.12 (16%) | 71.3 ± 11.2 (16%) |
| Rx 80945 Green 1 B | 10038-22-10 | 10 | 7.11 | 71.1 |
| Rx 80945 Green 2 B | 10038-22-10 | 10 | 5.81 | 58.1 |
| Rx 80945 Green 3 B | 10038-22-10 | 10 | 6.57 | 65.7 |
| | | Mean ± SD (%RSD) | 6.50 ± 0.653 (10%) | 65.0 ± 6.53 (10%) |
| | | **DOSE MEAN ± SD (%RSD) (N= 6)** | **6.82 ± 0.890 (13%)** | **68.2 ± 8.90 (13%)** |

(Continued)

| Study Lab Sample ID | RTI Log Numbers | Nominal Concentration[a] | Found Concentration[a,b] | Percent of Nominal |
|---|---|---|---|---|
| Rx 21297 Purple 1 T | 10038-22-11 | 20 | 18.4 | 92.0 |
| Rx 21297 Purple 2 T | 10038-22-11 | 20 | 19.4 | 97.0 |
| Rx 21297 Purple 3 T | 10038-22-11 | 20 | 19.8 | 99.0 |
| | | Mean ± SD (%RSD) | 19.2 ± 0.721 (3.8%) | 96.0 ± 3.61 (3.8%) |
| Rx 21297 Purple 1 B | 10038-22-12 | 20 | 16.8 | 84.0 |
| Rx 21297 Purple 2 B | 10038-22-12 | 20 | 15.6 | 78.0 |
| Rx 21297 Purple 3 B | 10038-22-12 | 20 | 15.5 | 77.5 |
| | | Mean ± SD (%RSD) | 16.0± 0.723 (4.5%) | 79.8 ± 3.62 (4.5%) |
| | | **DOSE MEAN ± SD (%RSD) (N=6)** | **17.6 ± 1.89 (11%)** | **87.9 ± 9.43 (11%)** |
| Rx 34208 Black 1 T | 10038-22-13 | 30 | 22.9 | 76.3 |
| Rx 34208 Black 2 T | 10038-22-13 | 30 | 24.3 | 81.0 |
| Rx 34208 Black 3 T | 10038-22-13 | 30 | 23.9 | 79.7 |
| | | Mean ± SD (%RSD) | 23.7 ± 0.721 (3.0%) | 79.0 ± 2.43 (3.1%) |
| Rx 34208 Black 1 B | 10038-22-14 | 30 | 24.6 | 82.0 |
| Rx 34208 Black 2 B | 10038-22-14 | 30 | 25.9 | 86.3 |
| Rx 34208 Black 3 B | 10038-22-14 | 30 | 24.2 | 80.7 |
| | | Mean ± SD (%RSD) | 24.9 ± 0.889 (3.6%) | 83.0 ± 2.93 (3.5%) |
| | | **DOSE MEAN ± SD (%RSD) (N=6)** | **24.3 ± 0.978 (4.0%)** | **81.0 ± 3.25 (4.0%)** |

[a]Concentration units: mg / mL.
[b]Calibration range was 0.0505 ug / mL to 5.05 ug / mL; Linear regression equation ( weighted 1 / x):
y = -668.7 + 1084656 x; r = 0.99988.
[c]ND = Not detected. LOD = 0.40 µg/mL; MDL = 4.02 mg/mL[d]NA = Not applicable.

COMMENT: No new curve was necessary, only continued calibration verification (CCV) standards. All doses had good reproducibility and the mean of the percent of nominal is within 70 to 130% in each dose formulation.

RTI INTERNATIONAL

Memorandum

**FORMULATION ANALYSIS INTERIM REPORT**
**DA2 Formulated October 05, 2005**

**Submission Date: October 06, 2005**
**Revised Date: November 18, 2005**

| | | | |
|---|---|---|---|
| Test Chemical (ID): | Linuron | Study Code: | Rt05-ED09 |
| Study Project No.: | 08055.004.040 | RTI ID No.: | 10038-21-07 to 10038-21-09<br>10038-20-09 |
| Lot No.(Vendor): | 348-8A (ChemServices) | Receipt Date: | 10 / 05 / 05 |
| Vehicle: | 0.25% Methyl Cellulose | Formulation Date: | 10 / 05 / 05 |
| No. of Samples: | 4 x 10 mL | Analysis Date: | 10 / 05 / 05 - 10 / 06 / 05 |

| Study Lab Sample ID | RTI Log Numbers | Nominal Concentration [a] | Found Concentration [a,b] | Percent of Nominal |
|---|---|---|---|---|
| Rx 75171 Red 1 | 10038-20-09 | 0 | ND[c] | NA[d] |
| Rx 75171 Red 2 | 10038-20-09 | 0 | ND | NA |
| Rx 80945 Green 1 | 10038-21-07 | 10 | 9.03 | 90.3 |
| Rx 80945 Green 2 | 10038-21-07 | 10 | 8.21 | 82.1 |
| | | Avg. (%RPD) | 8.62 (6.7%) | 86.2 (6.7%) |
| Rx 21297 Purple 1 | 10038-21-08 | 20 | 17.2 | 86.0 |
| Rx 21297 Purple 2 | 10038-21-08 | 20 | 19.0 | 95.0 |
| | | Avg. (%RPD) | 18.1 (7.0%) | 90.5 (7.0%) |
| Rx 34208 Black 1 | 10038-21-09 | 30 | 27.6 | 92.0 |
| Rx 34208 Black 2 | 10038-21-09 | 30 | 31.0 | 103 |
| | | Avg. (%RPD) | 29.3 (8.2%) | 97.5 (8.0%) |

[a]Concentration units: mg / mL.
[b]Calibration range was 0.0505 ug / mL to 5.05 ug / mL; Linear regression equation ( weighted 1 / x):
y = -668.7 + 1084656 x; r = 0.99988.
[c]ND = Not detected. LOD = 0.40 µg/mL; MDL = 4.02 mg/mL
[d]NA = Not applicable.

COMMENT: All the doses passed the requirements.

RTI INTERNATIONAL

Memorandum

**FORMULATION ANALYSIS INTERIM REPORT**
**HOMOGENEITY LAST DAY for DA2**

**Submission Date: November 03, 2005**
**Revision Date: November 18, 2005**

| | | | |
|---|---|---|---|
| Test Chemical (ID): | Linuron | Study Code: | Rt05-ED09 |
| Study Project No.: | 08055.004.040 | RTI ID No.: | 10038-26-01 to 10038-26-02<br>10038-26-09 to 10038-26-14 |
| Lot No.(Vendor): | 348-8A (ChemServices) | Receipt Date: | 10 / 13 / 05 |
| Vehicle: | 0.25% Methyl Cellulose | Formulation Date: | 10 / 05 / 05 |
| No. of Samples: | 8 x 10 mL | Analysis Date: | 10 / 17 / 05 to 10 / 18 / 05 |

| Study Lab Sample ID | RTI Log Numbers | Nominal Concentration[a] | Found Concentration[a,b] | Percent of Nominal |
|---|---|---|---|---|
| Rx 75171 Red 1 T | 10038-26-01 | 0 | ND[c] | NA[d] |
| Rx 75171 Red 2 T | 10038-26-01 | 0 | ND | NA |
| Rx 75171 Red 3 T | 10038-26-01 | 0 | ND | NA |
| Rx 75171 Red 1 B | 10038-26-02 | 0 | ND | NA |
| Rx 75171 Red 2 B | 10038-26-02 | 0 | ND | NA |
| Rx 75171 Red 3 B | 10038-26-02 | 0 | ND | NA |
| Rx 80945 Green 1 T | 10038-26-09 | 10 | 10.5 | 105 |
| Rx 80945 Green 2 T | 10038-26-09 | 10 | 11.1 | 111 |
| Rx 80945 Green 3 T | 10038-26-09 | 10 | 10.6 | 106 |
| | | Mean ± SD (%RSD) | 10.7 ±0.321 (3.0%) | 107 ± 3.21 (3.0%) |
| Rx 80945 Green 1 B | 10038-26-10 | 10 | 10.6 | 106 |
| Rx 80945 Green 2 B | 10038-26-10 | 10 | 10.3 | 103 |
| Rx 80945 Green 3 B | 10038-26-10 | 10 | 10.5 | 105 |
| | | Mean ± SD (%RSD) | 10.5 ± 0.153 (1.5%) | 105 ± 1.53 (1.5%) |
| | | **DOSE MEAN ± SD (%RSD) (N= 6)** | **10.6 ± 0.268 (2.5%)** | **106 ± 2.68 (2.5%)** |

(Continued)

| Study Lab Sample ID | RTI Log Numbers | Nominal Concentration[a] | Found Concentration[a,b] | Percent of Nominal |
|---|---|---|---|---|
| Rx 21297 Purple 1 T | 10038-26-11 | 20 | 20.4 | 102 |
| Rx 21297 Purple 2 T | 10038-26-11 | 20 | 20.5 | 103 |
| Rx 21297 Purple 3 T | 10038-26-11 | 20 | 20.4 | 102 |
| | | Mean ± SD (%RSD) | 20.4 ± 0.0577 (0.28%) | 102 ± 0.577 (0.57%) |
| Rx 21297 Purple 1 B | 10038-26-12 | 20 | 20.2 | 101 |
| Rx 21297 Purple 2 B | 10038-26-12 | 20 | 20.1 | 101 |
| Rx 21297 Purple 3 B | 10038-26-12 | 20 | 20.0 | 100 |
| | | Mean ± SD (%RSD) | 20.1 ± 0.100 (0.50%) | 101 ± 0.577 (0.6%) |
| | | **DOSE MEAN ± SD (%RSD) (N=6)** | **20.3 ± 0.197 (1.0%)** | **102 ± 1.05 (1.0%)** |
| Rx 34208 Black 1 T | 10038-26-13 | 30 | 32.3 | 108 |
| Rx 34208 Black 2 T | 10038-26-13 | 30 | 34.5 | 115 |
| Rx 34208 Black 3 T | 10038-26-13 | 30 | 32.3 | 108 |
| | | Mean ± SD (%RSD) | 33.0 ± 1.27 (3.8%) | 110 ± 4.04 3.7%) |
| Rx 34208 Black 1 B | 10038-26-14 | 30 | 33.3 | 111 |
| Rx 34208 Black 2 B | 10038-26-14 | 30 | 33.7 | 112 |
| Rx 34208 Black 3 B | 10038-26-14 | 30 | 28.1 | 93.7 |
| | | Mean ± SD (%RSD) | 31.7 ± 3.12 (9.8%) | 106 ± 10.3 9.7%) |
| | | **DOSE MEAN ± SD (%RSD) (N=6)** | **32.4 ± 2.25 (6.9%)** | **108 ± 7.46 (6.9%)** |

[a]Concentration units: mg / mL.
[b]Calibration range was 0.0505 ug / mL to 5.05 ug / mL; Linear regression equation ( weighted 1 / x):
y = -668.7 + 1084656 x; r = 0.99988.
[c]ND = Not detected. LOD = 0.40 µg / mL; MDL = 3.99 mg / mL
[d]NA = Not applicable.

COMMENT: No new calibration curve was necessary, only continued calibration verification (CCV) standards. All doses had good reproducibility and the mean of the percent of nominal is within 70 to 130% for each dose.

# DOSE FORMULATION AND ANALYSIS REPORT

RTI Project No.: 65U-08055.004.040
RTI Master Protocol No.: RTI-956
RTI Study Code.:Rt05-ED09

# PHENOBARBITAL IN 0.25 % METHYL CELLULOSE

November 28, 2005

Prepared by:

Nora P. Castillo 11/28/05

Nora ~~P. Castillo~~ Date
Task Leader

Approved by:

11/28/05

Jonathan S. Green, Ph.D. Date
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Prepared for:
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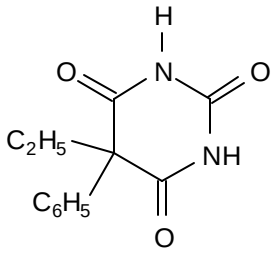
Endocrine Disruptor Screening Program
Battelle Memorial Institute
505 King Avenue
Columbus, OH 43201-2693

# PHENOBARBITAL IN 0.25 % METHYLCELLULOSE

| | |
|---|---|
| RTI Log Nos.:<br>10038-10-01 (Bulk Chemical)<br>10038-10-02 (Analytical aliquot) | Amount received:<br>100 g (Bulk chemical)<br>25 g (Analytical aliquot) |
| Cas No.: 50-06-6 | Received from: Battelle Marine Sciences. |
| RTI Receipt Date:<br>September 13, 2005 | Lot No: 104K2600 |
| Appearance: White solid | Purity: 99.1% (Sigma-Aldrich COA) |
| Storage: Ambient (RTI Vault) | |

| STRUCTURE | MOL. WT. | FORMULA |
|---|---|---|
|  | 232.2 | $C_{12}H_{12}N_2O_3$ |



**SUMMARY**

A neat sample of phenobarbital (RTI Log No. 10038-10-01) was submitted for dose formulation studies in support of RTI Project No. 65U-08055.004.040. The dose formulation studies included mixing studies of the test chemical in vehicle (0.25% (w/v) methylcellulose), concentration verification and homogeneity at day 1 and final day of the first dose formulations and concentration verification and homogeneity at the last day of administration for the second dose formulations.

The method validation and storage stability studies were conducted at Battelle.

The method: "Analysis of Phenobarbital in Methylcellulose Using HPLC with UV/VIS Detection" assigned as EDSP.H4-034-01 was provided by Battelle. The method transfer consisted in the preparation of a solvent standard curve and the preparation of an initial calibration verification sample (ICV). Linearity was confirmed for the solvent standards data (correlation coefficient = 0.9999). Accuracy was confirmed with relative errors within ± 3.8%. The initial calibration verification was demonstrated with a relative error of 4.1%.

Analyses were performed to determine the phenobarbital content of dose formulations mixed on September 21, 2005 and on October 05, 2005. Dose formulations of phenobarbital prepared in 0.25% (w/v) methylcellulose at 5, 10, and 20 mg/mL and used in the animal study (Rt05-ED09) were found to contain 85.9 to 93.8% of the nominal concentrations of the test chemical. The relative standard deviations for duplicate or triplicate analyses were less than or equal to 19%. No test chemical was detected in the matrix blanks. Estimated limit of detection (LOD) was 3.9 µg/mL and the method detection limit (MDL) was 1.05 mg/mL

Dose formulations used in animal studies were found to contain 77.0 to 120% of the nominal concentration of phenobarbital from homogeneity samples. The relative standard deviations for six sample analyses were less than or equal to 13%.

**TABLE OF CONTENTS**

## FIGURES



## TABLES

# PHENOBARBITAL

# DOSE FORMULATION IN 0.25% (w/v) METHYL CELLULOSE

## 1.0 INTRODUCTION

The purpose of this work was to confirm the homogeneity of dose formulations of the test chemical in 0.25% (w/v) methylcellulose and to determine the test chemical content in dose formulations to be used in the animal studies for the RTI Study Code Rt05-ED09 entitled: "Inter-Laboratory Validation of the 15-Day Adult Intact Male Rat Assay with Linuron and Phenobarbital".

## 2.0 CHEMICAL HANDLING

RTI Notebook No.: 10038 pp.: 10

The bulk chemical was received from Battelle Marine Sciences on September 13, 2005, and was stored at ambient temperature in the RTI Vault. One bottle aliquot (25 g) of the bulk chemical was removed for use in the dose formulation studies.

## 3.0 METHOD TRANSFER

For WA 5-15 under project No. 08055.004.040 the following method was received from Battelle: Method # EDSP.H4-034-01 "Analysis of Phenobarbital in Methylcellulose Using HPLC with UV/VIS Detection". The validation of this method and the stability in the vehicle were determined by Battelle. The following procedure was performed as method transfer.

RTI Notebook No.: 11595 pp.: 1-12, 23-25

### 3.1 Initial Calibration

Initial Calibration was performed preparing a calibration curve with 5 points from one stock solution, at a concentration range of 1 µg/ml to 200 µg/mL.

#### 3.1.1 Preparation of Stock Solution

A stock calibration solution was prepared by directly weighing 50.4 mg of phenobarbital into a 50-mL volumetric flask and dissolving the compound in acetonitrile. The solution was taken to the mark with acetonitrile and mixed by hand to yield a concentration of 1.01 mg of phenobarbital/mL of acetonitrile.

### 3.1.2 Dilution Scheme

The following dilution scheme was prepared to obtain solution in the range of 1 µg/mL to 200 µg/mL.

**Table 1 Preparation of Solvent Solutions for Initial Calibration**

| RTI ID | Sol. ID | Volume Stock Solution (mL) | Volume Solution A (mL) | Total Volume (mL) | Nominal Concentration (µg/mL) |
|---|---|---|---|---|---|
| 11595-6A | phenob A | 5 | - | 25 | 202 |
| 11595-6B | phenob B | - | 6 | 10 | 121 |
| 11595-6C | phenob C | - | 3 | 10 | 60.6 |
| 11595-6D | phenob D | - | 1 | 20 | 10.1 |
| 11595-6E | phenob E | - | 0.5 | 100 | 1.01 |

All volumetric flasks were taken to volume with mobile phase ( water/acetonitrile 50/50), mixed by hand and transferred to scintillation vials for storage under refrigeration conditions. An aliquot of each solvent standard solution was transferred into an autosampler vial and analyzed singly by HPLC as described in Section 3.4.

## 3.2 Initial Calibration Verification (ICV)

As part of the method transfer, an independent solution was prepared at a middle level range as ICV. This solution was prepared from the same neat material. A stock ICV solution was prepared by directly weighing 24.2 mg of phenobarbital into a 20-mL volumetric flask and dissolving the compound in acetonitrile. The solution was taken to the mark with acetonitrile and mixed by hand to yield a concentration of 1.21 mg of phenobarbital/mL of acetonitrile. A 1-mL aliquot of the stock ICV solution was transferred to a 20-mL volumetric flask and diluted to volume with mobile phase (50/50 water/acetonitrile). The concentration of the ICV solution was 60.5 µg/mL. An aliquot of the ICV solution was transferred into an autosampler vial and analyzed singly by HPLC as described in Section 3.4.

## 3.3 Method Detection Limit (MDL)

The Method Detection Limit (MDL) was determined by preparing 7 sample aliquots at the lowest concentration (5 mg/mL) as indicated in the method. The formulation used for this determination was prepared by RTI's material Handling Facilities (MHF) on September 21, 2005. In Section 4.1 are the details of this preparation.

The formulation was stirred for approximately one hour before it was sampled. The MDL samples were prepared by transferring a 1-mL aliquot of the formulation into a tared 25-mL volumetric flask and diluting to volume with acetonitrile. One hundred microliters of

this solution were combined with 0.99 mL of mobile phase (50/50 water/acetonitrile) in an autosampler vial and analyzed singly by HPLC as described in Section 3.4.

NOTE: The method required the final dilution to be 1 to 10 and not 1 to 10.9, but this error was corrected using a correction factor to account for the extra solvent added.

### 3.4 High Performance Liquid Chromatographic Analysis

Each blank, solvent standard, ICV solution and MDL sample was analyzed by single injection using HPLC as described in Table 2.

**Table 2 HPLC System**

| | |
|---|---|
| **Instrumentation** | |
| Pump : | Waters Alliance 2695 |
| Injector : | Waters Alliance 2695 |
| Column : | Synergy 4 µ Hydro-RP 80 Å (250 x 4.6 mm; 4 µm) (s/n 314628-13) |
| Detector : | Waters 2487 UV Detector |
| Data System : | Atlas 2000 (LabSystems Version R2) |
| **Conditions** | |
| Mobile Phase : | A: 100% water<br>B: 100% acetonitrile |
| Program : | Isocratic 50% water/50% acetonitrile |
| Flow Rate : | 1.0 mL/min |
| Detection : | 225 nm |
| Range : | 1.0 AUFS |
| Injection Volume : | 5 µL |
| Retention Times : | Analyte ~ 3.6 min |

### 3.5 Calculations

The peak area of phenobarbital was noted for each injection. A linear regression equation (nominal concentration of the solvent standards versus peak area) (weighted 1/x) was computed for the calibration standards. The correlation coefficient (r) was determined to examine the linearity. The found concentration of the phenobarbital in each solvent standard and sample expressed in µg/mL was determined from the linear regression equation and normalized to 1-g aliquots. For the MDL samples, the normalized found concentration in µg/mL was adjusted to mg/mL by applying a dilution factor of 250/1000* 1.09. The 1.09 was added as a factor to consider the different dilution for these samples. The results were presented in terms of percent recovery based on the nominal concentration. The precision of the analysis was examined in terms of the relative standard deviation expressed as percent of the mean (%RSD). The value was obtained by averaging actual concentrations of the seven

replicate dose formulations. To determine the MDL the standard deviation of the 7 aliquots expressed in mg/mL was multiplied by the student's T (3.143 for N = 7).

### 3.6 Results

Results for the initial calibration are listed in the Table 3, and for the MDL in Table 4.

**Table 3 Initial Calibration Results**

| Sample ID | Nominal Concentration (µg/mL) | Area Phenobarbital | Found Concentration (µg/mL)[a] | % Recovery[b] | % Error |
|---|---|---|---|---|---|
| phenob A | 202 | 2389843 | 201 | 99.5 | -0.5 |
| phenob B | 121 | 1426351 | 120 | 99.2 | -0.8 |
| phenob C | 60.6 | 743188 | 62.5 | 103 | 3 |
| phenob D | 10.1 | 121975 | 10.3 | 102 | 2 |
| phenob E | 1.01 | 11028 | 0.972 | 96.2 | -3.8 |
| ICV | 60.5 | 689449 | 58.0 | 95.9 | -4.1 |

[a]Quantitation was based on the linear regression equation y = - 533.0 + 11895 x ; r = 0.99988, expressed in µg/mL. The ICV sample was not included in the curve.
[b]Percent recovery = found concentration/nominal concentration * 100

**Table 4. Method Detection Limit**

| Sample ID | Nominal Conc. (mg/mL) | Area | Amount Weighed (g) | Found Concencentration (µg/mL) | Normalized (µg/mL) | Final (mg/mL) | % Recovery |
|---|---|---|---|---|---|---|---|
| MDL1 | 5 | 234570 | 1.3268 | 19.8 | 14.9 | 4.06 | 81.2 |
| MDL2 | 5 | 184469 | 0.9650 | 15.6 | 16.2 | 4.41 | 88.2 |
| MDL3 | 5 | 188168 | 0.9894 | 15.9 | 16.1 | 4.39 | 87.8 |
| MDL4 | 5 | 201411 | 0.9225 | 17.0 | 18.4 | 5.01 | 100 |
| MDL5 | 5 | 197490 | 0.9627 | 16.6 | 17.2 | 4.69 | 93.8 |
| MDL6 | 5 | 204037 | 1.0802 | 17.2 | 15.9 | 4.33 | 86.6 |
| MDL7 | 5 | 214562 | 1.0122 | 18.1 | 17.9 | 4.88 | 97.6 |
| | | | | | Mean | 4.54 | 90.7 |
| | | | | | SD | 0.335 | 6.65 |
| | | | | | % RSD | 7.4 | 7.3 |

### 3.7 Conclusions

The transfer of the method EDSP.H4-034-01 provided by Battelle was completed. Linearity was confirmed for the solvent standards data (correlation coefficient = 0.9999). Accuracy was confirmed with relative errors within ± 3.8%. The initial calibration verification was demonstrated with a relative error of 4.1%. The MDL was determined to be 1.05 mg/mL. See Figure 1 for chromatograms of solvent standards and Figure 2 for a plot of the solvent standards data for the calibration curve.

## 4.0 DOSE FORMULATION STUDIES

This section describes the procedures used in the mixing and storage of formulations of phenobarbital in 0.25% (w/v) methylcellulose. Also included are the procedures for analyzing dose formulations of phenobarbital in the 5 to 20 mg/mL concentration range for homogeneity and dose verification using the method EDSP.H4-034-01.

### 4.1 Mixing Procedure

The following procedure was used to prepare 500 mL of a 5 mg/mL formulation of phenobarbital in 0.25% (w/v) methylcellulose. For formulations at other concentrations, the amount of test material was adjusted accordingly.

A glass beaker was calibrated to a 500-mL mark including a magnetic stir bar. An amount of the vehicle was added to the beaker. The chemical was weighed in a small beaker also color coded. The chemical was added to the large beaker and the weighing beaker was rinsed 3 times with the vehicle for a quantitative transfer. The mixture was taken to volume with 0.25% (w/v) methylcellulose and stirred for at least 30 minutes, sonicated for 15 minutes and stirred an additional 10 minutes before sampling.

Duplicate 10-mL aliquots were taken from all dose formulations as an analytical and archive aliquot. The remaining formulation was transferred to 5 × 120 mL amber bottles as bulk formulations.

### 4.2 Storage Conditions

Formulations containing phenobarbital in 0.25% (w/v) methylcellulose at concentrations of 5 to 20 mg/mL can be stored for a period of 14 days at refrigerator temperature ( ~ 2 - 8°C) as indicated by the sponsor's stability study.

### 4.3 Concentration Verification

| | | |
|---|---|---|
| RTI Notebook No.: | 11595 | pp.: 7-12, 23-25, 28-29 |
| Vehicle: | 0.25% (w/v) methylcellulose | |
| Concentrations: | 5 mg/mL, 10 mg/mL, 20 mg/mL | |

Concentration verification was performed in dose formulations prepared in two

separate occasions, on September 21, 2005 and October 05, 2005. The lowest formulation from September 21, 2005 (5 mg/mL) was also used for the determination of the method detection limit as indicated in Section 3.3.

#### 4.3.1 Preparation of Calibration Standards

No calibration standards were prepared, solvent standards from the method transfer were used as continued calibration verification (CCV).

#### 4.3.2 Sample preparation

The formulations were removed from the refrigerator and stirred for approximately one hour prior to sample preparation. Duplicate 1-mL aliquots were transferred to tared 25-mL volumetric flasks using a 3-mL syringe equipped with a 3.5 inch needle of a wide bore (16 gauge). The weights were recorded and the formulations diluted with acetonitrile to the 25-mL mark. The samples were mixed by hand, and 0.1 mL were transferred to an autosampler vial and combined with 0.90 mL of mobile phase (50/50 water/acetonitrile). The samples were analyzed singly by HPLC as described in Section 4.3.3.

As indicated previously in Section 3.3, for the formulations prepared on September 21, 2005, the solvent added was 0.99 mL of mobile phase and a correction factor was applied.

#### 4.3.3 High Performance Liquid Chromatographic Analysis

Each blank, continued calibration verification (CCV), and sample was analyzed by single injection using HPLC as described in Table 2.

#### 4.3.4 Calculations

The peak area of phenobarbital was noted for each injection. The found concentration of the phenobarbital in each CCV and sample expressed in µg/mL was determined from the linear regression equation from the method transfer. The amounts found were normalized to 1-g aliquots. The normalized found concentration in µg/mL was adjusted to mg/mL by applying a dilution factor of 250/1000. For the samples formulated on September 21, 2005, the dilution factor was 250/1000* 1.09. The 1.09 was added as a factor to consider the different dilution for these samples. The results were presented in terms of percent recovery based on the nominal concentration and an average of the two aliquots.

#### 4.3.5 Results

The results of these analyses are presented as interim reports in the attachment. See Figures 3 and 4 for typical chromatograms of concentration verification samples.

### 4.4 Homogeneity Analysis

RTI Notebook No.: 11595 pp.: 26-27, 30-33

Vehicle: 0.25% (w/v) methylcellulose
Concentrations: 5 mg/mL, 10 mg/mL, 20 mg/mL

Homogeneity samples were analyzed for the following dose formulations: a) during the first day of dosing (September 29, 2005) for the formulations prepared on September 21, 2005, b) for the last day of dosing (October 11, 2005) for the formulations prepared on September 21, 2005 , and c) for the last day of dosing (October 13, 2005) for the formulations prepared on October 05, 2005.

On the days indicated above, homogeneity samples were provided by RTI Center for Life Sciences and Toxicology (CLST). There were two 10-mL samples from each formulation, collected on top 1/3 and bottom 1/3 of the formulations. The samples were in scintillation vials.

### 4.4.1 Preparation of Calibration Standards

No calibration standards were prepared, solvent standards from the method transfer were used as continued calibration verification (CCV).

### 4.4.2 Sample preparation

The formulations were removed from the refrigerator and stirred for approximately one hour prior to sample preparation. Some of the formulations were prepared for analysis the same day they were received, but they were also stirred approximately one hour prior to sample preparation. Triplicate 1-mL aliquots were transferred to tared 25-mL volumetric flasks using a 3-mL syringe equipped with a 3.5 inch needle of a wide bore (16 gauge). The weights were recorded and the formulations diluted with acetonitrile to the 25-mL mark. The samples were mixed by hand, and 0.1 mL were transferred to an autosampler vial and combined with 0.90 mL of mobile phase (50/50 water/acetonitrile). The samples were analyzed singly by HPLC as described in Section 3.4.

### 4.4.3 High Performance Liquid Chromatographic Analysis

Each blank, continued calibration verification (CCV), and sample was analyzed by single injection using HPLC as described in Table 2.

### 4.4.4 Calculations

The peak area of phenobarbital was noted for each injection. The found concentration of the phenobarbital in each CCV and sample expressed in µg/mL was determined from the linear regression equation from the method transfer. The amounts found were normalized to 1-g aliquots. The percent error for the CCV samples was determined in µg/mL. The normalized found concentration in the samples, expressed in µg/mL was adjusted to mg/mL by applying a dilution factor of 250/1000. The results were presented in terms of percent recovery based on the nominal concentration. The precision of the analysis was examined in terms of the relative standard deviation expressed as percent of the mean (%RSD). The value was obtained by averaging actual concentrations of the three replicate dose formulations at

each level for each homogeneity sample. An overall mean for each dose formulation was determined from the six replicates (three top and 3 bottom aliquots).

### 4.4.5 Results

The results of these analyses are presented as interim reports in the attachment.

### 4.4.6 Conclusions

Dose formulations used in animal studies were found to contain 77.0 to 120% of the nominal concentration of phenobarbital from homogeneity samples. The relative standard deviations for six sample analyses were less than or equal to 13%.

## 5.0 CONTRIBUTORS

Personnel contributing to the formulation and analysis of phenobarbital were Randy Price for the preparation of formulations and Nora Castillo for dose formulation analysis.

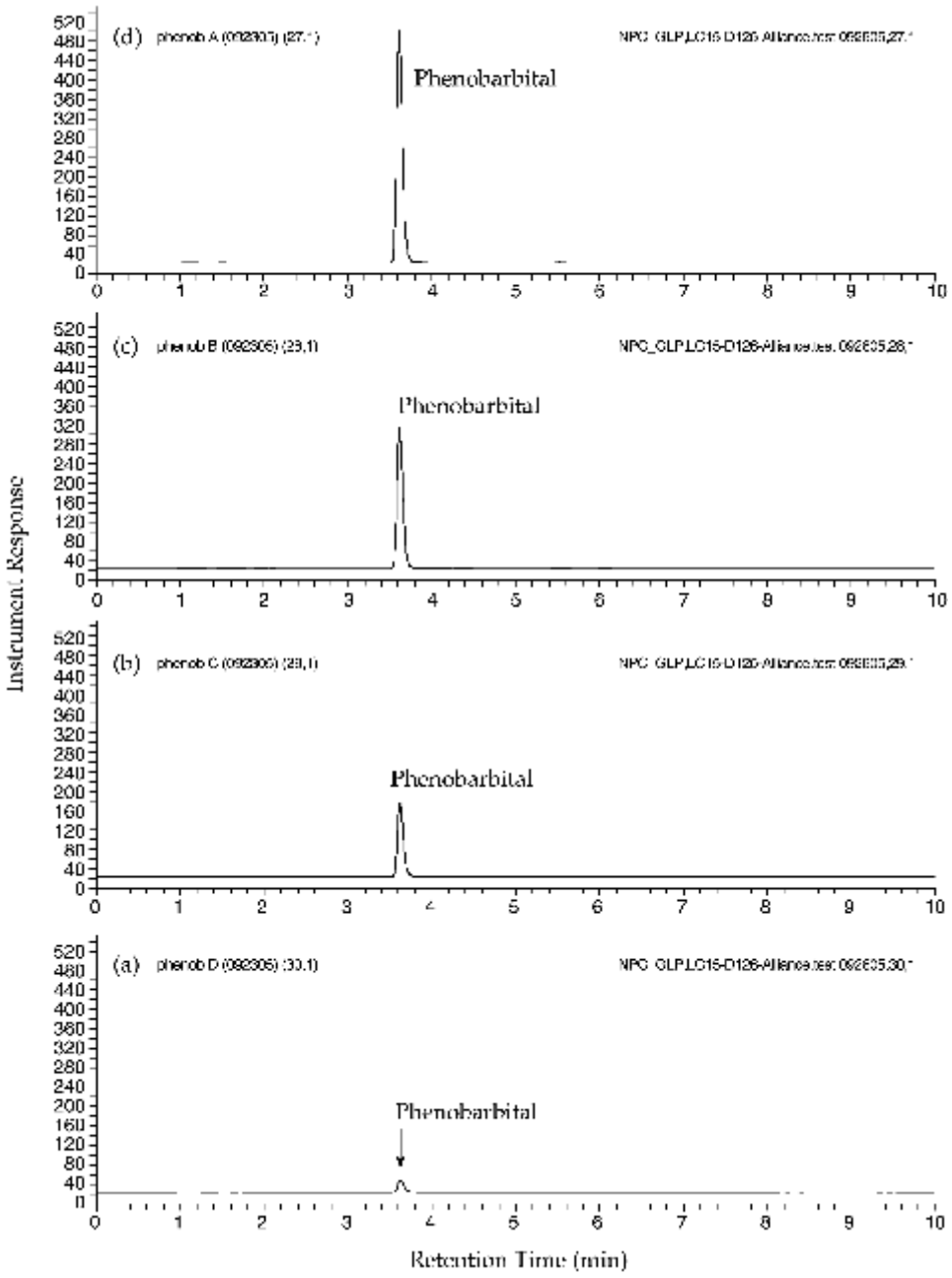



Figure 1. High Performance Liquid Chromatograms of Phenobarbital in 0.25% (w / v) Methylcellulose from Initial Calibration Standards

(a) 10.1 µg / mL; solvent standard
(b) 60.6 µg / mL; solvent standard
(c) 121 µg / mL; solvent standard
(d) 202 µg / mL; solvent standard

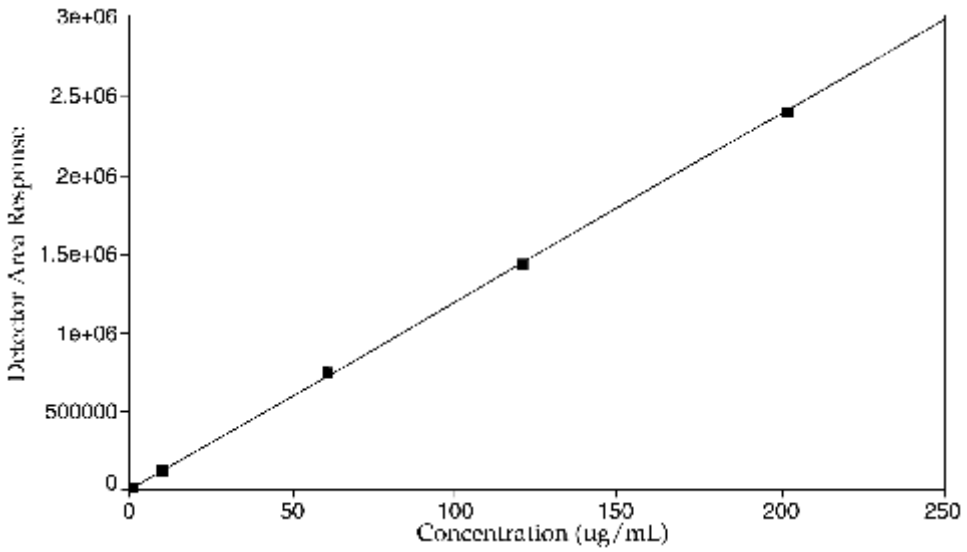



Figure 2. Plot of Initial Calibration Standards Data for Analysis of Dose Formulations of Phenobarbital in 0.25% (w / v) Methylcellulose.

Linear regression Equation: y = -533.0 + 11895 x
Correlation Coefficient (r) : r = 0.99989

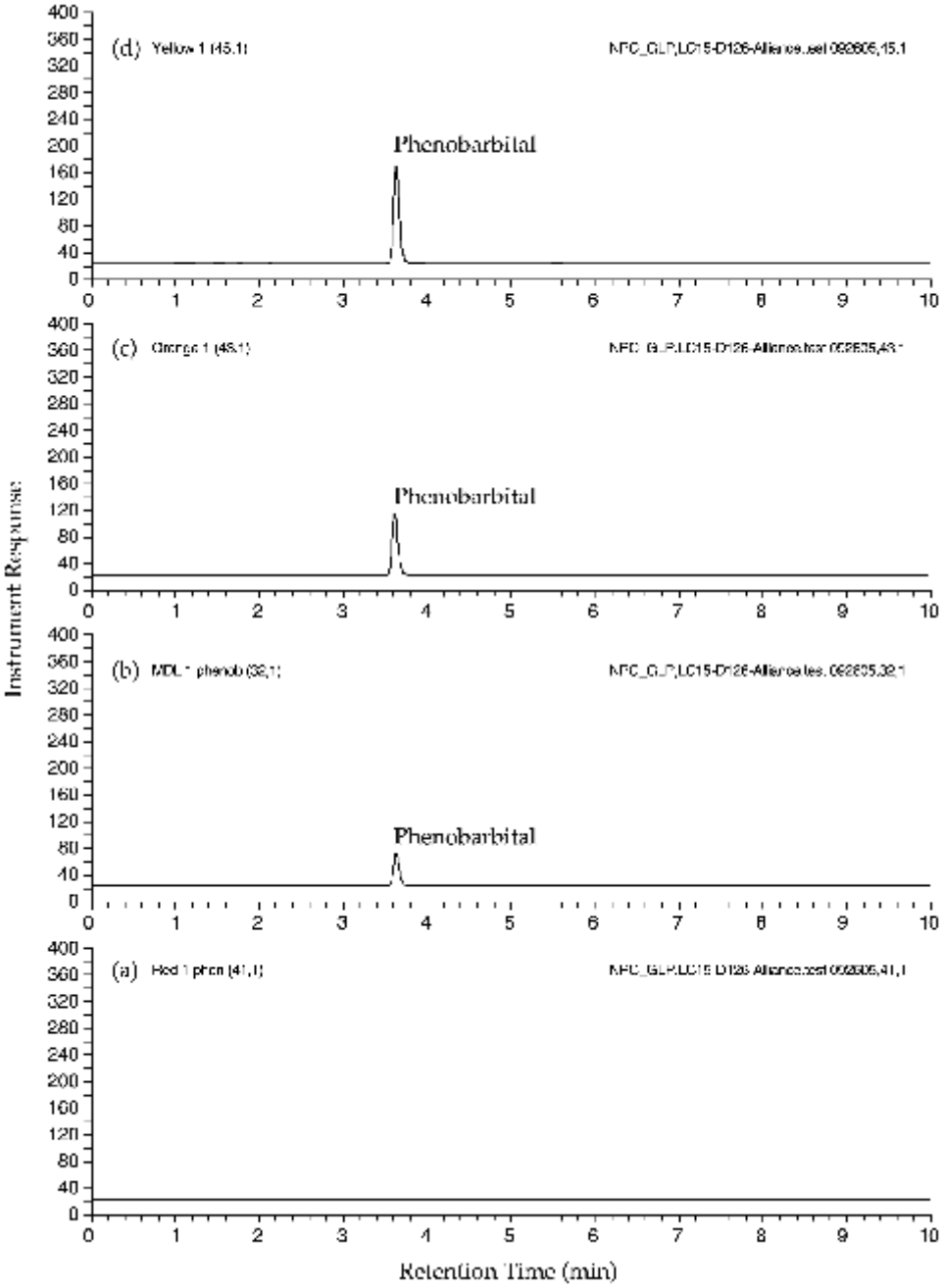



Figure 3. High Performance Liquid Chromatograms of Phenobarbital Dose Formulations in 0.25% (w / v) Methylcellulose (Mix Date: September 21, 2005)

(a) 0 mg / mL; RTI Log No. 10038-14-09
(b) 5.00 mg / mL; RTI Log No. 10038-14-10
(c) 10.0 mg / mL; RTI Log No. 10038-14-11
(d) 20.0 mg / mL; RTI Log No. 10038-14-12

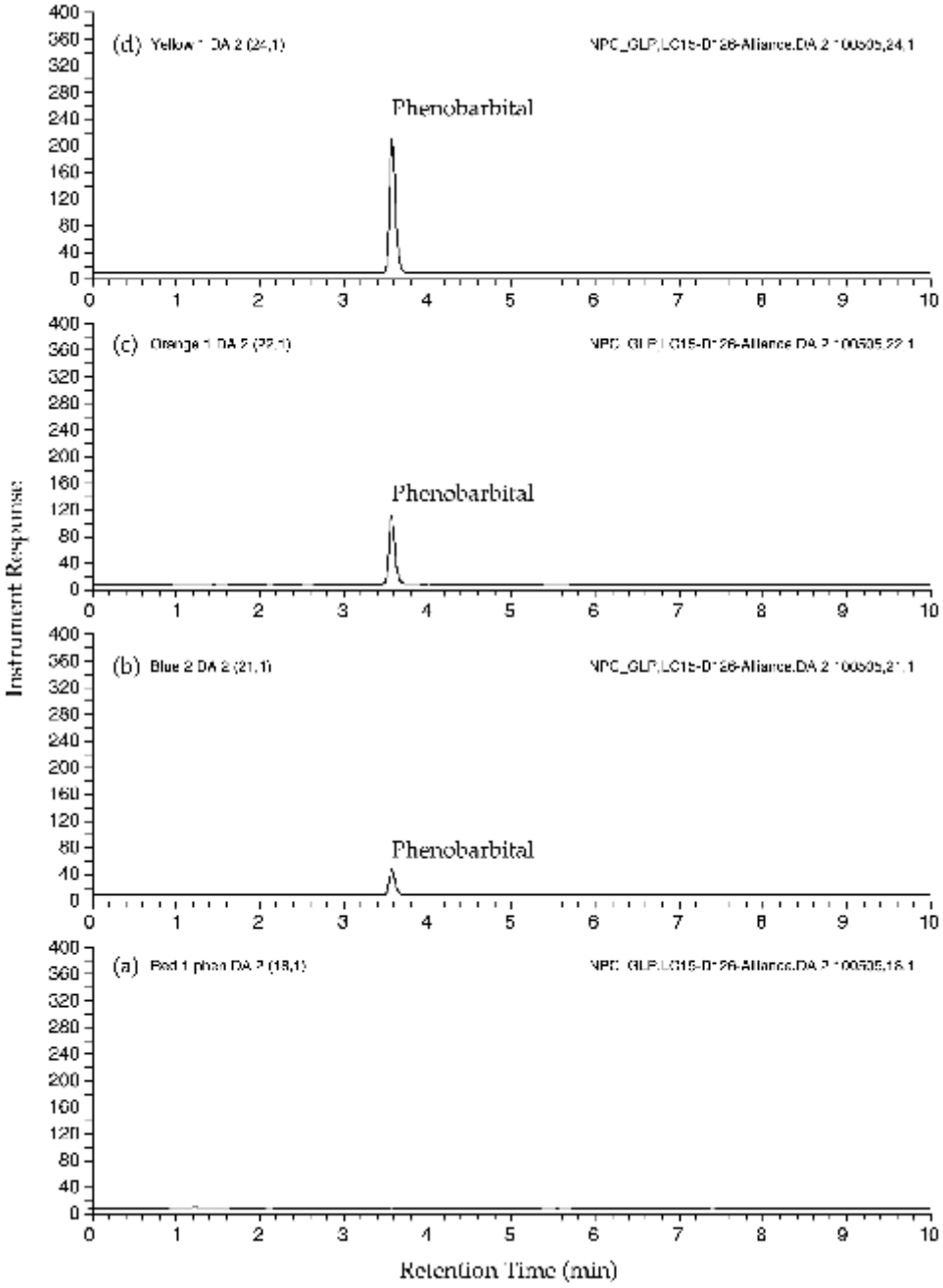



Figure 4. High Performance Liquid Chromatograms of Phenobarbital Dose Formulations in 0.25% (w / v) Methylcellulose (Mix Date: October 05, 2005)

(a) 0 mg / mL; RTI Log No. 10038-20-09
(b) 5.00 mg / mL; RTI Log No. 10038-20-10
(c) 10.0 mg / mL; RTI Log No. 10038-20-11
(d) 20.0 mg / mL; RTI Log No. 10038-20-12

**ATTACHMENT**

**Dosage Analysis Results**

RTI INTERNATIONAL

Memorandum

**FORMULATION ANALYSIS INTERIM REPORT**
**DA1 Formulated September 21, 2005**

**Submission Date: November 18, 2005**

| | | | |
|---|---|---|---|
| Test Chemical (ID): | Phenobarbital | Study Code: | Rt05-ED09 |
| Study Project No.: | 08055.004.040 | RTI ID No.: | 10038-14-09 to 10038-14-12 |
| Lot No.(Vendor): | 104K2600 (Sigma) | Receipt Date: | 09 / 22 / 05 |
| Vehicle: | 0.25% Methylcellulose | Formulation Date: | 09 / 21 / 05 |
| No. of Samples: | 4 x 10 mL | Analysis Date: | 09 / 23 / 05 - 09 / 26 / 05 |

| Study Lab Sample ID | RTI Log Numbers | Nominal Concentration[a] | Found Concentration[a,b] | Percent of Nominal |
|---|---|---|---|---|
| Rx 75171 Red 1 | 10038-14-09 | 0 | ND[c] | NA[d] |
| Rx 75171 Red 2 | 10038-14-09 | 0 | ND | NA |
| Rx 02766 Blue 1 | 10038-14-10 | 5 | 4.06 | 81.2 |
| Rx 02766 Blue 2 | 10038-14-10 | 5 | 4.41 | 88.2 |
| Rx 02766 Blue 3 | 10038-14-10 | 5 | 4.39 | 87.8 |
| Rx 02766 Blue 4 | 10038-14-10 | 5 | 5.01 | 100 |
| Rx 02766 Blue 5 | 10038-14-10 | 5 | 4.69 | 93.8 |
| Rx 02766 Blue 6 | 10038-14-10 | 5 | 4.33 | 86.6 |
| Rx 02766 Blue 7 | 10038-14-10 | 5 | 4.88 | 97.6 |
| | | Mean ± SD (%RSD) | 4.54 ± 0.335 (7.4%) | 90.7 ± 6.65 (7.3%) |
| Rx 64278 Orange 1 | 10038-14-11 | 10 | 8.67 | 86.7 |
| Rx 64278 Orange 2 | 10038-14-11 | 10 | 9.92 | 99.2 |
| | | Avg. (%RSD) | 9.30 (9.5%) | 93.0 (9.5%) |
| Rx 47491 Yellow 1 | 10038-14-12 | 20 | 16.7 | 83.5 |
| Rx 47491 yellow 2 | 10038-14-12 | 20 | 20.8 | 104 |
| | | Mean (%RSD) | 18.8 (15%) | 93.8 (15%) |

[a]Concentration units: mg / mL.
[b]Calibration range was 1.01 ug / mL to 202 ug / mL; Linear regression equation ( weighted 1 / x):
y = -533 + 11895 x; r = 0.9999.
[c]ND = Not detected. LOD = 3.9 µg / mL
[d]NA = Not applicable.

COMMENT: Rx 02766 Blue was used to determine the MDL.= Student's T * SD = 3.143 * 0.335 = 1.05 mg / mL

RTI INTERNATIONAL Memorandum

**FORMULATION ANALYSIS INTERIM REPORT**
**HOMOGENEITY DAY 1 for DA1**

**Submission Date: October 04, 2005**
**Revised Date: November 18, 2005**

| | | | |
|---|---|---|---|
| Test Chemical (ID): | Phenobarbital | Study Code: | Rt05-ED09 |
| Study Project No.: | 08055.004.040 | RTI ID No.: | 10038-17-01 to 10038-17-08 |
| Lot No.(Vendor): | 104K2600 (Sigma) | Receipt Date: | 09 / 29 / 05 |
| Vehicle: | 0.25% Methylcellulose | Formulation Date: | 09 / 21 / 05 |
| No. of Samples: | 8 x 10 mL | Analysis Date: | 09 / 29 / 05 to 09 / 30 / 05 |

| Study Lab Sample ID | RTI Log Numbers | Nominal Concentration[a] | Found Concentration[a,b] | Percent of Nominal |
|---|---|---|---|---|
| Rx 75171 Red 1 T | 10038-17-01 | 0 | ND[c] | NA[d] |
| Rx 75171 Red 2 T | 10038-17-01 | 0 | ND | NA |
| Rx 75171 Red 3 T | 10038-17-01 | 0 | ND | NA |
| Rx 75171 Red 1 B | 10038-17-02 | 0 | ND | NA |
| Rx 75171 Red 2 B | 10038-17-02 | 0 | ND | NA |
| Rx 75171 Red 3 B | 10038-17-02 | 0 | ND | NA |
| Rx 02766 Blue 1 T | 10038-17-03 | 5 | 4.05 | 81.0 |
| Rx 02766 Blue 2 T | 10038-17-03 | 5 | 4.68 | 93.6 |
| Rx 02766 Blue 3 T | 10038-17-03 | 5 | 5.13 | 103 |
| | | Mean ± SD (%RSD) | 4.62 ± 0.542 (12%) | 92.5 ± 11.0 (12%) |
| Rx 02766 Blue 1 B | 10038-17-04 | 5 | 4.65 | 93.0 |
| Rx 02766 Blue 2 B | 10038-17-04 | 5 | 3.48 | 69.6 |
| Rx 02766 Blue 3 B | 10038-17-04 | 5 | 4.63 | 92.6 |
| | | Mean ± SD (%RSD) | 4.25 ± 0.670 (16%) | 85.1 ± 13.4 (16%) |
| | | **DOSE MEAN ± SD (%RSD) (N= 6)** | **4.44 ± 0.581 (13%)** | **88.8 ± 11.7 (13%)** |

(Continued)

| Study Lab Sample ID | RTI Log Numbers | Nominal Concentration[a] | Found Concentration[a,b] | Percent of Nominal |
|---|---|---|---|---|
| Rx 64278 Orange 1 T | 10038-17-05 | 10 | 10.1 | 101 |
| Rx 64278 Orange 2 T | 10038-17-05 | 10 | 9.90 | 99.0 |
| Rx 64278 Orange 3 T | 10038-17-05 | 10 | 8.05 | 80.5 |
| | | Mean ± SD (%RSD) | 9.35 ± 1.13 (12%) | 93.5 ± 11.3 (12%) |
| Rx 64278 Orange 1 B | 10038-17-06 | 10 | 9.95 | 99.5 |
| Rx 64278 Orange 2 B | 10038-17-06 | 10 | 10.0 | 100 |
| Rx 64278 Orange 3 B | 10038-17-06 | 10 | 9.60 | 96.0 |
| | | Mean ± SD (%RSD) | 9.85 ± 0.218 (2.2%) | 98.5 ± 2.18 (2.2%) |
| | | **DOSE MEAN ± SD (%RSD) (N=6)** | **9.60 ± 0.778 (8.1%)** | **96.0 ±7.78 (8.1%)** |
| Rx 47491 Yellow 1 T | 10038-17-07 | 20 | 18.1 | 90.5 |
| Rx 47491 Yellow 2 T | 10038-17-07 | 20 | 18.1 | 90.5 |
| Rx 47491 Yellow 3 T | 10038-17-07 | 20 | 18.1 | 90.5 |
| | | Mean ± SD (%RSD) | 18.1 ± 0.0 (0%) | 90.5 ± 0.0 (0%) |
| Rx 47491 Yellow 1 B | 10038-17-08 | 20 | 17.8 | 89.0 |
| Rx 47491 Yellow 2 B | 10038-17-08 | 20 | 17.9 | 89.5 |
| Rx 47491 Yellow 3 B | 10038-17-08 | 20 | 18.2 | 91.0 |
| | | Mean ± SD (%RSD) | 18.0 ± 0.208 (1.2%) | 89.8 ± 1.04 (1.2%) |
| | | **DOSE MEAN ± SD (%RSD) (N=6)** | **18.0 ± 0.151 (0.84%)** | **90.2 ± 0.753 (0.83%)** |

[a]Concentration units: mg / mL.
[b]Calibration range was 1.01 ug / mL to 202 ug / mL; Linear regression equation ( weighted 1 / x):
y = -533 + 11895 x; r = 0.9999.
[c]ND = Not detected. LOD = 3.9 µg / mL
[d]NA = Not applicable.

RTI INTERNATIONAL

Memorandum

**FORMULATION ANALYSIS INTERIM REPORT**
**HOMOGENEITY DAY 12 for DA1**

**Submission Date: October 13, 2005**
**Revision date: November 18, 2005**

| | | | |
|---|---|---|---|
| Test Chemical (ID): | Phenobarbital | Study Code: | Rt05-ED09 |
| Study Project No.: | 08055.004.040 | RTI ID No.: | 10038-22-01 to 10038-22-08 |
| Lot No.(Vendor): | 104K2600 (Sigma) | Receipt Date: | 10 / 11 / 05 |
| Vehicle: | 0.25% Methylcellulose | Formulation Date: | 09 / 21 / 05 |
| No. of Samples: | 8 x 10 mL | Analysis Date: | 10 / 11 / 05 to 10 / 12 / 05 |

| Study Lab Sample ID | RTI Log Numbers | Nominal Concentration[a] | Found Concentration[a,b] | Percent of Nominal |
|---|---|---|---|---|
| Rx 75171 Red 1 T | 10038-22-01 | 0 | ND[c] | NA[d] |
| Rx 75171 Red 2 T | 10038-22-01 | 0 | ND | NA |
| Rx 75171 Red 3 T | 10038-22-01 | 0 | ND | NA |
| Rx 75171 Red 1 B | 10038-22-02 | 0 | ND | NA |
| Rx 75171 Red 2 B | 10038-22-02 | 0 | ND | NA |
| Rx 75171 Red 3 B | 10038-22-02 | 0 | ND | NA |
| Rx 02766 Blue 1 T | 10038-22-03 | 5 | 5.25 | 105 |
| Rx 02766 Blue 2 T | 10038-22-03 | 5 | 5.45 | 109 |
| Rx 02766 Blue 3 T | 10038-22-03 | 5 | 5.60 | 112 |
| | | Mean ± SD (%RSD) | 5.43 ± 0.176 (3.2%) | 109 ± 3.51 (3.2%) |
| Rx 02766 Blue 1 B | 10038-22-04 | 5 | 4.95 | 99.0 |
| Rx 02766 Blue 2 B | 10038-22-04 | 5 | 3.98 | 79.6 |
| Rx 02766 Blue 3 B | 10038-22-04 | 5 | 4.75 | 95.0 |
| | | Mean ± SD (%RSD) | 4.56 ± 0.512 (11%) | 91.2 ± 10.2 (11%) |
| | | **DOSE MEAN ± SD (%RSD) (N= 6)** | **5.00 ± 0.588 (12%)** | **100 ± 11.8 (12%)** |

(Continued)

| Study Lab Sample ID | RTI Log Numbers | Nominal Concentration[a] | Found Concentration[a,b] | Percent of Nominal |
|---|---|---|---|---|
| Rx 64278 Orange 1 T | 10038-22-05 | 10 | 7.78 | 77.8 |
| Rx 64278 Orange 2 T | 10038-22-05 | 10 | 7.83 | 78.3 |
| Rx 64278 Orange 3 T | 10038-22-05 | 10 | 7.55 | 75.5 |
| | | Mean ± SD (%RSD) | 7.72± 0.149 (1.9%) | 77.2 ± 1.49 (1.9%) |
| Rx 64278 Orange 1 B | 10038-22-06 | 10 | 7.30 | 73.0 |
| Rx 64278 Orange 2 B | 10038-22-06 | 10 | 7.88 | 78.8 |
| Rx 64278 Orange 3 B | 10038-22-06 | 10 | 7.83 | 78.3 |
| | | Mean ± SD (%RSD) | 7.67 ± 0.321 (4.2%) | 76.7 ± 3.21 (4.2%) |
| | | **DOSE MEAN ± SD (%RSD) (N=6)** | **7.70 ± 0.226 (2.9%)** | **77.0 ± 2.26 (2.9%)** |
| Rx 47491 Yellow 1 T | 10038-22-07 | 20 | 19.8 | 99.0 |
| Rx 47491 Yellow 2 T | 10038-22-07 | 20 | 21.1 | 106 |
| Rx 47491 Yellow 3 T | 10038-22-07 | 20 | 20.4 | 102 |
| | | Mean ± SD (%RSD) | 20.4 ± 0.651 (3.2%) | 102 ± 3.51 (3.4%) |
| Rx 47491 Yellow 1 B | 10038-22-08 | 20 | 19.6 | 98.0 |
| Rx 47491 Yellow 2 B | 10038-22-08 | 20 | 18.4 | 92.0 |
| Rx 47491 Yellow 3 B | 10038-22-08 | 20 | 20.2 | 101 |
| | | Mean ± SD (%RSD) | 19.4± 0.917 (4.7%) | 97.0 ± 4.58 (4.7%) |
| | | **DOSE MEAN ± SD (%RSD) (N=6)** | **19.9 ± 0.909 (4.6%)** | **99.7 ± 4.68 (4.7%)** |

[a]Concentration units: mg / mL.
[b]Calibration range was 1.01 ug / mL to 202 ug / mL; Linear regression equation ( weighted 1 / x): y = -533 + 11895 x; r = 0.9999.
[c]ND = Not detected. LOD = 3.9 µg/mL; MDL = 1.05 mg/mL.
[d]NA = Not applicable.

COMMENT: No new calibration curve was necessary, only continued calibration verification (CCV) standards. All doses had good reproducibility and the means are within 70 to 130% of nominal.

RTI INTERNATIONAL

Memorandum

**FORMULATION ANALYSIS INTERIM REPORT**
**DA2 Formulated October 05,2005**

**Submission Date: October 06, 2005**
**Revised Date: November 18, 2005**

| | | | |
|---|---|---|---|
| Test Chemical (ID): | Phenobarbital | Study Code: | Rt05-ED09 |
| Study Project No.: | 08055.004.040 | RTI ID No.: | 10038-20-09 to 10038-20-12 |
| Lot No.(Vendor): | 104K2600 (Sigma) | Receipt Date: | 10 / 05 / 05 |
| Vehicle: | 0.25% Methylcellulose | Formulation Date: | 10 / 05 / 05 |
| No. of Samples: | 4 x 10 mL | Analysis Date: | 10 / 05 / 05 - 10 / 06 / 05 |

| Study Lab Sample ID | RTI Log Numbers | Nominal Concentration[a] | Found Concentration[a,b] | Percent of Nominal |
|---|---|---|---|---|
| Rx 75171 Red 1 | 10038-20-09 | 0 | ND[c] | NA[d] |
| Rx 75171 Red 2 | 10038-20-09 | 0 | ND | NA |
| Rx 02766 Blue 1 | 10038-20-10 | 5 | 4.63 | 92.6 |
| Rx 02766 Blue 2 | 10038-20-10 | 5 | 3.65 | 73.0 |
| Rx 02766 Blue 3 | 10038-20-10 | 5 | 4.60 | 92.0 |
| | | Mean (%RSD) | 4.29 (13%) | 85.9 (13%) |
| Rx 64278 Orange 1 | 10038-20-11 | 10 | 8.80 | 88.0 |
| Rx 64278 Orange 2 | 10038-20-11 | 10 | 9.25 | 92.5 |
| | | Avg. (%RPD) | 9.03 (3.5%) | 90.3 (3.5%) |
| Rx 47491 Yellow 1 | 10038-20-12 | 20 | 18.3 | 91.5 |
| Rx 47491 Yellow 2 | 10038-20-12 | 20 | 13.5 | 67.5 |
| Rx 47491 yellow 3 | 10038-20-12 | 20 | 19.8 | 99.0 |
| | | Mean (%RSD) | 17.2 (19%) | 86.0 (19%) |

[a]Concentration units: mg / mL.
[b]Calibration range was 1.01 ug / mL to 202 ug / mL; Linear regression equation ( weighted 1 / x):
y = -533 + 11895 x; r = 0.9999.
[c]ND = Not detected. LOD = 3.9 µg / mL; MDL = 1.05 mg / mL.
[d]NA = Not applicable.

COMMENT: Another aliquot of Rx 02766 Blue and Rx 47491 Yellow was analyzed to improve the variation between sample aliquots. The three aliquots are included in the report.

RTI INTERNATIONAL

Memorandum

**FORMULATION ANALYSIS INTERIM REPORT**
**HOMOGENEITY LAST DAY for DA2**

**Submission Date: November 03, 2005**

| | | | |
|---|---|---|---|
| Test Chemical (ID): | Phenobarbital | Study Code: | Rt05-ED09 |
| Study Project No.: | 08055.004.040 | RTI ID No.: | 10038-26-01 to 10038-26-08 |
| Lot No.(Vendor): | 104K2600 (Sigma) | Receipt Date: | 10 / 13 / 05 |
| Vehicle: | 0.25% Methylcellulose | Formulation Date: | 10 / 05 / 05 |
| No. of Samples: | 8 x 10 mL | Analysis Date: | 10 / 17 / 05 to 10 / 18 / 05 |

| Study Lab Sample ID | RTI Log Numbers | Nominal Concentration[a] | Found Concentration[a,b] | Percent of Nominal |
|---|---|---|---|---|
| Rx 75171 Red 1 T | 10038-26-01 | 0 | ND[c] | NA[d] |
| Rx 75171 Red 2 T | 10038-26-01 | 0 | ND | NA |
| Rx 75171 Red 3 T | 10038-26-01 | 0 | ND | NA |
| Rx 75171 Red 1 B | 10038-26-02 | 0 | ND | NA |
| Rx 75171 Red 2 B | 10038-26-02 | 0 | ND | NA |
| Rx 75171 Red 3 B | 10038-26-02 | 0 | ND | NA |
| Rx 02766 Blue 1 T | 10038-26-03 | 5 | 4.65 | 93.0 |
| Rx 02766 Blue 2 T | 10038-26-03 | 5 | 4.90 | 98.0 |
| Rx 02766 Blue 3 T | 10038-26-03 | 5 | 5.15 | 103 |
| | | Mean ± SD (%RSD) | 4.90 ± 0.250 (5.1%) | 98.0 ± 5.00 (5.1%) |
| Rx 02766 Blue 1 B | 10038-26-04 | 5 | 5.28 | 106 |
| Rx 02766 Blue 2 B | 10038-26-04 | 5 | 5.25 | 105 |
| Rx 02766 Blue 3 B | 10038-26-04 | 5 | 5.18 | 104 |
| | | Mean ± SD (%RSD) | 5.24 ± 0.0513 (0.98%) | 105 ± 1.00 (0.95%) |
| | | **DOSE MEAN ± SD (%RSD) (N= 6)** | **5.07 ± 0.245 (4.8%)** | **102 ± 5.01 (4.9%)** |

(Continued)

| Study Lab Sample ID | RTI Log Numbers | Nominal Concentration[a] | Found Concentration[a,b] | Percent of Nominal |
|---|---|---|---|---|
| Rx 64278 Orange 1 T | 10038-26-05 | 10 | 9.73 | 97.3 |
| Rx 64278 Orange 2 T | 10038-26-05 | 10 | 10.1 | 101 |
| Rx 64278 Orange 3 T | 10038-26-05 | 10 | 10.1 | 101 |
| | | Mean ± SD (%RSD) | 9.98 ± 0.214 (2.1%) | 99.8 ± 2.14 (2.1%) |
| Rx 64278 Orange 1 B | 10038-26-06 | 10 | 9.45 | 94.5 |
| Rx 64278 Orange 2 B | 10038-26-06 | 10 | 9.60 | 96.0 |
| Rx 64278 Orange 3 B | 10038-26-06 | 10 | 10.3 | 103 |
| | | Mean ± SD (%RSD) | 9.78 ± 0.454 (4.6%) | 97.8 ± 4.54 (4.6%) |
| | | **DOSE MEAN ± SD (%RSD) (N=6)** | **9.88 ± 0.334 (3.4%)** | **98.8 ± 3.34 (3.4%)** |
| Rx 47491 Yellow 1 T | 10038-26-07 | 20 | 22.1 | 111 |
| Rx 47491 Yellow 2 T | 10038-26-07 | 20 | 24.4 | 122 |
| Rx 47491 Yellow 3 T | 10038-26-07 | 20 | 24.9 | 125 |
| | | Mean ± SD (%RSD) | 23.8 ±1.49 (6.3%) | 119 ± 7.37 (6.2%) |
| Rx 47491 Yellow 1 B | 10038-26-08 | 20 | 24.6 | 123 |
| Rx 47491 Yellow 2 B | 10038-26-08 | 20 | 23.0 | 115 |
| Rx 47491 Yellow 3 B | 10038-26-08 | 20 | 24.6 | 123 |
| | | Mean ± SD (%RSD) | 24.1 ± 0.920 (3.8%) | 120 ± 4.62 (3.9%) |
| | **DOSE MEAN ± SD (%RSD) (N=6)** | | **23.9 ± 1.12 (4.7%)** | **120 ± 5.53 (4.6%)** |

[a]Concentration units: mg / mL.
[b]Calibration range was 1.01 ug / mL to 202 ug / mL; Linear regression equation ( weighted 1 / x): y = -533 + 11895 x; r = 0.9999.
[c]ND = Not detected. LOD = 3.9 µg/mL; MDL = 1.05 mg/mL
[d]NA = Not applicable.

COMMENT: No new calibration curve was necessary, only continued calibration verification (CCV) standards. All doses had good reproducibility and the means are within 70 to 130% of nominal.

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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: 1/12/06

## 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<br>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

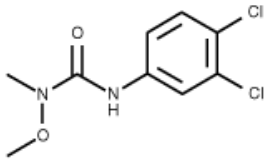
Experimental Start: September 14, 2005

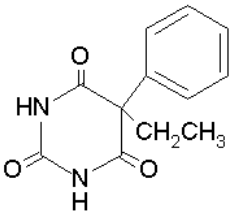
Experimental Termination: October 6, 2005

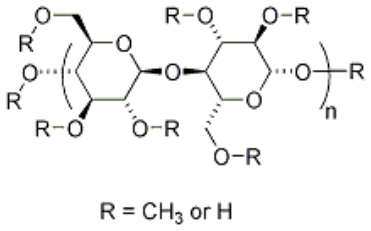
## 6.0 EXECUTIVE SUMMARY

# 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* | |

*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**

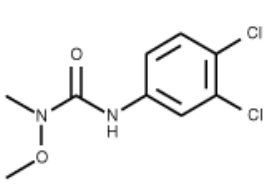
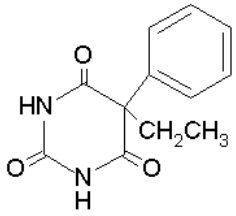
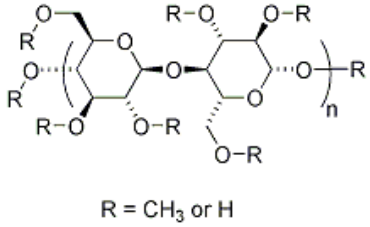
| Parameter | Test Substance | Linuron |
|---|---|---|
| Compound Name | Linuron |  |
| CAS # | 330-55-2 | |
| Central File No. | 2463-1 | |
| Initial Receipt Date | 08/24/2005 | |
| Expiration Date | August 2008 | |
| Supplier | Chem Service | |
| Lot Number | 348-8A | |
| Method | EDSP.H4-033 | |

**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 | |

* 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).

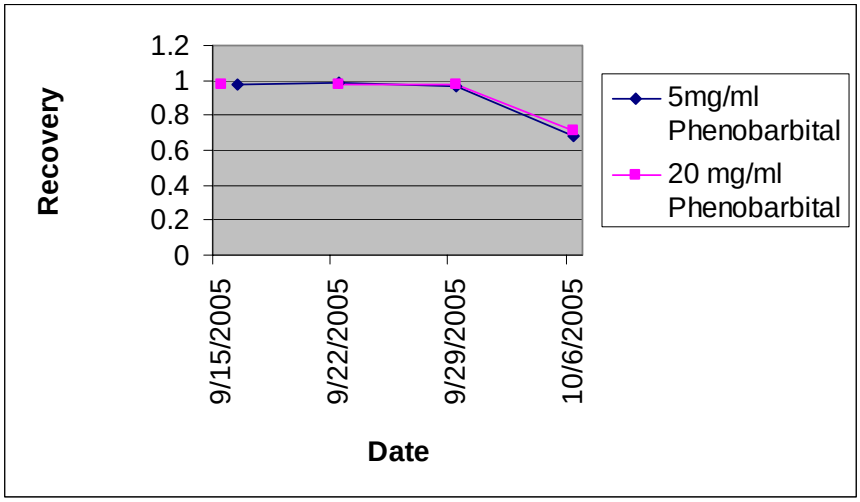



**Figure 1. Recoveries of Phenobarbital Plotted Against Time**

# 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.

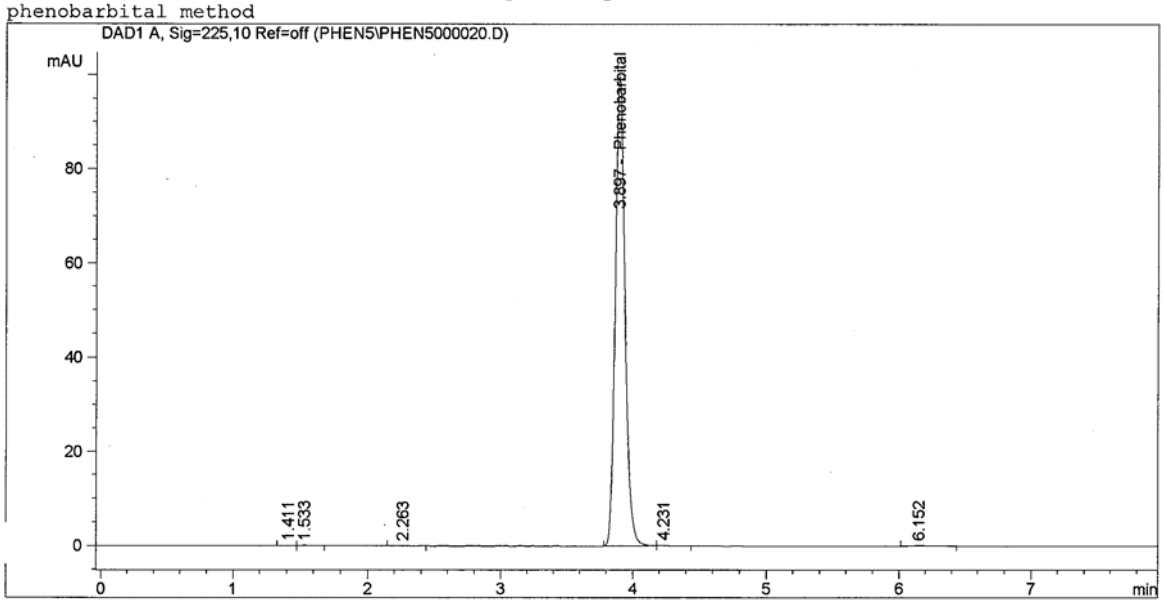
## 11.4 ARCHIVING

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
 cq. 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
```

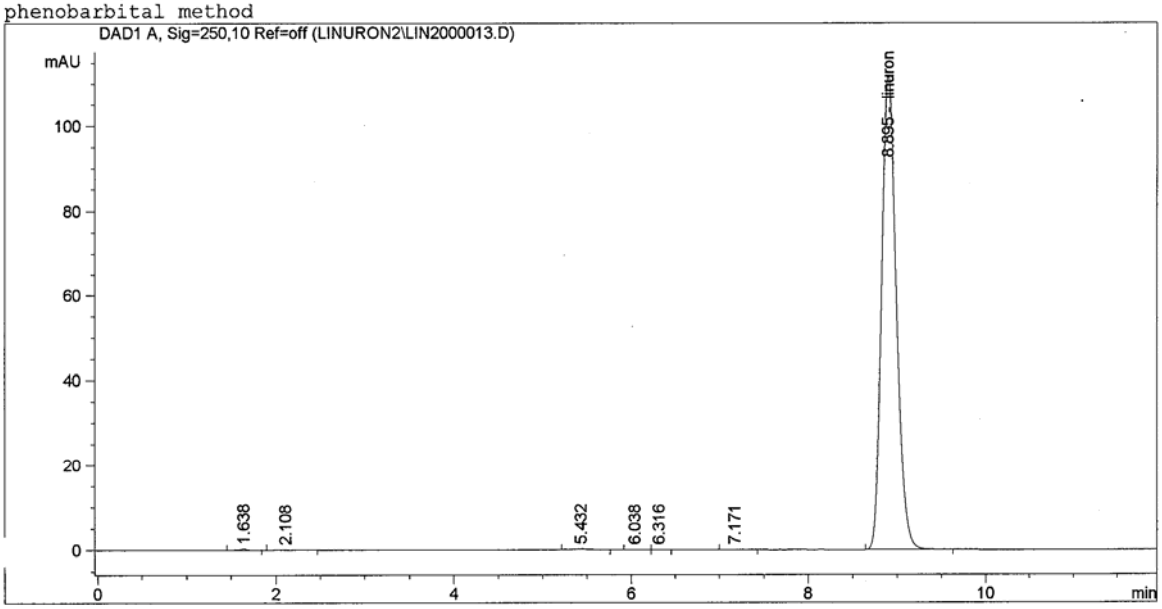



```
==========================================================================
                      External Standard Report
==========================================================================

Sorted By             :      Retention Time
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
```




```
==========================================================================
                      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

==========================================================================
```



**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

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

# 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:**

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

**Address of Sponsor's Representative**

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 $H_20$ | 0.25% Methylcellulose in $H_20$ |
| 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**

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.

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

Approvals:

| | | | |
|---|---|---|---|
| Work Assignment Leader | David P. Hendricks | Date | 9/8/05 |
| Study Director | | Date | 9/12/05 |
| EDSP QA Representative | Mary E Lynn | Date | 9/19/05 |
| MSL Laboratory Director | [illegible] | Date | 9/16/05 |
| EDSP Program Management | David P. Hendricks | Date | 9/8/05 |
| EDSP Battelle QAM | Terri 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
DEVIATION NUMBER: D-1
DATE: January 10, 2006
Page 2 of 3

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 | [signature] | Date 1/12/06 |
| Study Director | [signature] | Date 1/12/06 |
| EDSP QA Representative | [signature] | Date 1/12/06 |
| MSL Laboratory Director | [signature] | Date 1/12/06 |
| EDSP Program Management | [signature] | Date 1/12/06 |
| EDSP Battelle QAM | [signature] | 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:<br>Tim Fortman | Tim Fortman | 9-8-05 |
| | *Signature* | Date |
| TECHNICAL REVIEWER:<br>Linda Bingler | Linda S. Bingler | 9-8-05 |
| | *Signature* | Date |
| STUDY DIRECTOR:<br>Michael Cobb | MMC | 9-8-05 |
| | *Signature* | 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% acetontrile 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 acetontrile. 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 typo MM 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 250 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~~ equal to or than 0.995 is necessary before analysis can begin. inadvertently left out MM 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 ~~4.5.6~~ 4.5.5).

~~Typo~~ MM 1-11-06

typo MM 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

3 1/11/06

**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 $10^{th}$ 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.

Battelle
*The Business of Innovation*

**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:<br>Tim Fortman | Tim Fortman | 10-5-05 |
| | *Signature* | Date |
| TECHNICAL REVIEWER:<br>Linda Bingler | Linda S. Bingler | 10/5/05 |
| | *Signature* | Date |
| STUDY DIRECTOR:<br>Michael Cobb | | 10/05/05 |
| | *Signature* | 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 acetontrile. 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 4.5.5).

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

at least clarity reached 1/11/06

**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 acetontrile. 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 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 the 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.

# 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 VIII

# Histopathology Report

# INTER-LABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL

RTI NO.: 65U-08055.004.040

EPL PROJECT NO.: 229-143

PATHOLOGY REPORT

Submitted to

Research Triangle Institute
P.O. Box 12194
Research Triangle Park, NC 27709

Submitted by

Experimental Pathology Laboratories, Inc.
P. O. Box 12766
Research Triangle Park, NC 27709

January 12, 2006

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RTI Study No. 65U-08055.004.040

# INTER-LABORATORY VALIDATION OF THE 15-DAY ADULT INTACT MALE RAT ASSAY WITH LINURON AND PHENOBARBITAL

RTI NO.: 65U-08055.004.040

EPL PROJECT NO.: 229-143

PATHOLOGY SUMMARY

## INTRODUCTION

The objectives of this study were:

1. To determine if similar results for each endpoint could be obtained among three different laboratories using a similar protocol, compounds, and dose levels.
2. To determine if the observed results in the more current studies are comparable to the expected results established in earlier studies using the same protocol.
3. 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.

For this study, Linuron and Phenobarbital were tested. The testes, epididymides and thyroids were examined microscopically.

## SUMMARY

Administration of the test chemicals by gavage to male Sprague-Dawley CD® rats, under the conditions of this study, was associated with the following histopathologic changes:

1. Minimal seminiferous tubule degeneration within the testis was noted in some high-dose Linuron animals.

2. Minimal to mild mononuclear cell infiltration within the epididymides was noted in some high-dose Phenobarbital animals.

## DESIGN OF THE STUDY

Linuron and Phenobarbital were administered via gavage once daily for 15 consecutive days to male Sprague-Dawley (CD®) rats under the conditions outlined in the study protocol (RTI Master Protocol No.: RTI-956).

The study began with 15 weight-matched males/group. The study design, test chemicals and target dose levels are presented in Table 1.

Table 1. Experimental Design

| Group No. | Number of Males | Chemical | Dose (mg/kg/day)[b] | Concentration (mg/mL) | Dose Volume (mL/kg) |
|---|---|---|---|---|---|
| 1 | 15 | Vehicle Control[a] | 0 | 0.0 | 5 |
| 2 | 15 | Phenobarbital | 25 | 5.0 | 5 |
| 3 | 15 | Phenobarbital | 50 | 10.0 | 5 |
| 4 | 15 | Phenobarbital | 100 | 20.0 | 5 |
| 5 | 15 | Linuron | 50 | 10.0 | 5 |
| 6 | 15 | Linuron | 100 | 20.0 | 5 |
| 7 | 15 | Linuron | 150 | 30.0 | 5 |

[a] 0.25% aqueous methylcellulose, vehicle only
[b] Test compounds administered once daily by gavage on Test Days 1 through 15

Individual treatment groups within the study were given unique five digit codes that are presented in Table 2.

Table 2. Treatment Group Designations

| Group (mg/kg) | Five Digit Code |
|---|---|
| 0 | 75171 |
| Phenobarbital - 25 | 02766 |
| Phenobarbital - 50 | 64278 |
| Phenobarbital - 100 | 47491 |
| Linuron – 50 | 80945 |
| Linuron – 100 | 21297 |
| Linuron - 150 | 34208 |

According to the study protocol, tissues taken at necropsy were placed in fixative and transferred to Experimental Pathology Laboratories, Inc. for processing. Tissues were fixed in formalin except for the testes which were fixed in Bouin's fixative for 24 hours, rinsed, and stored in 70% alcohol. All tissues were trimmed, embedded in paraffin, sectioned and stained with Hematoxylin & Eosin (H&E).

Histopathological examination was conducted on the testes, epididymides and thyroids.

The gross and histopathologic data were entered in EPL's Computerized Pathology Reporting System. Each lesion was graded according to a four grade severity code (1-4).

## RESULTS

The individual animal data are presented by group in the Histopathology Incidence Table (HIT) and the group summary data in the Summary Incidence Table (SIT). Gross necropsy findings were correlated to the microscopic findings, if required. These findings are presented in the section "Correlation of Gross and Microscopic Findings Tables".

According to the organ weight data, the following pertinent organ weight changes were noted:

Phenobarbital

Thyroids – dose-related, increased absolute and relative weights
Liver – dose-related, increased absolute and relative weights
Paired Testes & Epididymides – high-dose, increased relative weights
Prostate, Seminal Vesicle, Accessory Sex Gland – high-dose, increased relative (in general) weights

Linuron

Thyroids – dose-related, increased relative weights

Liver – dose-related, decreased absolute weights

Paired Testes – dose-related, increased relative weights

Paired Epididymides – high-dose, increased relative weights

Prostate, Seminal Vesicle, Accessory Sex Gland – mid and high dose, decreased absolute weights

No related histopathology was detected in the thyroids, testes or epididymides which could account for the weight changes observed.

TREATED-RELATED FINDINGS BY CHEMICAL

Phenobarbital

Administration of 100 mg/kg Phenobarbital was associated with the presence and increased severity of mononuclear cell infiltration within the epididymides (bilateral).

Normally, the epididymis may have one or two small foci of perivascular, mononuclear inflammatory cell infiltrates which are generally not recorded. However, in nearly over half of the high-dose animals, a minimal to mild increased severity was noted. In these cases, the perivascular foci were more numerous, larger and widely distributed. In the mild cases, interstitial edema was also observed particularly in the cauda portion of the epididymis. No changes of the epididymal tubules or epithelium were noted. The pathogenesis of this change was unclear.

Treatment-associated lesions were not observed in the testes or thyroid glands.

The incidence and severity of the mononuclear cell infiltrates in the epididymides are presented in Table 3.

Table 3 – Incidence an Severity of Epididymal Mononuclear Cell Infiltration

| Dose (mg/kg) | 0 | 100 |
|---|---|---|
| Epididymis (No. Examined) | (15) | (13) |
| Normal | 15 | 6 |
| Infiltrative Cell, Mononuclear Cell, Bilateral | 0 | 7 |
| Minimal | 0 | 4 |
| Mild | 0 | 3 |

Linuron

Administration of 150 mg/kg Linuron was associated with a slight increased incidence of seminiferous tubule degeneration within the testes.

This change was characterized by a bilateral (2 out of 3 cases) degeneration of seminiferous tubules which was minimal in severity and consisted of one or more tubules which had thinning of spermatogenic epithelium and/or vacuolization within the spermatogenic epithelium which was accompanied by a few degenerative spermatogonia cells. This change involved only a few tubules. No decrease in sperm or desquamated degenerative spermatogonial cells was noted in the epididymal tubules.

The incidence and severity of the seminiferous tubule degeneration is presented in Table 4.

Table 4 – Incidence and Severity of Seminiferous Tubules Degeneration:

| Dose (mg/kg) | 0 | 150 |
|---|---|---|
| Testis (No. Examined) | (15) | (14) |
| Normal | 15 | 11 |
| Degeneration, Seminiferous Tubule, Bilateral | 0 | 2 |
| Minimal | 0 | 2 |
| Degeneration, Seminiferous Tubule, Unilateral | 0 | 1 |
| Minimal | 0 | 1 |

John Curtis Seely, DVM

JOHN CURTIS SEELY, D.V.M.
Diplomate, ACVP
Senior Pathologist

January 12, 2006
Date

**EXPERIMENTAL PATHOLOGY LABORATORIES, INC.**

**QUALITY ASSURANCE FINAL CERTIFICATION**

Study Title: Inter-Laboratory Validation of the 15-Day Adult Intact Male Rat Assay with Linuron and Phenobarbital

Client Study: 65U-08055.004.040; RTI-956; Rt05-ED09 EPL Project Coordinator: Dr. John Curtis Seely

EPL Project Number: 229-143 EPL Pathologist: Dr. John Curtis Seely

The following aspects of this study were inspected by the Quality Assurance Unit of Experimental Pathology Laboratories, Inc. Dates inspections were performed and findings reported to the EPL Project Coordinator and Management are indicated below.

| | Dates | |
|---|---|---|
| Area Inspected | Inspection | Reporting |
| EPL Project Sheets | October 7, 2005 | October 7, 2005 |
| Project Setup | October 21, 2005 | October 21, 2005 |
| Histology Setup | October 24, 2005 | October 24, 2005 |
| Data Review | November 16, 2005 | November 16, 2005 |
| Draft Report | December 1, 2005 | December 1, 2005 |
| Final Report | January 12, 2006 | January 12, 2006 |

Date Reported to Study Director/Management January 12, 2006

Date of last quarterly facility inspection: October 2005

James J. Hollingsworth
EPL Quality Assurance Unit

January 12, 2006
Date



Form No. 6-2 (09/10/99)

## SUMMARY INCIDENCE TABLES

## Day 15

# SUMMARY INCIDENCE TABLE

RTI-956
15-Day Sacrifice
Male Rat

| | GROUP 75171 | GROUP 47491 | GROUP 34208 | | | |
|---|---|---|---|---|---|---|
| EPIDIDYMIS (NO. EXAMINED) | (15) | (13) | (14) | | | |
| Infiltrative Cell, Mononuclear | | | | | | |
| Cell, Bilateral | | 7 | | | | |
| | | | | | | |
| TESTIS (NO. EXAMINED) | (15) | (13) | (14) | | | |
| Degeneration, Seminiferous | | | | | | |
| Tubule, Bilateral | | | 2 | | | |
| Degeneration, Seminiferous | | | | | | |
| Tubule, Unilateral | | 1 | 1 | | | |
| | | | | | | |
| THYROID (NO. EXAMINED) | (15) | (13) | (14) | | | |

# HISTOPATHOLOGY INCIDENCE TABLES

## Day 15

# HISTOPATHOLOGY INCIDENCE TABLE

RTI-956
15-Day Sacrifice
Male Rat

| | GROUP 75171 | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ANIMAL | 1 | 3 | 5 | 7 | 9 | 11 | 13 | 15 | 107 | 109 | 111 | 113 | 115 | 117 | 119 | | | | | |
| EPIDIDYMIS | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | | | | | |
| Infiltrative Cell, Mononuclear | | | | | | | | | | | | | | | | | | | | |
| Cell, Bilateral | | | | | | | | | | | | | | | | | | | | |
| TESTIS | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | | | | | |
| Degeneration, Seminiferous | | | | | | | | | | | | | | | | | | | | |
| Tubule, Bilateral | | | | | | | | | | | | | | | | | | | | |
| Degeneration, Seminiferous | | | | | | | | | | | | | | | | | | | | |
| Tubule, Unilateral | | | | | | | | | | | | | | | | | | | | |
| THYROID | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | | | | | |



EPL
Experimental Pathology Laboratories, Inc.

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=mild 3=moderate 4=severe
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

# HISTOPATHOLOGY INCIDENCE TABLE

RTI-956
15-Day Sacrifice
Male Rat

GROUP
47491

| ANIMAL | 47 | 49 | 53 | 55 | 57 | 59 | 151 | 153 | 155 | 157 | 159 | 161 | 163 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| EPIDIDYMIS | X | X | | X | X | X | | | | X | | | |
| Infiltrative Cell, Mononuclear | | | | | | | | | | | | | |
| Cell, Bilateral | | | 2 | | | | 1 | 2 | 1 | | 2 | 1 | 1 |
| | | | | | | | | | | | | | |
| TESTIS | | X | X | X | X | X | X | X | X | X | X | X | X |
| Degeneration, Seminiferous | | | | | | | | | | | | | |
| Tubule, Bilateral | | | | | | | | | | | | | |
| Degeneration, Seminiferous | | | | | | | | | | | | | |
| Tubule, Unilateral | 1 | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| THYROID | X | X | X | X | X | X | X | X | X | X | X | X | X |





Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=mild 3=moderate 4=severe
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

# HISTOPATHOLOGY INCIDENCE TABLE

RTI-956
15-Day Sacrifice
Male Rat

GROUP
34208

| ANIMAL | 91 | 93 | 95 | 97 | 99 | 101 | 103 | 105 | 197 | 199 | 201 | 205 | 207 | 209 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| EPIDIDYMIS | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Infiltrative Cell, Mononuclear | | | | | | | | | | | | | | |
| Cell, Bilateral | | | | | | | | | | | | | | |
| TESTIS | X | | | X | X | X | X | X | | X | X | X | X | X |
| Degeneration, Seminiferous | | | | | | | | | | | | | | |
| Tubule, Bilateral | | 1 | 1 | | | | | | | | | | | |
| Degeneration, Seminiferous | | | | | | | | | | | | | | |
| Tubule, Unilateral | | | | | | | | | 1 | | | | | |
| THYROID | X | X | X | X | X | X | X | X | X | X | X | X | X | X |





Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=mild 3=moderate 4=severe
P=Present B=Benign M=Malignant
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# CORRELATION OF GROSS AND MICROSCOPIC FINDINGS TABLES

## Day 15

RTI-956
15-Day Sacrifice

## CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rat Sex: Males Group Identification: 75171

| Animal Number | Client Topography / Site | Client Gross Observations | Microscopic Observations |
|---|---|---|---|
| 15 | KIDNEY | Right, hydronephrosis | No Comment Required |
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RTI-956
15-Day Sacrifice

## CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rat Sex: Males Group Identification: 47491

| Animal Number | Client Topography / Site | Client Gross Observations | Microscopic Observations |
|---|---|---|---|
| 155 | PROSTATE | Left lobe, missing, ventral | No Comment Required |
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RTI-956
15-Day Sacrifice

## CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rat Sex: Males Group Identification: 34208

| Animal Number | Client Topography / Site | Client Gross Observations | Microscopic Observations |
|---|---|---|---|
| 103 | KIDNEY | Right, hydronephrosis | No Comment Required |
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