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SafePharm Laboratories

DIBROMOMETHANE:

ORAL (GAVAGE) REPRODUCTION/DEVELOPMENT TOXICITY SCREENING TEST IN THE RAT

SPL PROJECT NUMBER: 0466/0261

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QUALITY ASSURANCE REPORT

The conduct of this study has been subject to periodic inspection by Safepharm Quality Assurance Unit.

This report has been audited by Safepharm Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

Unless otherwise indicated, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation and inspections were as follows:

	13 September 2006	Protocol Compliance Audit
	27 October 2006	Test Material Preparation
	26 October 2006	Dosing
	28 September 2006	Animal Preparation
	07 October 2006	Parental Observations
	20 October 2006	Smearing
	02 October 2006	Chemical Analysis
	21 November 2006	Litter Observations
	17 November 2006	Post Mortem
ϕ	13 June 2006	Histology (reported to management on 21 June 2006)
	21 March 2007	Draft Report Audit
	Date of QA Signature	Final Report Audit

ϕ Facility based audit by Propath UK Ltd QAU

..... DATE: 19 JUN 2007

For Safepharm Quality Assurance Unit*

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GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations (SI 1999/3106 as amended by SI 2004/0994)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 2004/9/EC and 2004/10/EC.

These international standards are acceptable to the Regulatory agencies of the following countries: Australia, Austria, Belgium, Canada, the Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Japan, Republic of Korea, Luxembourg, Mexico, The Netherlands, New Zealand, Norway, Poland, Portugal, Slovenia, South Africa, Spain, Sweden, Switzerland, Turkey, the United Kingdom, and the United States of America. Specifically, these GLP standards are acceptable to the United States Environmental Protection Agency, fulfilling the requirements of 40 CFR Part 792, as amended.

This report fully and accurately reflects the procedures used and data generated.

.....DATE: 19 JUN 2007

N K Dhinsa MSc BSc (Hons)
Study Director

Approved for issue:

.....DATE: 19 June 2007

E Wood CBiol MIBiol
Head of Toxicology

RESPONSIBLE PERSONNEL

The following scientific and supervisory personnel were involved in the study.

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DIBROMOMETHANE:

ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

SUMMARY

Introduction. The study was performed to screen for potential adverse effects on reproduction including embryo/foetal development and provides an initial hazard assessment for effect on reproduction. The study complies with the recommendations of the TSCA test guideline 40 CFR 799.9355, March 16, 2006 (comparable to the OECD Guidelines for Testing of Chemicals No. 421 "Reproduction/Developmental Toxicity Screening Test" (adopted 27 July 1996 and are in accordance with the EPA Health Effects Test Guidelines: OPPTS 870.3550 Reproduction/Developmental Toxicity Screening Test, July 2000).

Methods. The test material was administered by gavage to three groups each of ten male and ten female Sprague-Dawley Crl:CD® (SD) IGS BR strain rats, for approximately forty days (to include a two week maturation phase, pairing, gestation and early lactation) at dose levels of 500, 150 and 50 mg/kg/day. A control group of ten males and ten females was similarly dosed with vehicle alone (Polyethylene glycol 400).

Clinical signs, bodyweight development, food and water consumption were monitored during the study.

Pairing of animals within each dose group was undertaken on a one male: one female basis on Day 15 of the study, with females subsequently being allowed to litter and rear their offspring to Day 5 of lactation.

During the lactation phase, daily clinical observations were performed on all surviving offspring, together with litter size, offspring weights and assessment of righting reflex.

Surviving males were terminated on Day 43 of the study, with all the surviving females and offspring being killed on Day 5 *post partum*. All animals were subjected to a gross necropsy examination and histopathological evaluation of reproductive tissues was performed.

Results.

Mortality One male, receiving 500 mg/kg/day, was killed *in extremis* on Day 2 of the study and one control female was found dead on Day 41 of the study; neither of these deaths were considered to be a consequence of treatment.

Clinical observations There were no significant treatment-related clinical signs of toxicity observed at 50, 150 or 500 mg/kg/day.

Bodyweights At 500 mg/kg/day, lower bodyweight gain was apparent for females during gestation, compared to control, but this was considered to be a consequence of the lower litter size at this dosage. No similar effects were apparent for animals at 50 or 150 mg/kg/day.

Food consumption and food conversion efficiency At 500 mg/kg/day, lower food conversion efficiency was apparent for females during gestation, compared to control, reflecting the lower weight gain observed during this phase of the study. Food intake was lower during lactation and was attributed to the lower physiological demand on parent females due to the smaller litter size. No similar effects were apparent for animals at 50 or 150 mg/kg/day.

Mating performance, fertility and gestation length At 500 mg/kg/day, there was a clear increase in pre-coital interval, compared with control, with only four females mating during the first four days of pairing. Copulation plug count was generally lower than control, but fertility was not adversely affected with eight females achieving pregnancy. Gestation length of these females tended to be longer than control, possibly influenced by lower litter size.

At 50 or 150 mg/kg/day, mating performance, fertility and gestation length were considered to be unaffected by treatment.

Litter responses At 500 mg/kg/day, litter size was lower than control on Day 1 due to increased post-implantation loss; this loss probably representing death of the offspring *in utero*. In addition, one female at this dosage was considered to have lost her litter *in-utero*. No similar treatment related effects were apparent at 50 or 150 mg/kg/day.

At 50, 150 or 500 mg/kg/day, offspring bodyweight on Day 1 and subsequent survival, growth and development to Day 4 were unaffected by maternal treatment; lower litter weights at 500 mg/kg/day reflected the lower litter size. One control female and one female receiving 500 mg/kg/day showed total litter loss on Day 1 *post partum*.

Necropsy Necropsy findings among adult animals and their offspring did not indicate any effect of maternal treatment at dosages of 50, 150 or 500 mg/kg/day.

Organ weights At 500 mg/kg/day, males showed lower absolute and bodyweight-relative epididymal and testes weights compared to control. At 150 mg/kg/day, absolute and bodyweight-relative testes weights were also lower than control. In the absences of any treatment-related histopathological effects at 500 mg/kg/day, these differences in male reproductive organ weights were considered to be of equivocal toxicological significance.

Histopathology Microscopic examination of adult reproductive tissues did not reveal any treatment-related effects at dosages of 50, 150 or 500 mg/kg/day.

Conclusion Treatment with Dibromomethane at 500 mg/kg/day was associated with a clear effect on mating performance and a reduction in litter size at birth, most probably due to increased *in-utero* mortality. The 'No Observed Adverse Effect Level' (NOAEL) for reproduction observed in this study was considered to be 150 mg/kg/day.

DIBROMOMETHANE:

ORAL (GAVAGE) REPRODUCTION/DEVELOPMENT TOXICITY SCREENING TEST IN THE RAT

1. INTRODUCTION

The study was designed to screen for potential adverse effects of the test material on reproduction, including offspring development, following repeated oral administration to the Sprague-Dawley Crl:CD® rat for approximately forty days (including a two week maturation phase, pairing, gestation and early lactation), at dose levels of 500, 150 and 50 mg/kg/day.

The study was designed to comply with the recommendations of the TSCA test guideline 40 CFR 799.9355, March 16, 2006 (comparable to the OECD Guidelines for Testing of Chemicals, No. 421: "Reproduction/Developmental Toxicity Screening Test" (adopted 27 July, 1996) and are in accordance with the EPA Health Effects Test Guidelines: OPPTS 870.3550 Reproduction/Developmental Toxicity Screening Test, July 2000.

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations (SI 1999/3106 as amended by SI 2004/0994)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 2004/9/EC and 2004/10/EC. Specifically, these GLP standards are acceptable to the United States Environmental Protection Agency fulfilling the requirements of 40 CFR Part 792, as amended.

The rat was selected for this study as it is a readily available rodent species historically used in safety evaluation studies and is acceptable to appropriate regulatory authorities.

The dose levels were chosen based on the results of a preliminary range-finder performed as part of the study and presented in Appendix 21 of this report. The oral route was selected as the most appropriate route of exposure, based on the physical properties of the test material, and the results of the study are believed to be of value in screening potential adverse effects on reproduction.

The study was performed between 11 September 2006 and 24 January 2007.

2. TEST MATERIAL AND EXPERIMENTAL PREPARATION

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	Dibromomethane
Description	:	Clear colourless liquid
Purity	:	99.4%
CAS number	:	74-95-3
Lot numbers	:	DBM082506 and 20050298
Date received	:	1st September 2006 and 14th September 2006
Storage conditions	:	Room temperature in the dark

Individual samples of Dibromomethane were supplied by both Co-Sponsors. These two test materials were then mixed in equal proportions and the resulting mixture analysed to determine its purity at Safepharm Laboratories Ltd (SPL Project Number 0466/0267). This mixed test material was used for all experimental testing detailed in this report.

The integrity of supplied data relating to the identity and stability of the test material is the responsibility of the sponsor. The purity of the test material was determined by Safepharm Laboratories (SPL Project Number 0466/0267) and a Certificate of Analysis is presented in Addendum 3.

2.2 Preparation of Test Material

For the purpose of the study, the test material was prepared at the appropriate concentrations as a solution in polyethylene glycol 400. The stability and homogeneity of the test material formulations were determined by Safepharm Analytical Laboratory. Results are given in Appendix 20 and show the formulations to be stable for at least fourteen days. Formulations were therefore prepared weekly and stored at approximately 4°C in the dark.

Samples were taken of each test material formulation and were analysed for concentration of Dibromomethane at Safepharm Analytical Laboratory. The method used for analysis of formulations and the results obtained are given in Appendix 20. The results indicate that the prepared formulations were within ± 10% of the nominal concentration.

3. METHODS

3.1 Animals and Animal Husbandry

A sufficient number of male and female Sprague-Dawley Crl:CD (SD) IGS BR strain rats were obtained from Charles River (UK) Limited, Margate, Kent. On receipt, the animals were examined for signs of ill-health or injury. The animals were acclimatised for 14 days, during

which time their health status was assessed. A total of eighty animals (forty males and forty females) were accepted into the study. At the start of treatment, the males weighed 268 to 336g; the females weighed 176 to 226g and were approximately eight weeks old.

Initially, all animals were housed in groups of five in polypropylene cages with stainless steel grid floors and tops, suspended over polypropylene trays lined with absorbent paper. During the mating phase, animals were transferred to similar cages on a one male: one female basis. Following evidence of successful mating, the males were returned to their original cages. Mated females were housed individually during gestation and lactation, in polypropylene cages with solid floors and stainless steel lids, furnished with softwood flakes (Datesand Ltd. Cheshire, UK).

The animals were allowed free access to food and water. A pelleted diet (Rodent PMI 5002 (Certified) diet, BCM IPS Limited, London, UK) was used throughout the treatment period. A Certificate of Analysis of the batch used is given in Addendum 1. Mains drinking water was supplied from polycarbonate bottles attached to the cage. The diet and drinking water were considered not to contain any contaminant at a level that might have affected the purpose or integrity of the study. Environmental enrichment was provided in the form of wooden chew blocks (B & K Universal Ltd, Hull, UK) and cardboard fun tunnels (Datesand Ltd, Cheshire, UK) except for mated females during gestation and lactation. Mated females were also given softwood flakes, as bedding, throughout gestation and lactation.

The animals were housed in air-conditioned rooms within the Safepharm Laboratories Limited Barrier Maintained Rodent Facility. The rate of air exchange was at least fifteen air changes per hour and the low intensity fluorescent lighting was controlled to give twelve hours continuous light and twelve hours darkness. Environmental conditions were continuously monitored by a computerised system and print-outs of hourly mean temperatures and humidities are included in the study records. The temperature and relative humidity controls were set to achieve target values of 21±2°C and 55 ±15% respectively.

The animals were allocated to dose groups using a randomisation procedure based on stratified bodyweights and the group mean bodyweights were then determined to ensure similarity between the dose groups. The animals were uniquely identified within the study, by an ear punching system routinely used in these laboratories.

3.2 Procedure

Animals were allocated to treatment groups as follows:

Treatment Group	Dose Level (mg/kg/day)	Treatment Volume (ml/kg)	Concentration (mg/ml)	Animal Numbers	
				Male	Female
Control	0	4	0	10 (1 - 10)	10 (11 - 20)
Low	50	4	12.5	10 (21 - 30)	10 (31 - 40)
Intermediate	150	4	37.5	10 (41 - 50)	10 (51 - 60)
High	500	4	125	10 (61 - 70)	10 (71 - 80)

The numbers in parentheses ( ) show the individual animal numbers allocated to each treatment group.

The test material was administered daily by gavage using a stainless steel cannula attached to a disposable plastic syringe. Control animals were treated in an identical manner with 4 ml/kg/day of polyethylene glycol 400.

The volume of test and control material administered to each animal was based on the most recent scheduled bodyweight and was adjusted at regular intervals.

Chronological Sequence of Study

i) Groups of ten male and ten female animals were treated daily at the appropriate dose level throughout the study (except for females during parturition where applicable).

ii) On Day 15, all animals were paired on a 1 male:1 female basis within each dose group for a maximum of fourteen days. The scheduled mating partner for female 80 (500 mg/kg/day) died prior to pairing and this female was paired on Day 16 with a male (No. 63) who successfully mated the previous day.

iii) Following evidence of mating, the males (unless required for additional pairing) were returned to their original cages and females were transferred to individual cages.

iv) Pregnant females were allowed to give birth and maintain their offspring until Day 5 *post partum.* Evaluation of each litter size, litter weight, mean pup weight, clinical observations and landmark developmental signs were also performed during this period.

v) At Day 5 *post partum*, all surviving females and offspring were killed and examined macroscopically.

vi) The male dose groups were killed and examined macroscopically on Day 43.

3.3 Observations

3.3.1 Clinical Observations

All animals were examined for overt signs of toxicity, ill-health and behavioural change immediately before and after dosing, and one and five hours after dosing, during the working week. Animals were observed immediately before and after dosing, and one hour after dosing at weekends (except for females during parturition where applicable).

3.3.2 Bodyweight

Individual bodyweights were recorded on Day 0 (prior to dosing) and then weekly for males until termination and weekly for females until mating was evident. Bodyweights were then recorded for females on Days 0, 7, 14 and 20 *post coitum*, and on Days 1 and 4 *post partum*.

3.3.3 Food Consumption

During the maturation period, weekly food consumption was recorded for each cage of adults. This was continued for males after the mating phase. For females showing evidence of mating, food consumption was recorded for the periods covering Days 0-7, 7-14 and 14-20. For females with live litters, food consumption was recorded on Days 1 and 4 *post partum*.

3.3.4 Water Consumption

Water intake was observed daily by visual inspection of water bottles for any overt change.

3.3.5 Reproduction Screening

3.3.5.1 Mating

Animals were paired on a 1 male: 1 female basis within each dose group, for a period of up to fourteen days. Cage tray-liners were checked each morning for the presence of ejected copulation plugs and each female was examined for the presence of a copulation plug in the vagina. A vaginal smear was prepared for each female and the stage of the oestrous cycle or the presence of sperm was recorded. The presence of sperm within the vaginal smear and/or vaginal plug *in situ* was taken as positive evidence of mating (Day 0 of gestation) and the males were subsequently

returned to their original holding cages (unless required for additional pairing). Mated females were housed individually during the period of gestation and lactation.

3.3.5.2 Pregnancy and Parturition

Each pregnant female was observed at approximately 0830, 1230 and 1630 hours and around the period of expected parturition. Observations were carried out at approximately 0830 and 1230 hours at weekends and public holidays. The following was recorded for each female:

i) Date of mating

ii) Date and time of observed start of parturition

iii) Date and time of observed completion of parturition

iv) Duration of gestation

3.3.5.3 Litter Data

On completion of parturition (Day 0 *post partum*), the number of live and dead offspring was recorded.

For each litter the following was recorded:

i) Number of offspring born

ii) Number and sex of offspring alive recorded daily and reported on Day 1 and 4 *post partum*

iii) Clinical condition of offspring from birth to Day 4 *post partum*

iv) Individual offspring and litter weights on Day 1 and 4 *post partum*

3.3.5.4 Physical Development

All live offspring were assessed for surface righting reflex on Day 1 *post partum*.

3.3.6 Pathology

Adult males and females were killed by intravenous overdose of sodium pentobarbitone followed by exsanguination. Surviving males and a single female that failed to mate were killed on Day 43 of the study. One female that failed to litter was killed on Day 27 *post coitum*. Females that showed total litter loss were killed on the day the last offspring was found dead. Surviving females were killed on Day 5 of lactation. Surviving offspring were terminated via intracardiac overdose of sodium pentobarbitone at Day 5 of age.

All adult animals and offspring, including those dying during the study, were subjected to a full external and internal examination, and any macroscopic abnormalities were recorded.

In addition, the corpora lutea of all ovaries from pregnant females were counted at necropsy. The uterine implantation sites were counted. The procedure was enhanced (where necessary) by staining the uteri with a 1% ammonium polysulphide solution.

3.3.6.1 Organ Weights

The epididymides and testes from all adult males killed at the end of the study, were dissected free from fat and weighed before fixation.

3.3.6.2 Histopathology

Samples of the following tissues were preserved from all animals, in buffered 10% formalin.

Epididymides *	Coagulating gland
Gross lesions	Testes *
Ovaries	Seminal vesicles
Pituitary	Uterus/Cervix
Prostate	Vagina

All tissues were despatched to Propath UK Ltd Rotherwas, Hereford, UK (Principal Investigator: N Candy). The tissues from all control and 500 mg/kg/day dose group adult animals were prepared as paraffin blocks, sectioned at nominal thickness of 5μm and stained with haematoxylin and eosin for subsequent microscopic examination.

Microscopic examination was conducted by the Study Pathologist. All findings were entered into the ROELEE Pathology computerisation system for tabulation and report production.

3.4 Evaluation of Data

3.4.1 Treatment of Data

Data were processed to give group mean values and standard deviations where appropriate. Data shown in the appendices are frequently rounded values for presentation purposes. Group mean values are generally calculated using unrounded values therefore it is not always possible to calculate the exact group values from the individual values presented in the appendices.

* = preserved in Bouin's fluid and then in 70% IMS after forty-eight hours

For bodyweights and food consumptions during gestation, group mean values were calculated using data from females which were observed to give birth to offspring.

For bodyweights and food consumptions during lactation, group mean values were calculated using data from females with live young at Day 5 of lactation.

3.4.2 Statistical Analysis

The following parameters were subjected to statistical analysis:

Bodyweight and bodyweight change

Food consumption during gestation and lactation

Litter data

Implantation losses and viability indices

Offspring bodyweight and bodyweight change

Offspring surface righting

Adult absolute and bodyweight relative organ weights

The following statistical procedures were used:

Data were assessed for dose response relationships by linear regression analysis, followed by one way analysis of variance (ANOVA) incorporating Levene's test for homogeneity of variance. Where variances were shown to be homogenous, pairwise comparisons were conducted using Dunnett's test. Where Levene's test showed unequal variances the data were analysed using non-parametric methods: Kruskal-Wallis ANOVA and Mann-Whitney 'U' test.

Non-parametric methods were used to analyse implantation loss, offspring sex ratio and landmark developmental markers.

Probability values (p) are presented as follows:

$p < 0.001$ ***
$p < 0.01$ **
$p < 0.05$ *
$p \geq 0.05$ (not significant)

In addition, histopathological findings were analysed (excluding decedent animals) using the following methods:

Histopathology data were analysed using the following methods to determine significant differences between control and treatment groups for the individual sexes:

1. Chi-squared analysis for differences in the incidence of lesions occurring with an overall frequency of 1 or greater.

2. Kruskal-Wallis one-way non-parametric analysis of variance for the comparison of severity grades for the more frequently observed graded conditions.

Probability values (p) were calculated as follows:

$p < 0.001$	+++	---	***
$p < 0.01$	++	--	**
$p < 0.05$	+	-	*
$p < 0.1$	(+)	(-)	(*)
$p \geq 0.1$ N.S. (not significant)			

Plus (+) signs indicate positive differences from the control group and minus (-) signs indicate negative differences. Asterisks refer to overall differences between group variation which is non-directional.

3.4.3 Reproductive Indices

3.4.3.1 Mating Performance and Fertility

The following parameters were calculated from the individual data during the mating period of the parental generation.

i) Pre-coital Interval

Calculated as the time elapsing between initial pairing and the observation of positive evidence of mating.

ii) Fertility Indices

For each group the following were calculated:

$$\text{Mating Index (\%)} = \frac{\text{Number of animals mated}}{\text{Number of animals paired}} \times 100$$

$$\text{Pregnancy Index (\%)} = \frac{\text{Number of pregnant females}}{\text{Number of animals mated}} \times 100$$

3.4.3.2 Gestation and Parturition Data

The following parameters were calculated for individual data during the gestation and parturition period of the parental generation.

i) Gestation Length

Calculated as the number of days of gestation including the day for observation of mating and the start of parturition.

ii) Parturition Index

The following was calculated for each group:

$$\text{Parturition Index (\%)} = \frac{\text{Number of females delivering live offspring}}{\text{Number of pregnant females}} \times 100$$

3.4.3.3 Litter Responses

The standard unit of assessment was considered to be the litter, therefore values were first calculated for each litter and the group mean was calculated using their individual litter values. Group mean values included all litters reared to termination (Day 5 of age).

i) Implantation Losses (%)

Group mean percentile pre-implantation and post implantation loss were calculated for each female/litter as follows:

$$\text{\% pre - implantation loss} = \frac{\text{Number of Corpora Lutea - Number of implantation sites}}{\text{Number of corpora lutea}} \times 100$$

$$\text{\% post - implantation loss} = \frac{\text{Number of implantation sites - number of offspring}}{\text{Number of implantation sites}} \times 100$$

ii) Live Birth and Viability Indices

The following indices were calculated for each litter as follows:

$$\text{Live Birth Index (\%)} = \frac{\text{Number of offspring alive on Day 1}}{\text{Number of offspring born}} \times 100$$

$$\text{Viability Index (\%)} = \frac{\text{Number of offspring alive on Day 4}}{\text{Number of offspring alive on Day 1}} \times 100$$

iii) Sex Ratio (% males)

Sex ratio was calculated for each litter value on Day 1 and 4 *post partum*, using the following formula:

$$\frac{\text{Number of male offspring}}{\text{Number of offspring of determined sex}} \times 100$$

4. ARCHIVES

Unless instructed otherwise by the Sponsor, specimens and with the exception of unprocessed wet tissue samples, all original data and the final report will be retained in the Safepharm archives for five years, after which instructions will be sought as to further retention or disposal. Unprocessed wet tissue samples will be retained for two years prior to disposal.

5. RESULTS

5.1 Adult Responses

5.1.1 Mortality

There were two unscheduled deaths on the study:

Male 70, receiving 500 mg/kg/day, was killed for animal welfare considerations after showing noisy and laboured respiration, hunched posture and pilo-erection prior to dosing on Day 2 of the study. At necropsy examination, it was found that the thoracic cavity was filled with red fluid and the cranial, intermediate and caudal lobes of the right lung were encased in white fibrous tissue. Histopathological examination of these tissues revealed pleuritis and inflammatory cell infiltrates and these were considered to be the primary cause for the decline in clinical condition. In the absence of similar observations in other animals at this dosage, this early isolated death, following only a single daily administration, was considered to be incidental and unrelated to treatment.

Control Female 17 was found dead on Day 41 of the study having given birth to a litter the preceding day. No clinical signs had been apparent for this animal prior to death. Necropsy revealed red staining around the ano-genital region, reddened lungs, dark liver, gaseous distension of the gastro-intestinal tract and a dark uterus. Histopathological findings for these tissues included congestion and minimal peribronchiolar lymphoid aggregations in the lungs, congestion in the liver, moderate autolysis of intestinal tract and congestion of the uterus/cervix, however, the cause of death for this animal was not established.

5.1.2 Clinical Observations

Summary incidences of daily clinical observations are given in Tables 2 and 3. Individual clinical signs are presented in Appendices 1 and 2.

Among the surviving animals, the type, incidence and distribution of clinical signs did not indicate any obvious adverse effect of treatment for either sex at dosages of 50, 150 or 500 mg/kg/day.

For both sexes at 500 mg/kg/day there was a higher incidence of staining of the fur/mouth/snout compared to the control and lower dosage groups. Additionally for females at this dosage there was also a slight increase in the incidence of post-dose salivation, although these occurrences tended to be sporadic and only three females in total were affected.

5.1.3 Bodyweight

Group mean bodyweights and bodyweight changes for males are given in Tables 4 and 5. Group mean bodyweights and bodyweight changes during the maturation, gestation and lactation phases for females are given in Tables 6 and 7. Cumulative bodyweight changes are presented graphically in Figure 1 to Figure 4. Individual data are given in Appendices 3 and 4.

Males

There was considered to be no adverse effect on mean bodyweight gain of the males during the study at dosages of 50, 150 or 500 mg/kg/day.

At 500 mg/kg/day, higher bodyweight gain during Week 1 and lower bodyweight gain during Weeks 3 and 5 attained statistical significance when compared with control. However, overall mean bodyweight gain by termination was within 92% of control and did not attain statistical significance and these weekly variations in bodyweight gain were not considered to be of any toxicological significance.

Females

At 500 mg/kg/day bodyweight gain during the 2 week pre-pairing maturation period was unaffected by treatment with mean bodyweight being essentially similar or slightly superior to control. Bodyweight gains during gestation at this dosage, however, were clearly lower than control; these differences are considered to reflect the lower litter size apparent at this dosage rather than any effect on the females as mean bodyweight on Day 1 of lactation were essential similar to control. Bodyweight gain during the first four days of lactation was not statistically different from control.

At 50 and 150 mg/kg/day bodyweight and bodyweight gains during the pre-pairing phase, gestation and lactation were considered to be unaffected by treatment.

5.1.4 Food Consumption

Group mean food consumptions for males are given in Table 8 and group mean food consumptions during the maturation, gestation and lactation phases for females are given in Table 9. These are presented graphically in Figure 5 to Figure 7. Individual cage values during maturation and individual values for females during gestation and lactation are given in Appendices 5 to 7.

Males

There was no adverse effect on mean food intake of males during the pre-pairing and post-pairing phases of the study at dosages of 50, 150 or 500 mg/kg/day.

Females

At 500 mg/kg/day food intake during the two week pre-pairing period and during gestation was unaffected by treatment. Food consumption during lactation at this dosage was significantly lower than control; litter size was lower than control and these differences in food intake probably reflect the lower physiological demand on the lactating female at this dosage.

At 50 and 150 mg/kg/day food consumption during the pre-pairing phase and during gestation and lactation were unaffected by treatment.

5.1.5 Food Conversion Efficiency

Food conversion efficiencies are given in Table 10 and Table 11.

Males

Intergroup differences in the efficiency of food utilisation of males during the pre-pairing and post-pairing phases of the study did not indicate any adverse effect of treatment at dosages of 50, 150 or 500 mg/kg/day.

Females

Intergroup differences in the efficiency of food utilisation of females during the two week pre-pairing period did not indicate any adverse effect of treatment at dosages of 50, 150 or 500 mg/kg/day.

At 500 mg/kg/day food conversion efficiency of females appeared reduced during the second week of gestation, reflecting the lower bodyweight gain observed at this dosage during gestation. At 50 and 150 mg/kg/day conversion efficiency of females during gestation and lactation was unaffected by treatment.

5.1.6 Water Consumption

Daily visual inspection of water bottles revealed no intergroup differences.

5.1.7 Mating

A summary incidence of adult performance is given in Table 1. Group values for mating performance are presented in Table 12. Individual data are given in Appendix 8.

At 500 mg/kg/day there was a clear effect on mating performance compared with animals in the control and lower dosage groups.

The typical length of the female oestrous cycle in the rat is four days, and it therefore expected that the majority of females will mate within four days of pairing (i.e. at the first oestrus opportunity). Within the control, 50 and 150 mg/kg/day dosage groups, only one female (number 38 - 50 mg/kg/day) failed to show evidence of mating within this four day period. At 500 mg/kg/day, only four of the ten females paired showed evidence of mating at this stage of the study, although nine of the ten animals had mated by completion of the two week pairing period. Copulation plugs and sperm in the vagina are used to confirm that the paired animals have mated. At 500 mg/kg/day, the copulation plug count was generally lower than observed in the control and lower dosage groups, although sperm reading scores from the vaginal smear were not obviously affected.

Mating performance at 50 or 150 mg/kg/day were considered to be unaffected by treatment.

5.1.8 Fertility

A summary incidence of adult performance is given in Table 1. Group values for fertility, litter data and implantation losses are given in Tables 12, 14 and 15. Individual data are given in Appendices 8 to 10.

There was considered to be no adverse effect of treatment on fertility at dosages of 50, 150 or 500 mg/kg/day.

All matings amongst control, 50 and 150 mg/kg/day animals successfully lead to pregnancy. At 500 mg/kg/day, only one of the mated females failed to achieve pregnancy; in the absence of any effect on implantation count among the other females at this dosage, this was considered to be incidental and unrelated to treatment.

5.1.9 Gestation Length

Group values for gestation length are given in Table 13. Individual gestation lengths are given in Appendix 8.

At 500 mg/kg/day gestation length of pregnant females tended to be longer than control, with all females at this dosage showing a gestation length of 23 days or longer. Total litter size on Day 1 was generally lower than control and this lower litter size may have influenced the duration of pregnancy to some extent.

At 50 or 150 mg/kg/day there was no clear effect of treatment on gestation length. At 150 mg/kg/day, there was a slight increase in the number of females with a gestation length of 23 days, compared with control, and one female had a gestation length of 23.5 days; this female only had a small litter (six offspring). At 50 mg/kg/day there was a higher incidence of females with a gestation period of 22 days compared with control. Overall, however, the differences in the distribution of gestation lengths at these lower dosages were considered to reflect normal biological variation.

5.2 Litter Responses

Within the control group one female (No. 15) showed total litter loss on Day 1 *post partum* and another female was found dead (see mortalities) and the litter killed for reasons of animal welfare the day following parturition. The remaining eight control females reared their litter to termination on Day 5 of lactation/age. At 50 and 150 mg/kg/day all ten females respectively reared their young to termination on Day 5 of lactation/age. At 500 mg/kg/day one female failed to mate and another female was non-pregnant. One female was found to have implantations sites within the uterus at necropsy although no litter was observed to be born. It is considered likely that the loss of this litter occurred *in-utero* although the possibility that the female gave birth and subsequently ate all of the offspring without littering being detected cannot be discounted. Another female at this dosage showed total litter loss on Day 1 *post partum*. In total, only six females reared their young to termination on Day 5 of lactation/age at 500 mg/kg/day.

The following assessment of litter response is generally based on those litters reared to termination on Day 5 of lactation/age, although data available for females showing total litter loss has also been taken into consideration, where considered appropriate.

5.2.1 Offspring Litter Size and Viability

Group mean corpora lutea and implantation counts, litter size, implantation, survival indices and sex ratio given in Tables 14 to 16. Individual data are given in Appendices 9 to 11.

At 500 mg/kg/day there was no obvious adverse effect of treatment on mean corpora lutea or implantation count. However, subsequent mean litter size on Day 1 was notably lower than control due to clear increase in post-implantation loss; this loss most probably representing death of the offspring *in utero*. In addition, as previous discussed, one female (No. 72) at this dosage was found to have implantations sites at necropsy although parturition was not observed and it is considered that the loss of this litter probably occurred *in-utero*; this female also showed high pre-implantation loss. Sex ratio at birth was unaffected, indicating that the high post-implantation loss observed had not been due to a selective effect on survival for either sex. Post-natal survival of the offspring to Day 4 of age was good in the majority of litters, although this may have been influenced by the smaller litter size at this dosage. One female (No. 71), which had a litter of only two offspring, showed total litter loss on Day 1 of lactation/age; in the absence of post-natal losses in the remaining litters this litter loss was not attributed to treatment of the parent female. The sex ratio of live offspring on Day 1 and 4 were unaffected by treatment.

At 50 and 150 mg/kg/day, corpora lutea and implantation count, pre- and post implantation losses, litter size and sex ratio at birth and live litter size and sex ratio at Day 1 and 4, and post-natal survival were not adversely affected by treatment.

5.2.2 Offspring Growth and Development

Group values for offspring bodyweight and bodyweight change, surface righting reflex and the incidence of clinical signs are given in Tables 14, 17 and 18. Individual values and observations are given in Appendix 9, 12 and 13.

Bodyweight of the offspring on Day 1 and subsequent bodyweight gain to Day 4 of age were unaffected by treatment of the parent females at dosages of 50, 150 or 500 mg/kg/day. The percentage of offspring who successfully showed surface righting reflex on Day 1 and the type, incidence and distribution of clinical signs in the offspring to termination on Day 5 of age was similarly unaffected by maternal treatment.

At 500 mg/kg/day litter weight on Day 1 and 4 were notably lower than control as a consequence of the lower litter size at this dosage.

5.3 Pathology

5.3.1 Necropsy

A summary incidence of necropsy findings for offspring are given in Table 19, and for adults in Tables 20 and 21. Individual values are presented in Appendices 14 to 16.

Offspring

Neither the incidence, type or distribution of macroscopic findings observed at necropsy of decedent offspring or offspring killed at scheduled termination (Day 5 of age) indicated any adverse effect of maternal treatment at dosages of 50, 150 or 500 mg/kg/day.

Adults

Neither the incidence, type or distribution of macroscopic findings observed at necropsy of males or females surviving to scheduled termination indicated any adverse effect of treatment at dosages of 50, 150 or 500 mg/kg/day.

5.3.2 Organ Weights

Group mean absolute and bodyweight-relative male reproductive organ weight data are given in Table 22. Individual values are presented in Appendix 17.

At 500 and 150 mg/kg/day absolute and bodyweight-relative testes weights were lower than control, although statistical significance was only attained for absolute values. In addition, at 500 mg/kg/day absolute and bodyweight-relative epididymal weights were also lower than control. In the absence of any significant histopathological findings for these organ, the differences in organ weights observed were considered to be of equivocal toxicological importance.

There were no statistically significant differences from control in absolute and bodyweight-relative epididymal and testes weights at 50 mg/kg/day.

5.3.3 Histopathology

Summary incidences of histopathological findings are given in Table 23. Individual hisopathology is presented in Appendix 18 and 19.

Microscopic examination of reproductive tissues for adult males and females did not reveal any findings that indicated any adverse effect of treatment.

6. DISCUSSION

Treatment with Dibromomethane at 500 mg/kg/day was well tolerated by adults of either sex and was not associated with any adverse effect on bodyweight change, food intake, food conversion efficiency or clinical condition. Lower bodyweight gain and food conversion efficiency was apparent for females during gestation, compared to control, but this was considered to reflect a reduction in litter size at this dosage, rather than an effect on the parent females. Similarly, lower food intake during Days 1-4 of lactation was considered to reflect lower physiological demand on the dam due to the smaller litter size. One male was killed after showing noisy and laboured respiration, hunched posture and pilo-erection on Day 2. Necropsy revealed red fluid in the thoracic cavity and white fibrous tissue encasing the right lung with histopathological examination revealing pleuritis and inflammatory cell infiltrates. In the absence of any similar effects among other males at this dosage, this early isolated death, following only a single dose, was considered to be incidental and unrelated to treatment.

At 500 mg/kg/day, there was a clear effect on mating performance, with only four of the ten paired females showing evidence of mating during the first four days of pairing (compared to ten of the ten control females), although nine of the ten animals had mated by completion of the two week pairing period. The copulation plug count was also generally lower than observed in the control group, although vaginal smear sperm scores were not obviously affected. Despite the effect on mating performance, fertility was not adversely affected at this dosage and eight of the nine mated females achieved pregnancy. Gestation length of pregnant females tended to be longer than control, but may have been influenced by lower litter size at this dosage. One female was not observed to litter and it was considered that this litter had been lost *in-utero* and another female showed total litter loss on Day 1 *post partum*. In total, only six females at 500 mg/kg/day reared their young to termination on Day 5 of lactation/age compared to eight in the control group.

There was no obvious effect on the numbers of corpora lutea or implantations at 500 mg/kg/day, but litter size on Day 1 was lower than control due to increased post-implantation loss. This loss probably represents death of the offspring *in utero* although the possibility that the female gave birth and subsequently consumed the litter without parturition being detected, cannot be discounted. Sex ratio was unaffected, indicating that the apparent increase in post-implantation loss was not due to a selective effect on survival for either sex. Subsequent survival of offspring to Day 4 of age was unaffected by maternal treatment, however this may have been influenced by the smaller litter size, and one female did show total litter loss on Day 1 *post partum*. Bodyweight on Day 1 of age and subsequent growth and development (as assessed by surface

righting reflex) were also unaffected by maternal treatment; lower litter weights were attributed to lower litter size. Necropsy findings among decedent offspring and offspring surviving to Day 5 of age did not indicate any effect of maternal treatment.

Necropsy findings among adult males and females at 500 mg/kg/day did not indicate any adverse effect of treatment. For males, absolute and bodyweight-relative epididymal and testes weights were lower than control, however, microscopic examination of reproductive tissues for both adult males and females did not reveal any treatment effects, and the differences in organ weights were considered to be of equivocal toxicological significance.

Treatment at 50 or 150 mg/kg/day was well tolerated by both sexes and there were no clear effects observed on reproduction at these lower dosages. For males receiving 150 mg/kg/day, absolute and bodyweight-relative testes weights were lower than control, however, in the absence of any treatment-related histopathological effects at 500 mg/kg/day, the differences in organ weights were considered unlikely to be of any toxicological significance. The 'No Observed Adverse Effect Level' (NOAEL) for reproduction observed in this study was, therefore, considered to be 150 mg/kg/day.

7. CONCLUSION

Treatment with Dibromomethane at 500 mg/kg/day was associated with a clear effect on mating performance and a reduction in litter size at birth, most probably due to increased *in-utero* mortality. The 'No Observed Adverse Effect Level' (NOAEL) for reproduction observed in this study was considered to be 150 mg/kg/day.

TABULAR SUMMARY REPORT OF EFFECTS ON REPRODUCTION/DEVELOPMENT

Observations		Dose Level (mg/kg/day)			
		0 (Control)	50	150	500
Mated pairs	*n*	10	10	10	10
Females showing evidence of copulation	*n*	10	10	10	9
Pregnant females	*n*	10	10	10	8
Conception Days 1-5	*n*	10	9	10	5
Gestation = 22	*n*	2	4	1	0
Gestation = 22.5 days	*n*	5	3	4	0
Gestation = 23 days	*n*	3	3	4	6
Gestation = 23.5 days	*n*	0	0	1	1
Dams with live young born	*n*	9	10	10	7
Dams with live young at Day 5 *post partum*	*n*	8	10	10	6
Corpora lutea/dam	$\bar{x}$	17.8	17.4	16.8	15.7
Implants/dam	$\bar{x}$	15.1	15.3	14.0	14.2
Live offspring/dam Day 1 *post partum*	$\bar{x}$	14.4	12.9	12.8	6.8**
Live offspring/dam at Day 4 *post partum*	$\bar{x}$	14.4	12.8	12.7	6.8***
Sex ratio: % males Day 1 *post partum*	$\bar{x}$	53.6	50.7	45.6	50.5
Sex ratio: % males at Day 4 *post partum*	$\bar{x}$	53.6	50.2	45.0	50.5
Litter weight (g) at Day 1 *post partum*	$\bar{x}$	100.9	92.0	94.4	50.0**
Litter weight (g) at Day 4 *post partum*	$\bar{x}$	145.2	131.7	137.6	71.3**
Male - Offspring weight (g) at Day 1 *post partum*	$\bar{x}$	7.2	7.5	7.7	7.5
Male - Offspring weight (g) at Day 4 *post partum*	$\bar{x}$	10.2	10.8	11.4	10.7
Female offspring weight at Day 1 *Post Partum*	$\bar{x}$	6.8	7.1	7.3	7.3
Female offspring weight at Day 4 *Post Partum*	$\bar{x}$	9.8	10.3	10.8	10.3
ABNORMAL OFFSPRING/DAM					
0	*n*	10	10	10	7
LOSS OF OFFSPRING/DAM					
Pre-implantation (corpora lutea minus implantations)					
0	*n*	1	3	1	2
1	*n*	3	1	2	2
2	*n*	0	3	2	2
3	*n*	3	1	1	1
4	*n*	0	1	2	0
5	*n*	2	0	1	0
6	*n*	0	0	1	0
7	*n*	1	1	0	0
8	*n*	0	0	0	1
Pre-natal (implantations minus live births)					
0	*n*	3	2	3	0
1	*n*	4	2	3	0
2	*n*	2	4	3	0
3	*n*	0	1	1	0
4	*n*	0	0	0	3
5	*n*	0	0	0	1
8	*n*	0	0	0	1
11	*n*	0	1	0	1
12	*n*	0	0	0	1
16	*n*	1	0	0	1
Post natal (live births minus offspring alive on Day 4 *post partum*)					
0	*n*	8	9	9	6
1	*n*	0	1	1	0

n = Number
$\bar{x}$ = Mean
** = $p<0.01$
*** = $p<0.001$

TABLES

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Table 1 Summary of Reproductive Performance - Group Values

	Dose Group (mg/kg/day)			
	0 (Control)	50	150	500
Males				
Initial Group Size	10	10	10	10
Killed	0	0	0	1
Paired	10	10	10	9
Failed to mate	0	0	0	1
Failed to induce pregnancy in female partner	0	0	0	1
Induced pregnacy in female partner	10	10	10	7
Surived to terminal necropsy	10	10	10	9
Females				
Initial Group Size	10	10	10	10
Paired	10	10	10	10
Failed to mate	0	0	0	1
Non-pregnant	0	0	0	1
No young observed to be born (implantation sites detected in uterus at necropsy)	0	0	0	1
Animal found dead (post littering)	1	0	0	0
Total Litter Loss (*post partum*)	1	0	0	1
Reared young to Day 5 of age	8	10	10	6

Table 2 Clinical Observations for Males – Group Incidences

Dose Level (mg/kg/day)	Number of Animals	Clinical Observations	Number Showing Effects At Observation																				
			Day: 1			Day: 2			Day: 3			Day: 4			Day 5			Day: 6			Day: 7		
			Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h
0 (Control)	10	No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10
50	10	No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10
150	10	No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10
500	10/9	Hunched posture	0	0	0	1	0	0	0	0	0	0	0	*	0	0	*	0	0	0	0	0	0
		Noisy and laboured respiration	0	0	0	1	0	0	0	0	0	0	0	*	0	0	*	0	0	0	0	0	0
		Pilo-erection	0	0	0	1	0	0	0	0	0	0	0	*	0	0	*	0	0	0	0	0	0
		Red/brown stained mouth	0	0	0	1	0	0	0	0	0	0	0	*	0	0	*	0	0	0	0	0	0
		Red/brown stained snout	0	0	0	1	0	0	0	0	0	0	0	*	0	0	*	0	0	0	0	0	0
		Killed *in extremis*	0	0	0	1	0	0	0	0	0	0	0	*	0	0	*	0	0	0	0	0	0
		No abnormalities detected	10	10	10	9	9	9	9	9	9	9	9	*	9	9	*	9	9	9	9	9	9

Pre = immediately before dosing
1h = one hour after dosing
5h = five hours after dosing
* = five hour observation not performed at weekend

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Table 2 (continued) Clinical Observations for Males – Group Incidences

Dose Level (mg/kg/day)	Number of Animals	Clinical Observations	Number Showing Effects At Observation																				
			Day: 8			Day: 9			Day: 10			Day: 11			Day 12			Day: 13			Day: 14		
			Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h
0 (Control)	10	No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10
50	10	No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10
150	10	No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10
500	9	Increased salivation	0	0	0	0	0	0	0	0	0	0	0	*	0	1†	*	0	0	0	0	0	0
		No abnormalities detected	9	9	9	9	9	9	9	9	9	9	9	*	9	9	*	9	9	9	9	9	9

Pre = immediately before dosing
1h = one hour after dosing
5h = five hours after dosing
* = five hour observation not performed at weekend
† = increased salivation detected up to ten minutes after dosing

Table 2 (continued) **Clinical Observations for Males – Group Incidences**

Dose Level (mg/kg/day)	Number of Animals	Clinical Observations	Number Showing Effects At Observation																				
			Day: 15			Day: 16			Day: 17			Day: 18			Day 19			Day: 20			Day: 21		
			Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h
0 (Control)	10	Scratches under both eyes	0	0	0	0	0	0	0	0	0	0	0	*	1	1	*	1	1	1	1	1	1
		No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	9	9	*	9	9	9	9	9	9
50	10	No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10
150	10	No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10
500	9	Red/brown stained fur	0	0	0	3	3	3	0	0	0	1	1	*	4	4	*	4	4	4	4	4	4
		Red/brown stained mouth	0	0	0	0	0	0	0	0	0	0	0	*	0	0	*	0	0	1	1	1	1
		Noisy respiration	0	0	0	0	0	0	0	0	0	0	1#	*	0	0	*	0	0	0	0	0	0
		No abnormalities detected	9	9	9	6	6	6	9	9	9	9	7	*	5	5	*	5	5	5	5	5	5

Pre = immediately before dosing
1h = one hour after dosing
5h = five hours after dosing
* = five hour observation not performed at weekend
# = noisy respiration detected up to ten minutes after dosing

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Table 2 (continued) Clinical Observations for Males – Group Incidences

Dose Level (mg/kg/day)	Number of Animals	Clinical Observations	Number Showing Effects At Observation																				
			Day: 22			Day: 23			Day: 24			Day: 25			Day 26			Day: 27			Day: 28		
			Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h
0 (Control)	10	Scratches under both eyes	1	1	1	1	1	1	1	1	1	1	1	*	1	1	*	0	0	0	0	0	0
		No abnormalities detected	9	9	9	9	9	9	9	9	9	9	9	*	9	9	*	10	10	10	10	10	10
50	10	Increased salivation	0	0	0	0	0	0	0	0	0	0	0	*	1†	0	*	0	0	0	0	0	0
		No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	9	*	10	10	10	10	10	10
150	10	No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10
500	9	Red/brown stained fur	4	4	4	2	2	2	4	4	4	4	4	*	4	4	*	4	4	4	4	4	4
		Red/brown stained mouth	1	1	1	0	0	0	0	0	0	0	0	*	0	0	*	0	0	0	0	0	0
		Red/brown staining around eyes	0	0	0	0	0	0	0	0	0	0	0	*	0	0	*	0	0	0	0	0	1
		No abnormalities detected	5	5	5	7	7	7	5	5	5	5	5	*	5	5	*	5	5	5	5	5	5

Pre = immediately before dosing
1h = one hour after dosing
5h = five hours after dosing
* = five hour observation not performed at weekend
† = increased salivation detected up to ten minutes after dosing

Table 2 (continued) **Clinical Observations for Males – Group Incidences**

Dose Level (mg/kg/day)	Number of Animals	Clinical Observations	Number Showing Effects At Observation																				
			Day: 29			Day: 30			Day: 31			Day: 32			Day: 33			Day: 34			Day: 35		
			Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h
0 (Control)	10	No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10
50	10	No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10
150	10	No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10
500	9	Red/brown stained fur	1	1	3	3	3	3	3	3	3	3	3	*	7	7	*	4	4	4	4	4	4
		Wound/scab on dorsal surface	0	2	2	2	2	2	2	2	2	2	2	*	0	0	*	0	0	0	0	0	0
		No abnormalities detected	8	7	5	5	5	5	5	5	5	5	5	*	2	2	*	5	5	5	5	5	5

Pre = immediately before dosing
1h = one hour after dosing
5h = five hours after dosing
* = five hour observation not performed at weekend

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Table 2 (continued) Clinical Observations for Males – Group Incidences

Dose Level (mg/kg/day)	Number of Animals	Clinical Observations	Number Showing Effects At Observation																				
			Day: 36			Day: 37			Day: 38			Day: 39			Day 40			Day: 41			Day: 42		
			Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h
0 (Control)	10	No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10
50	10	No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10
150	10	No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10
500	9	Red/brown stained fur	4	4	4	4	4	4	4	4	4	4	4	*	4	4	*	4	4	4	4	4	4
		No abnormalities detected	5	5	5	5	5	5	5	5	5	5	5	*	5	5	*	5	5	5	5	5	5

Pre = immediately before dosing
1h = one hour after dosing
5h = five hours after dosing
* = five hour observation not performed at weekend

Table 3 Clinical Observations for Females – Group Incidences

Dose Level (mg/kg/day)	Number of Animals	Clinical Observations	Number Showing Effects At Observation																				
			Day: 1			Day: 2			Day: 3			Day: 4			Day 5			Day: 6			Day: 7		
			Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h
0 (Control)	10	No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10
50	10	No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10
150	10	No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10
500	10	No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10

Pre = immediately before dosing
1h = one hour after dosing
5h = five hours after dosing
* = five hour observation not performed at weekend

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Table 3 (continued) Clinical Observations for Females – Group Incidences

Dose Level (mg/kg/day)	Number of Animals	Clinical Observations	Number Showing Effects At Observation																				
			Day: 8			Day: 9			Day: 10			Day: 11			Day 12			Day: 13			Day: 14		
			Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h
0 (Control)	10	No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10
50	10	No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10
150	10	No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10
500	10	Increased salivation	0	0	0	0	0	0	0	0	0	0	0	*	0	1†	*	0	0	0	0	0	0
		Hunched posture	0	0	0	0	0	0	0	0	0	0	0	*	0	0	*	0	0	0	1	1	0
		No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	9	9	10

Pre = immediately before dosing
1h = one hour after dosing
5h = five hours after dosing
* = five hour observation not performed at weekend
† = increased salivation detected up to ten minutes after dosing

Table 3 (continued) Clinical Observations for Females – Group Incidences

Dose Level (mg/kg/day)	Number of Animals	Clinical Observations	Number Showing Effects At Observation																				
			Day: 15			Day: 16			Day: 17			Day: 18			Day 19			Day: 20			Day: 21		
			Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h
0 (Control)	10	No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10
50	10	No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10
150	10	No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10
500	10	Increased salivation	0	0	0	0	0	0	0	0	0	0	1†	*	0	2	*	0	0	0	0	0	0
		Red/brown stained fur	0	0	0	3	3	3	3	3	3	3	3	*	4	4	*	4	4	4	4	4	4
		Red/brown stained snout	0	0	0	2	1	1	3	3	3	3	3	*	3	3	*	3	3	4	4	4	4
		Red/brown stained mouth	0	0	0	0	0	0	0	0	0	0	0	*	0	0	*	0	0	1	1	1	1
		No abnormalities detected	10	10	10	6	7	7	7	7	7	7	7	*	6	6	*	6	6	5	5	5	5

Pre = immediately before dosing
1h = one hour after dosing
5h = five hours after dosing
* = five hour observation not performed at weekend
† = increased salivation detected up to ten minutes after dosing

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Table 3 (continued) Clinical Observations for Females – Group Incidences

Dose Level (mg/kg/day)	Number of Animals	Clinical Observations	Number Showing Effects At Observation																				
			Day: 22			Day: 23			Day: 24			Day: 25			Day 26			Day: 27			Day: 28		
			Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h
0 (Control)	10	No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10
50	10	Red/brown stained fur	0	0	0	1	1	1	0	0	0	0	0	*	0	0	*	0	0	0	0	0	0
		No abnormalities detected	10	10	10	9	9	9	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10
150	10	No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10
500	10	Increased salivation	0	0	0	0	1†	0	0	0	0	0	0	*	0	0	*	0	0	0	0	0	0
		Red/brown stained fur	4	4	0	0	0	0	0	0	0	0	0	*	0	0	*	0	0	0	0	0	2
		Red/brown stained snout	4	4	0	0	0	0	0	0	0	0	0	*	0	0	*	0	0	0	0	0	0
		Red/brown stained mouth	1	1	0	0	0	0	0	0	0	0	0	*	0	0	*	0	0	0	0	0	0
		No abnormalities detected	5	5	10	10	9	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	8

Pre = immediately before dosing
1h = one hour after dosing
5h = five hours after dosing
* = five hour observation not performed at weekend
† = increased salivation detected up to ten minutes after dosing

Table 3 (continued) **Summary Incidence of Daily Clinical Observations - Females**

Dose Level (mg/kg/day)	Number of Animals	Clinical Observations	Number Showing Effects At Observation																				
			Day: 29			Day: 30			Day: 31			Day: 32			Day 33			Day: 34			Day: 35		
			Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h
0 (Control)	10	No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10
50	10	No abnormalities detected	10	10	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10
150	10	Increased salivation	0	1†	0	0	0	0	0	0	0	0	0	*	0	0	*	0	0	0	0	0	0
		No abnormalities detected	10	9	10	10	10	10	10	10	10	10	10	*	10	10	*	10	10	10	10	10	10
500	10	Red/brown stained fur	2	2	2	2	2	2	2	2	2	2	2	*	1	1	*	1	1	1	1	1	1
		No abnormalities detected	8	8	8	8	8	8	8	8	8	8	8	*	9	9	*	9	9	9	9	9	9

Pre = immediately before dosing
1h = one hour after dosing
5h = five hours after dosing
* = five hour observation not performed at weekend
† = increased salivation detected up to ten minutes after dosing

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Table 3 (continued) Clinical Observations for Females – Group Incidences

Dose Level (mg/kg/day)	Number of Animals	Clinical Observations	Number Showing Effects At Observation																			
			Day: 36			Day: 37			Day: 38			Day: 39			Day 40			Day: 41			Day: 42		
			Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h
0 (Control)	10/9	Generalised Fur loss	0	0	0	0	0	0	0	0	0	1	1	*	1	1	*	1	1	1	1	1	1
		Littering ◊	0	0	0	0	0	1	1^1	1^1	3^1	2^2	2^2	*	6^6	6^6	*	0	2	2	0	0	0
		Death	0	0	0	0	0	0	0	0	0	0	0	*	0	0	*	1#	0	0	0	0	0
		No abnormalities detected	10	10	10	10	10	9	9	9	7	7	7	*	3	3	*	8	6	6	8	8	8
50	10	Littering ◊	0	0	0	0	0	2	2^2	2^2	3^2	2^2	2^2	*	4^4	4^4	*	0	2	2	0	0	0
		No abnormalities detected	10	10	10	10	10	8	8	8	7	8	8	*	6	6	*	10	8	8	10	10	10
150	10	Littering ◊	0	0	0	0	0	0	2^2	2^2	3^2	2^2	2^2	*	2^2	2^2	*	0	5	5	0	0	0
		No abnormalities detected	10	10	10	10	10	10	8	8	7	8	8	*	8	8	*	10	5	5	10	10	10
500	10	Red/brown stained fur	1	1	1	1	1	1	1	1	1	1	1	*	1	1	*	1	1	1	1	1	1
		Littering ◊	0	0	0	0	0	0	0	0	1	1^1	1^1	*	1^1	1^1	*	0	0	0	0	1	2
		No abnormalities detected	9	9	9	9	9	9	9	9	8	8	8	*	8	8	*	9	9	9	9	8	7

Pre = immediately before dosing
1h = one hour after dosing
5h = five hours after dosing
* = five hour observation not performed at weekend
◊ = clinical observations not performed during littering (superscript indicates animals that were also not dosed)
= animal found dead

Table 3 (continued) **Clinical Observations for Females – Group Incidences**

Dose Level (mg/kg/day)	Number of Animals	Clinical Observations	Number Showing Effects At Observation																				
			Day: 43			Day: 44			Day: 45			Day: 46			Day : 47			Day: 48			Day: 49		
			Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h
0 (Control)	9/0	Day 5 post partum – animal removed for necropsy	2	0	0	2	0	0	3	0	0	2											
		No abnormalities detected	7	7	7	5	5	5	2	2	2												
50	10/0	Day 5 post partum – animal removed for necropsy	2	0	0	1	0	0	4	0	0	2	0	*	0	0	*	0	0	0	1		
		Littering ◊	0	0	1	0	1	1	0	0	0	0	0	*	0	0	*	0	0	0			
		No abnormalities detected	8	8	7	7	6	6	3	3	3	1	1	*	1	1	*	1	1	1			
150	10/0	Day 5 post partum – animal removed for necropsy	2	0	0	2	0	0	1	0	0	5											
		Noisy respiration	0	0	0	0	1□	0	0	0	0	0											
		No abnormalities detected	8	8	8	6	5	6	5	5	5												
500	10/2	Day 5 post partum – animal removed for necropsy	2	0	0	1	0	0	1	0	0	0	0	*	1	0	*	2	0	0	1	0	0
		Littering ◊	0	1	2	0	1	2	0	1	1	0	0	*	0	0	*	0	0	0	0	0	0
		No abnormalities detected	8	7	6	7	6	5	6	5	5	6	6	*	5	5	*	3	3	3	2	2	2

Pre = immediately before dosing
1h = one hour after dosing
5h = five hours after dosing
* = five hour observation not performed at weekend
□ = noisy respiration observed up to ten minutes after dosing and one hour after dosing
◊ = clinical observations not performed during littering

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Table 3 (continued) Clinical Observations for Females – Group Incidences

Dose Level (mg/kg/day)	Number of Animals	Clinical Observations	Number Showing Effects At Observation																							
			Day: 50			Day: 51			Day: 52			Day: 53			Day: 54			Day: 55			Day: 56			Day: 57		
			Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h	Pre	1h	5h
500	2/0	Day 5 post partum – animal removed for necropsy	1	0	0	0	0	0	0	0	0	0	0	*	0	0	*	0	0	0	0	0	0	1		
		Littering ◊	0	0	0	0	0	0	0	1	1	0	0	*	0	0	*	0	0	0	0	0	0			
		Increased salivation	0	0	0	0	0	0	0	0	0	0	1†	*	0	1†	*	0	1†	0	0	1†	0			
		No abnormalities detected	1	1	1	1	1	1	1	0	0	1	0	*	1	0	*	1	0	1	1	0	1			

Pre = immediately before dosing
1h = one hour after dosing
5h = five hours after dosing
* = five hour observation not performed at weekend
† = increased salivation detected up to ten minutes after dosing

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Table 4 Bodyweights for Males – Group Mean Values

Dose Level (mg/kg/day)	Number of Animals		Bodyweight (g) at Day						
			1	8	15	22	29	36	43
0 (Control)	10	mean	308	349	370	396	422	447	451
		sd	22	26	29	29	30	36	37
50	10	mean	312	358	385	403	427	445	459
		sd	20	23	28	31	34	36	41
150	10	mean	314	356	384	406	426	448	461
		sd	17	19	20	20	23	26	28
500	10/9♦	mean	313	366	391	405	427	439	445
		sd	15	25	29	32	41	48	51

♦ = one male killed on Day 2
No statistical significance (p>0.05)

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Table 5 Bodyweight Change for Males - Group Mean Values

Dose Level (mg/kg/day)	Number of Animals		Increase in Bodyweight (g) during Week					
			1	2	3	4	5	6
0 (Control)	10	mean	41	21 *(62)*	26 *(88)*	27 *(115)*	25 *(139)*	4 *(144)*
		sd	8	6 *(10)*	9 *(11)*	5 *(13)*	7 *(18)*	13 *(23)*
50	10	mean	46	27 *(73)*	18 *(91)*	24 *(115)*	18 *(133)*	14 *(147)*
		sd	8	8 *(15)*	7 *(20)*	4 *(23)*	8 *(26)*	6 *(31)*
150	10	mean	42	28 *(70)*	22 *(92)*	21 *(112)*	22 *(134)*	13 *(147)*
		sd	8	5 *(8)*	6 *(10)*	7 *(14)*	5 *(15)*	6 *(19)*
500	10/9♦	mean	*53	25 *(79)*	**14 *(93)*	22 *(115)*	**12 *(127)*	6 *(133)*
		sd	11	10 *(19)*	9 *(20)*	11 *(28)*	11 *(36)*	4 *(39)*

♦ = one male killed on Day 2
( ) = cumulative bodyweight change relative to Day 1
* = significantly different from corresponding control group $p < 0.05$
** = significantly different from corresponding control group $p < 0.01$

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Table 6 Bodyweights for Females - Group Mean Values

Dose Level (mg/kg/day)	Number of Animals		Bodyweight (g) at Day								
			Maturation			Gestation				Lactation	
			1	8	15	0	7	14	20	1	4
0 (Control)	10/8♦	mean	197	215	226	232	271	306	381	293	303
		sd	11	14	16	15	14	18	26	22	21
50	10	mean	197	214	228	232	273	309	383	294	309
		sd	14	15	19	18	18	20	35	25	25
150	10	mean	203	218	233	237	274	312	384	304	320
		sd	16	14	17	18	20	20	29	23	20
500	10/7/6●	mean	199	220	232	247	280	307	**348	296	303
		sd	15	21	19	27	28	33	34	32	27

● = n = seven during gestation and six during lactation
♦ = n = eight during lactation
** = statistically significant from control group $p<0.01$

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Table 7 Bodyweight Change for Females - Group Mean Values

Dose Level (mg/kg/day)	Number of Animals		Increase in Bodyweight (g) during:					
			Maturation		Gestation			Lactation
			Week 1	Week 2	Days 0-7	Days 7-14	Days 14-20	Days 1-4
0 (Control)	10/8♦	mean	17	11 *(29)*	38	36 *(74)*	75 *(149)*	10
		sd	6	5 *(9)*	8	8 *(14)*	11 *(24)*	6
50	10	mean	17	14 *(31)*	41	36 *(77)*	73 *(151)*	15
		sd	7	6 *(7)*	8	6 *(12)*	18 *(28)*	7
150	10	mean	16	14 *(30)*	37	38 *(75)*	72 *(147)*	16
		sd	5	6 *(7)*	4	6 *(6)*	15 *(20)*	5
500	10/7/6●	mean	21	12 *(34)*	32	**27 *(60)*	***41 *(101)*	7
		sd	8	8 *(7)*	8	9 *(13)*	17 *(25)*	8

♦ = n =eight during lactation
● = n = seven during gestation and six during lactation
( ) = cumulative bodyweight change relative to commencement of phase (ie Day 1 of study, Day 0 of gestation)
** = statistically significant from control group $p<0.01$
*** = statistically significant from control group $p<0.001$

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Table 8 Food Consumption for Males - Group Mean Values

Dose Level (mg/kg/day)	Number of Animals	Mean Food Consumption (g/rat/day) during Week			
		1	2	5	6
0 (Control)	10	30	29	32	30
50	10	31 (+3)	29 (0)	30 (-5)	29 (-1)
150	10	31 (+4)	31 (+5)	32 (+1)	31 (+6)
500	10/9♦	33 (+10)	31 (+8)	32 (0)	30 (+3)

♦ = n = nine from Day 2
( ) = difference from Control as percentage of Control value

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Table 9 Food Consumption for Females - Group Mean Values

Dose Level (mg/kg/day)	Number of Animals		Mean Food Consumption (g/rat/day) during:					
			Maturation		Gestation			Lactation
			Week 1	Week 2	Days 0-7	Days 7-14	Days 14-20	Days 1-4
0 (Control)	10/8♦	mean	19	20	24	25	27	41
		sd			2	2	2	5
50	10	mean	18	20	26	27	27	41
		sd			2	2	4	7
		% C	(-5)	(+1)	(+7)	(+6)	(-1)	(0)
150	10	mean	17	20	24	27	29	41
		sd			2	2	3	7
		% C	(-12)	(-1)	(+2)	(+6)	(+7)	(+1)
500	10/7/6●	mean	19	22	24	27	27	**28
		sd			2	4	3	7
		% C	(0)	(+9)	(+2)	(+7)	(-1)	(-31)

♦ = n = eight during lactation
● = n = seven during gestation and six during lactation
% C = difference from Control as percentage of Control value
** = statistically significant from control group $p<0.01$ (statistical analysis only performed during gestation and lactation)

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Table 10 Food Efficiency for Males - Group Values

Dose Level (mg/kg/day)	Number of Animals	Food Efficiency* during Week			
		1	2	5	6
0 (Control)	10	0.20	0.10	0.11	0.02
50	10	0.21	0.13	0.08	0.07
150	10	0.19	0.13	0.10	0.06
500	10/9♦	0.23	0.11	0.05	0.03

$$* \text{ Food Efficiency} = \frac{\text{Group mean bodyweight gain (g/rat)}}{\text{Group mean food consumption (g/rat/day) x number of days}}$$

♦ = n=9 from Day 2

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Table 11 Food Efficiency for Females - Group Values

Dose Level (mg/kg/day)	Number of Animals	Food Efficiency* during:					
		Maturation		Gestation			Lactation
		Week 1	Week 2	Days 0-7	Days 7-14	Days 14-20	Days 1- 4
0 (Control)	10	0.13	0.08	0.23	0.20	-	-
50	10	0.13	0.10	0.23	0.19	-	-
150	10	0.13	0.10	0.22	0.20	-	-
500	10/7•	0.16	0.08	0.19	0.14	-	-

$$* \text{ Food Efficiency} = \frac{\text{Group mean bodyweight gain (g/rat)}}{\text{Group mean food consumption (g/rat/day) x number of days}}$$

- = not applicable
• = seven during gestation

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Table 12 Mating Performance and Fertility – Group Values

Dose Group (mg/kg/day)	Number of Males Paired	Number of Females			Pre-Coital Interval (Days)								Mating Index (%)	Pregnancy Index (%)
		Paired	Mated	Pregnant	1	2	3	4	5	6	7	14		
0 (Control)	10	10	10	10	1	4	4	1	0	0	0	0	100	100
50	10	10	10	10	2	3	3	1	0	0	1	0	100	100
150	10	10	10	10	3	2	2	3	0	0	0	0	100	100
500	9†	10	9	8	1	2	1	0	1	2	1	1	90	89

† = one male paired twice due to death of a male during pre-pairing phase of study

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Table 13 Gestation Length - Group Values

Dose Group (mg/kg/day)	Number of Pregnant Females	Gestation Lengths (Days)				Females with Live Offspring	Parturition Index (%)
		22	22½	23	23½		
0 (Control)	10	2	5	3	0	9#	90
50	10	4	3	3	0	10	100
150	10	1	4	4	1	10	100
500	8◊	0	0	6	1	7	88

= only dead offspring observed for one litter
◊ = one dam had implantation sites in uterus at necropsy but no offspring were observed to be born

Table 14 Litter and Offspring Bodyweight Data - Group Mean Litter Values

Dose Group (mg/kg/day)	Number of litters		Number of Corpora Lutea	Number of Implantation Sites	Total number of Offspring Born	Number of Live Offspring		Litter Weight (g)		Offspring Weight (g)				Mean Offspring Bodyweight Change (g)	
						Day 1	Day 4	Day 1	Day 4	Day 1		Day 4		Days 1 - 4	
										Male	Female	Male	Female	Male	Females
0 (Control)	8	mean	17.8	15.1	14.5	14.4	14.4	100.9	145.2	7.2	6.8	10.2	9.8	3.1	3.1
		sd	2.4	1.9	1.9	1.6	1.6	14.3	20.4	0.5	0.5	0.8	0.6	0.5	0.5
50	10	mean	17.4	15.3	13.6	12.9	12.8	92.0	131.7	7.5	7.1	10.8	10.3	3.3	3.3
		sd	2.4	1.4	3.4	3.9	3.9	26.1	38.2	0.9	0.8	1.4	1.3	0.6	0.6
150	10	mean	16.8	14.0	13.0	12.8	12.7	94.4	137.6	7.7	7.3	11.4	10.8	3.7	3.5
		sd	3.8	3.6	3.1	3.0	3.0	20.5	28.0	0.8	0.9	1.5	1.4	0.8	0.7
500	6	mean	15.7	14.2	***6.8	**6.8	***6.8	**50.0	**71.3	7.5	7.3	10.7	10.3	3.2	3.0
		sd	3.7	3.4	4.0	4.0	4.0	28.6	41.0	0.5	0.4	0.7	0.7	0.4	0.5

** = statistically significant from control group $p<0.01$
*** = statistically significant from control group $p<0.001$

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Table 15 Implantation Losses and Survival Indices – Group Mean Litter Values

Dose Group (mg/kg/day)	Number of litters		Pre- Implantation Loss (%)	Post -Implantation Loss (%)	Live Birth Index	Viability Index
0 (Control)	8	mean	14.1	4.0	99.3	100.0
		sd	11.4	4.7	2.0	0.0
50	10	mean	11.0	11.7	92.6	99.2
		sd	10.7	18.9	12.4	2.4
150	10	mean	17.0	6.3	98.6	99.3
		sd	11.3	5.1	3.0	2.1
500	6	mean	9.7	**52.2	100.0	100.0
		sd	7.0	20.8	0.0	0.0

** = statistically significant from control group $p<0.01$

Table 16 Sex Ratio – Group Mean Litter Values

Dose Group (mg/kg/day)	Number of litters		Sex Ratio (*Post Partum*) Day:								
			At birth			1			4		
			Male	Female	% Male	Male	Female	% Male	Male	Female	% Male
0 (Control)	8	mean	7.9	6.6	54.0	7.8	6.6	53.6	7.8	6.6	53.6
		sd	2.9	2.9	19.7	3.0	2.9	20.2	3.0	2.9	20.2
50	10	mean	6.6	6.9	49.4	6.4	6.5	50.7	6.3	6.5	50.2
		sd	2.0	2.1	8.0	2.2	2.2	8.7	2.2	2.2	9.1
150	10	mean	6.1	6.9	45.7	6.0	6.8	45.6	5.9	6.8	45.0
		sd	3.5	3.1	20.7	3.5	3.0	20.7	3.6	3.0	21.3
500	6	mean	3.3	3.5	50.5	3.3	3.5	50.5	3.3	3.5	50.5
		sd	2.2	2.4	26.2	2.2	2.4	26.2	2.2	2.4	26.2

% males - no statistical significance ($p>0.05$)

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Table 17 Clinical Observations for Offspring – Group Incidences

Dose Level (mg/kg/day)	Number of Females with Litters	Clinical Observations	Numbers of Litters and Offspring per Litter Affected *(Post Partum)* Day:											
			Pre-Day 1		1		2		3		4		5	
			Number of Litters	Number of Offspring	Number of Litters	Number of Offspring	Number of Litters	Number of Offspring	Number of Litters	Number of Offspring	Number of Litters	Number of Offspring	Number of Litters	Number of Offspring
0 (Control)	10†	Bruised and swollen left eye	-	-	-	-	-	-	1	1F	1	1F	1	1F
		Small	2	2F	2	2F	2	2F	1	1F	1	1F	1	1F
		Total litter loss	1	-	-	-	-	-	-	-	-	-	-	-
		Found dead	3	5M, 8F,1*	-	-	-	-	-	-	-	-	-	-
		No abnormalities detected	6	-	6	-	6	-	6	-	6	-	6	-
50	10	Bruising on nose/snout	1	1M	1	1M	-	-	-	-	-	-	-	-
		Small	-	-	1	1F	1	1F	1	1F	1	F	-	-
		Missing			1	1*	-			-	1	1M		
		Found dead	1	1F	-	-	-	-	-	-	-	-	1	1F
		No abnormalities detected	8	-	7	-	9	-	9	-	8	-	9	-
150	10	Bruising and/or scratch around eye	-	-	1	1M	1	1M	1	1M	-	-	-	-
		Bruised face	1	1M	-	-	-	-	-	-	-	-	-	-
		Small	1	1F	-	-	-	-	1	1F	1	1F	1	1F
		Swollen hind limb	-	-	-	-	-	-	1	1F	1	1F	1	1F
		Found dead	-	-	1	1F	-	-	1	1M	-	-	1	1F
		No abnormalities detected	8	-	8	-	9	-	7	-	9	-	8	-
500	7	Small	1	1M, 1F	1	1F	1	1F	1	1F	1	1F	1	1F
		No milk in stomach	1	1M, 1F	-	-	-	-	-	-	-	-	-	-
		Total litter loss	-	-	1	-	-	-	-	-	-	-	-	-
		Found Dead	1	1F	1	1M	-	-	-	-	-	-	-	-
		No abnormalities detected	6	-	5	-	5	-	5	-	5	-	5	-

† = one litter killed on Day 1 due to death of dam
- = not applicable
* = sex undetermined
M = male
F = female

Table 18 Reflexological Responses for Offspring – Group Mean Litter Values

Dose Level (mg/kg/day)	Number of Litters		Surface Righting Reflex (% passed)
0 (Control)	8	mean	74.5
		sd	13.2
50	10	mean	88.0
		sd	12.9
150	10	mean	92.6
		sd	7.9
500	6	mean	85.6
		sd	20.1

no statistical significance (p>0.05)

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Table 19 Necropsy Findings of Offspring – Group Incidences

	Dose Level (mg/kg/day)			
	0 (Control)	50	150	500
Interim death				
Number of animals examined	11M, 15F, 1* (3)†	2M, 5F (5)	2M, 2F (4)	1M, 1F (1)
Autolysis	1M, 4F(1)	1M, 3F(3)	1M, 1F(2)	1F(1)
Cannibalised	1M, 1F, 1*(1)	-	-	-
No milk in stomach	-	-	-	1M(1)
No abnormalities detected	9M, 10F(3)	1M, 2F(2)	1M, 1F(2)	-
Terminal kill				
Number of animals examined	62M, 53F (8)	63M, 64F (10)	59M, 68F (10)	20M, 21F (6)
Bruised and swollen left eye	1F (1)	-	-	-
Small	-	-	1F (1)	1F (1)
Swollen right hind limb	-	-	1F (1)	-
No abnormalites detected	62M, 52F (8)	63M, 64F (10)	59M, 67F (10)	20M, 20F (6)

( ) = litter affected
* = sex undetermined
- = not applicable
† = includes one litter (Number 17; six male and seven female) where offspring were killed due to death of dam

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Table 20 Necropsy Findings of Males – Group Incidences

	Dose Level (mg/kg/day)			
	0 (Control)	50	150	500
Interim Death				
Number of animals found dead:	0	0	0	1
Stained snout	-	-	-	1
Thoracic cavity: filled with red fluid	-	-	-	1
Cranial, intermediate and caudal lobes of right lung:				
encased in white fibrous tissue	-	-	-	1
Terminal Kill				
Number of animals examined at terminal kill	10	10	10	9
Staining of the fur	0	0	0	4
Left kidney: small	0	0	1	0
Right kidney: large, increased renal pelvic cavity	0	0	1	0
No abnormalities detected	10	10	9	5

External findings are presented in italics

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Table 21 Necropsy Findings of Females – Group Incidences

	Dose Level (mg/kg/day)			
	0 (Control)	50	150	500
Interim Death				
Number of animals Found Dead:	1	0	0	0
Red staining around ano-genital area	1	-	-	-
Lungs: reddened	1	-	-	-
Liver: dark	1	-	-	-
Gastro-intestinal tract: gaseous distension	1	-	-	-
Uterus: dark	1	-	-	-
Terminal Kill				
Number of animals examined at terminal kill	9	10	10	10
Staining of the fur	0	0	0	1
Generalised fur loss	1	0	0	0
Lungs: reddened	0	1	0	0
Right ovary encased in fluid filled sac	1	0	0	0
No abnormalities detected	7	9	10	9

External findings are presented in italics

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Table 22 Absolute and Bodyweight-Relative Reproductive Organ Weights for Males - Group Mean Values

Dose Level (mg/kg/day)	Number of Animals		Bodyweight (g) at Terminal Kill	Absolute Organ Weight (g)	
				Epididymides	Testes
0 (Control)	10	mean	451	1.3584	3.6147
		sd	37	0.1423	0.2237
50	10	mean	459	1.2745	3.4096
		sd	41	0.1206	0.3184
150	10	mean	461	1.2717	*3.2601
		sd	28	0.0896	0.2972
500	9	mean	445	***1.0683	**3.1841
		sd	51	0.0860	0.1905

Dose Level (mg/kg/day)	Number of Animals		Relative Organ Weight (%)	
			Epididymides	Testes
0 (Control)	10	mean	0.3014	0.8031
		sd	0.0243	0.0401
50	10	mean	0.2782	0.7487
		sd	0.0197	0.1060
150	10	mean	0.2765	0.7081
		sd	0.0209	0.0609
500	9	mean	***0.2422	0.7243
		sd	0.0254	0.0920

* = statistically significant from control group $p<0.05$
** = statistically significant from control group $p<0.01$
*** = statistically significant from control group $p<0.001$

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Table 23 Histopathological Findings for Males – Group Incidences

Histopathological Finding	Dose Level (mg/kg/day)	
	0 (Control)	500
Number of animals examined	10	9 (10)
	Pituitary	
Vacuolation pars anterior cells		
(minimal)	6	7 (8)
(slight)	4	2 (2)
	Non-Protocol Organs	
Lungs		
Pleuritis and peripheral acute inflammatory cell infiltrates	0	1†

( ) = includes decedent animal
No statistical significance ($p \geq 0.1$), (decedent animals excluded from statistical analysis)
† = decedent animal

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Table 24 Histopathological Findings for Females – Group Incidences

Histopathological Finding	Dose Level (mg/kg/day)	
	0 (Control)	500
Number of animals examined	9 (10)	10
	Uterus/Cervix	
Dilatation horn1		
absent	9 (10)	9
(slight)	0 (0)	1
Dilatation horn2		
absent	9 (10)	9
(slight)	0 (0)	1
Keratinisation cervix		
absent	8 (9)	8
present	1 (1)	2
Peripheral foam cells/ haemorrhage/pigment		
absent	0 (1)	3
present	9 (9)	7+
Congestion		
absent	9 (9)	10
present	0 (1)	0
	Vagina	
Epithelial keratinisation		
absent	7 (7)	8
present	1 (2)	2
	Non-Protocol Organs	
Intestinal tract		
Autolysis (moderate)	1†	0
Liver		
Congestion	1†	0
Lungs		
Peribronchiolar lymphoid aggregations (minimal)	1†	0
Congestion	1†	0

( ) = includes decedent animal
+ = $p<0.05$ (decedent animals excluded from statistical analysis)
† = decedent animal

FIGURES

Figure 1 **Bodyweight Change for Males – Group Mean Values**

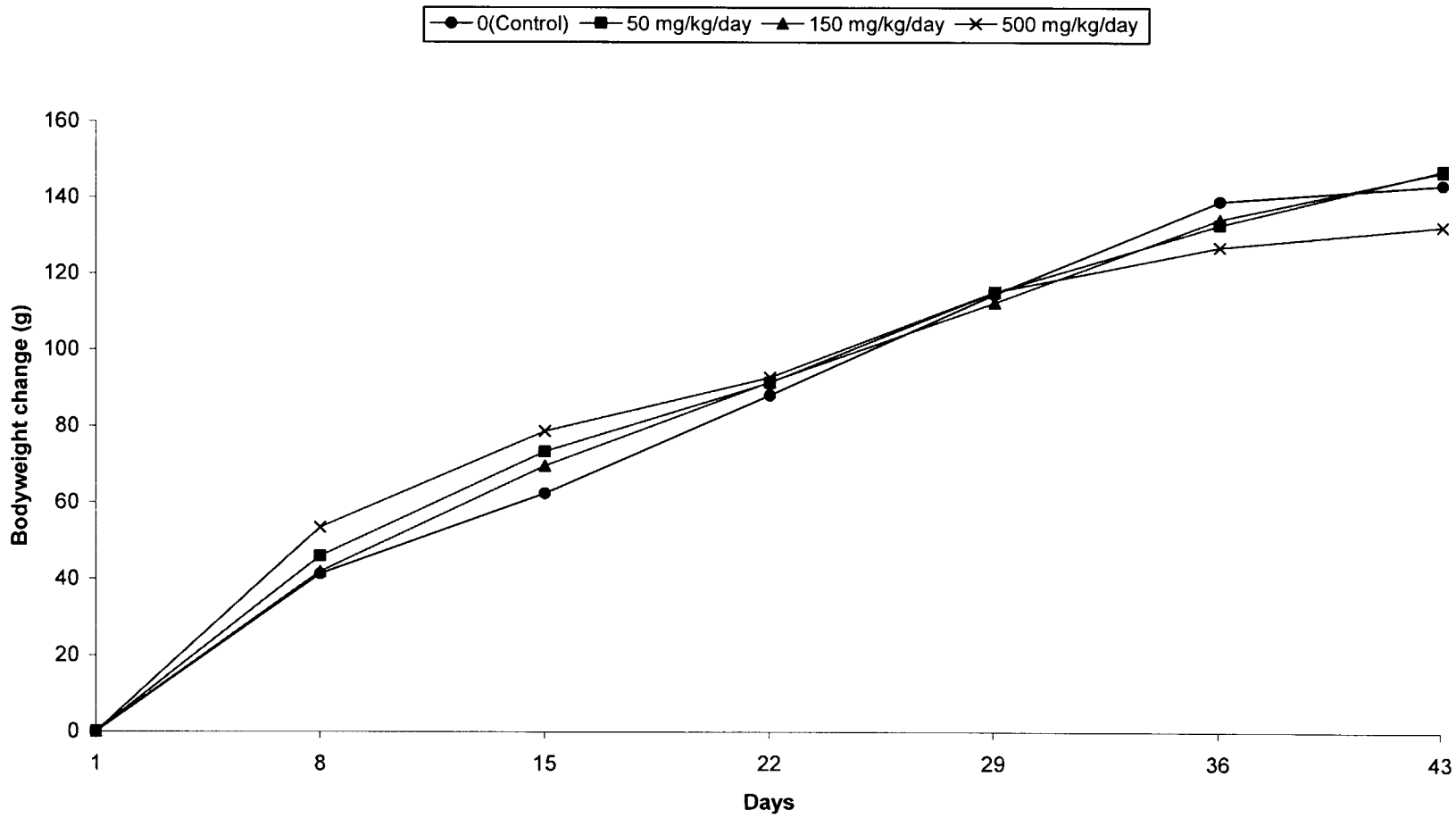


Figure 2 Bodyweight Change for Females – Group Mean Values

Maturation

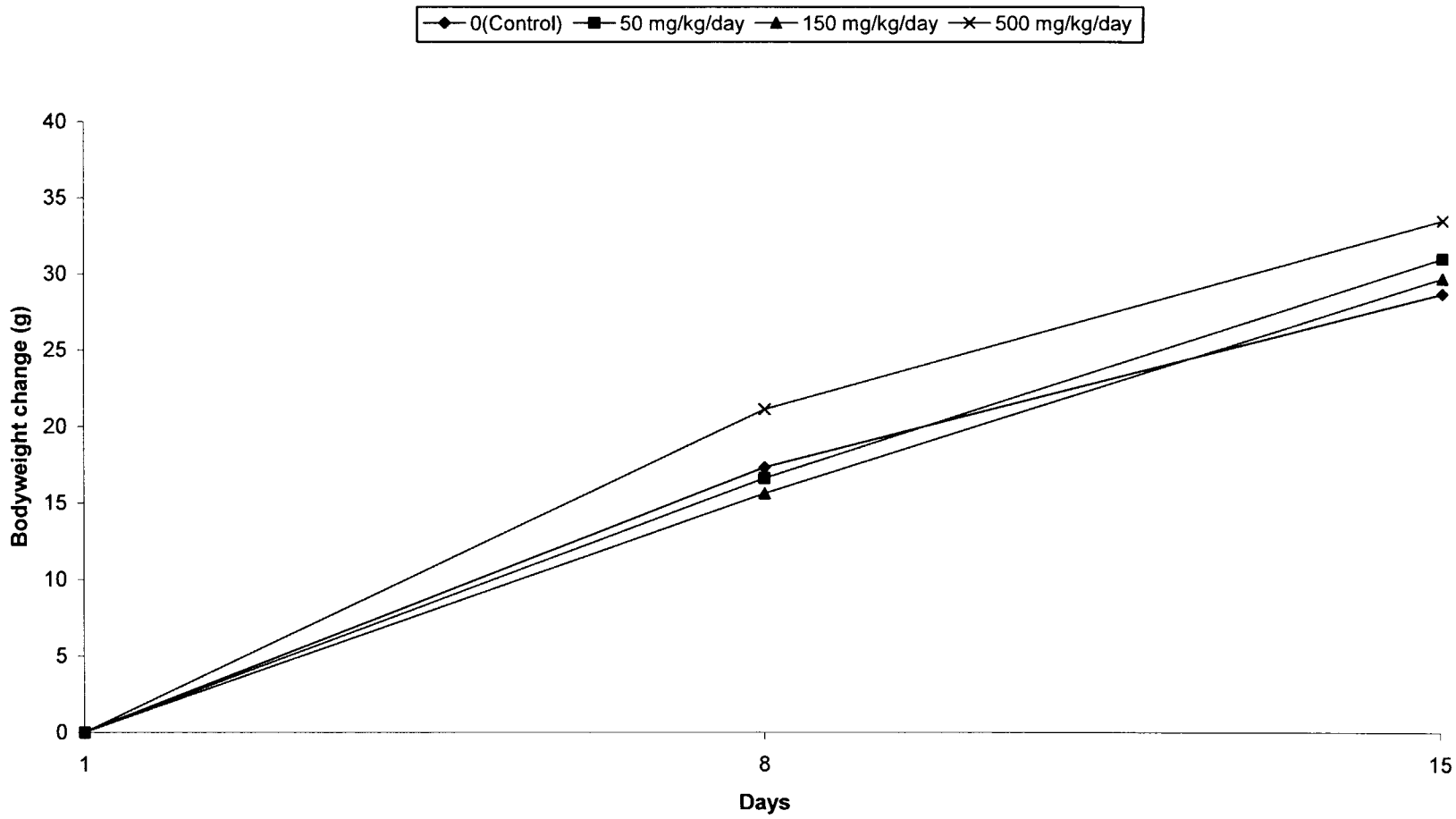


Figure 2 (continued) **Bodyweight Change for Females – Group Mean Values**

Gestation

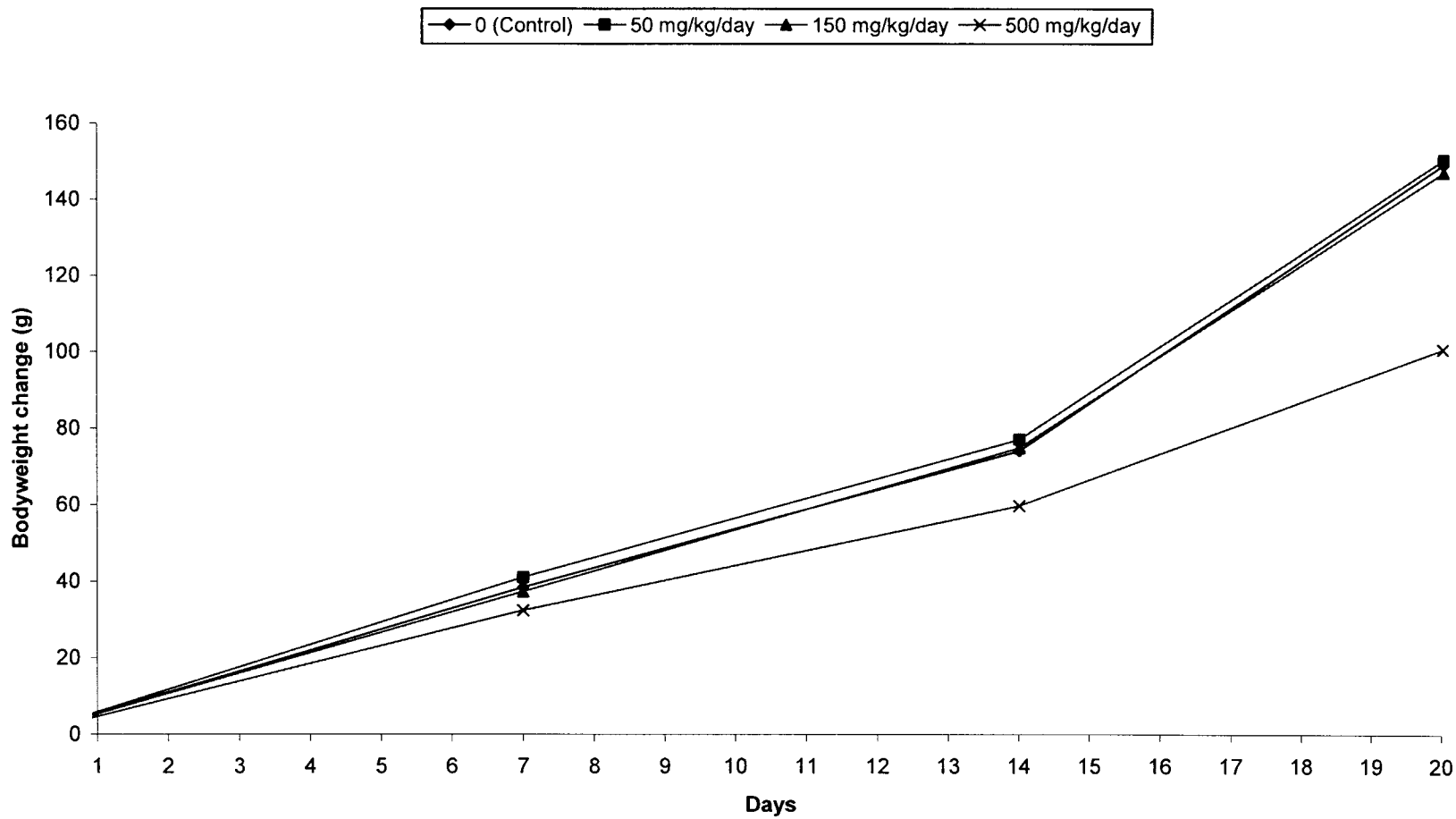


Figure 2 (continued) **Bodyweight Change for Females – Group Mean Values**

Lactation

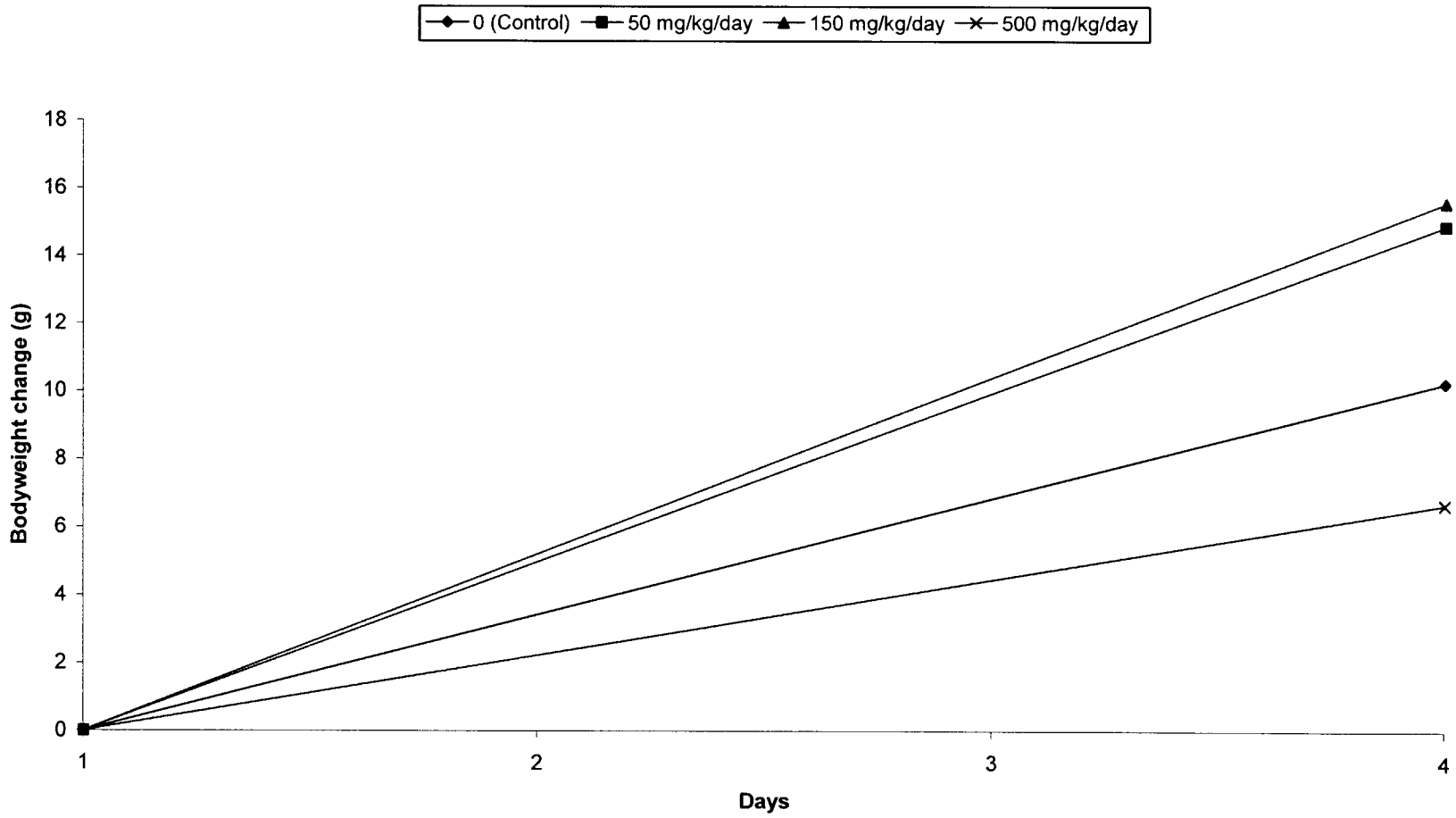


Figure 3 **Food Consumption for Males – Group Mean Values**

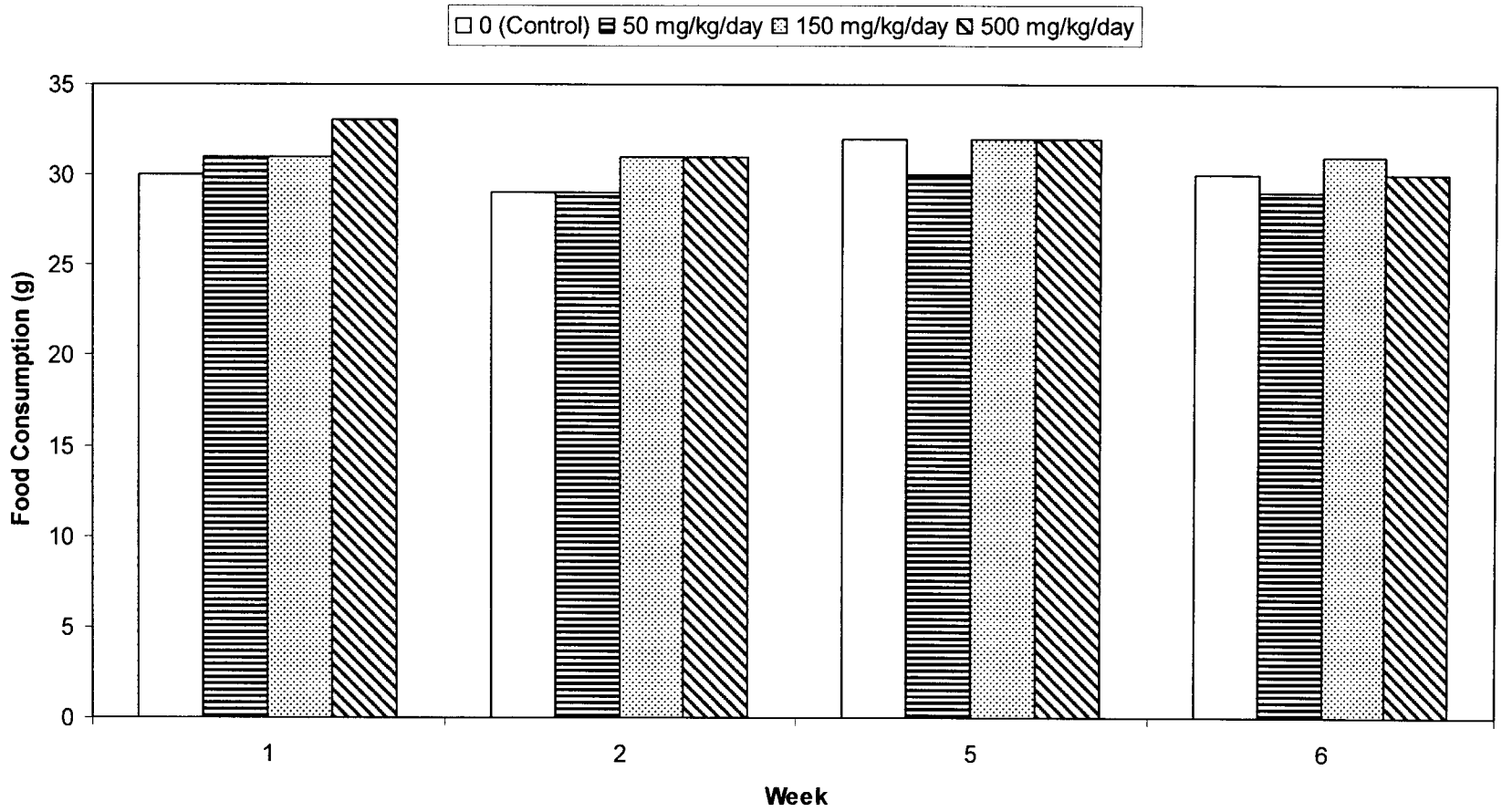


Figure 4 **Food Consumption for Females – Group Mean Values**

Maturation

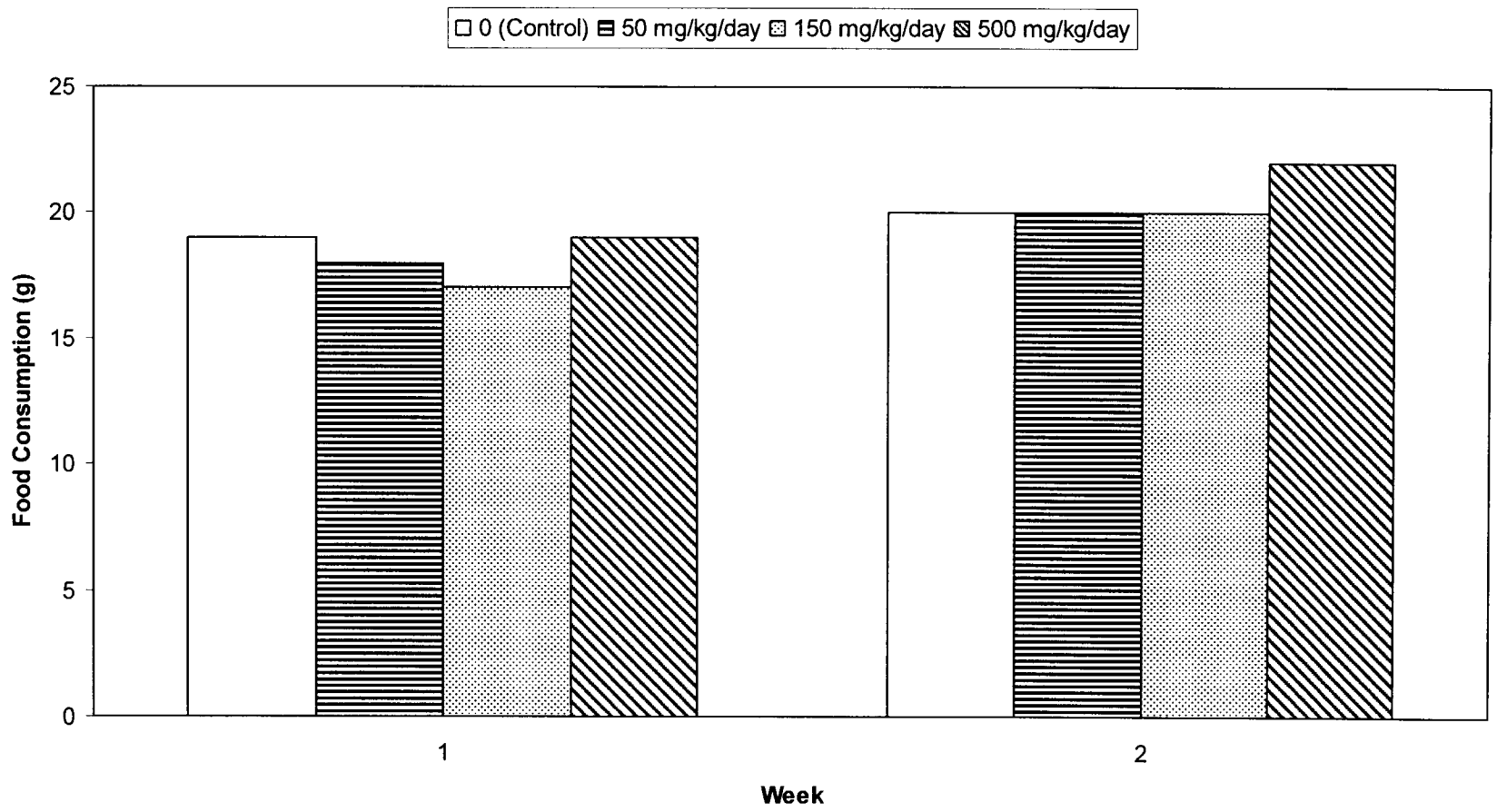
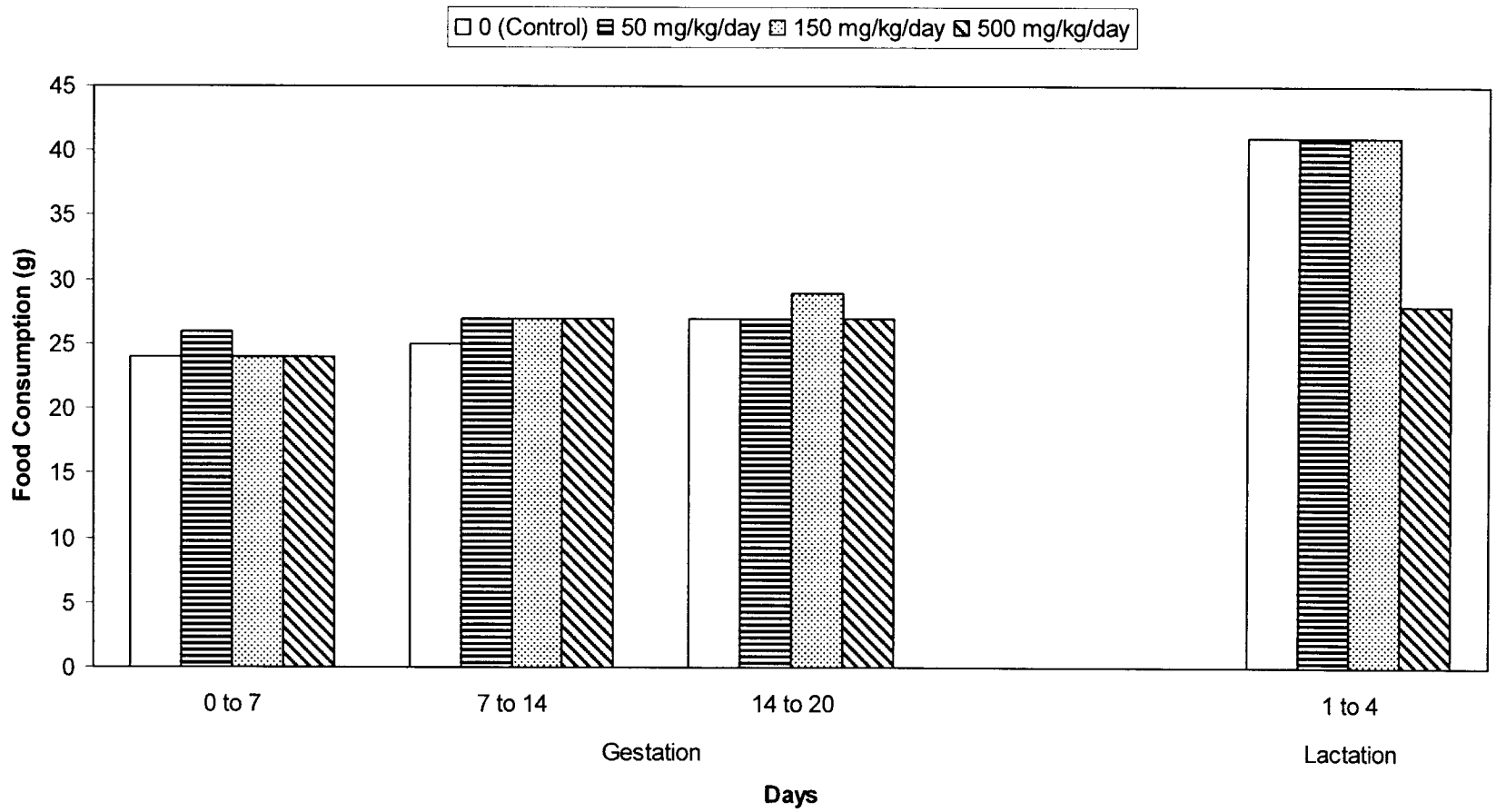


Figure 4 (continued) **Food Consumption during Gestation and Lactation – Group Mean Values**



APPENDICES

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Appendix 1 Clinical Signs for Males – Individual Observations

DOSE LEVEL: 0 (Control)

Animal Number	Day of Necropsy	Clinical Sign (Days of study observed)
1	43	No abnormalities detected
2	43	No abnormalities detected
3	43	No abnormalities detected
4	43	No abnormalities detected
5	43	No abnormalities detected
6	43	No abnormalities detected
7	43	No abnormalities detected
8	43	No abnormalities detected
9	43	No abnormalities detected
10	43	Scratches under both eyes (19-26)

Appendix 1 (continued) Clinical Signs for Males – Individual Observations

DOSE LEVEL: 50 mg/kg/day

Animal Number	Day of Necropsy	Clinical Sign (Days of study observed)
21	43	No abnormalities detected
22	43	No abnormalities detected
23	43	Increased salivation up to 10 minutes after dosing (26)
24	43	No abnormalities detected
25	43	No abnormalities detected
26	43	No abnormalities detected
27	43	No abnormalities detected
28	43	No abnormalities detected
29	43	No abnormalities detected
30	43	No abnormalities detected

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Appendix 1 (continued) Clinical Signs for Males – Individual Observations

DOSE LEVEL: 150 mg/kg/day

Animal Number	Day of Necropsy	Clinical Sign (Days of study observed)
41	43	No abnormalities detected
42	43	No abnormalities detected
43	43	No abnormalities detected
44	43	No abnormalities detected
45	43	No abnormalities detected
46	43	No abnormalities detected
47	43	No abnormalities detected
48	43	No abnormalities detected
49	43	No abnormalities detected
50	43	No abnormalities detected

Appendix 1 (continued) Clinical Signs for Males – Individual Observations

DOSE LEVEL: 500 mg/kg/day

Animal Number	Day of Necropsy	Clinical Sign (Days of study observed)
61	43	Red/brown staining of fur (24-33)
62	43	Increased salivation up to 10 minutes after dosing (12)
		Red/brown staining of fur (33-42)
63	43	Red/brown staining of fur (33)
64	43	Red/brown staining of fur (16, 18-42)
		Red/brown staining around mouth (20-22)
65	43	Noisy respiration up to 10 minutes after dosing (18)
		Red/brown staining of fur (19-22, 33)
66	43	No abnormalities detected
67	43	Red/brown staining of fur (24)
		Wound/Scab on dorsal body surface (29-32)
68	43	Red/brown staining of fur (16, 19-22, 25-42)
		Red/brown staining around eyes (28)
		Wound/Scab on dorsal body surface (29-32)
69	43	Red/brown staining of fur (16, 19-28, 33-42)
70KIE	2	Hunched posture (2)
		Noisy and laboured respiration (2)
		Pilo-erection (2)
		Red/brown staining of mouth and snout (2)
		Killed *in extremis* (2)

KIE = killed *in extremis*

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Appendix 2 Clinical Signs for Females – Individual Observations

DOSE LEVEL: 0 (Control)

Animal Number	Day of Necropsy	Clinical Sign (Days of study observed)
11	46	No abnormalities detected
12	45	No abnormalities detected
		Animal not dosed due to littering (40)
13	46	No abnormalities detected
		Animal not dosed due to littering (40-41)
14	44	No abnormalities detected
		Animal not dosed due to littering (39)
15TLL	43	No abnormalities detected
		Animal not dosed due to littering (40)
16	45	No abnormalities detected
		Animal not dosed due to littering (40)
17FD	41	No abnormalities detected
		Animal not dosed due to littering (40)
		Found dead (41)
18	44	No abnormalities detected
		Animal not dosed due to littering (39)
19	43	Generalised fur loss (39-42)
		Animal not dosed due to littering (38)
20	45	No abnormalities detected
		Animal not dosed due to littering (40)

TLL = total litter loss
FD = found dead

Appendix 2 (continued) Clinical Signs for Females – Individual Observations

DOSE LEVEL: 50 mg/kg/day

Animal Number	Day of Necropsy	Clinical Sign (Days of study observed)
31	45	No abnormalities detected *Animal not dosed due to littering (39-40)*
32	44	No abnormalities detected *Animal not dosed due to littering (39)*
33	45	No abnormalities detected *Animal not dosed due to littering (40)*
34	45	No abnormalities detected *Animal not dosed due to littering (40)*
35	43	No abnormalities detected *Animal not dosed due to littering (38)*
36	46	No abnormalities detected *Animal not dosed due to littering (41)*
37	45	No abnormalities detected *Animal not dosed due to littering (40)*
38	49	Red/brown staining of fur (23) *Animal not dosed due to littering (44)*
39	43	No abnormalities detected *Animal not dosed due to littering (38)*
40	46	No abnormalities detected *Animal not dosed due to littering (41)*

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Appendix 2 (continued) Clinical Signs for Females – Individual Observations

DOSE LEVEL: 150 mg/kg/day

Animal Number	Day of Necropsy	Clinical Sign (Days of study observed)
51	46	Increased salivation up to ten minutes after dosing (29)
		Animal not dosed due to littering (40-41)
52	46	Noisy respiration up to ten minutes after dosing and one hour after dosing (44)
		Animal not dosed due to littering (41)
53	46	No abnormalities detected
		Animal not dosed due to littering (41)
54	45	No abnormalities detected
		Animal not dosed due to littering (40)
55	46	No abnormalities detected
		Animal not dosed due to littering (41)
56	44	No abnormalities detected
		Animal not dosed due to littering (39)
57	44	No abnormalities detected
		Animal not dosed due to littering (38-39)
58	43	No abnormalities detected
		Animal not dosed due to littering (38)
59	46	No abnormalities detected
		Animal not dosed due to littering (41)
60	43	No abnormalities detected

Appendix 2 (continued) Clinical Signs for Females – Individual Observations

DOSE LEVEL: 500 mg/kg/day

Animal Number	Day of Necropsy	Clinical Sign (Days of study observed)
71 TLL	57	Increased salivation up to ten minutes after dosing (19, 53-56) Staining of fur (28-32) *Animal not dosed due to littering (52)*
72 NYB	48	Increased salivation up to ten minutes after dosing (12) Staining around snout (16, 20-22) Staining around mouth (20-22)
73	44	Hunched posture (14) Staining around snout (16-22) Staining of fur (16-22) *Animal not dosed due to littering (39)*
74	49	Staining of fur (16-22) Staining around snout (17-22) *Animal not dosed due to littering (44)*
75 NP	43	Staining of fur (16-22) Staining around snout (17-22)
76	48	No abnormalities detected *Animal not dosed due to littering (43)*
77 FTM	43	Increased salivation up to ten minutes after dosing (18) Staining of fur (28-42)
78	45	No abnormalities detected *Animal not dosed due to littering (40)*
79	50	Increased salivation up to ten minutes after dosing (19, 23) Staining of fur (19-22) *Animal not dosed due to littering (45)*
80	47	No abnormalities detected *Animal not dosed due to littering (42)*

TLL = total litter loss
NYB = implantation detected in uterus but no offspring observed to be born
NP = not pregnant
FTM = failed to mate

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Appendix 3 Bodyweights and Bodyweight Change for Males – Individual Values

DOSE LEVEL: 0 (Control)

Animal Number	Bodyweight (g) at Day						
	1	8	15 P	22	29	36	43
1	309	355	374	397	420	447	452
2	324	379	401	432	465	497	514
3	299	327	344	387	417	445	453
4	268	313	323	348	380	396	411
5	327	374	394	419	450	485	486
6	327	366	384	414	436	459	472
7	316	360	390	416	441	463	461
8	321	359	387	413	434	457	429
9	269	300	324	348	367	379	384
10	317	357	379	384	413	440	450

Animal Number	Bodyweight Change (g) during Week					
	1	2	3	4	5	6
1	46	19 *(65)*	23 *(88)*	23 *(111)*	27 *(138)*	5 *(143)*
2	55	22 *(77)*	31 *(108)*	33 *(141)*	32 *(173)*	17 *(190)*
3	28	17 *(45)*	43 *(88)*	30 *(118)*	28 *(146)*	8 *(154)*
4	45	10 *(55)*	25 *(80)*	32 *(112)*	16 *(128)*	15 *(143)*
5	47	20 *(67)*	25 *(92)*	31 *(123)*	35 *(158)*	1 *(159)*
6	39	18 *(57)*	30 *(87)*	22 *(109)*	23 *(132)*	13 *(145)*
7	44	30 *(74)*	26 *(100)*	25 *(125)*	22 *(147)*	-2 *(145)*
8	38	28 *(66)*	26 *(92)*	21 *(113)*	23 *(136)*	-28 *(108)*
9	31	24 *(55)*	24 *(79)*	19 *(98)*	12 *(110)*	5 *(115)*
10	40	22 *(62)*	5 *(67)*	29 *(96)*	27 *(123)*	10 *(133)*

P = animals paired for mating
( ) = cumulative bodyweight change relative to Day 1

Appendix 3 (continued) Bodyweights and Bodyweight Change for Males – Individual Values

DOSE LEVEL: 50 mg/kg/day

Animal Number	Bodyweight (g) at Day						
	1	8	15 P	22	29	36	43
21	308	356	384	402	420	450	464
22	317	370	404	434	462	488	513
23	307	356	387	409	436	459	475
24	332	378	408	423	450	464	482
25	276	317	345	364	383	404	414
26	332	389	425	440	469	489	505
27	283	321	338	350	373	381	388
28	327	376	410	431	457	464	483
29	304	352	376	399	419	433	443
30	331	362	373	379	399	414	423

Animal Number	Bodyweight Change (g) during Week					
	1	2	3 P	4	5	6
21	*48*	28 *(76)*	18 *(94)*	18 *(112)*	30 *(142)*	14 *(156)*
22	*53*	*34 (87)*	30 *(117)*	28 *(145)*	26 *(171)*	25 *(196)*
23	49	31 *(80)*	22 *(102)*	27 *(129)*	23 *(152)*	16 *(168)*
24	46	30 *(76)*	15 *(91)*	27 *(118)*	14 *(132)*	18 *(150)*
25	*41*	28*(69)*	19 *(88)*	19 *(107)*	21 *(128)*	10 *(138)*
26	57	36 *(93)*	15 *(108)*	29 *(137)*	20 *(157)*	16 *(173)*
27	*38*	17 *(55)*	12 *(67)*	23 *(90)*	8 *(98)*	*7 (105)*
28	49	34 *(83)*	21 *(104)*	26 *(130)*	7 *(137)*	19 *(156)*
29	48	24 *(72)*	23 *(95)*	20 *(115)*	14 *(129)*	10 *(139)*
30	31	11 *(42)*	6 *(48)*	20 *(68)*	15 *(83)*	9 *(92)*

P = animals paired for mating
( ) = cumulative bodyweight change relative to Day 1

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Appendix 3 (continued) Bodyweights and Bodyweight Change for Males – Individual Values

DOSE LEVEL: 150 mg/kg/day

Animal Number	Bodyweight (g) at Day						
	1	8	15 P	22	29	36	43
41	289	327	358	382	408	423	437
42	331	376	401	415	430	452	464
43	315	357	394	415	434	453	476
44	323	385	408	428	461	480	505
45	315	357	386	406	431	458	466
46	289	333	353	383	402	423	432
47	325	368	398	424	434	468	478
48	299	334	362	376	387	408	418
49	336	369	399	432	459	484	494
50	318	353	377	395	418	435	440

Animal Number	Bodyweight Change (g) during Week					
	1	2	3 P	4	5	6
41	*38*	31 *(69)*	24 *(93)*	26 *(119)*	15 *(134)*	14 *(148)*
42	*45*	*25 (70)*	14 *(84)*	15 *(99)*	22 *(121)*	12 *(133)*
43	42	37 *(79)*	21 *(100)*	19 *(119)*	19 *(138)*	23 *(161)*
44	62	23 *(85)*	20 *(105)*	33 *(138)*	19 *(157)*	25 *(182)*
45	*42*	29 *(71)*	20 *(91)*	25 *(116)*	27 *(143)*	8 *(151)*
46	44	20 *(64)*	30 *(94)*	19 *(113)*	21 *(134)*	9 *(143)*
47	*43*	30 *(73)*	26 *(99)*	10 *(109)*	34 *(143)*	*10 (153)*
48	35	28 *(63)*	14 *(77)*	11 *(88)*	21 *(109)*	10 *(119)*
49	33	30 *(63)*	33 *(96)*	27 *(123)*	25 *(148)*	10 *(158)*
50	35	24 *(59)*	18 *(77)*	23 *(100)*	17 *(117)*	5 *(122)*

P = animals paired for mating
( ) = cumulative bodyweight change relative to Day 1

Appendix 3 (continued) Bodyweights and Bodyweight Change for Males – Individual Values

DOSE LEVEL: 500 mg/kg/day

Animal Number	Bodyweight (g) at Day						
	1	8	15 P	22	29	36	43
61	307	353	374	385	396	405	411
62	329	393	418	434	454	469	481
63	304	361	390	389	408	412	409
64	318	387	432	454	479	506	516
65	316	370	402	411	446	464	470
66	278	315	338	344	345	349	352
67	320	365	371	398	425	418	423
68	307	353	377	403	429	442	448
69	330	393	415	426	464	487	492
70 KIE	317						

Animal Number	Bodyweight Change (g) during Week					
	1	2	3	4	5	6
61	46	21 *(67)*	11 *(78)*	11 *(89)*	9 *(98)*	6 *(104)*
62	64	25 *(89)*	16 *(105)*	20 *(125)*	15 *(140)*	12 *(151)*
63	57	29 *(86)*	-1 *(85)*	19 *(104)*	4 *(108)*	-3 *(105)*
64	69	45 *(114)*	22 *(136)*	25 *(161)*	27 *(188)*	10 *(198)*
65	54	32 *(86)*	9 *(95)*	35 *(130)*	18 *(148)*	6 *(154)*
66	37	23 *(60)*	6 *(66)*	1 *(67)*	4 *(71)*	3 *(78)*
67	45	6 *(51)*	27 *(78)*	27 *(105)*	-7 *(98)*	5 *(103)*
68	46	24 *(70)*	26 *(96)*	26 *(122)*	13 *(135)*	6 *(141)*
69	63	22 *(85)*	11 *(96)*	38 *(134)*	23 *(157)*	5 *(162)*
70 KIE						

P = animals paired for mating
KIE = killed *in extremis* - Day 2
( ) = cumulative bodyweight change relative to Day 1

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Appendix 4 Bodyweights and Bodyweight Change for Females – Individual Values

DOSE LEVEL: 0 (Control)

Animal Number	Bodyweight (g) at Day								
	Maturation			Gestation				Lactation	
	1	8	15	0	7	14	20	1	4
11	190	205	205	222	255	277	340	256	268
12	219	239	250	265	290	320	395	299	315
13	197	215	224	224	260	296	366	289	293
14	207	234	248	248	289	331	406	323	321
15 TLL	189	210	223	228	276	311	379	279	-
16	191	198	206	217	247	281	350	265	278
17FD	204	226	241	243	278	311	380	-	-
18	187	205	221	223	269	319	420	306	317
19	201	209	225	227	268	298	373	296	309
20	187	204	216	225	274	318	405	309	324

Animal Number	Bodyweight Change (g) during					
	Maturation		Gestation			Lactation
	Week 1	Week 2	Days 0 - 7	Days 7 - 14	Days 14 - 20	Days 1 - 4
11	15	0 *(15)*	33	22 *(55)*	63 *(118)*	12
12	20	11 *(31)*	25	30 *(55)*	75 *(130)*	16
13	18	9 *(27)*	36	36 *(72)*	70 *(142)*	4
14	27	14 *(41)*	41	42 *(83)*	75 *(158)*	-2
15 TLL	21	13 *(34)*	48	35 *(83)*	68 *(151)*	-
16	7	8 *(15)*	30	34 *(64)*	69 *(133)*	13
17 FD	22	15 *(37)*	35	33 *(68)*	69 *(137)*	-
18	18	16 *(34)*	46	50 *(96)*	101 *(197)*	11
19	8	16 *(24)*	41	30 *(71)*	75 *(146)*	13
20	17	12 *(29)*	49	44 *(93)*	87 *(180)*	15

TLL = total litter loss
FD = found dead - Day 1 lactation
- = not applicable
( ) = cumulative bodyweight change relative to start of phase (ie Day 1 of study, Day 0 of gestation)

Appendix 4 (continued) Bodyweights and Bodyweight Change for Females – Individual Values

DOSE LEVEL: 50 mg/kg/day

Animal Number	Bodyweight (g) at Day								
	Maturation			Gestation				Lactation	
	1	8	15	0	7	14	20	1	4
31	186	195	210	210	262	293	360	283	300
32	196	224	237	241	286	334	441	325	337
33	202	220	236	241	280	317	385	295	317
34	176	194	203	198	235	274	341	246	264
35	226	240	266	262	296	326	401	324	330
36	187	205	209	223	268	299	373	282	302
37	197	219	232	234	266	293	332	269	275
38	191	198	214	229	264	302	377	295	309
39	207	217	238	239	293	336	430	316	341
40	201	223	234	243	280	317	385	306	315

Animal Number	Bodyweight Change (g) during					
	Maturation		Gestation			Lactation
	Week 1	Week 2	Days 0 - 7	Days 7 - 14	Days 14 - 20	Days 1 - 4
31	9	15 *(24)*	52	31 *(83)*	67 *(150)*	17
32	28	13 *(41)*	45	48 *(93)*	107 *(200)*	12
33	18	16 *(34)*	39	37 *(76)*	68 *(144)*	22
34	18	9 *(27)*	37	39 *(76)*	67 *(143)*	18
35	14	26 *(40)*	34	30 *(64)*	75 *(139)*	6
36	18	4 *(22)*	45	31 *(76)*	74 *(150)*	20
37	22	13 *(35)*	32	27 *(59)*	39 *(98)*	6
38	7	16 *(23)*	35	38 *(73)*	75 *(148)*	14
39	10	21 *(31)*	54	43 *(97)*	94 *(191)*	25
40	22	11 *(33)*	37	37 (74)	68 (142)	9

( ) = cumulative bodyweight change relative to start of phase (ie Day 1 of study, Day 0 of gestation)

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Appendix 4 (continued) Bodyweights and Bodyweight Change for Females – Individual values

DOSE LEVEL: 150 mg/kg/day

Animal Number	Bodyweight (g) at Day								
	Maturation			Gestation				Lactation	
	1	8	15	0	7	14	20	1	4
51	224	246	259	269	304	346	417	337	346
52	200	217	226	239	276	318	406	315	335
53	183	206	219	219	251	284	322	261	284
54	203	213	218	219	252	292	367	296	308
55	206	222	233	251	290	337	413	306	328
56	182	202	215	210	248	291	374	283	298
57	196	210	233	236	276	312	389	322	337
58	222	232	253	249	291	323	408	323	330
59	191	206	217	230	265	299	368	284	302
60	221	230	252	249	291	318	378	312	327

Animal Number	Bodyweight Change (g) during					
	Maturation		Gestation			Lactation
	Week 1	Week 2	Days 0 - 7	Days 7 - 14	Days 14 - 20	Days 1 - 4
51	22	13 *(35)*	35	42 *(77)*	71 *(148)*	9
52	17	9 *(26)*	37	42 *(79)*	88 *(167)*	20
53	23	13 *(36)*	32	33 *(65)*	38 *(103)*	23
54	10	5 *(15)*	33	40 *(73)*	75 *(148)*	12
55	16	11 *(27)*	39	47 *(86)*	76 *(162)*	22
56	20	13 *(33)*	38	43 *(81)*	83 *(164)*	15
57	14	23 *(37)*	40	36 *(76)*	77 *(153)*	15
58	10	21 *(31)*	42	32 *(74)*	85 *(159)*	7
59	15	11 *(26)*	35	34 *(69)*	69 *(138)*	18
60	9	22 *(31)*	42	27 *(69)*	60 *(129)*	15

( ) = cumulative bodyweight change relative to start of phase (ie Day 1 of study, Day 0 of gestation)

Appendix 4 (continued) Bodyweights and Bodyweight Change for Females – Individual values

DOSE LEVEL: 500 mg/kg/day

Animal Number	Bodyweight (g) at Day								
	Maturation			Gestation				Lactation	
	1	8	15	0	7	14	20	1	4
71 TLL	218	244	248	275	299	320	341	305	308
72 NYB	176	190	212	215	240	259	275	-	-
73	195	220	219	215	255	271	301	270	275
74	196	221	230	243	269	298	341	288	301
75 NP	199	214	241	242	277	288	283	-	-
76	217	256	263	274	319	359	400	346	346
77 FTM	193	202	217	-	-	-	-	-	-
78	180	200	214	218	247	280	355	278	297
79	220	241	258	273	305	338	380	324	321
80	193	210	220	232	263	282	317	271	277

Animal Number	Bodyweight Change (g) during					
	Maturation		Gestation			Lactation
	Week 1	Week 2	Days 0 - 7	Days 7 - 14	Days 14 - 20	Days 1 - 4
71 TLL	26	4 *(30)*	24	21 *(45)*	21 *(66)*	3
72 NYB	14	22 *(36)*	25	19 *(44)*	16 *(60)*	-
73	25	-1 *(24)*	40	16 *(56)*	30 *(86)*	5
74	25	9 *(34)*	26	29 *(55)*	43 *(98)*	13
75 NP	15	27 *(42)*	35	11 *(46)*	-5 *(41)*	-
76	39	7 *(46)*	45	40 *(85)*	41 *(126)*	0
77 FTM	9	15 *(24)*	-	-	-	-
78	20	14 *(34)*	29	33 *(62)*	75 *(137)*	19
79	21	17 *(38)*	32	33 *(65)*	42 *(107)*	-3
80	17	10 *(27)*	31	19 *(50)*	35 *(85)*	6

TLL = total litter loss
NYB = implantation detected in uterus but no offspring observed to be born
NP = not pregnant
- = not applicable
FTM = failed to mate
( ) = cumulative bodyweight change relative to start of phase (ie Day 1 of study, Day 0 of gestation)

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Appendix 5 Food Consumption for Males

Dose Level (mg/kg/day)	Cage Number	Animal Numbers	Mean Food Consumption (g/rat/day) between Weeks:			
			1	2	5	6
0 (Control)	A1	1 - 5	30	29	32	32
	A2	6 - 10	29	29	31	27
50	C1	21 - 25	30	29	31	30
	C2	26 - 30	32	29	30	29
150	E1	41 - 45	32	31	33	32
	E2	46 - 50	30	30	31	30
500	G1	61 - 65	33	32	30	29
	G2	66 - 70	33	31	33	31

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Appendix 6 Food Consumption for Females

Dose Level (mg/kg/day)	Cage Number	Animal Numbers	Mean Food Consumption (g/rat/day) between Weeks:	
			1	2
0 (Control)	B1	11 - 15	21	21
	B2	16 - 20	17	19
50	D1	31 - 35	19	20
	D2	36 - 40	17	21
150	F1	51 - 55	17	19
	F2	56 - 60	16	21
500	H1	71 - 75	20	21
	H2	76 - 80	18	23

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Appendix 7 Food Consumption for Females during Gestation and Lactation – Individual Values

DOSE LEVEL: 0 (Control)

Animal Number	Mean Food Consumption (g/rat/day) during			
	Gestation			Lactation
	Days 0 - 7	Days 7 - 14	Days 14 - 20	Days 1-4
11	21	22	24	33
12	26	26	30	44
13	22	24	27	43
14	26	29	29	36
15 TLL	26	26	28	-
16	22	24	25	37
17 FD	24	25	26	-
18	24	27	30	44
19	23	24	26	45
20	25	27	31	45

TLL = total litter loss
FD = found dead - Day 1 lactation
- = not applicable

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Appendix 7 (continued) Food Consumption for Females during Gestation and Lactation – Individual Values

DOSE LEVEL: 50 mg/kg/day

Animal Number	Mean Food Consumption (g/rat/day) during			
	Gestation			Lactation
	Days 0 - 7	Days 7 - 14	Days 14 - 20	Days 1-4
31	30	30	18	38
32	27	28	30	46
33	23	27	29	43
34	24	23	25	35
35	25	28	31	38
36	23	25	27	41
37	28	28	28	27
38	23	25	27	42
39	27	28	30	50
40	26	26	28	48

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Appendix 7 (continued) Food Consumption for Females during Gestation and Lactation – Individual Values

DOSE LEVEL: 150 mg/kg/day

Animal Number	Mean Food Consumption (g/rat/day) during			
	Gestation			Lactation
	Days 0 - 7	Days 7 - 14	Days 14 - 20	Days 1-4
51	29	31	36	49
52	22	27	31	47
53	23	26	28	28
54	21	23	26	50
55	24	28	29	35
56	23	26	30	43
57	24	27	27	47
58	25	26	29	39
59	23	26	29	41
60	27	28	28	35

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Appendix 7 (continued) Food Consumption for Females during Gestation and Lactation – Individual Values

DOSE LEVEL: 500 mg/kg/day

Animal Number	Mean Food Consumption (g/rat/day) during			
	Gestation			Lactation
	Days 0 - 7	Days 7 - 14	Days 14 - 20	Days 1-4
71 TLL	25	27	25	22
72 NYB	20	23	23	-
73	25	24	25	22
74	23	26	26	34
75 NP	23	23	21	-
76	28	34	34	25
77 FTM	-	-	-	-
78	22	24	26	38
79	25	31	28	26
80	23	24	25	23

TLL = total litter loss
NYB = implantation detected in uterus but no offspring observed to be born
NP = not pregnant
FTM = failed to mate
- = not applicable

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Appendix 8 Mating Performance, Fertility and Gestation Length – Individual Values

DOSE LEVEL: 0 (Control)

Mated Animal Number		Pre-Coital Interval (Days)	Copulation Plug Count	Sperm Reading Score	Pregnancy Status	Gestation Length (Days)
11 F	1 M	4	3 PT H	2+	P	22½
12 F	2 M	3	5 PT	2+	P	22½
13 F	3 M	3	4 PT H	2+	P	23
14 F	4 M	2	2 PT	2+	P	22
15 F	5 M	2	4 PT H	2+	P	23
16 F	6 M	3	5 PT	1+	P	22½
17 F	7 M	2	4 PT H	3+	P	23
18 F	8 M	2	5 PT H	1+	P	22½
19 F	9 M	1	5 PT H	3+	P	22
20 F	10 M	3	5 PT	2+	P	22½

1+ = few spermatozoa present
2+ = continuous few spermatozoa in all fields
3+ = many spermatozoa in all fields
H = haemorrhage
P = pregnant
M = male
F = female
PT = plug detected on tray liner

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Appendix 8 (continued) Mating Performance, Fertility and Gestation Length – Individual Values

DOSE LEVEL: 50 mg/kg/day

Mated Animal Number		Pre-Coital Interval (Days)	Copulation Plug Count	Sperm Reading Score	Pregnancy Status	Gestation Length (Days)
31 F	21 M	2	5 PT H	1+	P	23
32 F	22 M	2	4 PT	3+	P	22
33 F	23 M	3	2 PT H	2+	P	22½
34 F	24 M	3	1 PT	2+	P	22½
35 F	25 M	1	5 PT H	3+	P	22
36 F	26 M	4	3 PT	3+	P	22½
37 F	27 M	2	0	2+	P	23
38 F	28 M	7	3 PT	2+	P	22
39 F	29 M	1	5 PT H	2+	P	22
40 F	30 M	3	2 PT	2+	P	23

1+ = few spermatozoa present
2+ = continuous few spermatozoa in all fields
3+ = many spermatozoa in all fields
H = haemorrhage
P = pregnant
M = male
F = female
PT = plug detected on tray liner

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Appendix 8 (continued) **Mating Performance, Fertility and Gestation Length – Individual Values**

DOSE LEVEL: 150 mg/kg/day

Mated Animal Number		Pre-Coital Interval (Days)	Copulation Plug Count	Sperm Reading Score	Pregnancy Status	Gestation Length (Days)
51 F	41 M	3	5 PT H	3+	P	23
52 F	42 M	4	1 PT	3+	P	22½
53 F	43 M	3	1 PT PV	2+	P	23½
54 F	44 M	2	4 PT H	3+	P	23
55 F	45 M	4	PV	2+	P	22½
56 F	46 M	2	4 PT	1+	P	22
57 F	47 M	1	4 PT	3+	P	23
58 F	48 M	1	4 PT H	2+	P	22½
59 F	49 M	4	3 PT PV	1+	P	22½
60 F	50 M	1	6 PT	3+	P	23

1+ = few spermatozoa present
2+ = continuous few spermatozoa in all fields
3+ = many spermatozoa in all fields
H = haemorrhage
P = pregnant
M = male
F = female
PT = plug detected on tray liner
PV = vaginal plug

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Appendix 8 (continued) Mating Performance, Fertility and Gestation Length – Individual Values

DOSE LEVEL: 500 mg/kg/day

Mated Animal Number		Pre-Coital Interval (Days)	Copulation Plug Count	Sperm Reading Score	Pregnancy Status	Gestation Length (Days)
71 F	61 M	14	1 PT H	1+	P	23½
72 F	62 M	6	PV	1+	P	◊
73 F	63 M	1	0	2+	P	23
74 F	64 M	6	0	2+	P	23
75 F	65 M	2	1 PT	2+	NP	-
76 F	66 M	5	3 PT	2+	P	23
77 F	67 M	-	-	-	FTM	-
78 F	68 M	2	2 PT	3+	P	23
79 F	69 M	7	0	2+	P	23
80 F	63 M	3	1 PT	2+	P	23

1+ = few spermatozoa present
2+ = continuous few spermatozoa in all fields
3+ = many spermatozoa in all fields
H = haemorrhage
P = pregnant
M = male
F = female
PT = plug detected on tray liner
PV = vaginal plug
◊ = dam had implantation sites in uterus at necropsy but no offspring observed to be born
FTM = failed to mate
NP = not pregnant

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Appendix 9 Litter and Offspring Bodyweight Data – Individual Values

DOSE LEVEL: 0 (Control)

Animal/Litter Number	Number of Corpora Lutea	Number of Implantation Sites	Total number of Offspring Born	Number of Live Offspring		Litter Weight (g)		Offspring Weight (g)				Offspring Bodyweight Change (g)	
								Day 1		Day 4		Days 1- 4	
				Day 1	Day 4	Day 1	Day 4	Males	Females	Males	Females	Males	Females
11	17	12	12	12	12	77.1	106.5	6.4	6.4	8.7	8.9	2.3	2.5
12	18	15	14	14	14	102.3	143.1	7.3	7.3	10.2	10.3	2.9	3.0
13	15	14	14	14	14	111.9	154.4	8.1	7.7	11.2	10.5	3.1	2.8
14	17	14	13	13	13	84.8	130.9	7.0	6.2	10.4	9.9	3.4	3.7
15 TLL	21	16	12	-	-	-	-	-	-	-	-	-	-
16	16	15	15	15	15	101.9	140.3	6.9	6.4	9.5	8.9	2.6	2.6
17 FD	18	15	14	13	-	-	-	-	-	-	-	-	-
18	18	18	18	17	17	116.4	169.6	7.1	6.7	10.4	9.7	3.4	3.0
19	23	16	14	14	14	96.8	149.6	7.1	6.6	10.9	10.3	3.8	3.7
20	18	17	16	16	16	116.0	167.2	7.4	7.1	10.6	10.3	3.3	3.1

TLL = total litter loss
FD = found dead - Day 1 lactation
- = not applicable

Appendix 9 (continued) Litter and Offspring Bodyweight Data – Individual Values

DOSE LEVEL: 50 mg/kg/day

Animal/Litter Number	Number of Corpora Lutea	Number of Implantation Sites	Total number of Offspring Born	Number of Live Offspring		Litter Weight (g)		Offspring Weight (g)				Offspring Bodyweight Change (g)	
								Day 1		Day 4		Days 1- 4	
				Day 1	Day 4	Day 1	Day 4	Males	Females	Males	Females	Males	Females
31	18	14	12	12	12	97.6	137.3	8.7	7.7	12.1	11.0	3.4	3.3
32	20	18	18	18	18	124.8	182.1	7.3	6.5	10.5	9.7	3.2	3.2
33	16	16	15	14	14	98.2	138.3	7.4	6.7	10.6	9.3	3.2	2.6
34	14	14	14	14	14	90.0	121.8	6.5	6.3	8.7	8.7	2.2	2.4
35	16	14	13	13	12	84.0	111.2	6.5	6.4	9.1	9.4	2.6	3.0
36	16	15	14	12	12	86.0	127.2	7.4	7.0	10.9	10.4	3.5	3.4
37	21	14	5	3	3	27.3	40.4	9.3	8.8	13.6	13.2	4.4	4.4
38	15	15	14	13	13	93.8	144.8	7.3	7.1	11.2	11.1	3.9	3.9
39	20	17	16	15	15	102.3	147.8	7.0	6.6	10.1	9.6	3.1	3.0
40	18	16	15	15	15	116.1	166.3	7.9	7.6	11.2	11.0	3.4	3.3

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Appendix 9 (continued) Litter and Offspring Bodyweight Data – Individual Values

DOSE LEVEL: 150 mg/kg/day

Animal/Litter Number	Number of Corpora Lutea	Number of Implantation Sites	Total number of Offspring Born	Number of Live Offspring		Litter Weight (g)		Offspring Weight (g)				Offspring Bodyweight Change (g)	
								Day 1		Day 4		Days 1- 4	
				Day 1	Day 4	Day 1	Day 4	Males	Females	Males	Females	Males	Females
51	20	16	14	14	14	118.5	171.7	8.5	8.3	12.3	12.1	3.8	3.8
52	17	17	16	16	16	100.7	146.4	6.4	6.0	9.2	8.9	2.9	2.9
53U	8	6	6	6	6	51.0	76.5	8.4	8.6	12.6	12.8	4.3	4.3
54	18	15	14	14	14	115.0	162.0	8.4	8.2	12.0	11.5	3.6	3.3
55	20	14	14	14	14	98.2	134.8	7.3	6.7	9.9	9.4	2.6	2.6
56	21	17	15	15	14	99.3	142.4	6.7	6.6	10.2	10.2	3.5	3.6
57	16	15	14	14	14	110.5	166.9	8.2	7.6	12.5	11.3	4.3	3.8
58	16	15	13	12	12	89.2	126.1	7.7	7.3	11.6	10.0	3.9	2.7
59	18	16	15	14	14	89.1	132.6	6.7	6.2	9.9	9.3	3.2	3.1
60	14	9	9	9	9	72.4	117.0	8.4	7.8	13.5	12.6	5.2	4.8

U = unilateral implantation

Appendix 9 (continued) Litter and Offspring Bodyweight Data – Individual Values

DOSE LEVEL: 500 mg/kg/day

Animal/Litter Number	Number of Corpora Lutea	Number of Implantation Sites	Total number of Offspring Born	Number of Live Offspring		Litter Weight (g)		Offspring Weight (g)				Offspring Bodyweight Change (g)	
								Day 1		Day 4		Days 1- 4	
				Day 1	Day 4	Day 1	Day 4	Males	Females	Males	Females	Males	Females
71 TLL	16	16	2	-	-	-	-	-	-	-	-	-	-
72 NYB	12	4	-	-	-	-	-	-	-	-	-	-	-
73	10	8	4	4	4	29.5	38.7	7.9	7.2	10.6	9.4	2.7	2.2
74	15	14	9	9	9	69.5	101.7	8.2	7.4	11.8	10.9	3.6	3.6
75 NP	-	-	-	-	-	-	-	-	-	-	-	-	-
76	18	16	4	4	4	31.9	44.7	8.0	8.0	11.3	11.1	3.3	3.1
77 FTM	5	-	-	-	-	-	-	-	-	-	-	-	-
78	21	18	14	14	14	99.1	139.6	7.1	7.0	10.0	9.9	2.9	2.9
79	14	14	6	6	6	42.3	62.9	7.2	7.0	10.7	10.4	3.6	3.4
80	16	15	4	4	4	27.9	40.4	7.0	-	10.1	-	3.1	-

TLL = total litter loss
NYB = implantation detected in uterus but no offspring observed to be born
NP = not pregnant
FTM = failed to mate
- = not applicable

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Appendix 10 Implantation Losses and Survival Indices – Individual Litter Values

DOSE LEVEL: 0 (Control)

Litter Number	Pre-Implantation Loss (%)	Post-Implantation Loss (%)	Live Birth Index	Viability Index
11	29	0	100	100
12	17	7	100	100
13	7	0	100	100
14	18	7	100	100
15 TLL	24	25	0	-
16	6	0	100	100
17 FD	17	7	93	-
18	0	0	94	100
19	30	13	100	100
20	6	6	100	100

TLL = total litter loss
FD = found dead
- = not applicable

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Appendix 10 (continued) Implantation Losses and Survival Indices – Individual Litter Values

DOSE LEVEL: 50 mg/kg/day

Litter Number	Pre-Implantation Loss (%)	Post-Implantation Loss (%)	Live Birth Index	Viability Index
31	22	14	100	100
32	10	0	100	100
33	0	6	93	100
34	0	0	100	100
35	13	7	100	92
36	6	7	86	100
37	33	64	60	100
38	0	7	93	100
39	15	6	94	100
40	11	6	100	100

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Appendix 10 (continued) Implantation Losses and Survival Indices – Individual Litter Values

DOSE LEVEL: 150 mg/kg/day

Litter Number	Pre-Implantation Loss (%)	Post-Implantation Loss (%)	Live Birth Index	Viability Index
51	20	13	100	100
52	0	6	100	100
53U	25	0	100	100
54	17	7	100	100
55	30	0	100	100
56	19	12	100	93
57	6	7	100	100
58	6	13	92	100
59	11	6	93	100
60	36	0	100	100

U = unilateral implantation

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Appendix 10 (continued) Implantation Losses and Survival Indices – Individual Litter Values

DOSE LEVEL: 500 mg/kg/day

Litter Number	Pre- Implantation Loss (%)	Post -Implantation Loss (%)	Live Birth Index	Viability Index
71 TLL	0	88	0	-
72 NYB	67	100	-	-
73	20	50	100	100
74	7	36	100	100
75 NP	-	-	-	-
76	11	75	100	100
77 FTM	-	-	-	-
78	14	22	100	100
79	0	57	100	100
80	6	73	100	100

TLL = total litter loss
NYB = implantation detected in uterus but no offspring observed to be born
NP = not pregnant
FTM = failed to mate
- = not applicable

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Appendix 11 Sex Ratio – Individual Litter Values

DOSE LEVEL: 0 (Control)

Litter Number	Sex Ratio (*Post Partum*) Day:								
	At birth			1			4		
	Male	Female	% Male	Male	Female	% Male	Male	Female	% Male
11	3	9	25	3	9	25	3	9	25
12	8	6	57	8	6	57	8	6	57
13	11	3	79	11	3	79	11	3	79
14	5	8	38	5	8	38	5	8	38
15 TLL	4 †	7†	36	-	-	-	-	-	-
16	12	3	80	12	3	80	12	3	80
17 FD	6	8	43	6	7	46	-	-	-
18	7	11	39	6	11	35	6	11	35
19	9	5	64	9	5	64	9	5	64
20	8	8	50	8	8	50	8	8	50

TLL = total litter loss
FD = found dead
- = not applicable
† = excludes one pup sex undetermined

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Appendix 11 (continued) Sex Ratio – Individual Litter Values

DOSE LEVEL: 50 mg/kg/day

Litter Number	Sex Ratio (*Post Partum*) Day:								
	At birth			1			4		
	Male	Female	% Male	Male	Female	% Male	Male	Female	% Male
31	5	7	42	5	7	42	5	7	42
32	10	8	56	10	8	56	10	8	56
33	6	9	40	6	8	43	6	8	43
34	9	5	64	9	5	64	9	5	64
35	6	7	46	6	7	46	5	7	42
36	7	7	50	6	6	50	6	6	50
37	3	2	60	2	1	67	2	1	67
38	6	7	46	6	7	46	6	7	46
39	7	9	44	7	8	47	7	8	47
40	7	8	47	7	8	47	7	8	47

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Appendix 11 (continued) Sex Ratio – Individual Litter Values

DOSE LEVEL: 150 mg/kg/day

Litter Number	Sex Ratio (*Post Partum*) Day:								
	At birth			1			4		
	Male	Female	% Male	Male	Female	% Male	Male	Female	% Male
51	12	2	86	12	2	86	12	2	86
52	12	4	75	12	4	75	12	4	75
53 U	2	4	33	2	4	33	2	4	33
54	3	11	21	3	11	21	3	11	21
55	7	7	50	7	7	50	7	7	50
56	4	11	27	4	11	27	3	11	21
57	7	7	50	7	7	50	7	7	50
58	4	9	31	4	8	33	4	8	33
59	6	9	40	5	9	36	5	9	36
60	4	5	44	4	5	44	4	5	44

U = unilateral implantation

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Appendix 11 (continued) Sex Ratio – Individual Litter Values

DOSE LEVEL: 500 mg/kg/day

Litter Number	Sex Ratio (*Post Partum*) Day:								
	At birth			1			4		
	Male	Female	% Male	Male	Female	% Male	Male	Female	% Male
71 TLL	1	1	50	-	-	-	-	-	-
72 NYB	-	-	-	-	-	-	-	-	-
73	1	3	25	1	3	25	1	3	25
74	4	5	44	4	5	44	4	5	44
75 NP	-	-	-	-	-	-	-	-	-
76	2	2	50	2	2	50	2	2	50
77 FTM	-	-	-	-	-	-	-	-	-
78	7	7	50	7	7	50	7	7	50
79	2	4	33	2	4	33	2	4	33
80	4	0	100	4	0	100	4	0	100

TLL = total litter loss
NYB = implantation detected in uterus but no offspring observed to be born
NP = not pregnant
FTM = failed to mate
- = not applicable

Appendix 12 Clinical Signs – Individual Litter Observations

DOSE LEVEL: 0 (Control)

Litter Number	Offspring Affected	Clinical Observation	Day *post partum*
11	Whole litter	NAD	1-5
12	Whole litter	NAD	1-5
13	F13 Remaining litter	Bruised and swollen left eye NAD	3-5 1-5
14	F 12 Remaining litter	Small NAD	Pre-Day 1, 1-5 1-5
15 TLL	Whole litter	All pups found dead (4M, 7F, 1U)	Pre-Day 1
16	Whole litter	NAD	1-5
17 FD†	F* Remaining litter	Found dead NAD	Pre-Day 1 1
18	M* F11 Remaining litter	Found dead Small NAD	Pre-Day 1 Pre-Day 1, 1-2 1-5
19	Whole litter	NAD	1-5
20	Whole litter	NAD	1-5

M = male
F = female
TLL = total litter loss
U = sex undetermined
FD = found dead - Day 1 lactation
† = litter killed Day 1 due to death of dam
* = prior to allocation of within litter identity
NAD = no abnormalities detected
- = not applicable

Appendix 12 (continued) Clinical Signs – Individual Litter Observations

DOSE LEVEL: 50 mg/kg/day

Litter Number	Offspring Affected	Clinical Observation	Day *post partum*
31	Whole litter	NAD	1-5
32	F18 F18 Remaining litter	Small Found dead NAD	1-4 5 1-5
33	Whole litter	NAD	1-5
34	Whole litter	NAD	1-5
35	M1 M4 Remaining litter	Bruising on nose/snout Missing NAD	Pre-Day1, 1 4 1-5
36	Whole litter	NAD	1-5
37	Whole litter	NAD	1-5
38	U* Remaining litter	Missing NAD	1 1-5
39	F* Remaining litter	Found dead NAD	Pre-Day 1 1-5
40	Whole litter	NAD	1-5

M = male
F = female
U = sex undetermined
* = prior to allocation of within litter identity
NAD = no abnormalities detected

DIBROMOMETHANE : ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN THE RAT

Appendix 12 (continued) Clinical Signs – Individual Litter Observations

DOSE LEVEL: 150 mg/kg/day

Litter Number	Offspring Affected	Clinical Observation	Day *post partum*
51	Whole litter	NAD	1-5
52	M1 M1 Remaining litter	Bruising and scratch around eye Bruised right eye NAD	1-2 3 1-5
53	M* Remaining litter	Bruised face NAD	Pre-Day 1 1-5
54	F8 Remaining litter	Found dead NAD	5 1-5
55	Whole litter	NAD	1-5
56	M4 Remaining litter	Found dead NAD	3 1-5
57	Whole litter	NAD	1-5
58	F* F* F10 Remaining litter	Small Found dead Small, swollen right hindlimb NAD	Pre-Day 1 1 3-5 1-5
59	Whole litter	NAD	1-5
60	Whole litter	NAD	1-5

M = male
F = female
* = prior to allocation of within litter identity
NAD = no abnormalities detected

Appendix 12 (continued) Clinical Signs – Individual Litter Observations

DOSE LEVEL: 500 mg/kg/day

Litter Number	Offspring Affected	Clinical Observation	Day *post partum*
71 TLL	F* M* M*	Small, no milk in stomach (later found dead) Small, no milk in stomach Found dead	Pre-Day 1 Pre-Day 1 1
72 NYB	-	-	-
73	Whole litter	NAD	1-5
74	Whole litter	NAD	1-5
75 NP	-	-	-
76	Whole litter	NAD	1-5
77 FTM	-	-	-
78	Whole litter	NAD	1-5
79	F5 Remaining litter	Small NAD	1-5 1-5
80	Whole litter	NAD	1-5

M = male
F = female
- = not applicable
* = prior to allocation of within litter identity
TLL = total litter loss
NYB = implantation detected in uterus but no offspring observed to be born
FTM = failed to mate
NP = not pregnant
NAD = no abnormalities detected