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Albert Brightwell, Jr.
Vice President
Environment, Health, Safety &
and Product Regulatory Compliance

May 2, 2005

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Document Processing Center (7407)
Attention: TSCA Section 8(e) Coordinator
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
1200 Constitution Avenue, N.W.
Washington, DC 20460-0001

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05 MAY -3 PM 1:02

Re: TSCA 8(e) Submission of N-oxidiethylenethiocarbamoyl-N'-oxidiethylenesulfenamide Oral Dietary Reproductive/Developmental Toxicity Screening Study with Rats: Unaudited Draft Report

Dear Sir or Madam:

Noveon, Inc. (Noveon) is submitting the unaudited draft final report on the oral dietary reproductive/developmental toxicity screening study with rats with N-oxidiethylenethiocarbamoyl-N'-oxidiethylenesulfenamide (OTOS; CAS# 13752-51-7) pursuant to Section 8(e) of the Toxic Substance Act (TSCA). This study is being sponsored under the auspices of the American Chemistry Council's Rubber Additives (RAPA) Panel as a stand alone chemical.

A statistically significant reduction in group mean pup weight was observed on day 1 and day 4 *post partum* in the study with OTOS. However, this finding is considered incidental and not biologically significant because these values are within historical control ranges, and the litter sizes and litter weights are comparable between all groups.

Noveon has concluded that this finding does not indicate that OTOS poses a reproductive or developmental hazard. However, Noveon is submitting this information because it may be information that EPA considers to be reportable under TSCA 8(e).

The final report will be submitted to EPA when it is available.

None of the information in this submission is claimed as confidential business information.

If you have any questions, please contact Dr. Robert K. Hinderer at 216-447-5181 or robert.hinderer@noveon.com.

Sincerely,

Al Brightwell
Vice President Environmental, Health, Safety & Product Regulatory Compliance

CC: Robert K. Hinderer, Ph.D.
Anne LeHuray, Ph.D. (ACC)



285958

**SafePharm
Laboratories**

OTOS:

**ORAL DIETARY
REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

SPL PROJECT NUMBER: 826/150

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QUALITY ASSURANCE REPORT

The conduct of this study has been subjected to periodic inspections by Safepharma Quality Assurance Unit.

This report has been audited by Safepharma 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 appropriate to the type and schedule of this study were as follows:

06 May 2004	Protocol Compliance Audit
10 June 2004	Smearing
17 June 2004	Dosing
22 June 2004	Parental Observations
28 May 2004	Test Material Preparation
09 June 2004	Animal Preparation
25 June 2004	Litter Observations
30 June 2004	Post mortem
φ 08 April 2004	Audit by Propath UK Ltd QAU (reported to management on 09 April 2004) Draft Report Audit
Date of QA Signature	Final Report Audit

..... DATE:

For Safepharma Quality Assurance Unit*

*** Authorised QA Signatures:**

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Deputy Head of Department:	JM Crowther MIScT MRQA
Senior Audit Staff:	JV Johnson BSc MRQA; G Wren ONC MRQA

GLP COMPLIANCE STATEMENT

I, the undersigned, hereby declare that the objectives laid down in the protocol were achieved and as nothing occurred to adversely affect the quality or integrity of the study, I consider the data generated to be valid. This report fully and accurately reflects the procedures used and the data generated.

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (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.

These international standards are acceptable to the United States Environmental Protection Agency and Food and Drug Administration, and fulfil the requirements of 40 CFR Part 160, 40 CFR Part 792 and 21 CFR Part 58 (as amended).

..... DATE:

K A Knox BSc (Hons)
Study Director

AUTHENTICATION

The microscopic pathology data presented in this report were compiled by me and the results reported herein accurately reflect the data obtained.

..... DATE:

P N Brooks MSc BSc EurProBiol CBiol MIBiol
EUROTOX Registered Toxicologist
Study Pathologist

The analytical data presented in this report were compiled by me or under my supervision and the results reported herein accurately reflect the data obtained.

..... DATE:

J McKenzie PhD CChem MRSC
Head of Analytical Services

Approved for issue:

..... DATE:

E Wood
Head of Toxicology

CONTENTS

QUALITY ASSURANCE REPORT	3
GLP COMPLIANCE STATEMENT	5
AUTHENTICATION	7
CONTENTS	9
ORAL DIETARY REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING STUDY IN THE RAT	13
SUMMARY	15
1. INTRODUCTION	17
2. TEST MATERIAL AND EXPERIMENTAL PREPARATION	17
2.1 Description, Identification and Storage Conditions	17
2.2 Experimental Preparation	18
3. METHODS	18
3.1 Animals and Animal Husbandry	18
3.2 Procedure	19
3.3 Observations	20
3.4 Post Mortem Studies	22
3.5 Evaluation of Data	23
3.6 Lactation Data	25
3.7 Statistical Evaluation	25
3.8 Histopathology	26
4. ARCHIVES	26
5. RESULTS	27
5.1 Mortality	27
5.2 Clinical Observations	27
5.3 Bodyweight	27
5.4 Food Consumption	27
5.5 Mating Performance	28
5.6 Necropsy Data	28
5.7 Organ Weights	28
5.8 Offspring	29
5.9 Histopathology	29
6. DISCUSSION	31
7. CONCLUSION	31
TABLES	33
Table 1 Summary Incidence of Daily Clinical Observations - Males	34
Table 2 Summary Incidence of Daily Clinical Observations - Females	35
Table 3 Group Mean Weekly Bodyweights (g) and Standard Deviations (SD) During Maturation - Males	36
Table 4 Group Mean Bodyweights (g) and Standard Deviations (SD) - Females	37
Table 5 Group Mean Weekly Bodyweight Gains and Standard Deviations (SD) - Males	38
Table 6 Group Mean Bodyweight Gains and Standard Deviations (SD) - Females	39
Table 7 Group Mean Food Consumption (g/rat/day) - Males	40

Table 8	Group Mean Food Consumption (g/rat/day) - Females	41
Table 9	Group Mean Food Conversion Ratio During Maturation - Males	42
Table 10	Group Mean Food Conversion Ratio - Females	43
Table 11	Group Mean Chemical Intake - Males	44
Table 12	Group Mean Chemical Intake - Females	45
Table 13	Group Summary of Mating Performance, Fertility and Gestation Length	46
Table 14	Group Summary of Live Birth and Viability Indices - Females	47
Table 15	Group Mean Litter Sizes During Lactation	48
Table 16	Group Mean Litter Weights (g) During Lactation	49
Table 17	Group Mean Offspring Weights (g) During Lactation	50
Table 18	Group Summary of Landmarks of Offspring Development and Reflexological Responses	51
Table 19	Group Mean Sex Ratios of Offspring	52
Table 20	Group Summary of Clinical Observations – Offspring	53
Table 21	Summary Incidence of Necropsy Findings - Males	54
Table 22	Summary Incidence of Necropsy Findings - Females	55
Table 23	Summary Incidence of Macroscopic Findings - Offspring	56
Table 24	Group Mean Organ Weights and Standard Deviations (SD) - Males	57
Table 25	Group Mean Relative Organ Weights (% of Bodyweight) and Standard Deviations (SD) - Males	58
Table 26	Group Summary of Corpora Lutea and Implantation Sites	59
Table 27	Summary Incidence of Histopathological Findings - Males	60
Table 28	Summary Incidence of Histopathological Findings - Females	61
FIGURES		63
Figure 1	Group Mean Weekly Bodyweights - Males	64
Figure 2	Group Mean Weekly Bodyweights – Females	65
Figure 3	Group Mean Weekly Food Consumption - Males	66
Figure 4	Group Mean Weekly Food Consumption - Females	67
Figure 5	Group Mean Bodyweights During Gestation - Females	68
APPENDICES		69
Appendix 1	Individual and Group Mean Weekly Bodyweight and Standard Deviations (SD) - Males	70
Appendix 2	Individual and Group Mean Weekly Bodyweights and Standard Deviations (SD) - Females	74
Appendix 3	Individual and Group Mean Weekly Bodyweight Gains and Standard Deviations (SD) - Males	78
Appendix 4	Individual and Group Mean Weekly Bodyweight Gains and Standard Deviations (SD) - Females	82
Appendix 5	Cage Mean Food Consumption (g/rat/day) - Males	86
Appendix 6	Cage Mean Food Consumption (g/rat/day) Before Pairing - Females	87
Appendix 7	Individual Mating Performance, Fertility and Gestation Length - Females	88
Appendix 8	Individual Food Consumption (g/rat/day) During Gestation and Lactation - Females	92
Appendix 9	Individual Litter Size During Lactation	96

Appendix 10	Individual Litter Weights with Mean Pup Bodyweights and Standard Deviations (SD)	100
Appendix 11	Individual Litter Age on Onset and Completion of Landmarks of Offspring	104
Appendix 12	Development and Reflexological Responses	108
Appendix 13	Individual Litter Ratios	112
Appendix 14	Individual Clinical Observations - Offspring	116
Appendix 15	Individual Macroscopic Post Mortem Findings - Offspring	120
Appendix 16	Individual Macroscopic Post Mortem Findings - Males	124
Appendix 17	Individual Macroscopic Post Mortem Findings - Females	128
Appendix 18	Individual and Group Mean Organ Weights with Corresponding Relative Organ Weights (% of Bodyweight) and Standard Deviations (SD) - Males	132
Appendix 19	Individual Corpora Lutea and Implantation Sites	136
Appendix 20	Individual Histopathological Findings	140
Appendix 21	Chemical Analysis of Dietary Admixtures, Methods and Results	145
Appendix 22	Normal Ranges for litter Sizes, Litter Weights and Mean Offspring Weight Values in the Sprague-Dawley CrI:CD (SD) IGS BR Strain Rat	154
Appendix 23	Protocol	155
Appendix 24	Study Time Plan	170
Appendix 25	Statement of GLP Compliance in Accordance with Directive 88/320/EEC	171

**ORAL DIETARY REPRODUCTION/DEVELOPMENTAL TOXICITY
SCREENING STUDY IN THE RAT**

OTOS:**ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****SUMMARY**

Introduction. The study was designed to screen for potential adverse effect on reproduction including embryo/foetal development in the rat and complies with OECD Guidelines for Testing of Chemicals No 421, 27 July 1995.

Methods. The test material was administered in the diet to groups of ten male and ten female rats throughout maturation, mating, gestation and up to Day 4 *post partum*. The dose levels were 60, 200 and 600 parts per million (ppm) of OTOS with a same sized group receiving vehicle (PMI powdered diet) alone.

Following fourteen days of dosing, male and female rats were paired within their dose groups to produce litters. On Day 5 *post partum*, all surviving animals were killed and examined macroscopically.

Parental animals were observed weekly for clinical signs. Bodyweights and food consumption were recorded weekly during the maturation phase which was continued for males after the mating phase. Mated females were weighed on Days 0, 7, 14 and 20 *post coitum* and Days 1 and 4 *post partum* and food consumptions recorded between Days 1 to 7, 7 to 14 and 14 to 20 *post coitum* and 1 to 4 *post partum*.

The offspring were observed daily for clinical signs. The litter signs and individual pup bodyweights were recorded on Days 1 and 4 *post partum*. During the lactation period the offspring were observed for intra-litter onset and duration of landmarks of physical development. On specific days of lactation, reflexological assessment of offspring was performed.

Post mortem macroscopic examinations were performed on all adults and offspring including decedents. Reproductive and potential target organs and any significant abnormalities from all parental animals were preserved in fixative. Histopathology was carried out on reproductive and target organs from control and high dose group parental animals.

Results.**Adults**

At 600 ppm there were no adult mortalities or clinical signs of toxicity. An initial reduction in food consumption lead to a transient reduction in bodyweight gain. No macroscopic or microscopic abnormalities were observed at post mortem examination.

At 200 ppm there were no signs of test material toxicity.

At 60 ppm there were no signs of test material toxicity.

There were no treatment-related effects on fertility, mating performance, gestation length and subsequent offspring pre or post -natal viability growth or development.

Offspring

There were no treatment-related effects upon litter size at birth or on subsequent offspring survival throughout lactation. At 600 ppm there was a statistically significant reduction in group mean offspring weight on Day 1 and Day 4 *post partum*. However, as litter sizes and group mean total litter weight was comparable between all dose groups, at this dose level, this was considered to be incidental and not related to treatment. There were no effects on offspring reflexological responses and no effect on the intra-litter sex ratios.

Conclusion. Administration of OTOS to male and female rats throughout maturation, mating, gestation and lactation phases of reproduction resulted in an initial reduction in food consumption leading to a transient reduction in bodyweight gain at 600 ppm. This is not considered to be an adverse effect as food consumptions and bodyweight gain were comparable to controls by the second week of treatment. There were no histopathological changes in the tissues examined. There was no effect on offspring viability, growth and development.

The No Observed Adverse Effect Level for adults was 600 ppm. The No Observed Effect Level for offspring was 600 ppm.

OTOS:**ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****1. INTRODUCTION**

The study was designed to screen for potential adverse effects on reproduction including embryo/foetal development in the rat when administered to groups of male and female rats throughout maturation, mating, gestation and lactation.

The study was designed to comply with the OECD Guidelines for Testing of Chemicals No 421, 27 July 1995.

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 available toxicity data. The dietary 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 predicting the potential reproductive toxicity of the test material to man.

The study was performed in accordance with internationally accepted general principles of Good Laboratory Practice and Safepharm Laboratories Standard Operating Procedures. A statement of Compliance with UK Good Laboratory Practice issued by the Department of Health is given in Appendix 25.

The in-life phase of the study was performed between 18 May 2004 and 27 June 2004 (see Appendix 24).

2. TEST MATERIAL AND EXPERIMENTAL PREPARATION**2.1 Description, Identification and Storage Conditions**

Sponsor's identification : OTOS
Description : off-white solid
Batch number : HY3OZCC18
Date received : 19 January 2004
Storage conditions : 4°C in the dark

Data relating to the identity, purity and stability of the test material are the responsibility of the Sponsor.

2.2 Experimental Preparation

For the purpose of this study the test material was prepared at the appropriate concentration as an admixture in PMI 5002 powdered diet. For each dose level a separate aliquot of the test material was weighed into the appropriate container. The vehicle (PMI 5002 powdered diet) was added and mixed using a hobart mixer for approximately 19 minutes to ensure a homogenous mixture was formed.

Prior start of study samples were analysed for stability and homogeneity. The results of these analyses showed OTOS was homogeneous in the PMI 5002 powdered diet and was stable in this vehicle for at least fourteen days.

Samples of each admixture were taken once per fortnight throughout the study (representing the start, middle and end of the dosing period) and analysed for achieved concentration of OTOS at Safepharm Analytical Laboratory. The results indicate that the prepared admixtures were within acceptable limits of the nominal concentration.

The analytical methods used in this study and the results of these analyses are presented in Appendix 23.

3. METHODS

3.1 Animals and Animal Husbandry

A sufficient number of male and female Sprague-Dawley CrI:CD (SD) IGS BR strain rats were obtained from Charles River (UK) Limited, Manston Road, Margate, Kent. On receipt the animals were examined for signs of ill-health or injury. The animals were acclimatised for seven 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 the treatment the males weighed 293g to 334g and the females weighed 173g to 223g.

Upon arrival, the animals were housed in groups of five by sex in polypropylene cages with stainless steel grid floors and tops, suspended over paper-lined polypropylene trays. During the mating period animals were transferred to a similar type cage on a one male to one female per cage basis.

Following evidence of successful mating, the males were returned to their original cages. The females were housed, individually, in polypropylene cages with solid floors and stainless steel tops. Mated females were given softwood chips, as bedding, throughout gestation and lactation.

The animals were allowed free access to food and water. A powdered diet (Certified Rodent Diet PMI 5002, supplied by International Product Supplies Ltd, Wellingborough, Northants, UK) was used. Mains water was supplied from polycarbonate bottles attached to the cage. The drinking water was considered not to contain any contaminant at a level that might have affected the purpose or integrity of the study.

The animals were housed in air-conditioned rooms within the Safepharm Laboratories 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.

The temperature was maintained to operate within a target range of 21 (± 2)°C and a relative humidity of 55 (± 15)%. On isolated occasions the room temperature and/or humidity fell outside the protocol limits but this was considered not to have affected the purpose or integrity of the study.

The animals were allocated to four dose groups, each of twenty rats (ten males and ten females) 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 at these laboratories. Colour coded cage labels were used to assist recognition of dose groups according to the following schedule:

Group Number	Dose Level ppm	Colour Code	Animal Numbers	
			Male	Female
1	0 (Control)	Buff	1 – 10	41 – 50
2	60	Green	11 – 20	51 – 60
3	200	Blue	21 – 30	61 – 70
4	600	Pink	31 – 40	71 – 80

3.2 Procedure

The test material was administered continuously in the diet. Control animals were given vehicle alone (PMI 5002 powdered diet). Male and female rats were exposed to the test material during maturation, mating, gestation and early lactation. This study was not designed to show proof of test material absorption.

3.2.1 Chronological Sequence of Study

- i) Both male and female animals were dosed for fourteen days at their appointed dose levels, prior to pairing.
- ii) On Day 14 parental males and females were paired within their respective dose groups for up to fourteen days.
- iii) Following evidence of mating, the animals were separated and males returned to their holding cages.
- iv) The pregnant females were allowed to deliver their offspring. The offspring were observed for growth and development during lactation up to Day 4 *post partum*.
- v) On Day 5 *post partum* the surviving adults and offspring were killed and examined macroscopically *post mortem*.

Subject to confirmation of successful mating males were killed and examined macroscopically.

3.3 Observations**3.3.1 Morbidity/Mortality**

All animals were checked twice daily during the normal working week and once daily on weekends and public holidays.

3.3.2 Clinical Observations

All animals were observed weekly for clinical signs of toxicity.

3.3.3 Bodyweight

During the maturation and mating period the parental generation animals were weighed weekly. Following mating the parental males were weighed weekly until termination. Parental generation females showing evidence of mating were weighed on Days 0, 7, 14 and 20 *post coitum*. Parental generation females with a live litter were weighed on Days 1 and 4 *post partum*.

3.3.4 Food Consumption

During the maturation period (which continued following mating for males) dietary intake was recorded weekly for each cage of parental generation adults. For parental generation females showing evidence of mating, dietary intake was recorded for the periods covering Days 1 to 7, 7 to 14 and 14 to 20 *post coitum*. For parental generation females with live litters, food consumption was recorded for the period covering Days 1 to 4, *post partum*.

3.3.5 Reproductive Screening

3.3.5.1 Mating

After the maturation period, the parental generation adults were paired on a one male to one female basis for a period of up to fourteen days. Following pairing, the polypropylene trays beneath each cage were checked each morning for the presence of ejected copulation plugs. Additionally each female was checked 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. Mated females were then separated from the male and housed individually during the period of gestation and lactation. The males were returned to their original holding cages.

3.3.5.2 Pregnancy and Parturition

Each pregnant female was observed at 0830, 1230 and 1630 hours at or around the period of expected parturition. At weekends, observations were carried out at 0830 and 1230 hours only. 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 Observations

At the observation of completion of parturition, the number of live and dead offspring was recorded. The subsequent date and time of Day 1 *post partum* litter observations were standardised according to the following:

Day 1 Observations – Weekdays

Littering Complete	0830 Hours	1230 Hours	1630 Hours
Day 1 litter observations performed	1630 Hours same day	0830 Hours next day	1230 Hours next day

Day 1 Observations – Weekends/Public Holidays

Littering Started	Overnight	1230 Hours
Littering complete	0830 Hours	1630 Hours same day assumed
Day 1 litter observations performed	1230 Hours same day	1230 Hours next day

For each litter the following was recorded:

- i) Number of pups born
- ii) Number and sex of pups alive recorded daily and reported on Day 1 and 4 *post partum*
- iii) Clinical condition of pups from birth to Day 4 *post partum*

Individual litter weights on Day 1 and 4 *post partum*

3.3.5.4 Physical Development

All live offspring were observed for the detachment of pinna - as noted by the separation of the edges and subsequent unfolding of both pinnae.

3.3.5.5 Offspring Reflexological Assessment

All live offspring were assessed for surface righting reflex – on Day 1 *post partum*, offspring were tested for their ability to turn over to a normal resting position when placed on their back on a flat surface.

3.4 Post Mortem Studies**3.4.1 Terminal Necropsy**

- a) **Adult Animals** – On Day 5 *post partum* all the surviving adults, including non-fertile animals, were killed by carbon dioxide asphyxiation; followed by cervical dislocation. All animals were examined macroscopically for both internal and external abnormalities. Selected organs and tissues were retained in fixative.

- b) **Offspring** – All offspring alive on Day 5 were killed by intracardiac overdose of sodium pentobarbitone. All these offspring were examined macroscopically for internal and external abnormalities.

3.4.2 Organ Weights

The following list of organs were weighed and preserved in bouins solution for all adult males at necropsy:

Testes

Epididymides

3.4.3 Histology/Histopathology

The following list of organs were preserved in buffered 10% formalin and examined at histopathology for all adult males and females from the control and high dose levels:

Coagulating glands

Pituitary

Epididymides

Ovaries

Prostate

Uterus/cervix

Seminal vesicles

Vagina

Testes

3.4.3.1 Additional Procedures

- a) The corpora lutea of all ovaries from pregnant females were counted at necropsy.
- b) The uterine implantation sites were counted. In addition the uteri of apparently non-pregnant females were examined.

3.5 Evaluation of Data

The data were processed to give litter mean values, group mean values and standard deviations.

The following sections describe the methods of evaluation data.

3.5.1 Food Conversion Ratio

Calculated weekly during the maturation period of the parental generation.

$$\text{Food Conversion Ratio} = \frac{\text{Group mean bodyweight gain (g/rat/day) during week}}{\text{Group mean food consumption (g/rat/day)}}$$

3.5.2 Chemical Intake

$$\text{Chemical intake} = \frac{\text{grams/rat/day}}{\text{Group mean bodyweight} \frac{\text{week 1} + \text{week 2}}{2}} \times \text{Dose level (ppm)}$$

3.5.3 Mating Performance and Fertility

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

3.5.3.1 Pre-Coital Interval

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

3.5.3.2 Fertility Indices

For each group the following were calculated:

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

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

3.5.4 Gestation and Parturition Data

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

3.5.4.1 Gestation Length

Calculated as the number of days of gestation including the day for observation of mating and the start of parturition. Where the start of parturition occurred overnight, the total was adjusted by subtracting half a day.

3.5.4.2 Gestation and Parturition Index

The following was calculated for each group:

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

3.6 Lactation Data

3.6.1 Live Birth and Viability Indices

The following indices were calculated for each group from individual data:

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

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

3.6.2 Sex Ratio

Group mean values calculated from each litter value on Day 1 and 4 using the following formula:

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

3.6.3 Offspring Physical Development

A continuity correction of half a day was subtracted from the age of appearance of physical landmarks of development for those litters born overnight.

3.7 Statistical Evaluation

The following parameters were analysed statistically, where appropriate using the test methods outlined as follows:

Adult male and female bodyweight during the maturation, gestation and lactation periods, adult male food consumption, female food consumption during maturation, gestation and lactation, litter size, litter weight, individual offspring bodyweight, offspring landmarks of physical development, reproductive and viability indices and adult organ weights.

Values were analysed to establish homogeneity of group variances using Bartlett's chi-square test followed by one-way analysis of variance. If the variances were unequal subsequent comparisons between control and treated groups were performed using student's t-test assuming unequal variances. If variances were equal subsequent comparisons between control and treated groups were performed using Dunnett's Multiple Comparison Method.

Adult pre-coital intervals, female gestation lengths, offspring reflexological responses and litter sex ratios, relative organ weights. Individual values were compared using Kruskal-Wallis non-parametric rank sum test. Where significant differences were seen, pairwise comparison of control values against treated group values was performed using Mann-Whitney "U" test.

3.8 Histopathology

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

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

p < 0.001	+++	---***
p < 0.01	++	--**
p < 0.05	+	-*
p < 0.01	(+)	(-)*
p ≥ 0.1	NS	(not significant)
	NE	(statistical test not performed)

Where plus signs indicate positive differences from the control group, and minus signs negative differences. Asterisks refer to overall between group variation which is non-directional

4. ARCHIVES

Unless instructed otherwise by the Sponsor, specimens, all original data (including test site generated 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.

5. RESULTS

5.1 Mortality

There were no mortalities through the course of the study at any dose level.

5.2 Clinical Observations

A summary incidence of daily clinical observations is given in Table 1 and Table 2.

There were no clinical signs of toxicity observed throughout the course of the study at any dose level.

5.3 Bodyweight

Group mean weekly bodyweights and standard deviations are given in Table 3 and Table 4 and are presented graphically in Figure 1 and Figure 2. Group mean weekly bodyweight gains and standard deviations are given in Table 5 and Table 6. Individual data are given in Appendix 1 to Appendix 4.

At 600 ppm there was a statistically significant, $p < 0.001$ and $p < 0.05$, reduction in bodyweight gain for males and females during the first week of treatment compared to control values. This resulted in a lower group mean bodyweight which was statistically significant for females during maturation and for males throughout the study.

At 200 ppm bodyweight gain was comparable to controls.

At 60 ppm there was a statistically significant increase in bodyweight gain, $p < 0.01$, for females during lactation. This increase in bodyweight gain did not result in a statistically significant increase in group mean bodyweight.

5.4 Food Consumption

Group mean food consumptions and standard deviations are given in Tables 7 and 8 and presented graphically in figures 3 and 4. Individual data are given in Appendices 5, 6 and 8.

At 600 ppm there was a significant reduction in food consumption values during the first week of treatment for both males and females. In addition, food consumption values for males continued to be significantly lower than control values during the second week of treatment.

Female food consumption values were significantly ($p < 0.05$) reduced between Day 7 and 14 *post coitum*.

At 200 ppm there was a reduction in male food consumption during the second week of treatment.

At 60 ppm there was an increase in female food consumption between Day 1 and 7 *post coitum*. In the absence of a dose related response this is unlikely to be treatment related.

5.5 Mating Performance

A summary incidence of mating performance, fertility and gestation length is given in Tables 13 and 14. Individual data are given in Appendix 7.

There were no treatment-related effects on fertility or mating performance.

All females at all dose levels including controls had a pre-coital interval of four days or less.

There was no treatment-related increase in gestation times at any dose level.

5.6 Necropsy Data

A summary incidence of macroscopic findings is given in Tables 21 and 22. Individual findings are given in Appendices 16 and 17.

There were no treatment related macroscopic abnormalities observed at *post mortem* examination.

At 200 ppm one female had a dark liver but, in isolation, this is considered not to be of toxicological significance.

5.7 Organ Weights

Group mean organ weights are given in Table 24. Individual data are given in Appendix 18.

There were no treatment related differences in organ weights either absolute or relative to terminal bodyweight.

5.8 Offspring

Group mean litter size, litter and offspring weight, sex ratios, landmarks of physical development and reflexological responses are given in Tables 14 to 19. A summary incidence of offspring clinical observations and macroscopic findings are given in Tables 20 and 23. Individual data are given in Appendices 9 to 15.

At 600 ppm there was a statistically significant reduction in group mean pup weight on Day 1 and 4 *post partum*. However, as litter sizes and group mean total litter weight was comparable between all dose groups, at this dose level, this was considered to be incidental and not related to treatment.

There were no statistically significant differences in litter size, weight of pup weight at 200 ppm or 60 ppm.

There were no treatment related effects on offspring development. The clinical signs observed throughout lactation and the macroscopic findings observed at *post mortem* examination were comparable across all dose groups.

5.9 Histopathology

A summary incidence of histopathological findings is given in Tables 27 and 28. Individual data are given in Appendix 21.

No treatment-related changes were observed at histopathological examination.

Other Histopathology:

Prostate: Interstitial chronic inflammatory cell infiltrates are a commonly observed background finding in laboratory maintained rats.

Uterus: Areas of haemorrhage and fibrosis were seen in the myometrium of the uterus in the majority of female animals examined from control and high dose groups. These conditions are consistent with normal *post partum* uterine changes in the rats.

All remaining morphological changes were those commonly observed in laboratory maintained rats of the age and strain employed and, since there were no differences in incidence or severity

between control and treatment groups, all were considered to be without toxicological significance.

6. DISCUSSION

There was no evidence of treatment-related toxicity at 600 ppm. The reduction in food consumption values during the first week of treatment is indicative of an unpalatable test material. This resulted in a reduction in bodyweight gain over this period leading to lower group mean bodyweights throughout the study. Food consumptions and bodyweight gains were comparable to control values for the remainder of the treatment period.

At *post mortem* examination, there were no treatment related macroscopic changes observed. One female at 200 ppm showed a dark liver. This was considered not to be treatment-related due to the lack of a dose response. No treatment-related macroscopic changes were observed at histopathological examination. All morphological changes were those commonly observed in laboratory maintained rats of the age and strain employed, or were entirely consistent with *post partum* physiology or pathology in the case of females and, since there were no differences in incidence of severity between control and treatment groups, all were considered to be without toxicological significance.

There were no treatment-related effects seen on the fertility of male or female rats, as shown by the high pregnancy rate for all treatment groups and the lack of significant differences in the distribution of pre-coital intervals for all dose groups.

Generally for reproduction studies, the analysis of litter responses is normal practice in order that any effect on maternal performance can be evaluated. There were no significant treatment-related effects upon offspring. The live litter size and their subsequent viability during lactation were comparable to controls across all dose groups. The growth and physical development of offspring was not affected by the test material as evidenced by offspring bodyweight gain between birth and Day 4 *post partum* being comparable across all dose groups. Although group mean offspring weight was significantly lower compared to control values for litters from females treated with 600 ppm on Days 1 and 4 *post partum* total litter weight was unaffected (Tables 16 and 17). It was therefore considered to be incidental and of no toxicological significance. Group mean offspring weights for all treatment groups were within normal control ranges. Control ranges are given in Appendix 22.

7. CONCLUSION

Administration of OTOS to male and female rats throughout maturation, mating, gestation and early lactation phases of reproduction resulted in an initial reduction in food consumption leading to a transient reduction in bodyweight gain at 600 ppm. This is not considered to be an adverse effect as food consumptions and bodyweight gain were comparable to controls by the second

week of treatment. There were no histopathological changes in the tissues examined. There was no effect on offspring viability, growth and development.

The No Observed Adverse Effect Level for adults was 600 ppm. The No Observed Effect Level for offspring was 600 ppm.

TABLES

OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING STUDY IN THE RAT

Table 2 Summary Incidence of Daily Clinical Observations - Females

Dose Level ppm	Number of Animals	Clinical Observations	Number Showing Effects At Observation (Week)						
			1	2	3	4	5	6	
0 (Control)	10	No abnormalities detected	0	-	0	0	0	0	0
60	10	No abnormalities detected	0	-	0	0	0	0	0
200	10	No abnormalities detected	0	-	0	0	0	0	0
600	10	No abnormalities detected	0	-	0	0	0	0	0

- = observations not performed in error

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

**Table 3 Group Mean Weekly Bodyweights (g) and Standard Deviations (SD)
During Maturation - Males**

Dose Level (ppm)	Number of Animals		Bodyweight (g) at Day						
			0	7	14	22	28	35	42
0 (Control)	10	mean	314	369	419	443	473	488	499
		sd	9	10	13	13	17	25	25
60	10	mean	314	367	416	436	465	476	492
		sd	14	18	24	25	30	31	37
200	10	mean	309	356	401	421	450	463	481
		sd	11	13	19	20	23	26	30
600	10	mean	313	***335	**387	**405	**438	*450	470
		sd	11	18	24	27	27	32	37

* = p<0.05

** = p<0.01

*** = p<0.001

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Table 4 Group Mean Bodyweights (g) and Standard Deviations (SD) - Females

Dose Level (ppm)	Number of Animals		Bodyweight (g) at Day								
			Maturation			Gestation				Lactation	
			0	7	14	0	7	14	20	1	4
0 (Control)	10/9●	mean	204	221	235	245	279	313	384	293	308
		sd	9	12	15	18	17	19	26	20	21
60	10	mean	207	226	242	249	288	321	397	299	322
		sd	8	12	16	19	23	26	35	31	30
200	10/9■	mean	205	224	239	244	279	311	383	291	307
		sd	15	10	15	10	14	16	20	24	25
600	10/9/8▲	mean	200	*206	221	227	262	290	356	264	282
		sd	10	9	10	13	13	20	20	21	17

-
- = 9 during gestation and lactation
 - = 9 during final day of lactation
 - ▲ = 8 during gestation, 9 during lactation
 - * = p<0.05

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Table 5 Group Mean Weekly Bodyweight Gains and Standard Deviations (SD) - Males

Dose Level (ppm)	Number of Animals		Increase in Bodyweight (g) during Week					
			1	2	3	4	5	6
0 (Control)	10	mean	55	50	24	31	15	11
		sd	5	6	7	7	11	7
60	10	mean	53	49	21	29	11	16
		sd	9	7	7	7	6	8
200	10	mean	47	45	20	29	13	18
		sd	7	8	7	7	6	9
600	10	mean	***23	52	19	33	12	20
		sd	11	7	6	8	10	6

*** = p<0.001

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Table 6 Group Mean Bodyweight Gains and Standard Deviations (SD) - Females

Dose Level (ppm)	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)	10/9●	mean	17	14	34	34	70	15
		sd	6	4	9	6	13	5
60	10	mean	19	16	40	32	76	**24
		sd	6	6	9	7	11	7
200	10/9■	mean	20	15	35	32	72	14
		sd	10	6	8	9	10	17
600	10/9/8▲	mean	***5	15	35	28	66	18
		sd	4	6	8	10	9	12

● = 9 during gestation and lactation
 ■ = 9 during final day of lactation
 ▲ = 8 during gestation, 9 during lactation
 ** = p<0.01
 *** = p<0.001

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Table 7 Group Mean Food Consumption (g/rat/day) - Males

Dose Level (ppm)	Number of Animals		Mean Food Consumption (g/rat/day) during Week			
			1	2	5	6
0 (Control)	10	mean	28	29	29	28
		sd	2	0	1	0
60	10	mean	30	30	28	28
		sd	1	0	0	1
200	10	mean	28	*28	28	28
		sd	1	0	0	1
600	10	mean	*23	*28	28	28
		sd	0	0	0	0

* = p<0.05

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Table 8 Group Mean Food Consumption (g/rat/day) - Females

Dose Level (ppm)	Number of Animals		Mean Food Consumption (g/rat/day) during:					
			Maturation		Gestation			Lactation
			Week 1	Week 2	Days 1-7	Days 7-14	Days 14-20	Days 1-4
0 (Control)	10/9□/9#	mean	18	18	22	27	30	35
		sd	0	1	4	2	7	6
60	10/10□/10#	mean	18	19	*28	28	29	43
		sd	0	1	4	3	4	11
200	10/10□/9#	mean	18	18	23	27	28	34
		sd	0	0	3	1	3	9
600	10/9□/8#	mean	**14	16	23	*24	28	32
		sd	1	1	1	2	4	4

□ = Number of females showing positive evidence of mating

= Number of females with live offspring

* = p<0.05

** = p<0.01

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Table 9 Group Mean Food Conversion Ratio During Maturation - Males**

Dose Level (ppm)	Number of Animals	Food Efficiency* during Week					
		1	2	3	4	5	6
0 (Control)	10	0.28	0.24	-	-	0.08	0.05
60	10	0.26	0.24	-	-	0.06	0.08
200	10	0.24	0.23	-	-	0.07	0.09
600	10	0.14	0.26	-	-	0.06	0.10

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

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Table 10 Group Mean Food Conversion Ratio - Females

Dose Level (ppm)	Number of Animals	Food Efficiency* during:					
		Maturation		Gestation			Lactation
		Week 1	Week 2	Days 1-7	Days 7-14	Days 14-20	Days 1-4
0 (Control)	10/9	0.13	0.11	0.25	0.18	0.39	0.14
60	10	0.15	0.12	0.24	0.17	0.44	0.18
200	10	0.16	0.12	0.25	0.17	0.42	0.16
600	10/8	0.05	0.13	0.25	0.17	0.39	0.19

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

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Table 11 Group Mean Chemical Intake - Males**

Dose Level (ppm)	Study Week			
	1	2	5	6
60	5.22	4.52	3.60	3.50
200	16.86	14.87	12.33	11.90
600	43.35	46.52	38.18	36.88

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Table 12 Group Mean Chemical Intake - Females**

Dose Level (ppm)	Maturation Week:		Gestation Day:			Lactation Days 1-4
	1	2	1-7	7-14	14-20	
60	5.12	4.84	6.24	5.50	4.78	8.35
200	16.63	15.93	17.89	18.23	16.39	22.56
600	42.30	45.70	55.73	52.04	41.57	70.38

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Table 13 Group Summary of Mating Performance, Fertility and Gestation Length

Dose Level (ppm)	Number of Males Paired	Number of Females			Pre-Coital Interval (Days)			
		Paired	Mated	Pregnant	1	2	3	4
0	10	10	9	9	1	3	2	3
60	10	10	10	10	3	1	4	2
200	10	10	10	10	4	1	3	2
600	10	10	9*	9*	1	2	4	1

Dose Level (ppm)	Mating Index (%)	Pregnancy Index (%)	Gestation Lengths (Days)				With Live Offspring	Parturition Index (%)
			21.5	22	22.5	23		
0	90	100	0	7	1	1	9	100
60	100	100	0	8	2	0	10	100
200	100	100	1	5	1	3	10	100
600	90	100	0	5	2	1	9	100

* = Female 72 showed no positive evidence of mating but subsequently gave birth to live young (See Appendix 7).

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Table 14 Group Summary of Live Birth and Viability Indices - Females**

Dose Level (ppm)	Number of Animals	Mating Index	Live Birth Index	Viability Index
0 (Control)	10	90	98.5	100.0
60	10	100	98.1	96.2
200	10	100	98.6	83.9
600	10	90	99.2	96.2

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Table 15 Group Mean Litter Sizes During Lactation

Dose Level (ppm)	Number of Animals		Number Born	Mean litter size at Day <i>Post Partum</i>	
				1	4
0 (Control)	9	mean	14.7	14.4	14.4
		sd	1.6	1.5	1.5
60	10	mean	15.9	15.6	15.0
		sd	1.4	1.4	2.4
200	10■	mean	13.9	13.7	11.5
		sd	1.4	1.2	4.1
600	9	mean	14.6	14.4	13.9
		sd	1.6	1.4	2.6

■ = 10 born on Day 1, 8 on Day 4

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Table 16 Group Mean Litter Weights (g) During Lactation

Dose Level (ppm)	Number of Animals		Mean Litter Weight at Day(s)	
			1	4
0 (Control)	9	mean	89.6	122.0
		sd	10.5	14.0
60	10	mean	94.2	122.8
		sd	11.3	22.6
200	10■	mean	87.0	102.8
		sd	13.4	40.8
600	9	mean	83.9	106.2
		sd	7.5	25.5

■ = 10 born on Day 1, 8 on Day 4

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Table 17 Group Mean Offspring Weights (g) During Lactation

Dose Level (ppm)	Number of Animals		Mean Offspring Weight at Day(s)	
			1	4
0 (Control)	9	mean	6.2	8.5
		sd	0.3	0.5
60	10	mean	6.0	8.2
		sd	0.4	0.6
200	10/8■	mean	6.4	8.5
		sd	0.9	1.8
600	9	mean	**5.8	*7.6
		sd	0.5	1.1

■ = 10 at Day 1, 8 at Day 4

* = p<0.05

** = p<0.01

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

**Table 18 Group Summary of Landmarks of Offspring Development and
Reflexological Responses**

Dose Level (ppm)		Pinna Unfolding		Mean percentage of offspring with successful response
		Onset	Completion	Surface Righting Reflex
0 (Control)	mean	3.1	4.1	92.5
	sd	0.7	0.6	9.5
	N	9	8	9
60	mean	3.2	4.1	85.9
	sd	0.7	0.5	16.8
	N	10	10	9
200	mean	2.9	3.9	84.5
	sd	0.4	0.4	21.9
	N	7	7	10
600	mean	3.4	4.3	90.0
	sd	0.8	0.5	12.9
	N	9	7	9

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Table 19 Group Mean Sex Ratios of Offspring

Dose Level (ppm)	Number of Animals		Day 1			Day 4		
			Males	Females	% Males	Males	Females	% Males
0 (Control)	9	mean	8	7	52.6	8	7	52.6
		sd	1	2	8.7	1	2	8.7
60	9/10■	mean	7	9	44.6	7	8	47.1
		sd	2	2	10.6	2	2	11.8
200	10/7△	mean	6	8	44.2	6	7	45.3
		sd	2	1	8.6	1	1	8.2
600	9	mean	7	7	51.9	7	7	49.6
		sd	2	2	11.2	2	2	11.2

■ = 9 on Day 1, 10 on Day 5 (technical error)

△ = 10 on Day 1, 7 on Day 5

OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING STUDY IN THE RAT

Table 20 Group Summary of Clinical Observations – Offspring

Clinical Observation	Number of litters with observation			
	0 ppm	60 ppm	200 ppm	600 ppm
1 pup – No tail, non patent anus	1	0	0	0
1 pup small	0	1	1	1
More than 1 pup small	1	1	1	2
1 pup cold	0	1	0	0
More than 1 pup cold	0	0	2	1
1 pup weak	0	1	0	1
More than 1 pup weak	0	0	1	0
1 pup pale	0	1	0	2
More than 1 pup pale	0	0	0	0
1 pup – No milk in stomach	0	1	0	0
More than 1 pup – No milk in stomach	0	0	1	1
More than 1 pup lethargic	0	0	1	0
Litter scattered	0	0	1	0

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Table 21 Summary Incidence of Necropsy Findings - Males**

	Dose Level (ppm)			
	0 (Control)	60	200	600
Number of animals examined at terminal kill	10	10	10	10
No abnormalities detected	10	10	10	10

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Table 22 Summary Incidence of Necropsy Findings - Females**

	Dose Level (ppm)			
	0 (Control)	60	200	600
Number of animals examined at terminal kill	10	10	10	10
Liver dark	0	0	1	0
No abnormalities detected	10	10	9	10

Table 24 **Group Mean Organ Weights and Standard Deviations (SD) - Males**

Dose Level (ppm)	Number of Animals		Bodyweight (g) at Terminal Kill	Organ Weight (g)	
				Testes	Epididymides
0 (Control)	10	mean	505	3.43	1.25
		sd	28	0.33	0.09
60	10	mean	498	3.53	1.25
		sd	36	0.24	0.05
200	10	mean	488	3.58	1.29
		sd	34	0.30	0.14
600	10	mean	475	3.52	1.28
		sd	37	0.28	0.08

Table 25 Group Mean Relative Organ Weights (% of Bodyweight) and Standard Deviations (SD) - Males

Dose Level (ppm)	Number of Animals	Bodyweight (g) at Terminal Kill	Relative Organ Weight (%)		
			Testes	Epididymides	
0 (Control)	10	mean	505	0.68	0.25
		sd	28	0.07	0.02
60	10	mean	498	0.71	0.25
		sd	36	0.08	0.02
200	10	mean	488	0.73	0.26
		sd	34	0.06	0.03
600	10	mean	475	0.75	0.27
		sd	37	0.09	0.03

Table 26 **Group Summary of Corpora Lutea and Implantation Sites**

Dose Level (ppm)	Number of Animals		Total Number Corpora Lutea	Total No. Implantation sites	Pre Implantation Loss (%)	Post Implantation Loss (%)
0 (Control)	8	mean	16	15	1.36	6.35
		sd	2	2	2.53	4.76
60	10	mean	17	17	2.66	4.18
		sd	1	1	6.69	3.92
200	10	mean	15	15	2.66	6.02
		sd	2	2	6.69	6.75
600	9	mean	16	15	1.44	5.73
		sd	1	2	2.85	5.21

Table 27 Summary Incidence of Histopathological Findings - Males

Histopathological Finding	Dose Level (ppm)	
	0 (Control)	600
Number of animals examined at terminal kill	10	10
	Pituitary	
Developmental cysts		
absent	9	10
present	1	0
	Prostate	
Chronic inflammatory cell foci		
absent	5	7
(minimal)	3	1
(moderate)	1	2
(marked)	1	0
	Statistical Information	
Mode of death		
Terminal kill	10	10

Table 28 Summary Incidence of Histopathological Findings - Females

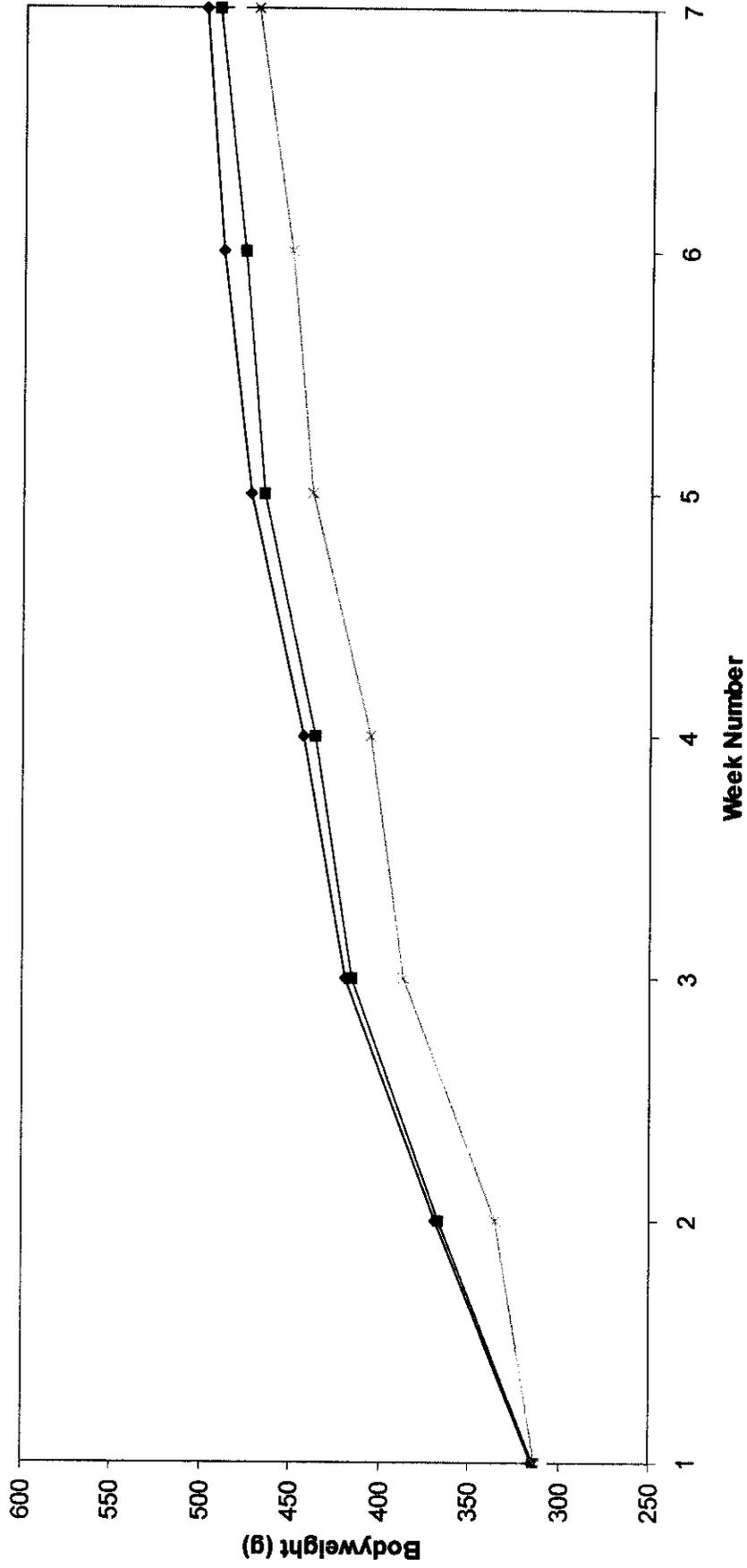
Histopathological Finding	Dose Level (ppm)	
	0 (Control)	600
Number of animals examined at terminal kill	10	10
	Uterus/cervix	
Peripheral fibrosis/haemorrhage		
absent	1	1
present	9	9
	Statistical Information	
Mode of death		
Terminal kill	9	9
Killed Day 25	1	1

FIGURES

OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING STUDY IN THE RAT

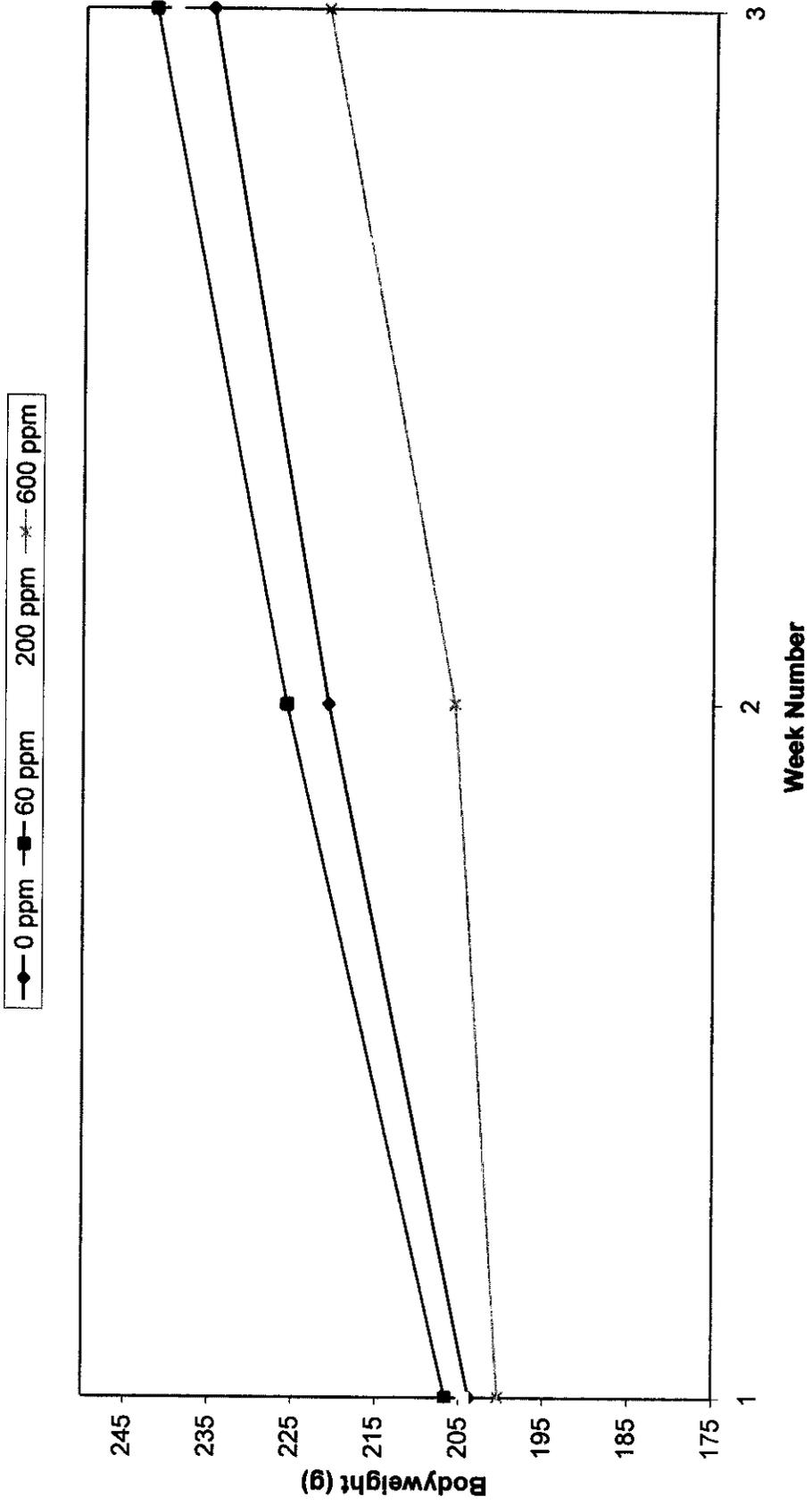
Figure 1 Group Mean Weekly Bodyweights - Males

—◆— 0 ppm —■— 60 ppm —*— 200 ppm —x— 600 ppm



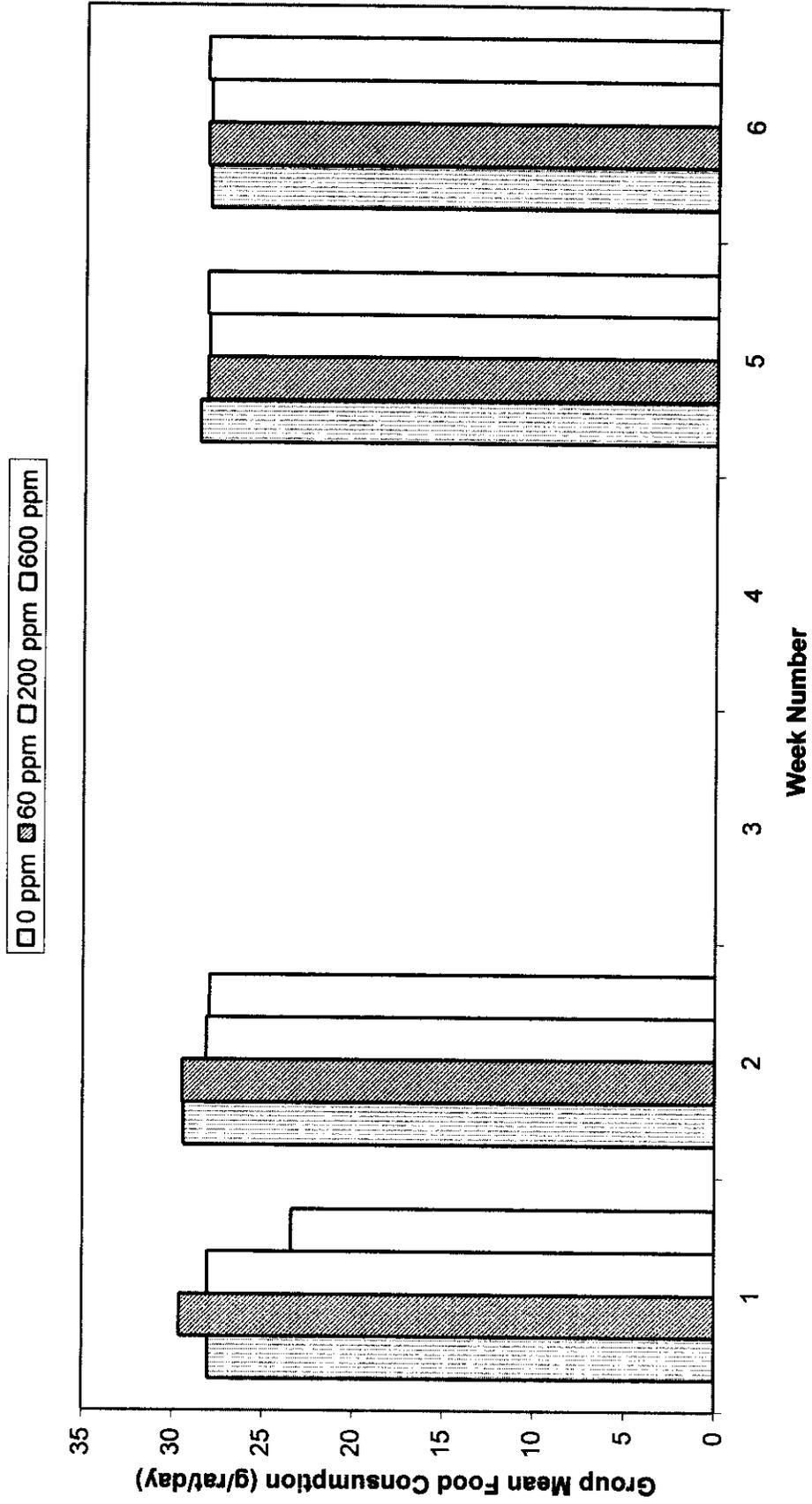
OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING STUDY IN THE RAT

Figure 2 Group Mean Weekly Bodyweights - Females



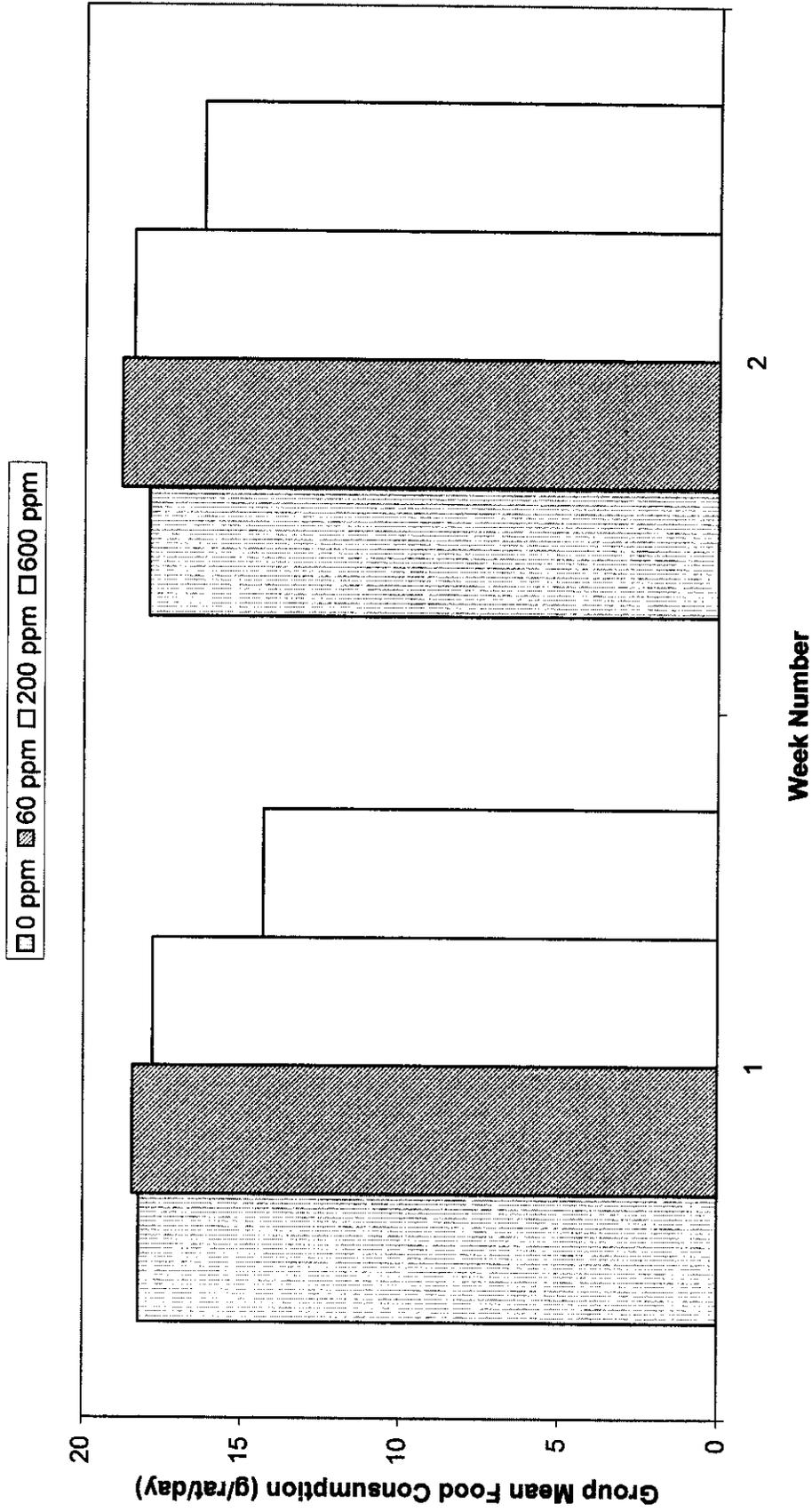
OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING STUDY IN THE RAT

Figure 3 Group Mean Weekly Food Consumption - Males



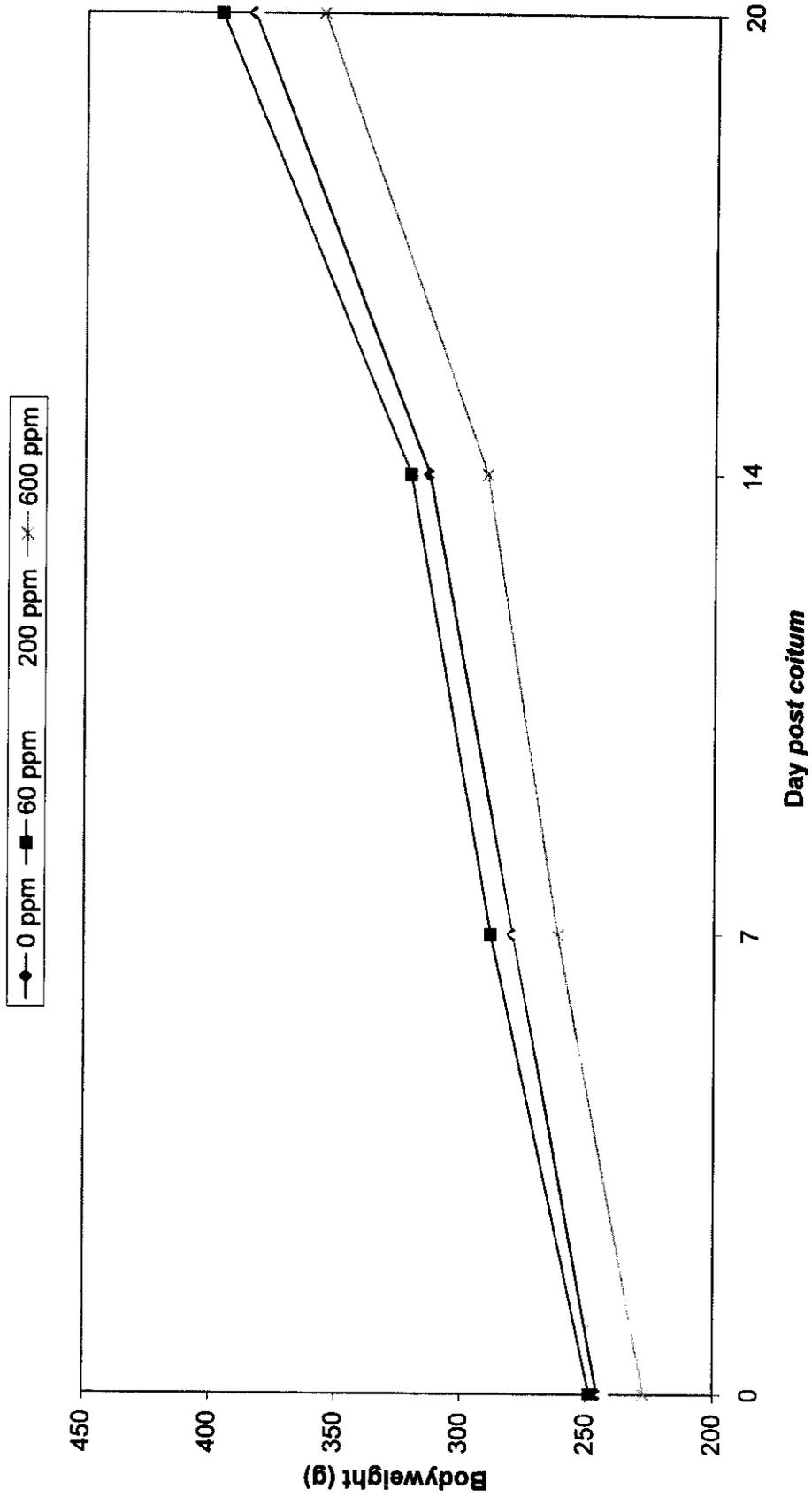
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Figure 4 Group Mean Weekly Food Consumption - Females



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Figure 5 Group Mean Bodyweights During Gestation - Females



APPENDICES

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

**Appendix 1 Individual and Group Mean Weekly Bodyweight and Standard Deviations
(SD) - Males**

DOSE LEVEL: 0 (Control)

Animal Number and Sex	Increase in Bodyweight (g) at Week						
	1	2	3	4	5	6	7
1 M	313	377	429	458	492	512	512
2 M	319	369	420	442	485	514	520
3 M	298	347	392	424	441	453	462
4 M	319	374	427	452	489	510	525
5 M	325	376	415	441	462	471	485
6 M	324	382	434	453	487	504	508
7 M	315	366	413	441	469	480	487
8 M	321	374	434	459	488	509	532
9 M	308	364	420	427	458	479	499
10 M	302	361	406	429	460	450	459
mean	314	369	419	443	473	488	499
sd	9	10	13	13	17	25	25

M = male

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 1 (continued) Individual and Group Mean Weekly Bodyweights and Standard Deviations (SD) - Males

DOSE LEVEL: 60 ppm

Animal Number and Sex	Increase in Bodyweight (g) at Week						
	1	2	3	4	5	6	7
11 M	299	346	394	417	439	460	477
12 M	323	362	409	438	463	474	501
13 M	329	390	446	476	514	531	558
14 M	317	373	425	434	458	463	468
15 M	298	345	385	400	424	431	442
16 M	302	346	384	405	430	438	443
17 M	294	357	404	427	464	476	488
18 M	331	385	436	452	486	495	513
19 M	319	383	443	469	507	513	529
20 M	327	383	432	446	464	482	500
mean	314	367	416	436	465	476	492
sd	14	18	24	25	30	31	37

M = male

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 1 (continued) Individual and Group Mean Weekly Bodyweights and Standard
Deviations (SD) - Males****DOSE LEVEL:** 200 ppm

Animal Number and Sex	Increase in Bodyweight (g) at Week						
	1	2	3	4	5	6	7
21 M	305	347	383	407	442	452	470
22 M	299	357	392	410	449	456	476
23 M	316	353	397	415	432	451	467
24 M	308	362	405	427	456	467	485
25 M	323	379	431	459	497	521	549
26 M	306	351	410	435	463	471	506
27 M	316	356	392	416	443	462	466
28 M	301	346	390	416	445	455	464
29 M	293	335	376	385	408	417	435
30 M	326	376	432	438	460	476	491
mean	309	356	401	421	450	463	481
sd	11	13	19	20	23	26	30

M = male

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 1 (continued) Individual and Group Mean Weekly Bodyweights and Standard Deviations (SD) - Males

DOSE LEVEL: 600 ppm

Animal Number and Sex	Increase in Bodyweight (g) at Week						
	1	2	3	4	5	6	7
31 M	334	357	412	443	479	495	522
32 M	317	357	413	440	481	496	520
33 M	299	323	374	394	423	435	451
34 M	301	319	374	388	426	447	470
35 M	299	315	357	374	398	416	428
36 M	316	345	400	412	441	460	484
37 M	312	315	354	373	419	409	420
38 M	311	345	394	406	427	430	447
39 M	311	321	369	388	425	432	453
40 M	325	355	420	435	462	480	505
mean	313	335	387	405	438	450	470
sd	11	18	24	26	27	32	37

M = male

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

**Appendix 2 Individual and Group Mean Weekly Bodyweights and Standard Deviations
(SD) - Females**

DOSE LEVEL: 0 (Control)

Animal Number and Sex	Bodyweight (g) at Day										
	Maturation			Gestation				Lactation			
	0	7	14	0	7	14	20	1	4		
41 F	214	237	261	272	306	345	423	321	340		
42 F	201	215	232	241	286	317	394	309	320		
43 F	192	206	217	221	253	292	354	263	280		
44 F	203	219	229	244	259	288	353	269	278		
45 F	197	217	233	238	280	322	395	292	310		
46 F	194	213	222	241	277	314	357	295	315		
47 F	220	247	262	277	301	333	410	314	322		
48 F	215	221	236	239	280	313	399	297	315		
49 F	199	213	227	236	272	294	367	275	289		
50 F	203	219	229	Not Mated							
mean	204	221	235	245	279	313	384	293	308		
sd	9	12	15	18	17	19	26	20	21		

F = female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 2 (continued) Individual and Group Mean Weekly Bodyweights and Standard Deviations (SD) - Females

DOSE LEVEL: 60 ppm

Animal Number and Sex	Bodyweight (g) at Day								
	Maturation			Gestation				Lactation	
	0	7	14	0	7	14	20	1	4
51 F	212	241	262	277	314	348	435	319	354
52 F	205	219	229	238	259	287	348	262	283
53 F	207	223	236	248	288	336	421	308	325
54 F	195	211	225	234	276	309	388	279	310
55 F	210	226	250	250	287	309	371	289	314
56 F	210	236	248	256	313	347	429	334	351
57 F	205	217	236	233	275	305	374	290	309
58 F	190	209	217	216	254	282	345	245	270
59 F	215	232	243	255	301	332	425	322	352
60 F	217	243	270	278	316	351	429	337	352
mean	207	226	242	249	288	321	397	299	322
sd	8	12	16	19	23	26	35	31	30

F = female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 2 (continued) Individual and Group Mean Weekly Bodyweights and Standard Deviations (SD) - Females

DOSE LEVEL: 200 ppm

Animal Number and Sex	Bodyweight (g) at Day								
	Maturation			Gestation				Lactation	
	0	7	14	0	7	14	20	1	4
61 F	204	226	235	245	268	309	367	271	265
62 F	220	236	260	257	298	324	405	315	345
63 F	173	219	231	234	268	285	367	272	280
64 F	202	213	224	225	260	299	378	271	
65 F	212	229	246	246	282	307	374	272	308
66 F	206	213	221	243	262	300	379	277	308
67 F	189	210	225	240	278	310	367	292	306
68 F	208	227	238	238	281	304	367	281	292
69 F	223	241	263	255	295	338	423	335	322
70 F	208	226	250	253	297	332	402	323	334
mean	205	224	239	244	279	311	383	291	307
sd	15	10	15	10	14	16	20	24	25

F = female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 2 (continued) Individual and Group Mean Weekly Bodyweights and Standard Deviations (SD) - Females

DOSE LEVEL: 600 ppm

Animal Number and Sex	Bodyweight (g) at Day								
	Maturation			Gestation				Lactation	
	0	7	14	0	7	14	20	1	4
71 F	197	201	216	231	265	287	360	277	288
72 F	202	209	226	-	-	-	-	245	280
73 F	210	209	218	231	273	295	355	275	286
74 F	187	197	210	218	259	283	347	261	262
75 F	197	197	224	221	266	309	388	282	302
76 F	186	196	212	213	248	276	338	240	267
77 F	202	210	218	Not Mated					
78 F	215	219	240	252	280	326	377	294	313
79 F	196	200	213	214	238	259	326	234	268
80 F	212	219	233	238	265	285	355	272	276
mean	200	206	221	227	262	290	356	264	282
sd	10	9	10	13	13	20	20	21	17

F = female

- = female showed no positive evidence of mating but subsequently gave birth to live young

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 3 Individual and Group Mean Weekly Bodyweight Gains and Standard
Deviations (SD) - Males**

DOSE LEVEL: 0 (Control)

Animal Number and Sex	Increase in Bodyweight (g) during Week					
	1	2	3	4	5	6
1 M	64	52	29	34	20	0
2 M	50	51	22	43	29	6
3 M	49	45	32	17	12	9
4 M	55	53	25	37	21	15
5 M	51	39	26	21	9	14
6 M	58	52	19	34	17	4
7 M	51	47	28	28	11	7
8 M	53	60	25	29	21	23
9 M	56	56	7	31	21	20
10 M	59	45	23	31	-10	9
mean	55	50	24	31	15	11
sd	5	6	7	7	11	7

M = male

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 3 (continued) Individual and Group Mean Weekly Bodyweight Gains and
Standard Deviations (SD) - Males****DOSE LEVEL: 60 ppm**

Animal Number and Sex	Increase in Bodyweight (g) during Week					
	1	2	3	4	5	6
11 M	47	48	23	22	21	17
12 M	39	47	29	25	11	27
13 M	61	56	30	38	17	27
14 M	56	52	9	24	5	5
15 M	47	40	15	24	7	11
16 M	44	38	21	25	8	5
17 M	63	47	23	37	12	12
18 M	54	51	16	34	9	18
19 M	64	60	26	38	6	16
20 M	56	49	14	18	18	18
mean	53	49	21	29	11	16
sd	9	7	7	7	6	8

M = male

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 3 (continued) Individual and Group Mean Weekly Bodyweight Gains and
Standard Deviations (SD) - Males****DOSE LEVEL:** 200 ppm

Animal Number and Sex	Increase in Bodyweight (g) during Week					
	1	2	3	4	5	6
21 M	42	36	24	35	10	18
22 M	58	35	18	39	7	20
23 M	37	44	18	17	19	16
24 M	54	43	22	29	11	18
25 M	56	52	28	38	24	28
26 M	45	59	25	28	8	35
27 M	40	36	24	27	19	4
28 M	45	44	26	29	10	9
29 M	42	41	9	23	9	18
30 M	50	56	6	22	16	15
mean	47	45	20	29	13	18
sd	7	8	7	7	6	9

M = male

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 3 (continued) Individual and Group Mean Weekly Bodyweight Gains and
Standard Deviations (SD) - Males**

DOSE LEVEL: 600 ppm

Animal Number and Sex	Increase in Bodyweight (g) during Week					
	1	2	3	4	5	6
31 M	23	55	31	36	16	27
32 M	40	56	27	41	15	24
33 M	24	51	20	29	12	16
34 M	18	55	14	38	21	23
35 M	16	42	17	24	18	12
36 M	29	55	12	29	19	24
37 M	3	39	19	46	-10	11
38 M	34	49	12	21	3	17
39 M	10	48	19	37	7	21
40 M	30	65	15	27	18	25
mean	23	52	19	33	12	20
sd	11	7	6	8	10	6

M = male

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 4 Individual and Group Mean Weekly Bodyweight Gains and Standard Deviations (SD) - Females

DOSE LEVEL: 0 (Control)

Animal Number and Sex	Increase in Bodyweight (g) during					
	Maturation		Gestation			Lactation
	Week 1	Week 2	Days 0-7	Days 7-14	Days14-20	Days1-4
41 F	23	24	34	39	78	19
42 F	14	17	45	31	77	11
43 F	14	11	32	39	62	17
44 F	16	10	15	29	65	9
45 F	20	16	42	42	73	18
46 F	19	9	36	37	43	20
47 F	27	15	24	32	77	8
48 F	6	15	41	33	86	18
49 F	14	14	36	22	73	14
50 F	16	10	Not mated			
mean	17	14	34	34	70	15
sd	6	4	9	6	13	5

F = female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

**Appendix 4 (continued) Individual and Group Mean Weekly Bodyweight Gains and
Standard Deviations (SD) - Females**

DOSE LEVEL: 60 ppm

Animal Number and Sex	Increase in Bodyweight (g) during					
	Maturation		Gestation			Lactation
	Week 1	Week 2	Days 0-7	Days 7-14	Days 14-20	Days 1-4
51 F	29	21	37	34	87	35
52 F	14	10	21	28	61	21
53 F	16	13	40	48	85	17
54 F	16	14	42	33	79	31
55 F	16	24	37	22	62	25
56 F	26	12	57	34	82	17
57 F	12	19	42	30	69	19
58 F	19	8	38	28	63	25
59 F	17	11	46	31	93	30
60 F	26	27	38	35	78	15
mean	19	16	40	32	76	24
sd	6	6	9	7	11	7

F = female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

**Appendix 4 (continued) Individual and Group Mean Weekly Bodyweight Gains and
Standard Deviations (SD) - Females**

DOSE LEVEL: 200 ppm

Animal Number and Sex	Increase in Bodyweight (g) during					
	Maturation		Gestation			Lactation
	Week 1	Week 2	Days 0-7	Days 7-14	Days14-20	Days1-4
61 F	22	9	23	41	58	-6
62 F	16	24	41	26	81	30
63 F	46	12	34	17	82	8
64 F	11	11	35	39	79	
65 F	17	17	36	25	67	36
66 F	7	8	19	38	79	31
67 F	21	15	38	32	57	14
68 F	19	11	43	23	63	11
69 F	18	22	40	43	85	-13
70 F	18	24	44	35	70	11
mean	20	15	35	32	72	14
sd	10	6	8	9	10	17

F = female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

**Appendix 4 (continued) Individual and Group Mean Weekly Bodyweight Gains and
Standard Deviations (SD) - Females**

DOSE LEVEL: 600 ppm

Animal Number and Sex	Increase in Bodyweight (g) during					
	Maturation		Gestation			Lactation
	Week 1	Week 2	Days 0-7	Days 7-14	Days 14-20	Days 1-4
71 F	4	15	34	22	73	11
72 F	7	17	-	-	-	-
73 F	-1	9	42	22	60	11
74 F	10	13	41	24	64	1
75 F	0	27	45	43	79	20
76 F	10	16	35	28	62	27
77 F	8	8	Not mated			
78 F	4	21	28	46	51	19
79 F	4	13	24	21	67	34
80 F	7	14	27	20	70	4
mean	5	15	35	28	66	18
sd	4	6	8	10	9	12

F = female

- = female showed no positive evidence of mating but subsequently gave birth to live young

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 5 Cage Mean Food Consumption (g/rat/day) - Males**

Dose Level (ppm)	Cage Number	Number of Animals	Mean Food Consumption (g/rat/day) during Week			
			1	2	5	6
0 (Control)	1	5	30	29	29	28
	2	5	26	29	28	28
60	3	5	30	29	28	27
	4	5	29	30	28	29
200	5	5	28	28	28	28
	6	5	28	28	28	28
600	7	5	23	28	28	28
	8	5	24	28	29	28

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 6 Cage Mean Food Consumption (g/rat/day) Before Pairing - Females**

Dose Level (ppm)	Cage Number	Number of Animals	Mean Food Consumption (g/rat/day) during Week	
			1	2
0 (Control)	9	5	18	18
	10	5	18	18
60	11	5	18	18
	12	5	19	19
200	13	5	18	18
	14	5	18	19
600	15	5	14	16
	16	5	15	17

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 7 Individual Mating Performance, Fertility and Gestation Length - Females

DOSE LEVEL: 0 (Control)

Animal Number		Pre-Coital Interval (Days)	Copulation Plug Count	Sperm Reading Score	Pregnancy Status	Gestation Length (Days)
41 F	1 M	3	3H	2+	P	22
42 F	2 M	2	4	2+	P	22
43 F	3 M	2	2	2+	P	22
44 F	4 M	4	3	3+	P	23
45 F	5 M	2	5	2+	P	22
46 F	6 M	4	5	3+	P	22.5
47 F	7 M	4	6H	3+	P	22
48 F	8 M	1	3	2+	P	22
49 F	9 M	3	7	3+	P	22
50 F	10 M			Not Mated		

2+ = continuous few spermatozoa in all fields

3+ = many spermatozoa in all fields

H = haemorrhage

P = pregnant

M = male

F = female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 7 (continued) Individual Mating Performance, Fertility and Gestation Length

DOSE LEVEL: 60 ppm

Animal Number		Pre-Coital Interval (Days)	Copulation Plug Count	Sperm Reading Score	Pregnancy Status	Gestation Length (Days)
51 F	11 M	3	5	1+	P	22.5
52 F	12 M	4	4 PV	3+	P	22.5
53 F	13 M	4	5	3+	P	22
54 F	14 M	3	4	3+	P	22
55 F	15 M	1	5	1+	P	22
56 F	16 M	2	3	1+	P	22
57 F	17 M	1	4H	2+	P	22
58 F	18 M	1	3H	3+	P	22
59 F	19 M	3	4H	3+	P	22
60 F	20 M	3	2	1+	P	22

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

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 7 (continued) Individual Mating Performance, Fertility and Gestation Length

DOSE LEVEL: 200 ppm

Animal Number		Pre-Coital Interval (Days)	Copulation Plug Count	Sperm Reading Score	Pregnancy Status	Gestation Length (Days)
61 F	21 M	4	3H	3+	P	22
62 F	22 M	1	5	3+	P	22.5
63 F	23 M	3	5H	3+	P	23
64 F	24 M	1	2H	1+	P	21.5
65 F	25 M	3	3H	3+	P	22
66 F	26 M	4	5	2+	P	22
67 F	27 M	3	7H	1+	P	23
68 F	28 M	1	3	2+	P	22
69 F	29 M	2	5	1+	P	22
70 F	30 M	1	3H	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

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 7 (continued) Individual Mating Performance, Fertility and Gestation Length

DOSE LEVEL: 600 ppm

Animal Number		Pre-Coital Interval (Days)	Copulation Plug Count	Sperm Reading Score	Pregnancy Status	Gestation Length (Days)
71 F	31 M	3	5H	3+	P	23
72 F	32 M	*	*	*	P	*
73 F	33 M	3	5	3+	P	22
74 F	34 M	3	6	1+	P	22
75 F	35 M	2	5	1+	P	22.5
76 F	36 M	2	2	2+	P	22
77 F	37 M			Not Mated		
78 F	38 M	4	4H PV	3+	P	22.5
79 F	39 M	1	4	2+	P	22
80 F	40 M	3	2	3+	P	22

1+ = few spermatozoa present

2+ = continuous few spermatozoa in all fields

3+ = many spermatozoa in all fields

H = haemorrhage

PV = vaginal plug

P = pregnant

M = male

F = female

* = Showed no positive evidence of mating but subsequently gave birth to live young

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

**Appendix 8 Individual Food Consumption (g/rat/day) During Gestation and Lactation -
Females**

DOSE LEVEL: 0 (Control)

Animal Number and Sex	Gestation			Lactation
	Days 1-7	Days 7-14	Days 14-20	Days 1-4
41 F	28	30	27	38
42 F	29	28	48	39
43 F	19	24	26	26
44 F	18	28	28	27
45 F	17	28	28	39
46 F	23	26	27	35
47 F	25	26	29	41
48 F	23	27	30	39
49 F	21	24	27	34
50 F	Not mated			
mean	22	27	30	35
sd	4	2	7	6

F = Female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 8 (continued) Individual Food Consumption (g/rat/day) During Gestation and
Lactation - Female**

DOSE LEVEL: 60 ppm

Animal Number and Sex	Gestation			Lactation
	Days 1-7	Days 7-14	Days 14-20	Days 1-4
51 F	31	29	33	39
52 F		23	22	35
53 F	24	26	27	39
54 F	28	28	28	64
55 F	28	26	28	45
56 F	37	33	34	35
57 F	28	27	26	34
58 F	23	26	25	57
59 F	27	31	33	50
60 F	26	30	31	33
mean	28	28	29	43
sd	4	3	4	11

F = Female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 8 (continued) Individual Food Consumption (g/rat/day) During Gestation and Lactation - Female

DOSE LEVEL: 200 ppm

Animal Number and Sex	Gestation			Lactation
	Days 1-7	Days 7-14	Days 14-20	Days 1-4
61 F	22	26	24	16
62 F	23	28	29	39
63 F	25	26	26	33
64 F	19	27	30	-
65 F	25	27	28	35
66 F	24	26	32	35
67 F	25	27	25	34
68 F	19	27	33	26
69 F	27	28	30	43
70 F	26	27	30	43
mean	23	27	28	34
sd	3	1	3	9

F = Female

- = data not available

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 8 (continued) Individual Food Consumption (g/rat/day) During Gestation and Lactation - Female

DOSE LEVEL: 600 ppm

Animal Number and Sex	Gestation			Lactation
	Days 1-7	Days 7-14	Days 14-20	Days 1-4
71 F	23	23	24	37
72 F	-	-	-	33
73 F	25	26	27	35
74 F	22	24	29	24
75 F	23	24	28	33
76 F	22	23	24	34
77 F		Not mated		
78 F	23	27	37	32
79 F	23	21	25	34
80 F	21	23	29	27
mean	23	24	28	32
sd	1	2	4	4

F = Female

- = female showed no positive evidence of mating but subsequently gave birth to live young

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 9 Individual Litter Size During Lactation

DOSE LEVEL: 0 (Control)

Animal Number and Sex	Number Born	Day		
		1	4	5
41 F	18	17	17	17
42 F	14	14	14	14
43 F	13	12	12	12
44 F	13	13	13	13
45 F	15	15	15	15
46 F	15	15	15	15
47 F	14	14	14	14
48 F	16	16	16	16
49 F	14	14	14	14
50 F		Not mated		
mean	14.7	14.4	14.4	14.4
sd	1.6	1.5	1.5	1.5

F = Female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 9 (continued) Individual Litter Size During Lactation****DOSE LEVEL: 60 ppm**

Animal Number and Sex	Number Born	Day		
		1	4	5
51 F	18	18	18	18
52 F	15	15	15	15
53 F	17	17	16	16
54 F	16	16	16	16
55 F	14	14	14	14
56 F	14	14	14	14
57 F	16	16	16	16
58 F	15	15	15	15
59 F	17	17	17	17
60 F	17	14	9	8
mean	15.9	15.6	15.0	14.9
sd	1.4	1.4	2.4	2.7

F = Female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 9 (continued) Individual Litter Size During Lactation

DOSE LEVEL: 200 ppm

Animal Number and Sex	Number Born	Day		
		1	4	5
61 F	15	15	2	-
62 F	14	14	14	14
63 F	15	15	13	13
64 F	14	14	-	-
65 F	12	12	12	12
66 F	15	15	15	15
67 F	13	13	13	13
68 F	12	12	10	10
69 F	16	14	-	-
70 F	13	13	13	13
mean	13.9	13.7	11.5	12.9
sd	1.4	1.2	4.1	1.6

F = Female

- = total litter loss

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 9 (continued) Individual Litter Size During Lactation****DOSE LEVEL:** 600 ppm

Animal Number and Sex	Number Born	Day		
		1	4	5
71 F	14	14	14	14
72 F	13	13	13	13
73 F	15	15	15	15
74 F	13	13	8	5
75 F	17	17	17	17
76 F	17	16	16	16
77 F			Not mated	
78 F	13	13	13	13
79 F	14	14	14	14
80 F	15	15	15	15
mean	14.6	14.4	13.9	13.6
sd	1.6	1.4	2.6	3.5

F = Female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 10 Individual Litter Weights with Mean Pup Bodyweights and Standard Deviations (SD)

DOSE LEVEL: 0 (Control)

Animal Number	Total Litter Weight (g)		Mean Pup Bodyweight (g)	
	(Post Partum) Day:		(Post Partum) Day:	
	1	4	1	4
41 F	100.1	135.2	5.9	8.0
42 F	88.9	123.4	6.4	8.8
43 F	69.0	93.8	5.8	7.8
44 F	85.8	116.9	6.6	9.0
45 F	98.8	136.9	6.6	9.1
46 F	86.4	113.4	5.8	7.6
47 F	87.3	119.9	6.2	8.6
48 F	104.2	138.4	6.5	8.7
49 F	86.2	120.2	6.2	8.6
50 F				
mean	89.6	122.0	6.0	8.2
sd	10.5	14.0	0.4	0.6

F = female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

**Appendix 10 (continued) Individual Litter Weights with Mean Pup Bodyweights and
Standard Deviations (SD)**

DOSE LEVEL: 60 ppm

Animal Number	Total Litter Weight (g)		Mean Pup Bodyweight (g)	
	(Post Partum) Day:		(Post Partum) Day:	
	1	4	1	4
51 F	106.0	143.8	5.9	8.0
52 F	90.1	116.3	6.0	7.8
53 F	96.2	122.6	5.7	7.7
54 F	94.7	133.4	5.9	8.3
55 F	80.3	105.5	5.7	7.5
56 F	91.3	130.9	6.5	9.4
57 F	97.1	130.2	6.1	8.1
58 F	97.2	124.0	6.5	8.3
59 F	114.3	150.8	6.7	8.9
60 F	75.0	70.0	5.4	7.8
mean	94.2	122.8	6.0	8.2
sd	11.3	22.6	0.4	0.6

F = female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

**Appendix 10 (continued) Individual Litter Weights with Mean Pup Bodyweights and
Standard Deviations (SD)**

DOSE LEVEL: 600 ppm

Animal Number	Total Litter Weight (g)		Mean Pup Bodyweight (g)	
	(Post Partum) Day:		(Post Partum) Day:	
	1	4	1	4
71 F	96.7	122.6	6.9	8.8
72 F	81.4	110.4	6.3	8.5
73 F	88.7	116.9	5.9	7.8
74 F	71.8	39.5	5.5	4.9
75 F	90.5	118.9	5.3	7.0
76 F	82.0	119.1	5.1	7.4
77 F	Not mated			
78 F	79.2	108.3	6.1	8.3
79 F	78.4	111.1	5.6	7.9
80 F	86.7	109.0	5.8	7.3
mean	83.9	106.2	5.8	7.6
sd	7.5	25.5	0.5	1.1

F = female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 11 Individual Litter Age on Onset and Completion of Landmarks of Offspring****DOSE LEVEL: 0 (Control)**

Animal Number and Sex	Pinnae Unfolding	
	Onset	Completion
41 F	4.0	5.0
42 F	2.5	3.5
43 F	3.0	4.0
44 F	2.5	3.5
45 F	2.5	*
46 F	3.5	4.5
47 F	3.5	4.5
48 F	4.0	4.0
49 F	2.5	3.5
50 F	Not mated	
mean	3.1	4.1
sd	0.7	0.6

F = Female

* = not complete

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 11 (continued) Individual Litter Age of Onset and Completion of Landmarks
of Offspring****DOSE LEVEL:** 60 ppm

Animal Number and Sex	Pinnae Unfolding	
	Onset	Completion
51 F	3.5	4.5
52 F	2.5	3.5
53 F	3.5	4.5
54 F	4.0	4.0
55 F	4.0	5.0
56 F	2.5	4.5
57 F	2.5	3.5
58 F	4.0	4.0
59 F	2.5	3.5
60 F	3.0	4.0
mean	3.2	4.1
sd	0.7	0.5

F = Female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 11 (continued) Individual Litter Age of Onset and Completion of Landmarks
of Offspring****DOSE LEVEL:** 200 ppm

Animal Number and Sex	Pinnae Unfolding	
	Onset	Completion
61 F	-	-
62 F	3.5	4.5
63 F	3.0	4.0
64 F	-	-
65 F	2.5	3.5
66 F	2.5	3.5
67 F	3.0	4.0
68 F	3.5	4.5
69 F	-	-
70 F	2.5	3.5
mean	2.9	3.9
sd	0.4	0.4

F = Female

- = Total litter loss

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 11 (continued) Individual Litter Age of Onset and Completion of Landmarks
of Offspring****DOSE LEVEL: 600 ppm**

Animal Number and Sex	Pinnae Unfolding	
	Onset	Completion
71 F	3.0	4.0
72 F	2.5	4.5
73 F	3.0	4.0
74 F	5.0	*
75 F	3.5	4.5
76 F	3.5	*
77 F		Not mated
78 F	2.5	3.5
79 F	3.5	4.5
80 F	4.0	5.0
mean	3.4	4.3
sd	0.8	0.5

F = Female

* = not complete

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 12 Development and Reflexological Responses****DOSE LEVEL:** 0 (Control)

Animal Number and Sex	Surface Righting		
	Examined	Passed	% Passed
41 F	17	16	94.1
42 F	14	14	100.0
43 F	12	11	91.7
44 F	13	12	92.3
45 F	15	15	100.0
46 F	15	11	73.3
47 F	14	14	100.0
48 F	16	13	81.3
49 F	14	14	100.0
50 F		Not mated	
mean	14.4	13.3	92.5
sd	1.5	1.7	9.5

F = Female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 12 (continued) Development and Reflexological Responses****DOSE LEVEL:** 60 ppm

Animal Number and Sex	Examined	Surface Righting	
		Passed	% Passed
51 F	18	17	94.4
52 F	15	15	100.0
53 F	17	8	47.1
54 F	16	14	87.5
55 F	14	11	78.6
56 F	14	12	85.7
57 F	16	16	100.0
58 F	15	12	80.0
59 F	17	17	100.0
60 F	-	-	-
mean	15.8	13.6	85.9
sd	1.4	3.0	16.8

F = Female

- = no data available

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 12 (continued) Development and Reflexological Reponses****DOSE LEVEL:** 200 ppm

Animal Number and Sex	Surface Righting		
	Examined	Passed	% Passed
61 F	15	9	60.0
62 F	14	14	100.0
63 F	15	14	93.3
64 F	14	10	71.4
65 F	12	12	100.0
66 F	15	15	100.0
67 F	13	12	92.3
68 F	12	12	100.0
69 F	14	5	35.7
70 F	13	12	92.3
mean	13.7	11.5	84.5
sd	1.2	2.9	21.9

F = Female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 12 (continued) Development and Reflexological Reponses****DOSE LEVEL:** 600 ppm

Animal Number and Sex	Examined	Surface Righting	
		Passed	% Passed
71 F	14	13	92.9
72 F	13	13	100.0
73 F	15	14	93.3
74 F	13	9	69.2
75 F	17	16	94.1
76 F	16	15	93.8
77 F		Not mated	
78 F	13	13	100.0
79 F	14	14	100.0
80 F	15	10	66.7
mean	14.4	13.0	90.0
sd	1.4	2.2	12.9

F = Female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 13 Individual Litter Ratios

DOSE LEVEL: 0 (Control)

Animal Number and Sex	Day 1			Day 5		
	Males	Females	% Males	Males	Females	% Males
41 F	7	10	41.2	7	10	41.2
42 F	7	7	50.0	7	7	50.0
43 F	6	6	50.0	6	6	50.0
44 F	7	6	53.8	7	6	53.8
45 F	9	6	60.0	9	6	60.0
46 F	7	8	46.7	7	8	46.7
47 F	10	4	71.4	10	4	71.4
48 F	8	8	50.0	8	8	50.0
49 F	7	7	50.0	7	7	50.0
50 F	Not mated					
mean	8	7	52.6	8	7	52.6
sd	1	2	8.7	1	2	8.7

F = Female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 13 (continued) Individual Litter Sex Ratios****DOSE LEVEL:** 60 ppm

Animal Number and Sex	Day 1			Day 5		
	Males	Females	% Males	Males	Females	% Males
51 F	10	8	55.6	10	8	55.6
52 F	7	8	46.7	7	8	46.7
53 F	-	-	-	9	7	56.3
54 F	5	11	31.3	5	11	31.3
55 F	7	7	50.0	7	7	50.0
56 F	8	6	57.1	8	6	57.1
57 F	6	10	37.5	6	10	37.5
58 F	4	11	26.7	4	11	26.7
59 F	8	9	47.1	8	9	47.1
60 F	7	7	50.0	5	3	62.5
mean	7	9	44.6	7	8	47.1
sd	2	2	10.6	2	2	11.8

F = Female

- = data not available

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 13 (continued) Individual Litter Sex Ratios

DOSE LEVEL: 200 ppm

Animal Number and Sex	Day 1			Day 5		
	Males	Females	% Males	Males	Females	% Males
61 F	8	7	53.3		Total litter loss	
62 F	6	8	42.9	6	8	42.9
63 F	8	7	53.3	7	6	53.8
64 F	6	8	42.9		Total litter loss	
65 F	6	6	50.0	6	6	50.0
66 F	8	7	53.3	8	7	53.3
67 F	6	7	46.2	6	7	46.2
68 F	4	8	33.3	4	6	40.0
69 F	5	9	35.7		Total litter loss	
70 F	4	9	30.8	4	9	30.8
mean	6	8	44.2	6	7	45.3
sd	2	1	8.6	1	1	8.2

F = Female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 13 (continued) Individual Litter Sex Ratios

DOSE LEVEL: 600 ppm

Animal Number and Sex	Day 1			Day 5		
	Males	Females	% Males	Males	Females	% Males
71 F	6	8	42.9	6	8	42.9
72 F	8	5	61.5	8	5	61.5
73 F	5	10	33.3	5	10	33.3
74 F	8	5	61.5	2	3	40.0
75 F	8	9	47.1	8	9	47.1
76 F	10	6	62.5	10	6	62.5
77 F			Not mated			
78 F	8	5	61.5	8	5	61.5
79 F	8	6	57.1	8	6	57.1
80 F	6	9	40.0	6	9	40.0
mean	7	7	51.9	7	7	49.6
sd	2	2	11.2	2	2	11.2

F = Female

OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING STUDY IN THE RAT

Appendix 14 Individual Clinical Observations - Offspring

GROUP: 1

Female Number	Number and Sex of Offspring Affected	Observation	Day post partum
41	1 F	No tail. Non patent anus	1
42		No abnormalities detected	
43	1 F	Small	1
	1 F	Small	4
44		No abnormalities detected	
45		No abnormalities detected	
46		No abnormalities detected	
47		No abnormalities detected	
48		No abnormalities detected	
49		No abnormalities detected	
50		Not Mated	

F = female

OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING STUDY IN THE RAT

Appendix 14(continued) Individual Clinical Observations – Offspring

GROUP: 2

Female Number	Number and Sex of Offspring Affected	Observation	Day post partum
51		No abnormalities detected	
52	1 F	Cold	PD1
53		No abnormalities detected	
54		No abnormalities detected	
55	1 F	Small	4
56		No abnormalities detected	
57		No abnormalities detected	
58		No abnormalities detected	
59		No abnormalities detected	
60	1 M	Small	1
	3 F	Small	1
	1 F	Small and weak	2
	1 M	Pale, weak. No milk in stomach	5

M = male
F = female

OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING STUDY IN THE RAT

Appendix 15 (continued) Individual Clinical Observations – Offspring

GROUP: 3

Female Number	Number and Sex of Offspring Affected	Observation	Day post partum
61		No abnormalities detected	
62		No abnormalities detected	
63	1 M	Small	1
64	Whole Litter	Cold, lethargic, scattered	2
65		No abnormalities detected	
66		No abnormalities detected	
66		No abnormalities detected	
67		No abnormalities detected	
68		No abnormalities detected	
69	Whole Litter	Cold and weak	1
	3 F	Cold and weak	2
	2 F	Small, weak, cold. No milk in stomach	3
70		No abnormalities detected	

M = male
F = female

OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING STUDY IN THE RAT

Appendix 14 (continued) Individual Clinical Observations – Offspring

GROUP: 4

Female Number	Number and Sex of Offspring Affected	Observation	Day post partum
71		No abnormalities detected	
72		No abnormalities detected	
73		No abnormalities detected	
74	1 F Whole Litter	Weak and pale	3
75	1 F	Cold, small. No milk in stomach	4
		Small	4
76	1 M 1 M 1 M	Small Small and pale Small	1 3 4
77		Not Mated	
78		No abnormalities detected	
79		No abnormalities detected	
80		No abnormalities detected	

M = male
F = female

OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING STUDY IN THE RAT

Appendix 15 Individual Macroscopic Post Mortem Findings - Offspring

GROUP: 1

DOSE LEVEL: 0 (Control)

Female Number	Number and Sex of Offspring Affected	Interim Deaths		Day of Death <i>post partum</i>	Terminal Kill	
		Observations	Observations		Observation	Observation
41		-			1 F - No milk in stomach	
42		-			No abnormalities detected	
43	1 M	No abnormalities detected		PDI	No abnormalities detected	
44		-			No abnormalities detected	
45		-			No abnormalities detected	
46		-			No abnormalities detected	
47		-			No abnormalities detected	
48		-			No abnormalities detected	
49		-			No abnormalities detected	
50		-			No abnormalities detected	
Not Mated						

M = male
F = female

OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING STUDY IN THE RAT

Appendix 15 (continued) Individual Macroscopic Post Mortem Findings - Offspring

GROUP: 2

DOSE LEVEL: 60 ppm

Female Number	Number and Sex of Offspring Affected	Interim Deaths		Day of Death <i>post partum</i>	Terminal Kill	
		Observations	Observations		Observation	Observation
51		-			No abnormalities detected	No abnormalities detected
52		-			No abnormalities detected	No abnormalities detected
53		-			No abnormalities detected	No abnormalities detected
54		-			No abnormalities detected	No abnormalities detected
55		-			No abnormalities detected	No abnormalities detected
56		-			No abnormalities detected	No abnormalities detected
57		-			No abnormalities detected	No abnormalities detected
58		-			No abnormalities detected	No abnormalities detected
59		-			No abnormalities detected	No abnormalities detected
60		-			No abnormalities detected	No abnormalities detected

OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING STUDY IN THE RAT
Appendix 15 (continued) Individual Macroscopic Post Mortem Findings - Offspring

GROUP: 3

Female Number	Number and Sex of Offspring Affected	Interim Deaths		Day of Death <i>post partum</i>	Terminal Kill	
		Observations	Observations		Observation	Observation
61	2 F	No abnormalities detected	-	2	Total litter loss	
	3 F			3		
	2 M			3		
	1 M			3		
62	2 M	No milk in stomach	-	4	Total litter loss	
	2 M			4		
	3 F			4		
63						
64	1 M	Autolysed. No milk in stomach Autolysed. No milk in stomach No abnormalities detected Autolysed Lower body crushed. Autolysed Autolysed. No milk in stomach Lower body crushed. Autolysed	-	2	Total litter loss	
	2 F			2		
	2 F			2		
	3 F			3		
	1 M			3		
	4 M			3		
	1 F			4		
65						
66						
67						
68	2 F	Autolysed Autolysed Autolysed No milk in stomach Autolysed No milk in stomach Autolysed Lower body crushed. Autolysed	-	2	Total litter loss	
	1 M			1		
	1 F			1		
	2 M			2		
	3 M			2		
	1 F			2		
	5 F			2		
1 F	3					
69						
70						

M = male
F = female

OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING STUDY IN THE RAT

Appendix 15 (continued) Individual Macroscopic Post Mortem Findings - Offspring

GROUP: 4

DOSE LEVEL: 600 ppm

Female Number	Number and Sex of Offspring Affected	Interim Deaths		Day of Death <i>post partum</i>	Terminal Kill	
		Observations			Observation	
71		-			No abnormalities detected	
72		-			No abnormalities detected	
73		-			No abnormalities detected	
74		-			No abnormalities detected	
75		-			No abnormalities detected	
76	1 F	Autolysed		1	No abnormalities detected	
77		Not mated				
78		-			No abnormalities detected	
79		-			No abnormalities detected	
80		-			1 F - Right kidney: increased renal pelvic cavitation	

F = female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 16 Individual Macroscopic Post Mortem Findings - Males****DOSE LEVEL:** 0 (Control)

Animal Number and Sex	Macroscopic Observations
1 M	No abnormalities detected
2 M	No abnormalities detected
3 M	No abnormalities detected
4 M	No abnormalities detected
5 M	No abnormalities detected
6 M	No abnormalities detected
7 M	No abnormalities detected
8 M	No abnormalities detected
9 M	No abnormalities detected
10 M	No abnormalities detected

M = male

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 16 (continued) Individual Macroscopic Post Mortem Findings - Males****DOSE LEVEL:** 60 ppm

Animal Number and Sex	Macroscopic Observations
11 M	No abnormalities detected
12 M	No abnormalities detected
13 M	No abnormalities detected
14 M	No abnormalities detected
15 M	No abnormalities detected
16 M	No abnormalities detected
17 M	No abnormalities detected
18 M	No abnormalities detected
19 M	No abnormalities detected
20 M	No abnormalities detected

M = male

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 16 (continued) Individual Macroscopic Post Mortem Findings - Males****DOSE LEVEL:** 200 ppm

Animal Number and Sex	Macroscopic Observations
21 M	No abnormalities detected
22 M	No abnormalities detected
23 M	No abnormalities detected
24 M	No abnormalities detected
25 M	No abnormalities detected
26 M	No abnormalities detected
27 M	No abnormalities detected
28 M	No abnormalities detected
29 M	No abnormalities detected
30 M	No abnormalities detected

M = male

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 16 (continued) Individual Macroscopic Post Mortem Findings - Males****DOSE LEVEL:** 600 ppm

Animal Number and Sex	Macroscopic Observations
31 M	No abnormalities detected
32 M	No abnormalities detected
33 M	No abnormalities detected
34 M	No abnormalities detected
35 M	No abnormalities detected
36 M	No abnormalities detected
37 M	No abnormalities detected
38 M	No abnormalities detected
39 M	No abnormalities detected
40 M	No abnormalities detected

M = male

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 17 Individual Macroscopic Post Mortem Findings - Females****DOSE LEVEL:** 0 (Control)

Animal Number and Sex	Macroscopic Observations
41 F	No abnormalities detected
42 F	No abnormalities detected
43 F	No abnormalities detected
44 F	No abnormalities detected
45 F	No abnormalities detected
46 F	No abnormalities detected
47 F	No abnormalities detected
48 F	No abnormalities detected
49 F	No abnormalities detected
50 F	No abnormalities detected

F = female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 17 (continued) Individual Macroscopic Post Mortem Findings - Females****DOSE LEVEL:** 60 ppm

Animal Number and Sex	Macroscopic Observations
51 F	No abnormalities detected
52 F	No abnormalities detected
53 F	No abnormalities detected
54 F	No abnormalities detected
55 F	No abnormalities detected
56 F	No abnormalities detected
57 F	No abnormalities detected
58 F	No abnormalities detected
59 F	No abnormalities detected
60 F	No abnormalities detected

F = female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 17 (continued) Individual Macroscopic Post Mortem Findings - Females****DOSE LEVEL:** 200 ppm

Animal Number and Sex	Macroscopic Observations
61 F	No abnormalities detected
62 F	No abnormalities detected
63 F	No abnormalities detected
64 F	No abnormalities detected
65 F	No abnormalities detected
66 F	No abnormalities detected
67 F	No abnormalities detected
68 F	No abnormalities detected
69 F	Liver - Dark
70 F	No abnormalities detected

F = female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 17 (continued) Individual Macroscopic Post Mortem Findings - Females****DOSE LEVEL:** 600 ppm

Animal Number and Sex	Macroscopic Observations
71 F	No abnormalities detected
72 F	No abnormalities detected
73 F	No abnormalities detected
74 F	No abnormalities detected
75 F	No abnormalities detected
76 F	No abnormalities detected
77 F	No abnormalities detected
78 F	No abnormalities detected
79 F	No abnormalities detected
80 F	No abnormalities detected

F = female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 18 Individual and Group Mean Organ Weights with Corresponding Relative Organ Weights (% of Bodyweight) and Standard Deviations (SD) - Males

DOSE LEVEL: 0 (Control)

Animal Number and Sex	Bodyweight (g) at Terminal Kill	Organ Weight (g)	
		Testes	Epididymides
1 M	517	3.04	1.14
2 M	521	3.57	1.29
3 M	465	3.06	1.20
4 M	534	3.69	1.40
5 M	486	3.29	1.15
6 M	515	3.06	1.23
7 M	494	4.00	1.34
8 M	544	3.48	1.27
9 M	510	3.73	1.29
10 M	461	3.33	1.14
mean	505	3.43	1.25
sd	28	0.33	0.09

Animal Number and Sex	Bodyweight (g) at Terminal Kill	Relative Organ Weight (%)	
		Testes	Epididymides
1 M	517	0.59	0.22
2 M	521	0.69	0.25
3 M	465	0.69	0.26
4 M	534	0.69	0.26
5 M	486	0.68	0.24
6 M	515	0.59	0.24
7 M	494	0.81	0.27
8 M	544	0.64	0.23
9 M	510	0.73	0.25
10 M	461	0.72	0.25
mean	505	0.68	0.25
sd	28	0.07	0.02

M = male

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

**Appendix 18 (continued) Individual and Group Mean Organ Weights with
Corresponding Relative Organ Weights (% of Bodyweight) and
Standard Deviations (SD) - Males**

DOSE LEVEL: 60 ppm

Animal Number and Sex	Bodyweight (g) at Terminal Kill	Organ Weight (g)	
		Testes	Epididymides
11 M	479	3.76	1.25
12 M	506	3.60	1.24
13 M	566	3.13	1.22
14 M	469	3.68	1.18
15 M	451	3.69	1.20
16 M	455	3.24	1.30
17 M	492	3.66	1.29
18 M	521	3.44	1.28
19 M	530	3.81	1.22
20 M	510	3.32	1.33
mean	498	3.53	1.251
sd	36	0.24	0.05

Animal Number and Sex	Bodyweight (g) at Terminal Kill	Relative Organ Weight (%)	
		Testes	Epididymides
11 M	479	0.79	0.26
12 M	506	0.71	0.25
13 M	566	0.55	0.22
14 M	469	0.79	0.25
15 M	451	0.82	0.27
16 M	455	0.71	0.29
17 M	492	0.74	0.26
18 M	521	0.66	0.25
19 M	530	0.72	0.23
20 M	510	0.65	0.26
mean	498	0.714	0.25
sd	36	0.08	0.02

M = male

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

**Appendix 18 (continued) Individual and Group Mean Organ Weights with
Corresponding Relative Organ Weights (% of Bodyweight) and
Standard Deviations (SD) - Males**

DOSE LEVEL: 200 ppm

Animal Number and Sex	Bodyweight (g) at Terminal Kill	Organ Weight (g)	
		Testes	Epididymides
21 M	469	3.42	1.210
22 M	478	3.78	1.230
23 M	476	3.82	1.410
24 M	495	3.69	1.350
25 M	567	3.58	1.130
26 M	512	3.62	1.310
27 M	470	3.78	1.450
28 M	466	3.17	1.180
29 M	442	2.99	1.110
30 M	504	3.93	1.490
mean	488	3.58	1.29
sd	34	0.30	0.14

Animal Number and Sex	Bodyweight (g) at Terminal Kill	Relative Organ Weight (%)	
		Testes	Epididymides
21 M	469	0.73	0.26
22 M	478	0.79	0.26
23 M	476	0.80	0.30
24 M	495	0.75	0.27
25 M	567	0.63	0.20
26 M	512	0.71	0.26
27 M	470	0.80	0.31
28 M	466	0.68	0.25
29 M	442	0.68	0.25
30 M	504	0.78	0.30
mean	488	0.74	0.27
sd	34	0.06	0.0311

M = male

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

**Appendix 18 (continued) Individual and Group Mean Organ Weights with
Corresponding Relative Organ Weights (% of Bodyweight) and
Standard Deviations (SD) - Males**

DOSE LEVEL: 600 ppm

Animal Number and Sex	Bodyweight (g) at Terminal Kill	Organ Weight (g)	
		Testes	Epididymides
31 M	519	3.31	1.31
32 M	536	3.77	1.23
33 M	453	4.01	1.38
34 M	468	3.71	1.21
35 M	435	3.54	1.40
36 M	490	3.42	1.28
37 M	429	3.73	1.15
38 M	453	3.28	1.26
39 M	452	3.37	1.34
40 M	510	3.09	1.21
mean	475	3.52	1.28
sd	37	0.28	0.08

Animal Number and Sex	Bodyweight (g) at Terminal Kill	Relative Organ Weight (%)	
		Testes	Epididymides
31 M	519	0.64	0.252
32 M	536	0.70	0.229
33 M	453	0.89	0.305
34 M	468	0.79	0.259
35 M	435	0.81	0.322
36 M	490	0.70	0.261
37 M	429	0.87	0.268
38 M	453	0.72	0.278
39 M	452	0.75	0.296
40 M	510	0.61	0.237
mean	475	0.75	0.27
sd	37	0.09	0.03

M = male

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 19 Individual Corpora Lutea and Implantation Sites

DOSE LEVEL: 0 (Control)

Animal Number	Total Number Corpora Lutea	Total No. Implantation sites	Pre Implantation Loss (%)	Post Implantation Loss (%)
41 F	20	19	5.0	5.3
42 F	17	16	5.9	12.5
43 F	14	14	0.0	7.1
44 F	14	14	0.0	7.1
45 F	15	15	0.0	0.0
46 F	16	16	0.0	6.3
47 F	14	14	0.0	0.0
48 F	-	-	-	-
49 F	16	16	0.0	12.5
50 F		Not mated		
mean	16	16	1.36	6.35
sd	2	2	2.53	4.76

F = female

- = data not available

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 19 (continued) Individual Corpora Lutea and Implantation Sites**

DOSE LEVEL: 60 ppm

Animal Number	Total Number Corpora Lutea	Total No. Implantation sites	Pre Implantation Loss (%)	Post Implantation Loss (%)
51 F	18	18	0.0	0.0
52 F	16	16	0.0	6.3
53 F	18	18	0.0	5.6
54 F	18	18	0.0	11.1
55 F	15	15	0.0	6.7
56 F	15	15	0.0	6.7
57 F	16	16	0.0	0.0
58 F	19	15	21.1	0.0
59 F	18	17	5.6	0.0
60 F	18	18	0.0	5.6
mean	17	17	2.66	4.18
sd	1	1	6.69	3.92

F = female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 19 (continued) Individual Corpora Lutea and Implantation Sites**

DOSE LEVEL: 200 ppm

Animal Number	Total Number Corpora Lutea	Total No. Implantation sites	Pre Implantation Loss (%)	Post Implantation Loss (%)
61 F	19	15	21.1	0.0
62 F	15	15	0.0	6.7
63 F	16	16	0.0	6.3
64 F	14	14	0.0	0.0
65 F	15	15	0.0	20.0
66 F	15	15	0.0	0.0
67 F	13	13	0.0	0.0
68 F	14	14	0.0	14.3
69 F	18	17	5.6	5.9
70 F	14	14	0.0	7.1
mean	15	15	2.66	6.02
sd	2	1	6.69	6.75

F = female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 19 (continued) Individual Corpora Lutea and Implantation Sites

DOSE LEVEL: 600 ppm

Animal Number	Total Number Corpora Lutea	Total No. Implantation sites	Pre Implantation Loss (%)	Post Implantation Loss (%)
71 F	15	15	0.0	6.7
72 F	14	14	0.0	7.1
73 F	15	15	0.0	0.0
74 F	15	15	0.0	13.3
75 F	18	18	0.0	5.6
76 F	18	18	0.0	5.6
77 F		Not mated		
78 F	15	15	0.0	13.3
79 F	15	14	6.7	0.0
80 F	16	15	6.3	0.0
mean	16	15	1.44	5.73
sd	1	2	2.85	5.21

F = female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 20 Individual Histopathological Findings

The histopathology report following concerns these routinely processed organs:

Coagulating glands	Seminal vesicles
Epididymides	Testes
Ovaries	Uterus/cervix
Pituitary	Vagina
Prostate	

Where no comment is made for an organ, no abnormality was detected

Tissue	Observation	
Epididymides	Reduced spermatozoal content - epidid 1	graded 1
	Reduced spermatozoal content - epidid 2	graded 1
Pituitary	Developmental cysts	presence/absence 6
Prostate	Chronic inflammatory cell foci	graded 1
Seminal vesicles	Reduced secretory content - vesicle 1	presence/absence 6
	Reduced secretory content - vesicle 2	graded 6
Testes	Atrophy gonad 1	graded 1
	Atrophy gonad 2	graded 1
Uterus/cervix	Dilatation horn1	graded 1
	Dilatation horn2	graded 1
	Peripheral fibrosis/haemorrhage	presence/absence 6

The grading systems used are as follows:

**Grading Level
System**

- | | | |
|---|---|------------|
| 1 | 1 | (minimal) |
| | 2 | (slight) |
| | 3 | (moderate) |
| | 4 | (marked) |
| | 5 | (severe) |
| 6 | 1 | [present] |

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 20 (continued) Individual Histopathological Findings****DOSE LEVEL: 0 (Control)**

Animal Number and Sex	Mode of Death	Tissue	Observation
1 M	Scheduled kill	Pituitary Prostate	Developmental cysts Chronic inflammatory cell foci (minimal)
2 M	Scheduled kill	Prostate	Chronic inflammatory cell foci (marked)
3 M	Scheduled kill		No abnormality detected
4 M	Scheduled kill		No abnormality detected
5 M	Scheduled kill		No abnormality detected
6 M	Scheduled kill	Prostate	Chronic inflammatory cell foci (minimal)
7 M	Scheduled kill		No abnormality detected
8 M	Scheduled kill	Prostate	Chronic inflammatory cell foci (moderate)
9 M	Scheduled kill		No abnormality detected
10 M	Scheduled kill	Prostate	Chronic inflammatory cell foci (minimal)

M = male

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 20 (continued) Individual Histopathological Findings****DOSE LEVEL: 0 (Control)**

Animal Number and Sex	Mode of Death	Tissue	Observation
41 F	Scheduled kill	Uterus/cervix	Peripheral fibrosis/haemorrhage
42 F	Scheduled kill	Uterus/cervix	Peripheral fibrosis/haemorrhage
43 F	Scheduled kill	Uterus/cervix	Peripheral fibrosis/haemorrhage
44 F	Scheduled kill	Uterus/cervix	Peripheral fibrosis/haemorrhage
45 F	Scheduled kill	Uterus/cervix	Peripheral fibrosis/haemorrhage
46 F	Scheduled kill	Uterus/cervix	Peripheral fibrosis/haemorrhage
47 F	Scheduled kill	Uterus/cervix	Peripheral fibrosis/haemorrhage
48 F	Scheduled kill	Uterus/cervix	Peripheral fibrosis/haemorrhage
49 F	Scheduled kill	Uterus/cervix	Peripheral fibrosis/haemorrhage
50 F	Killed Day 25		No abnormality detected

F = female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 20 (continued) Individual Histopathological Findings****DOSE LEVEL:** 600 ppm

Animal Number and Sex	Mode of Death	Tissue	Observation
31 M	Scheduled kill		No abnormality detected
32 M	Scheduled kill		No abnormality detected
33 M	Scheduled kill	Prostate	Chronic inflammatory cell foci (minimal)
34 M	Scheduled kill	Prostate	Chronic inflammatory cell foci (moderate)
35 M	Scheduled kill		No abnormality detected
36 M	Scheduled kill		No abnormality detected
37 M	Scheduled kill	Prostate	Chronic inflammatory cell foci (moderate)
38 M	Scheduled kill		No abnormality detected
39 M	Scheduled kill		No abnormality detected
40 M	Scheduled kill		No abnormality detected

M = male

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 20 (continued) Individual Histopathological Findings**

DOSE LEVEL: 600 ppm

Animal Number and Sex	Mode of Death	Tissue	Observation
71 F	Scheduled kill	Uterus/cervix	Peripheral fibrosis/haemorrhage
72 F	Scheduled kill	Uterus/cervix	Peripheral fibrosis/haemorrhage
73 F	Scheduled kill	Uterus/cervix	Peripheral fibrosis/haemorrhage
74 F	Scheduled kill	Uterus/cervix	Peripheral fibrosis/haemorrhage
75 F	Scheduled kill	Uterus/cervix	Peripheral fibrosis/haemorrhage
76 F	Scheduled kill	Uterus/cervix	Peripheral fibrosis/haemorrhage
77 F	Killed Day 25		No abnormality detected
78 F	Scheduled kill	Uterus/cervix	Peripheral fibrosis/haemorrhage
79 F	Scheduled kill	Uterus/cervix	Peripheral fibrosis/haemorrhage
80 F	Scheduled kill	Uterus/cervix	Peripheral fibrosis/haemorrhage

F = female

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 21 Chemical Analysis of Dietary Admixtures, Methods and Results

1. METHOD OF ANALYSIS

1.1 Summary

The concentration of OTOS in the dietary admixtures was determined by high performance liquid chromatography (HPLC) using an external standard technique.

1.2 Samples

The dietary admixtures were extracted with acetonitrile to give a final, theoretical test material concentration of approximately 5 ppm.

1.3 Standards

Standard solutions of test material were prepared in acetonitrile at a nominal concentration of 5 ppm.

1.4 Procedure

The standard and sample solutions were analysed by HPLC using the following conditions:

HPLC	:	Agilent Technologies 1050 or 1100, incorporating autosampler and workstation
Column	:	Prodigy (150 x 4.6 mm id)
Mobile phase	:	acetonitrile:water (50:50 v/v)
Flow-rate	:	1.0 ml/min
UV detector wavelength	:	282nm
Injection volume	:	10 µl
Retention time	:	~ 4 mins

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 21 (continued) Chemical Analysis of Dietary Admixtures, Methods and Results

1.5 Homogeneity Determinations

The dietary admixtures were sampled from the middle and two opposite sites in triplicate and analysed.

1.6 Stability Determinations

The dietary admixtures were sampled and analysed initially and then after storage at approximately -18°C in the dark for fourteen days.

1.7 Verification of Test Material Formulation Concentrations

The dietary admixtures were sampled and analysed within four days of preparation.

2. RESULTS

2.1 Homogeneity of Test Material Formulations

Nominal Concentration (ppm)	Sampling Location	Concentration Found (ppm)			
		1	2	3	Mean
60	Side	63.6	63.0	65.1	61.9
	Middle	60.5	62.9	64.0	63.2
	Side	61.5	63.7	64.4	64.5
200	Side	209	209	214	210
	Middle	212	213	213	210
	Side	208	209	209	212
600	Side	638	626	627	635
	Middle	639	633	626	629
	Side	629	630	628	627

2.2 Stability of Test Material Formulations

Nominal Concentration (ppml)	Concentration Found Initially (ppm)	Concentration Found After Storage For Fourteen Days	
		(ppm)	(expressed as % of initial)
60	49.4	47.1	95
200	185	175	95
600	559	533	95

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 21 (continued) Chemical Analysis of Dietary Admixtures, Methods and Results

2.3 Verification of Dietary Admixture Concentrations

Analysis	Nominal Concentration (ppm)	Sampling Location	Concentration Found		Mean	
			(ppm)	(expressed as % of nominal)	(ppm)	(expressed as % of nominal)
1	60	Side	61.9	103	63.2	105
		Middle	63.2	105		
		Side	64.5	108		
	200	Side	210	105	211	105
		Middle	210	105		
		Side	212	106		
	600	Side	635	106	631	105
		Middle	629	105		
		Side	627	105		
2	60	Side	59.0	98	58.7	98
		Middle	59.0	98		
		Side	58.1	97		
	200	Side	199	99	199	99
		Middle	199	100		
		Side	198	99		
	600	Side	595	99	589	98
		Middle	590	98		
		Side	583	97		
3	60	Side	61.9	103	62.0	103
		Middle	62.5	104		
		Side	61.6	103		
	200	Side	202	101	203	101
		Middle	204	102		
		Side	203	102		
	600	Side	607	101	608	101
		Middle	606	101		
		Side	609	102		

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 21 (continued) Chemical Analysis of Dietary Admixtures, Methods and Results

3. METHOD VALIDATION

3.1 Linearity

A range of standard solutions covering the concentration range 0 to 10 ppm, were prepared and analysed.

The detector response was shown to be linear up to 10 ppm.

Standard Concentration (ppm)	Peak Area (units)
0.000	0.000
2.5	48.780
4	78.651
5	98.784
6	116.921
10	192.281
Slope	19.242
Intercept	1.043
Correlation Coefficient	1.000

The results are presented graphically in Figure 1.

3.2 Specificity

The diluent solvent acetonitrile and a blank Basal laboratory diet (control) were analysed. The results are shown in the following table:

Sample	Concentration Found
Acetonitrile	None detected
Basal laboratory diet (control)	None detected

Analysis of the solvent and a blank Basal laboratory diet (control) produced no signal that interfered with the signal due to the test material.

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 21 (continued) Chemical Analysis of Dietary Admixtures, Methods and Results****3.3 Accuracy**

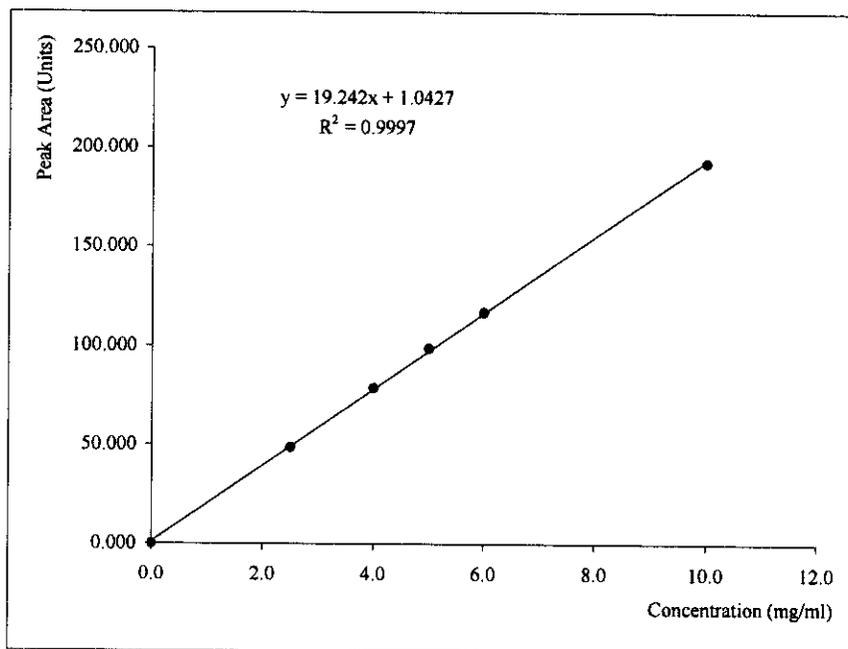
Samples of CH₃CN were accurately fortified with known amounts of test material, and analysed:

Fortification (ppm)	Concentration Found (ppm)	% Recovered	Mean Recovery (%)
59.9	56.0	93	94
60.8	57.8	95	
203	197	97	96
203	192	95	
641	646	101	99
638	618	97	

The analytical method has been considered to be sufficiently accurate for the purpose of this study. The test sample results have not been corrected for recovery.

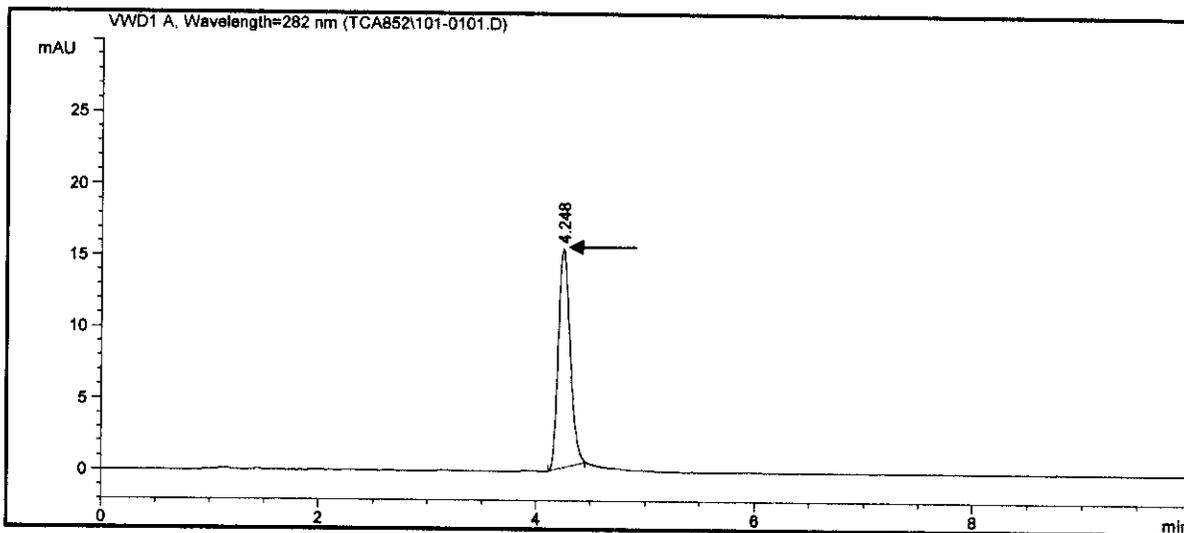
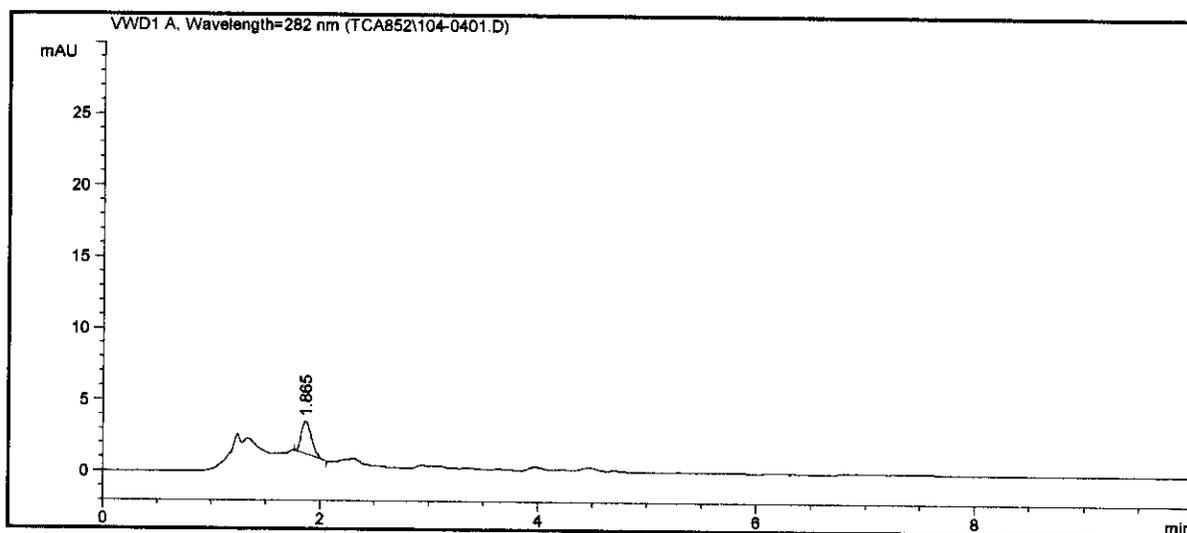
3.4 Conclusion

The analytical method has been satisfactorily validated in terms of linearity, specificity and accuracy for the purposes of the study.

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 21 (continued) Chemical Analysis of Dietary Admixtures, Methods and Results****Figure 1 Linearity of Detector Response**

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 21 (continued) Chemical Analysis of Dietary Admixtures, Methods and Results**

Examples of the typical chromatography generated during this study are given below:

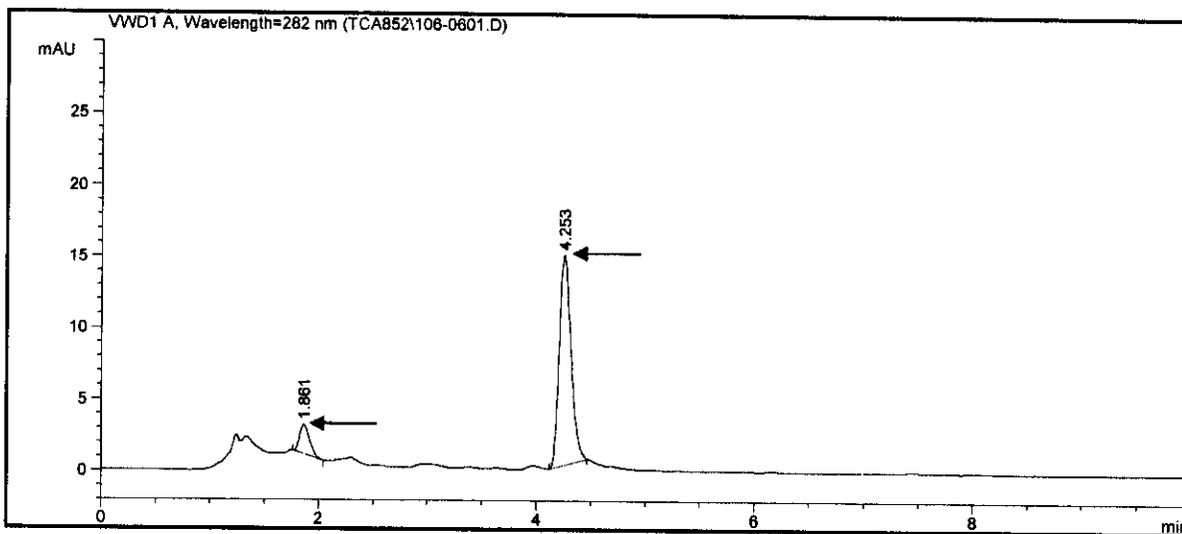
Standard Solution 5 ppm**Control Admixture**

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

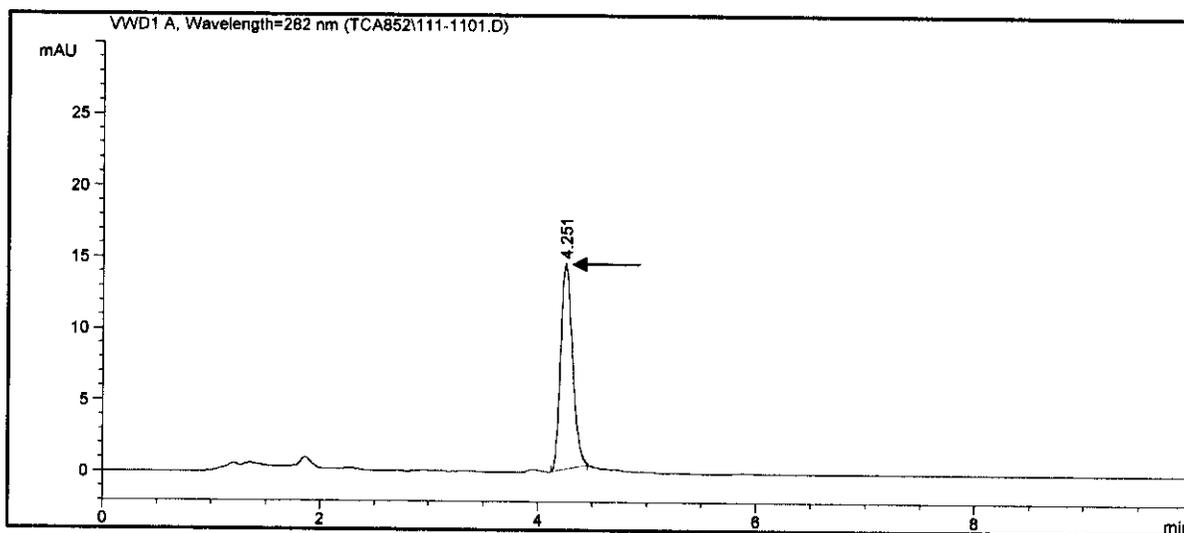
Appendix 21 (continued) Chemical Analysis of Dietary Admixtures, Methods and Results

Examples of the typical chromatography generated during this study are given below:

Test Material Admixture 60 ppm



Test Material Admixture 200 ppm

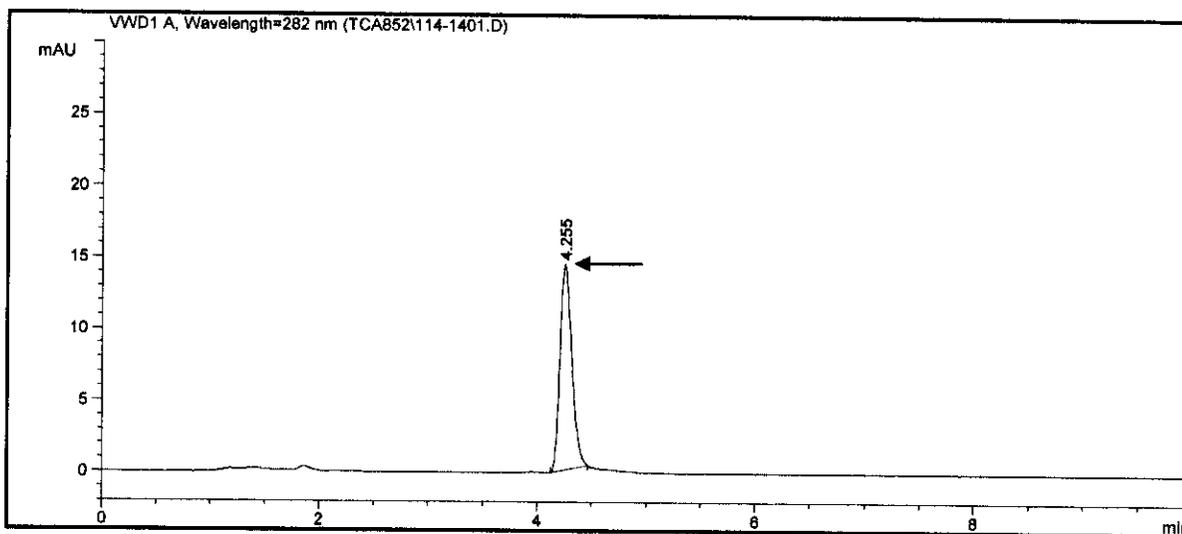


**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 21 (continued) Chemical Analysis of Dietary Admixtures, Methods and Results

Examples of the typical chromatography generated during this study are given below:

Test Material Admixture 600 ppm



**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

**Appendix 22 Normal Ranges for litter Sizes, Litter Weights and Mean Offspring Weight
Values in the Sprague-Dawley Crl:CD (SD) IGS BR Strain Rat**

Offspring Weight	Range *		No. of Animals
Litter size (at birth)	7.6 (14.2)	- (3.3)	201
Total Litter Weight (g)			
Day 1	50.99 (89.61)	- (19.31)	199
Day 4	67.21 (121.10)	- (26.95)	199
Mean Offspring Weight (g)			
Day 1	5.23 (6.45)	- (0.61)	200
Day 4	6.53 (8.93)	- (1.20)	199

* Range = mean \pm 2 standard deviations
(values in brackets indicate group mean and standard deviation respectively)

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 23 Protocol****SafePharm
Laboratories****PROTOCOL**

TEST MATERIAL : OTOS
STUDY TYPE : Dietary Reproduction/Developmental Toxicity
Screening Test in the Rat
PROJECT NUMBER : 826/150
PROPOSED START DATE : March 2004
PROPOSED COMPLETION DATE : May 2004
TARGET (DRAFT) REPORT DATE : October 2004
SPONSOR : Noveon, Inc.
9911 Brecksville Road
Cleveland
OHIO 44141-3247
USA

APPROVED FOR
SPONSOR BY:

DATE: 2/13/04

AUTHORISED BY:

K Knox BSc (Hons)
STUDY DIRECTOR

DATE: 3rd March 2004

This protocol is issued without signature by the Study Director to enable changes to be made if necessary prior to authorisation. Sponsors should sign and return the document to indicate approval and GLP authorisation will be confirmed by the Study Director's signature prior to the start of the study.

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 23 (continued) Protocol

**DIETARY REPRODUCTION/DEVELOPMENTAL TOXICITY
SCREENING TEST IN THE RAT
(OECD 421)**

1. INTRODUCTION AND OBJECTIVES

This protocol details a study designed to comply with the recommendations of the OECD Guidelines for Testing of Chemicals No 421 "Reproduction/developmental toxicity screening test" (adopted 27.07.95).

The study is designed to screen for potential adverse effects on reproduction including embryo/foetal development and therefore provides an initial hazard assessment for effects on reproduction.

The work will be performed in compliance with the UK Principles of Good Laboratory Practice (The United Kingdom Compliance Programme, Department of Health, 1989). These Principles are in accordance with GLP standards published as OECD Environment Monograph No. 45 (OCDE/GD(92)32); and are in conformity with, and implement, the requirements of Directives 87/18/EEC and 88/320/EEC.

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, Israel, Italy, Japan, Republic of Korea, Luxembourg, Mexico, The Netherlands, New Zealand, Norway, Poland, Portugal, Slovenia, Spain, Sweden, Switzerland, Turkey, the United Kingdom, and the United States of America.

2. STUDY FACILITIES

Test Facility

Safepharma Laboratories Ltd
Shardlow Business Park
Shardlow
Derbyshire
DE72 2GD

Histopathology elements of the study will be conducted at external, GLP compliant, facilities monitored by Safepharma Laboratories Ltd.

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 23 (continued) Protocol

3. ANIMALS

Specification

Sprague Dawley CrI:CD® BR strain rats obtained from Charles River (UK) Limited, Margate, Kent. At the start of the study animals will be aged nine to ten weeks. The weight variation will not exceed $\pm 20\%$ of the mean weight for either sex.

Justification

Preferred species of choice as historically used for safety evaluation studies and specified by appropriate regulatory authorities.

4. ANIMAL HUSBANDRY

Environment

Target temperature: $21 \pm 2^{\circ}\text{C}$

Target humidity: $55 \pm 15\%$

Lighting: Twelve hours of continuous artificial light in each twenty-four hour period.

Ventilation: At least fifteen air changes per hour.

Housing

Groups of five by sex in polypropylene cages with stainless steel lids and grid bases, suspended over trays containing absorbent paper. During mating animals will be housed on a one male:one female basis. Mated females will be housed individually in solid floor polypropylene cages fitted with stainless steel mesh lids. Mated females will be provided with softwood chips for bedding during gestation and lactation.

Diet and Water

Certified Rodent Diet PMI 5002 (supplied by International Product Supplies Ltd, Wellingborough, Northants, UK) with batch analysis.

The water for this study will be tap water, as provided to all rooms within the barrier-maintained animal facility at Safepharm Laboratories Ltd.

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 23 (continued) Protocol

The diet and drinking water are routinely analysed and are considered not to contain any contaminant that could reasonably be expected to affect the purpose or integrity of the study.

5. ANIMAL WELFARE

The study was designed and will be conducted to cause the minimum suffering or distress to the animals consistent with the scientific objectives and in accordance with the Safepharm policy on animal welfare. This standard test method is subject to review and the conduct of the study may be retrospectively reviewed, as part of the Safepharm Ethical Review Process.

6. PRE-TEST PROCEDURES

Acclimatisation Period

At least seven days.

Allocation

Animals will be allocated to dose groups using a randomisation procedure based on bodyweight.

Identification

Each animal, selected at random, will be uniquely identified within the study by ear-punch. A colour-coded cage card will be prepared with details of test material, project number, dose level, sex, numbers of animals, route of administration and Study Director responsible for the study.

7. TEST MATERIAL AND EXPERIMENTAL PREPARATION

Identification

Supplied by Sponsor with details of purity, stability and hazardous properties if known.

Storage

Room temperature unless otherwise specified by Sponsor.

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TOXICITY SCREENING STUDY IN THE RAT**

Appendix 23 (continued) Protocol

Preparation

The test material will be prepared as a direct dietary admixture. The method of preparation will be recorded in the appropriate study records. Subject to suitable stability the test material preparations will be performed weekly.

Analysis

Details of identification of the test material will be supplied by the Sponsor. The test material preparations will be analysed for homogeneity, concentration and stability by Safepharm Analytical Laboratory, prior to the start of the study. The achieved concentration of the test material preparations will be analysed at three time points during the course of the study, representing the start, middle and end of the dosing phase.

8. STUDY DESIGN

Administration

The test material will be administered in the diet as a direct dietary admixture.

Dose Groups

Four dose groups (control, low, intermediate and high) each comprising twenty animals (ten male and ten female) will be used. Dose levels will be based on available toxicity data. The dose levels to be used in the study will be documented as a protocol addendum. The control group will be handled in an identical manner to the test group.

Chronological Sequence of Study

- i) Groups of ten male and ten female rats will be dosed according to dose group for fourteen days prior to pairing.
- ii) On Day 14 animals will be paired on a one male:one female basis within each dose group for a maximum of fourteen days.
- iii) At the end of the mating period males will be returned to their original cages and females will be transferred to individual cages. Dosing will continue for both sexes during subsequent female gestation and lactation phases.

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 23 (continued) Protocol

- iv) Pregnant females are allowed to give birth and maintain their offspring until day four *post partum*. Evaluation of each litter will be performed during this period.
- v) At day five *post partum* all females and offspring will be killed and examined macroscopically.
- vi) Subject to confirmation of successful mating males are killed and examined macroscopically.

9. OBSERVATIONS

Morbidity/Mortality Inspection

Twice daily, early and late during the working period.

Clinical Observations

Individual clinical observations will be performed immediately before dosing and one hour after dosing. All observations will be recorded.

Bodyweights

Individual bodyweights will be recorded on Day 0 and at weekly intervals thereafter. Mated females will be weighed on Day 0, 7, 14, and 20 of gestation and Day 1 and Day 4 of lactation.

Food Consumption

Dietary intake will be recorded weekly prior to mating for each cage group. Weekly food efficiency (bodyweight gain/food intake) will be calculated. Dietary intake for mated females will be recorded on Day 1, 7, 14 and 20 of gestation and Day 1 and 4 of lactation.

Water Consumption

Visual inspection of water consumption will be performed daily.

Mating

One male and one female paired up for fourteen days. For females, stage of the oestrous cycle will be recorded during this period.

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 23 (continued) Protocol

Mating confirmed by the presence of sperm in a vaginal smear. The day on which sperm are observed is taken as Day 0 of gestation. Smearing of individual females discontinued when sperm are found. Mated females are removed from the mating cage and housed individually.

Pregnancy and Parturition

For each pregnant female the following will be recorded:

- i) Date of mating
- ii) Date of parturition
- iii) Duration of gestation

Litter Data

For each litter the following will be recorded:

- i) Number of pups born
- ii) Number and sex of pups alive recorded daily and reported on Day 1 and 4 *post partum*.
- iii) Clinical condition of pups from birth to day 4 *post partum*
- iv) Individual litter weights on Day 1 and 4 *post partum*

Post Mortem Studies

Carried out on animals that die or are killed in extremis during the study and on all adult animals killed by carbon dioxide asphyxiation followed by cervical dislocation. Offspring will be killed by intracardiac overdose of sodium pentobarbitone.

Gross Examination

Full external and internal examination of all animals including decedent adults and offspring.

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 23(continued) Protocol

Organ Weights

(Terminal kill only)

Epididymides

Testes

Carried out on all surviving males where appropriate.

Additional Procedures

- a) The corpora lutea of all ovaries from pregnant females will be counted at necropsy.
- b) The uterine implantation sites will be counted. The procedure may be enhanced using the technique proposed by Salewski [1]. Additionally the uteri of apparently non-pregnant females will be examined.

Histopathology

Samples of the following tissues will be preserved, from all animals, in buffered 10% formalin except where stated:

Epididymides †	Seminal vesicles
Gross lesions	Coagulating gland
Ovaries	Testes †
Pituitary	Uterus with Cervix
Prostate	Vagina

† = preserved in bouins fluid

Initially all tissues listed from any decedents plus all control and high dose animals will be routinely processed to paraffin wax, sectioned, stained with haematoxylin and eosin and examined microscopically. Special staining techniques may be used, where appropriate, at the discretion of the Study Pathologist.

Where treatment-related lesions are seen in the high dose group the affected tissues will be similarly processed and examined from all animals of each sex, from intermediate and low dose animals. Examinations may be extended to the remaining animals at the discretion of the Study Pathologist.

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 23 (continued) Protocol

10. EVALUATION OF DATA

All data will be summarised in tabular form and analysed statistically, where appropriate, to assess the significance of intergroup differences.

Reproductive indices will be prepared as presented in Appendix I.

Where appropriate the data will be statistically analysed using the following; parametric test for homogeneity of variance followed by either one way analysis of variance and pairwise comparison using students "t" test; or Kruskal-Wallis non parametric one way analysis of variance and Mann Whitney "U" test/Wilcoxon signed rank test.

Reproductive and viability indices by Fisher's exact test or chi-squared probability test, where applicable.

11. QUALITY ASSURANCE

Study procedures will be inspected and the final report will be audited by Safepharm Quality Assurance Unit, in accordance with QAU Standard Operating Procedures.

12. PROTOCOL AMENDMENTS

Amendments to this protocol will be made only by completion of an Amendment to Protocol form authorised by the Study Director.

13. REPORT

The Sponsor will be informed immediately of all relevant findings. A full report containing a description of the test material, detailed description of the experimental procedures, summary of the observations together with tabulated group mean and individual animal data, discussion and interpretation of the results will be presented. A draft report will be sent to the Sponsor for review and comments before issue of the final report.

14. ARCHIVE

Unless instructed otherwise by the Sponsor, 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. Further retention or return of the data will be charged at extra cost to the Sponsor.

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 23 (continued) Protocol

15. REFERENCE

Salewski E (1964) Färbemethode zum makroskopischen Nachweis von Implantationsstellen am Uterus der Ratte. Naunyn - Schmiedebergs Arch Exp Path Pharmacol 247 367.

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 23 (continued) Protocol****Appendix 1 Reproductive Indices**

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

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

$$\text{Parturition Index} = \frac{\text{Number of females delivering live pups}}{\text{Number of pregnant females}} \times 100$$

$$\text{Live Birth Index} = \frac{\text{Number of pups alive on Day 1}}{\text{Number of pups born}} \times 100$$

$$\text{Viability Index} = \frac{\text{Number of pups alive on Day 4}}{\text{Number of pups alive on Day 1}} \times 100$$

$$\text{Pre - implantation Loss} = \frac{\text{Number of corpora lutea} - \text{number of implantations}}{\text{Number of corpora lutea}} \times 100$$

$$\text{Post - implantation Loss} = \frac{\text{Number of implantation} - \text{number of live foetuses}}{\text{Number of implantations}} \times 100$$

% Male pups (Sex Ratio) at birth will be calculated as:

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

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 23 (continued) Protocol

RESPONSIBLE PERSONNEL

PROJECT NUMBER: 826/150	ISSUE NUMBER: 1
--------------------------------	------------------------

HOME OFFICE PROJECT LICENCE NUMBER: PPL 40/2572
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TITLE	NAME	REPLACEMENT DATE
STUDY DIRECTOR	K KNOX	
STUDY PATHOLOGIST	P N BROOKS	
PROJECT LICENCE HOLDER	E WOOD	
ANIMAL HUSBANDRY	N R STATHAM	
ANIMAL HEALTH	M L LJUBOJEVIC	
HISTOLOGY		
: LABORATORY	PROPATH UK LTD	
: PRINCIPAL INVESTIGATOR	H YOUNG	
FORMULATION	N R STATHAM	
CHEMICAL ANALYSIS	P WATSON	
DATA PROCESSING	K KNOX	

QUALITY ASSURANCE		
TEST FACILITY	SAFEPHARM LABORATORIES LTD	
TEST SITE	PROPATH UK LTD	

PROPOSED DATES			
ANIMALS ON SITE	MARCH 2004	STUDY TERMINATION	MAY 2004
FIRST TREATMENT	MARCH 2004	DRAFT REPORT	OCTOBER 2004

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 23 (continued) Protocol****SAFEPHARM LABORATORIES LIMITED****ADDENDUM TO PROTOCOL**

ADDENDUM NUMBER: 1

PROTOCOL TITLE: Dietary Reproduction/Developmental Toxicity Screening Test
in the Rat

TEST MATERIAL: OTOS

PROJECT NUMBER: 826/150

SPONSOR: Noveon, Inc
9911 Brecksville Road
Cleveland
OHIO 44141-3247
USA

ADDENDUM:

Protocol page 6 – Dose groups

Groups of 10 males and 10 females will be dosed at the following dose levels:

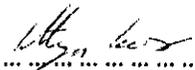
Low – 60 ppm
Intermediate – 200 ppm
High – 600 ppm

A further group of 10 males and 10 females will be dosed with the vehicle (PMI 5002 powdered diet) alone.

RATIONALE:

Dose levels were based on available toxicity data.

**AUTHORISED FOR SAFEPHARM
LABORATORIES LIMITED BY:**


..... DATE: 16th April 2004
K. Knox BSc (hons)
STUDY DIRECTOR

APPROVED FOR SPONSOR BY:


..... DATE: 20th 5 2004
SIGNATURE

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TOXICITY SCREENING STUDY IN THE RAT**

**Appendix 23 (continued) Protocol
SAFEPHARM LABORATORIES LIMITED**

AMENDMENT TO PROTOCOL

AMENDMENT NUMBER:

PROTOCOL TITLE: Dietary Reproduction/Developmental Toxicity Screening Test in the Rat

TEST MATERIAL: OTOS

PROJECT NUMBER: 826/150

SPONSOR: Noveon, Inc.
9911 Brecksville Road
Cleveland
OHIO 44141-3247
USA

AMENDMENT

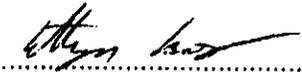
Page 6: Clinical Observations

Individual clinical observations will be performed daily during the dosing period.

JUSTIFICATION

Dosing of the test material is continuous in the diet.

AUTHORISED FOR SAFEPHARM
LABORATORIES LIMITED BY:



K. Knox BSc (hons)
STUDY DIRECTOR

DATE: *5th March 2004*

APPROVED FOR SPONSOR BY:



SIGNATURE

DATE: *2 March 2004*

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 23(continued) Protocol

Deviations to Protocol Documented as File Notes During the Study

1. Clinical observations were performed weekly rather than daily as stated in the Protocol in error. No signs of ill-health were seen at daily mortality checks or weekly clinical observations.
2. Organ weights were weighed at 2 decimal places in error. This did not affect the integrity of the study.
3. Histology site not included in Protocol in error. Should read:

ProPath UK Ltd
Willow Court
Netherwood Road
Rotherwas
HEREFORD
HR2 6JU

Clinical observations were performed for all animals on 2nd June and recorded as Day 17 in error. The correct study day was Day 16. This study day was corrected on Day 29 (15/06/04). This did not affect the integrity of the study.

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT****Appendix 24 Study Time Plan**

	DATE
STUDY DIRECTOR'S APPROVAL	03 March 2004
SPONSOR'S APPROVAL	13 February 2004
ANIMALS ARRIVE	11 May 2004
START OF DOSING	18 May 2004
START OF MATING PHASE	01 June 2004
START OF LACTATION PHASE - OFFSPRING	22 June 2004
COMPLETION OF NECROPSY OF PARENTAL ANIMALS	27 June 2004

**OTOS : ORAL DIETARY REPRODUCTION/DEVELOPMENTAL
TOXICITY SCREENING STUDY IN THE RAT**

Appendix 25 Statement of GLP Compliance in Accordance with Directive 88/320/EEC



**THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM**

GOOD LABORATORY PRACTICE

**STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC**

**LABORATORY
SafePharm Limited
Shardlow Business Park,
London Road,
Shardlow,
Derbyshire,
DE72 2GD**

**TEST TYPE
Analytical/Clinical
Chemistry
Environmental tox.
Environmental fate
Mutagenicity
Phys./Chem. tests
Toxicology**

DATE OF INSPECTION

2nd December 2002

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

A handwritten signature in black ink, appearing to read 'Roger G. Alexander', with the date '13/2/03' written below it.

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority