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OFFICE OF TOXIC SUBSTANCES
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Document Control Officer (TS-790)
Attn: Section 8 (e) Coordinator
Information Management Division
Office of Toxic Substances
Environmental Protection Agency
401 M Street, SW
Washington, DC 20460

Subject: 8EHQ-99-14506

To Whom It May Concern:

Enclosed is a copy of the final report for a study that our company submitted to the Environmental Protection Agency under Section 8(e) of the Toxic Substances Control Act on July 13, 1999. The study is a one-generation reproduction study in the rat with the test substance [

]. The generic name for the test substance is "long chain alkenyl amide borate".

A copy of this letter and of the final report, with confidential business information deleted, is attached for EPA's use when responding to public inquiries on this matter.

If there are any questions on this submission, please contact me at the number shown on the top of this page.

Sincerely,

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[]

Final report []

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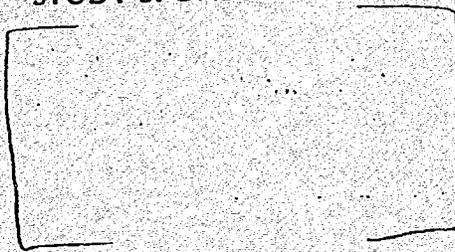
ORAL GAVAGE ONE GENERATION
REPRODUCTION STUDY IN THE RAT

SPL PROJECT NUMBER:



AUTHORS: E Wood
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STUDY SPONSOR:



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RTWPR00009

Company Sanitized

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QUALITY ASSURANCE REPORT

The conduct of this study has been subjected to periodic inspections by Safeparm Laboratories Quality Assurance Unit. The dates of inspection and reporting are given below:

28 July 1998

05 October 1998

17, 24 August 1998

04, 19, 24 November 1998

22 September 1998

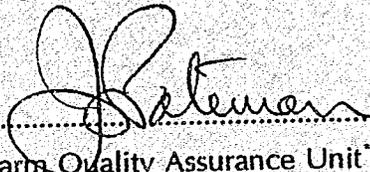
01, 08 December 1998

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

Date(s) of Report Audit:

21 June 1999

17 September 1999



DATE: 27 SEP 1999

For Safeparm Quality Assurance Unit*

* Authorised QA Signatures:

- Head of Department: JR Pateman CBiol MIBiol DipRQA
- Deputy Head of Department: JM Crowther MIScT
- Senior Audit Staff: JV Johnson BSc; G Wren ONC; RJ Gilbert BSc

[] []

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 data generated.

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1997 (SI 1997/654)). 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 87/18/EEC and 88/320/EEC.

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).



27 SEP 1999

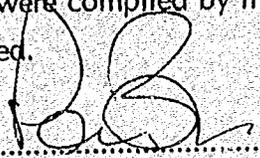
DATE:

Eric Wood CBiol MIBiol
Study Director
for Safeparm Laboratories

SPL PROJECT NUMBER:

AUTHENTICATION

I, the undersigned, hereby declare that the microscopic pathology data presented in this report were compiled by me or under my supervision, and accurately reflect the data obtained.

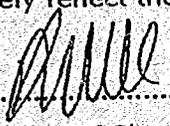


27 SEP 1999

..... DATE:

P N Brooks MSc BSc (Hons) EurBiol CBiol MIBiol
EUROTOX Registered Toxicologist
Study Pathologist

I, the undersigned, hereby declare that the analytical data presented in this report accurately reflect the data obtained.



27 SEP 1999

..... DATE:

D M Mullee CChem MRSC
Head of Analytical Chemistry

Approved for issue:



27 SEP 1999

..... DATE:

Eric Wood CBiol MIBiol
Head of Reproductive Toxicology

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REPRODUCTION STUDY IN THE RAT

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SUMMARY

STUDY SPONSOR

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[] []

STUDY TYPE

:

ORAL GAVAGE ONE GENERATION
REPRODUCTION STUDY IN THE RAT

TEST MATERIAL

:

[] []

The study was designed to investigate the effects of the test material on the growth and reproductive performance of the rat and complies with OECD Guidelines for Testing of Chemicals, Section 4: Health Effects, Test Guidelines No. 415, 26 May 1983.

METHODS

The test material was administered orally, by gavage, to groups of twenty-four male and twenty-four female rats throughout maturation, mating, gestation and lactation. The dose levels were 50, 250 and 1000 mg/kg of [] [] with a similar sized control group receiving vehicle alone.

Following at least ten and two weeks of dosing respectively, male and female rats were paired within their dose groups to produce litters. At weaning of the offspring, all surviving animals were killed and examined macroscopically.

Parental animals were observed daily 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 and food consumption recorded on specific days *post coitum* and *post partum*.

The offspring were observed daily for clinical signs. The litter signs and individual pup bodyweights were recorded on specific days *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. At necropsy of adult males a semen sample was collected from the *vas deferens* of the left testis for sperm evaluation. Reproductive and potential target organs and any significant abnormalities from all parental animals were preserved in fixative. Histopathology was carried out on reproductive organs from control and high dose group parental animals. Additional testicular histopathology, involving staging of testicular spermatogenesis, was performed on control and high dose males.

RESULTS

Reproduction was unaffected by treatment. The incidence of non-treatment related total litter losses seen at 1000 mg/kg and 50 mg/kg is a recognised aberration with the particular strain and source of rat used in this study.

At 1000 mg/kg there was evidence of minor systemic toxicity. Males showed slightly reduced bodyweight gain compared with that of controls during the maturation phase. In addition, there was a slight increase in male and female kidney weight at this dose level, a slight increase in male liver weight and prostrate weight was slightly reduced. There were no associated histopathological changes. There were no toxicologically significant findings at the remaining dose levels, although there were a number of unscheduled deaths at dose levels of 250 and 1000 mg/kg. These were attributable to dosing trauma and were not related to test material toxicity.

CONCLUSION

The administration of [] to adult male and female rats throughout the reproductive cycle resulted in no effects on reproduction that could be attributed to the test material. There was evidence of minor systemic toxicity at a dose level of 1000 mg/kg. The No Observed Effect Level for reproductive effects was 1000 mg/kg and the No Observed Effect Level for adult toxicity was 250 mg/kg.

**ORAL GAVAGE ONE GENERATION
REPRODUCTION STUDY IN THE RAT**

1. INTRODUCTION

The study was designed to investigate the effects of the test material on the growth and reproductive performance of 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, Section 4: Health Effects, Test Guidelines No. 415, 26 May 1983.

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

The dose levels were chosen based on the results of previous toxicology studies. The oral route was selected as the most appropriate route of exposure, based on the physical properties of the test material, and the results of the study are believed to be of value in 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 XXXII.

The in-life phase of the study was performed between 28 July 1998 and 22 December 1998 (see Appendix XXVIII).

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

The test material was supplied by the Sponsor as follows:

Sponsor's identification :

Order : 175840-001

Description : amber viscous liquid

Storage conditions : room temperature in the dark

Date received : 12 January 1998

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 a solution in Arachis oil BP. For each dose level a separate aliquot of the test material was weighed into the appropriate container. The vehicle was added and mixed using a Silverson homogeniser to ensure a homogeneous solution was formed.

Samples of each formulation were taken once per week during the first four weeks and approximately once every month until study completion and analysed for achieved concentration of at Safepharm Analytical Laboratory. The results indicate that the prepared formulations 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 XXIX.

3. METHODS**3.1 Animals and Animal Husbandry**

A sufficient number of male and female Sprague-Dawley CD 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 up to sixteen days during which time their health status was assessed. A total of 192 animals (96 males and 96 females) were accepted into the study. At the start of the treatment the males weighed 158 to 205g and the females weighed 170 to 210g.

Upon arrival, the animals were housed in groups of four 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 and transferred to a separate animal room of comparable conditions. 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 pelleted diet (Rat and Mouse SQC Expanded Diet No. 3, Special Diets Services Limited, Witham, Essex, UK) was used. Mains water was supplied from polycarbonate bottles attached to the cage. The diet and drinking water were considered not to contain any contaminant at a level that might have affected the purpose or integrity of the study.

The animals were housed in air-conditioned rooms within the Safepharm Laboratories Limited Barrier Maintained Rodent Facility. The rate of air exchange was at least fifteen air changes per hour and the low intensity fluorescent lighting was controlled to give twelve hours continuous light and twelve hours darkness.

Environmental conditions were continuously monitored by a computerised system. For both animal rooms used for this study 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 target limits but this was considered not to have affected the purpose or integrity of the study.

The animals were allocated to dose groups using a randomisation procedure based on stratified bodyweights and the group mean bodyweights were then determined to ensure similarity between the dose groups.

The animals were uniquely identified within the study, by an ear punching system routinely used at these laboratories. Colour coded cage labels were used to assist recognition of dose groups according to the following schedule.

GROUP NUMBER	DOSE LEVEL (mg/kg)	COLOUR CODE	ANIMAL NUMBERS	
			MALE	FEMALE
1	0 (Control)	Buff	1 - 24	97 - 120
2	50	Green	25 - 48	121 - 144
3	250	Blue	49 - 72	145 - 168
4	1000	Pink	73 - 96	169 - 192

3.2 Procedure

Four groups, each of 48 rats (24 males and 24 females) were dosed as follows:

GROUP NUMBER	DOSE LEVEL (mg/kg)	DOSE VOLUME (ml/kg)	CONCENTRATION (mg/ml)
1	0 (Control)	2	0
2	50	2	25
3	250	2	125
4	1000	2	500

The test material was administered once daily, by gavage, using a plastic dosing catheter attached to a disposable plastic syringe. Control animals were given vehicle alone (Arachis oil BP).

Male and female animals were dosed during maturation, mating, gestation and lactation. This study was not designed to show proof of test material absorption. One female (number 170) from 1000 mg/kg dose group was not dosed for one day (day 17 of gestation) during gestation due to her clinical condition.

3.2.1 Chronological Sequence of Study

- 3.2.1.1** Male animals were dosed for seventy four days and female rats were dosed for eighteen days, at their appointed dose levels, prior to pairing.
- 3.2.1.2** Parental males and females were paired within their respective dose groups for up to sixteen days.
- 3.2.1.3** Following evidence of mating, the animals were separated and males returned to their holding cages.
- 3.2.1.4** The pregnant females were allowed to deliver their offspring. The offspring were observed for growth and development during lactation.
- 3.2.1.5** At weaning on Day 21 (or as near to this date as possible) *post partum* the surviving offspring were killed and examined macroscopically *post mortem*.
- 3.2.1.6** The surviving adult Parental animals were killed and examined macroscopically *post mortem*. Selected reproductive tissues and organs together with potential target organs were retained in fixative. Reproductive tissues and organs from the high dose and

control animals were processed and subsequently examined microscopically by a pathologist.

4. OBSERVATIONS

4.1 Mortality/Morbidity

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

4.2 Clinical Observations

All animals were observed daily, immediately before, immediately after and one hour after dosing, for clinical signs of toxicity.

4.3 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 1, 4, 7, 14 and 21 *post coitum*. Parental generation females with a live litter were weighed on Days 1, 4, 7, 14 and 21 *post partum*.

4.4 Food Consumption

During the maturation periods food consumption was recorded weekly for each cage of parental generation adults. For parental generation females showing evidence of mating, food consumption was recorded for the periods covering Days 1 to 7, 7 to 14 and 14 to 21 *post coitum*. For parental generation females with live litters, food consumption was recorded for the periods covering Days 1 to 7 and 7 to 14 *post partum*.

4.5 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 sixteen 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.

4.6 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

4.7 Litter Data

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

The following observations were recorded for all individual offspring alive on the particular day of observation.

4.7.1 Bodyweight

Individual offspring weights were recorded on Days 1, 4, 7, 14 and 21 *post partum*.

4.7.2 Offspring Numbers and Sex

The number of offspring was recorded daily, up to weaning, and reported for Days 1, 4, 7, 14 and 21 *post partum*. On Days 1 and 21 the sex of the individual offspring was recorded.

4.7.3 Clinical Signs

The clinical condition of individual offspring was observed daily and any findings recorded.

4.7.4 Physical Development

All live offspring were observed for the following landmarks of development.

- a) Detachment of pinna - as noted by the separation of the edges and subsequent unfolding of both pinnae.

- b) **Tooth Eruption** - as noted by the eruption of the upper incisors through the gum.
- c) **Eye Opening** - as noted by the separation of the upper and lower eyelids of both eyes.

4.7.5 Offspring Reflexological Assessment

All live offspring were assessed for reflexological response to various stimuli as follows:

- a) **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.
- b) **Mid-air Righting Reflex** - on Day 17 *post partum*, offspring were tested for their ability to turn their body to a normal upright position, in mid-air, when dropped from a standard height above a bed of sawdust.
- c) **Startle Reflex** - on Day 21 *post partum*, auditory function was assessed by the reaction of offspring to a short auditory stimulus.
- d) **Pupil Reflex** - on Day 21 *post partum*, offspring pupil reaction to light shone on each eye, was assessed.

4.8 *Post Mortem* Studies

4.8.1 Decedents

All adult animals found dead during the course of the study, were examined macroscopically for internal and external abnormalities. All significant abnormalities were retained in fixative for possible further study. All offspring that died, or were killed *in extremis* during the lactation period, were examined macroscopically internally and externally.

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4.8.2 Terminal Necropsy

- a) **Adult Animals** - following successful weaning of offspring, 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 at weaning were killed by carbon dioxide asphyxiation, followed by cervical dislocation. All these offspring were examined macroscopically for internal and external abnormalities.

4.8.3 Organ Weights

The following list of organs were weighed for all adult males and females at terminal necropsy were applicable:

- Testes with epididymides
- Prostate
- Seminal vesicles/coagulating gland
- Uterus with cervix
- Ovaries
- Pituitary
- Liver
- Kidneys

4.8.4 Tissue Preservation

The following list of tissues and organs, from all adult animals at terminal necropsy (Section 4.8.2a) were preserved in fixative.

- | | |
|----------|--------------------|
| Ovaries* | Coagulating gland* |
| Uterus* | Pituitary gland* |
| Cervix* | Liver |

Vagina*	Lungs
Testes*	Thyroid gland
Epididymides*	Mesenteric lymph nodes
Seminal Vesicles*	Kidneys
Prostate*	
Significant abnormalities	

* organs subsequently processed for histopathological evaluation.

All the above tissues and organs were preserved in 10% formalin except the testes with epididymides which were retained in Bouin's fluid for 48 hours and then transferred to 70% industrial methylated spirits.

4.8.5 Histology/Histopathology

The organs (highlighted *) from the list presented in Section 4.8.4 from high dose and control animals were despatched to Propath UK Ltd, Rotherwas, Hereford. The tissues were processed and embedded in paraffin wax BP (mp 56 °C). Sections of the tissues were taken at 5 µm thickness, mounted on glass slides and stained with haematoxylin and eosin.

The sections of reproductive organs from control and high dose animals were examined microscopically by a pathologist. Examination included an identification and characterisation of the individual cell stages of the seminiferous epithelium from sections of the testis.

4.8.6 Semen Assessment

At necropsy of adult males a sample of semen was collected from the left epididymis/vas deferens. The semen sample was mixed with 0.5 ml 0.9% sterile saline at 35 °C. The following evaluations were performed:

--	--

4.8.6.1 Sperm Motility

Immediately upon collection, the semen sample was evaluated for motility using a microscope. The sample was graded using the following scale:

- 0 = no sperm motile
- 1+ = occasional sperm motile
- 2+ = few sperm motile
- 3+ = moderate numbers of sperm motile
- 4+ = majority of sperm motile and normally displaying head to head agglutination
- W = wave motion in semen sample

4.8.6.2 Sperm Concentration

A subsample of the semen sample collected at necropsy was preserved and diluted in neutral buffered formalin. An aliquot of the preserved sperm sample was examined under a microscope using a modified Fuchs-Rosenthal haemocytometer. The number of individual sperm in each of the two counting chambers was recorded and a mean of the two values presented. In order to present the concentration of sperm in the actual semen sample (in millions per ml of semen), values were corrected to take into account the dilution factor used. Each animal was sampled twice to reduce bias.

4.8.6.3 Sperm Morphology

A subsample of the preserved semen sample (see Section 4.8.6.2) was mixed with an equal volume of 0.25% aqueous eosin solution. A slide preparation was then examined microscopically. One hundred individual sperm were examined for morphological variation and the following coding scheme was used:

- A = normal
- B = misshapen
- C = abnormal hook
- D = reversed head
- E = head only
- F = other abnormality

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 of data.

5.1 Food Conversion Ratio

Calculated weekly during the maturation period of the Parental generation.

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

5.2 Mating Performance and Fertility

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

5.2.1 Pre-coital Interval

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

5.2.2 Fertility Indices

For each group the following were calculated.

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

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

5.3 Gestation and Parturition Data

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

5.3.1 Gestation Length

Calculated as the number of days of gestation including the days 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.

5.3.2 Gestation and Parturition Index

The following was calculated for each group.

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

5.4 Lactation Data

5.4.1 Live Birth and Viability Indices

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

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

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

$$\text{Viability Index 2 (\%)} = \frac{\text{Number of pups alive on Day 7}}{\text{Number of pups alive on Day 4}} \times 100$$

$$\text{Viability Index 3 (\%)} = \frac{\text{Number of pups alive on Day 14}}{\text{Number of pups alive on Day 7}} \times 100$$

$$\text{Viability Index 4 (\%)} = \frac{\text{Number of pups alive on Day 21}}{\text{Number of pups alive on Day 14}} \times 100$$

$$\text{Viability Index 5 (\%)} = \frac{\text{Number of viable pups at weaning}}{\text{Number of pups on Day 1}} \times 100$$



5.4.2 Sex Ratio

Group mean values calculated from each litter value on Day 1 and 21 using the following formula:

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

5.4.3 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.

5.5 Statistical Evaluation

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

- 5.5.1 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.

Values were analysed to establish homogeneity of group variances using Levene's test followed by one-way analysis of variance. If the variances were unequal subsequent comparisons between control and treated groups were performed using Dunnett's T3 Multiple Comparison Method. If variances were equal subsequent comparisons between control and treated groups were performed using Dunnett's Multiple Comparison Method.

- 5.5.2 Adult pre-coital intervals, female gestation lengths, offspring reflexological responses and litter sex ratios. Individual values

were compared using the 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.

5.5.3 Histopathology

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

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

Probability values were calculated as follows:

$P < 0.001$	+++	---	***
$p < 0.01$	++	-	**
$p < 0.05$	+	-	*
$p < 0.1$	(+)	(-)	(*)
$p \geq 0.1$	NS	(not significant)	

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.

6. ARCHIVES

Unless instructed otherwise by the sponsor, all original data, specimens and a copy of the final report will be retained in the archives of Safeparm Laboratories for a period of five years. After this period, the Sponsor's instructions will be sought.

SPL PROJECT NUMBER:

Specimens will be taken to include test material, any tissues or tissue blocks derived from the test system for examination or analysis.

Primary data will be taken to include laboratory data sheets, computer print-outs, records, memoranda and file notes that are a result of the original observations and activities of the study and which are necessary for the reconstruction and evaluation of the report of the study.

7. RESULTS

7.1 Mortality/Morbidity (Tables 1,2,3, Appendices I,II)

There were no unscheduled deaths that could be attributed to test material toxicity.

There were several deaths that were considered to be due to dosing trauma. At 1000 mg/kg one female (number 185) was found dead during the maturation phase of the study. Four further females (numbers 178, 180, 184 and 187) were found dead during the gestation period of the study and one male (number 93) was found dead during the post mating phase of the study. With the exception of respiratory distress and lethargy, which was evident for animal number 93, these animals showed no significant clinical findings prior to death. Female 187 was found to have a mass on the right flank that was subsequently found to be a mammary adenocarcinoma but this was considered not to be the cause of death. One female (number 161) dosed at 250 mg/kg was also found dead during the gestation phase of the study.

For all animals that were found dead, the principal post mortem macroscopic findings were associated with the thorax and lungs. Typically fluid or blood filled thorax was observed with incidents of lung congestion and/or adhesions involving organs within the thorax. With the exception of female 185, histopathology of the lungs showed evidence of pleuritis.

At 50 and 0 mg/kg there were no unscheduled deaths throughout the study.

7.2 Clinical Observations (Tables 2,3, Appendices I,II)

There were no toxicologically significant clinical findings throughout the study.

Transient increased salivation was observed pre- and post-dosing for all animals dosed at 1000 mg/kg and was seen post-dosing in some animals from the 250 mg/kg dose group. This is a characteristic response to the oral

administration of an unpleasant tasting material by gavage and, in isolation, is of no toxicological importance. Other clinical findings included respiratory abnormalities, hunched posture, chromodacryorrhea and pallor of the extremities.

These were invariably transient in nature and were usually confined to a single animal within a particular dose group. Such sporadic findings were, therefore, considered unlikely to be indicative of systemic toxicity.

7.3 Bodyweight

7.3.1 Maturation (Figures I,II, Tables 4,6, Appendices IV,V)

At 1000 mg/kg there was a slight reduction in male bodyweight gain from Week 5 until the end of maturation phase compared to controls. Although male group mean bodyweights were slightly lower than control values the differences were not significant statistically. Female bodyweight gain was not affected by treatment at this dose level.

At 250 and 50 mg/kg there were no significant differences in male and female bodyweight gain throughout the respective maturation phases.

7.3.2 Post Maturation (Table 5, Appendix IV)

At 1000 mg/kg male group mean bodyweight gain post maturation was slightly lower than that of controls, although the intergroup difference was not significant statistically.

At 250 and 50 mg/kg there were no significant differences in male bodyweight throughout this period.

7.3.3 Gestation (Figure V, Table 14, Appendix IX)

There were no significant intergroup differences in bodyweight gain for females throughout the gestation period.

7.3.4 Lactation (Figure VI, Table 15, Appendix X)

There were no significant intergroup differences in bodyweight gain for females throughout the lactation period.

7.4 Food Consumption

7.4.1 Maturation (Figures III, IV, Tables 7,8, Appendices VI,VII)

There were no treatment related effects on food consumption for males and females throughout their respective maturation periods.

7.4.2 Post Maturation (Figure III, Table 7, Appendix VI)

There were no effects on male food consumption during the post maturation phase of the study.

7.4.3 Gestation (Table 16, Appendix XI)

There were no effects on female food consumption throughout the gestation phase of the study.

7.4.4 Lactation (Table 16, Appendix XII)

There were no effects on female food consumption throughout the lactation phase of the study.

7.4.5 Food Conversion Ratio (Tables 9,10)

The variances in food conversion ratios, for males and females throughout their respective maturation phases, showed no treatment related trends.

7.5 Reproductive Performance

7.5.1 Fertility (Tables 11,12, Appendix VIII)

There were no treatment-related effects on male and female mating performance. The mating and pregnancy indices were comparable for all treated groups. The pre-coital intervals were comparable for all groups, with mating invariably occurring within the first four days after pairing.

7.5.2 Gestation and Parturition (Table 13, Appendix VIII)

There was no convincing treatment-related effect on gestation length.

At 1000 mg/kg the proportion of females with a gestation length of twenty-three days and above was slightly higher than amongst controls ($p < 0.05$). The actual values reported do not represent an anomalous gestation length however, based on historical data for this strain of rat, (Appendix XXXII) and this finding was therefore considered not to represent an adverse effect of treatment.

At 250 and 50 mg/kg there were no significant differences in the distribution of gestation lengths when compared to control values.

7.6 Litter Data

7.6.1 Litter Size, Sex and Offspring Viability (Tables 1,17,18, Appendices III,XIII)

There was no adverse effect on litter size, sex or viability that could be convincingly attributed to test material toxicity.

At 1000 mg/kg there were five females with total litter loss. Four incidents of total litter loss occurred between birth and Day 4 of lactation. For the remainder, the majority of pups died during this period but one pup survived until Day 11 of lactation. The nature of these total litter losses are consistent with findings observed for other Reproduction studies with this strain of rat, both within this laboratory and at other United Kingdom Contract Research Laboratories. This phenomenon predominated during 1998 and was apparent with one particular strain of rat from one particular supplier. This was the source of the animals used in this study. Although relatively high, the incidence of the total litter losses is consistent with the numbers seen in other Reproduction studies performed at this laboratory (Appendix XXXIII) and at other United Kingdom Contract Research Laboratories [1] during the same period, using the same strain

of rat and animal husbandry regime. These total litter losses have resulted in reduced live birth and viability indices during the first four days of lactation, compared to controls.

Between Days 1 and 7 *post partum* the group mean live litter size for females dosed at 1000 mg/kg was also slightly lower than controls. The intergroup difference was notable but not statistically significant and was exacerbated by a combination of the relatively high number of total litter losses at this dose level and two 1000 mg/kg females (numbers 173 and 179) with small numbers of surviving pups on Day 1 *post partum*. One of these animals eventually showed total litter loss by Day 11 of lactation. The disproportionate effect these small litters have on the group mean can be illustrated by excluding the data for these two females. When these data are excluded, the group mean litter size on Day 1 *post partum* for females dosed at 100 mg/kg is 12.9. This value is much closer to the control group mean value of 14.0 than the group mean litter size (11.8) when these two litters are included in the analysis (Table 18).

At 250 mg/kg there were no females with total litter loss and the offspring live birth and viability indices were comparable to controls. Group mean live litter size at birth and throughout lactation were also comparable to control values.

At 50 mg/kg there were three females with total litter loss and no animals with small numbers of surviving pups. These total litter losses did not result in a difference in live litter size either at birth or throughout lactation compared to controls. Viability index at weaning was slightly lower than controls due to these total litter losses.

7.6.2 Offspring Clinical Condition (Appendix III)

Surviving offspring showed no treatment-related clinical abnormalities.

Unusually small pups and pups that were cold, weak and had no milk in their stomach were frequently observed amongst litters from all dose groups, including controls. The latter finding was often observed for the entire litter, especially during the first few days *post-partum*, but most pups generally recovered. Other, more sporadic, clinical findings were observed for individual pups during this period. These included pups that were unusually pale and several pups with signs of physical damage.

Similar clinical findings were also evident for those litters where the entire litter was lost. However, for those seven litters where all pups had died by Day 4 *post partum* (four from the 1000 mg/kg group and three from the 50 mg/kg group), clinical findings also included scattering of the pups and offspring not cleared of amnion and placenta after birth. These latter findings are indicative of maternal neglect and strongly suggest that this was responsible for the total litter losses rather than a toxic effect of the test material on the offspring. The litter from animal number 173 did not show the characteristic lack of maternal care evident amongst the other litters but the majority of this litter died within 24 hours of birth and there were only two surviving pups by Day 1 *post partum*. One of these died between Days 1 and 4 *post partum* and the other, a female, died on Day 11. This pup was observed to be small and weak on Day 7 *post partum*.

7.6.3 Offspring Bodyweight and Development (Figure VII, Tables 19,20,21, Appendices XIV,XV,XVI)

There were no significant differences in the offspring development of animals treated with the test material compared to controls, as indicated by the group mean age of start and completion of the appearance of landmarks of offspring development. Similarly, there were no convincing differences in offspring bodyweight between treated animals and controls during lactation.

At 1000 mg/kg the group mean litter weights were slightly lower than controls at Day 1 of lactation. This was not statistically significant and reflects the significant variation in litter size noted for this group. Again this can be demonstrated by the exclusion of the two females (173 and 179) with small numbers of surviving pups from the group mean values. Excluding these animals, Day 1 *post partum* group mean litter weight at 1000 mg/kg is 81.0g (as opposed to 74.1g when these two litters are included). This is similar to the Day 1 *post partum* control group mean litter weight and demonstrates the marked effect that a number of unusually small litters can have on such group mean values. The disproportionate effect of these two unusually small litters on group mean litter weight is also demonstrated by the absence of any intergroup difference for individual offspring bodyweight throughout lactation.

7.6.4 Offspring Reflexological Assessment (Table 22, Appendix XVII)

There were no significant treatment related differences in offspring reflexological responses compared to controls.

7.6.5 Offspring Sex Ratios (Table 23, Appendix XVIII)

There were no significant treatment related differences in litter mean sex ratios on Days 1 and 21 of lactation compared to controls.

7.7 Post Mortem Studies

7.7.1 Adult Necropsy Findings (Appendices XIX,XX)

At terminal necropsy the incidence and distribution of macroscopic *post mortem* findings show no treatment related trends. The gross abnormalities are those commonly observed in this study type.

7.7.2 Offspring Necropsy Findings (Appendix XXI)

The overall incidence of necropsy findings did not show a significant treatment related trend. The predominant macroscopic finding was no milk in the stomach, particularly for those offspring from non-viable litters.

7.7.3 Organ Weights (Tables 24,25, Appendices XXII,XXIII)

At 1000 mg/kg there was a slight increase in male liver and kidney weight which, as a percentage of bodyweight, showed a statistically significant ($p < 0.01$) difference compared to controls. There was also a reduction in absolute prostate weight and prostate weight relative to bodyweight with the intergroup difference again achieving statistical significance compared to controls ($p < 0.05$). Female liver and kidney weights (absolute and relative to bodyweight) were slightly increased compared to control values although only the increased kidney weight achieved statistical significance ($p < 0.05$).

At 250 and 50 mg/kg there were no significant differences compared to controls.

7.7.4 Semen Assessment (Table 26, Appendix XXIV)

There were no significant treatment related differences for the parameters evaluated. The distribution of motility scores was comparable for all dose groups including controls. The intergroup differences in sperm concentration show no treatment related trends. The proportion of abnormal individual sperm is within the expected incidence for this strain of rat (Appendix XXXIV).

7.7.5 Histopathology (Tables 27 to 32, Appendices XXV to XXVII)

There were no significant treatment related findings from the selected reproduction organs examined.

The findings observed at histopathology are those commonly observed amongst animals of this strain and age, and the distribution of these findings showed no treatment related trend.

The histopathological staging of spermatogenesis, from sections of testicular tissue, showed no treatment related effects. All commonly characterised stages of spermatogenesis were identifiable.

8. DISCUSSION

At 1000 mg/kg there was evidence of minor systemic toxicity during the in-life phase of the study. Male rats showed a slight reduction in bodyweight gain and animals of either sex showed slightly elevated liver and kidney weight compared to controls. No associated macroscopic or histological abnormalities were identified however. There were a number of unscheduled deaths at this dose level during the course of the study. These were all attributed to dosing trauma and were considered not to be indicative of test material toxicity.

At 1000 mg/kg, mating performance was unaffected by administration of the test material with pregnancy rates and pre-coital intervals comparable to control values. There was a greater number of females that showed a slightly increased gestation length compared to controls. The gestation length observed is within the normal range for this strain of rat however and the intergroup difference was considered not to be toxicologically significant. Offspring individual bodyweight and development were unaffected at this dose level and no toxicologically significant macroscopic or microscopic anomalies were detected amongst offspring at terminal kill.

Following the birth of offspring there were five females dosed at 1000 mg/kg with total litter loss. Three females dosed at 50 mg/kg also showed total litter loss but there were no incidents of total litter loss amongst animals dosed at 250 mg/kg. The clinical and post mortem findings for offspring from these litters were symptomatic of maternal neglect, possibly due to maternal stress. In particular there were no clinical or pathological findings to suggest that a toxic insult was responsible for these total litter losses. This relatively high level of non-treatment related total litter loss is now a recognised aberration associated with this particular strain of rat under the prevailing study conditions. It was observed for three other Reproduction studies performed at this laboratory during 1998 and was evident at other United Kingdom Contract Research Laboratories during the same period for studies of this type, performed using the same strain of rat and supplier [1]. Studies performed at this laboratory before the introduction of this strain of rat and several studies performed subsequent to a change in animal husbandry (notably a change of diet) have shown a more

acceptable, substantially lower background incidence of total litter loss of between 2% and 4% (Appendix XXXIII). Although the aetiology of this aberration appears to be inherent within this strain of rat under the conditions of this present study, there does appear to be a slightly higher incidence of total litter loss amongst treated animals (13.8%) compared with controls (9.5%). This may indicate that the inherent maternal neglect can be slightly exacerbated by maternal stress, such as might be expected by the administration of an unpleasant tasting test material. In this present study, the finding that the highest incidence of total litter loss was observed for the 1000 mg/kg dose group may be fortuitous or it may indicate that the test material is compounding the inherent trait of a higher degree of maternal neglect. Regardless of the reason for this finding, the historical data presented in Appendix XXXIII provides a strong indication that the total litter losses seen in this study were not associated with a direct toxic effect upon reproduction. In particular, the absence of a true dose response relationship for the total litter losses seen in this study strongly indicates that an increased incidence of maternal neglect is an inherent behavioural trait of the strain of rat used in this study.

At 250 mg/kg there were no toxicologically significant effects on any of the reproduction parameters evaluated and there were no incidents of total litter loss. In addition, there was no evidence of systemic toxicity amongst the adults at this dose level. There was one unscheduled death but again macroscopic and microscopic findings were consistent with dosing trauma and this was considered not to represent test material toxicity.

At 50 mg/kg there were no toxicologically significant effects on the reproduction parameters during the course of the study. The three incidents of total litter loss observed at this dose level were considered to be unrelated to treatment.

The test material is produced by a reaction between boric acid, tall oil fatty acids and an alkanolamine. There are literature reports that boric acid can have an adverse effect on the male reproductive tract of rats and dogs when administered for a chronic period of up to two years, and that rats can become sterile following chronic administration of this substance. There is, however, no

epidemiological evidence of an adverse effect in humans to date. Particular attention was therefore focused on the male reproductive tract during this study and additional parameters associated with male fecundity, but not normally investigated as part of a One Generation Reproduction Study, were included in the study design at the request of the United Kingdom Health and Safety Executive.

Male fertility was unaffected by treatment. Organ weight measurement at terminal kill showed a slight reduction in prostate weight but there were no associated morphological abnormalities and there was no reduction in semen functions. Sperm concentration amongst animals treated with the test material were comparable with controls, and sperm morphology and mortality were unaffected by treatment. Histopathological analysis of the stages of testicular spermatogenesis also showed no treatment-related effects. These data demonstrate that there were no adverse effects on the male reproductive tract following sub-chronic repeated administration of the test material at up to 1000 mg/kg.

9. CONCLUSION

The administration of [] to adult male and female rats throughout the reproductive cycle resulted in no effects on reproduction that could be attributed to the test material. There was evidence of minor systemic toxicity at a dose level of 1000 mg/kg. The No Observed Effect Level for reproductive effects was 1000 mg/kg and the No Observed Effect Level for adult toxicity was 250 mg/kg.

10. REFERENCES

- [1] Bailey, GP, Diggins, GP and Biggs, LD. Teratology. 59 6 410 (1999).
- [2] Weir, R J, and Fisher, RS, Toxicology and Applied Pharmacology 23, 351-364 (1972)