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ORIGINAL

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April 26, 1996

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(Attn.: Section 8(e) Coordinator)
Office of Pollution Prevention and Toxics
U. S. Environmental Protection Agency
401 M Street, SW
Washington, DC 20460

RE.: **TSCA Section 8(e) Notice; 8EHQ-0883-0490; Araldite PT-810, Supplemental Submission, 13-Week Oral Reproduction Study in Male Rats**

Dear Section 8(e) Coordinator:

Ciba-Geigy Corporation (Ciba) claims no information in this letter or the enclosed report as Confidential Business Information.

In accordance with EPA's March 16, 1978, policy statement on Section 8(e) reporting under the Toxic Substances Control Act and EPA's June 1991 TSCA Section 8(e) Reporting Guide, Ciba wishes to bring to your attention results from a 13-week oral reproduction study in male rats with Araldite PT-810. Araldite PT-810 is 1,3,5-Triglycidylisocyanurate (CAS No. 2451-62-9).

In this study, male rats received dietary concentrations in food of either 0, 10, 30, or 100 ppm for 13 weeks (0, 0.72, 2.08, or 7.32 mg/kg/day, respectively). No signs of adverse effects were noted in any of the numerous parameters assayed for, except a slight, although dose related, decrease in mean spermatozoa concentrations.

Since the protocol for this study follows the normal 90-day subchronic study standards, evaluations of numerous endpoints (i.e., pathology, clinical chemistry, mating and fertility outcomes, etc.) were assessed.

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Concerning the pathologic outcomes, no gross differences occurred in organ weights in any treatment group. However, a few rats in the 100 ppm exposure group developed a reddish coloration of the mesentery lymph nodes. Upon microscopic examination of these tissues, four of the ten animals had hemosiderosis and/or congestion in these specific lymph nodes. No other treatment related effects were noted.

For the reproductive (i.e., mating) success in this study, 100 percent fertility was seen in the 10 and 30 ppm treated animals and a 90 percent success rate occurred in the 100 ppm males. The control group fertility index was similar to the highest dosed animals. The females showed no mortalities, adverse clinical signs, abortions, body weight changes, gross pathology, or food consumption compared to the controls.

An additional subgroup of pregnant females was hysterectomized to determine the effects, if any, on litter parameters. No changes or effects were seen compared to controls in corporea lutea, pre- and post-implantation losses, fetal death, live fetuses, body weights, or sex ratios. Concerning the live pup data, no differences were found in the number of live born, viability (21 days postpartum) and pup weight from day 1-21. For clinical signs and gross observation in these pups, several effects were seen, although the observations were presented at low incidences and were not considered to be treatment related.

Also, several measurements were made on these pups for physical development, indicating no change from controls. These included hair growth, tooth eruption, and eye and auditory canal openings. Further measurements on reflex development (*i.e.*, surface righting, cliff avoidance, and air righting) were normal.

Therefore, the only effect seen in all the areas tested was a slight but dose-related decrease in the mean number of spermatozoa. This slight decrease did not impact fertility outcomes or embryonic and pup development.

Since the findings of some slight reduction in mean sperm values corroborate similar findings via other routes of exposure, the data are not new. However, because the route of exposure in this case was dietary and not tested before, this information is being submitted to EPA to supplement the existing 8(e) file on this material.

A copy of the final report, entitled "13-Week Toxicity Study and Fertility Study by Oral Route (Dietary Admixture) in Male Rats," is enclosed (2 volumes, 466 pages).

In response to these findings, Ciba will do the following:

1. Modify the Material Safety Data Sheet to reflect these findings.

2. Notify persons working with this compound of the new findings in accordance with the notification requirements of OSHA's Hazard Communication Standard (29 CFR 1910.1200).

Please contact the undersigned if you require additional information.

Very truly yours,

A. Di Battista

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Encl.

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STUDY TITLE

13-WEEK TOXICITY STUDY AND FERTILITY STUDY BY ORAL ROUTE (DIETARY ADMIXTURE) IN MALE RATS

TEST SUBSTANCE

PT 810® (TGIC)

DATA REQUIREMENT
. O.E.C.D. guideline No. 408, 12th May 1981,
. U.S.A./E.P.A./T.S.C.A. Federal Register,
Vol 50 No. 188, 27th September 1985.

AUTHOR
Catherine Fabreguettes

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EXPERIMENTAL COMPLETION DATE
24th April 1995

STUDY COMPLETION DATE
12th December 1995

PERFORMING LABORATORY
Centre International de Toxicologie (C.I.T.)
Miserey - 27005 Evreux - France

LABORATORY STUDY NUMBER
11099 TCR

Volume 1

STATEMENT OF CONFIDENTIALITY OR NO CONFIDENTIALITY CLAIMS

(to be completed by the Sponsor)

STATEMENT OF THE STUDY DIRECTOR

The study was conducted in compliance with the Principles of Good Laboratory Practice Regulations:

. O.E.C.D. Principles of Good Laboratory Practice, C(81)30(final) Annex 2. May 12, 1981,

. US Environmental Protection Agency, Federal Register, 40 CFR Part 792; Toxic Substances Control Act; Good Laboratory Practice Standards, November 29, 1983 (and subsequents amendments).

I declare that this report constitutes a true and faithful record of the procedures undertaken and the results obtained in the performance of the study.

The study was performed at the Centre International de Toxicologie (C.I.T.), Miserey, 27005 Evreux, France.

C. Fabreguettes

C. Fabreguettes Date: 12.12.95
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Doctor of Pharmaceutical Sciences
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Director of Toxicology

STATEMENT OF QUALITY ASSURANCE UNIT

Type of inspections	Dates (day/month/year)		
	Inspections	Report to Study Director (*)	Report to Management (*)
Protocol	6.12.94	8.12.94	12.12.94
Study	29.12.94	5.1.95	6.1.95
Study	29.12.94	5.1.95	6.1.95
Study	24.1.95	26.1.95	27.1.95
Study	24.1.95	26.1.95	27.1.95
Study	14.2.95	17.2.95	17.2.95
Study	7.3.95	13.3.95	13.3.95
Study	14.3.95	23.3.95	23.3.95
Study	24.3.95	27.3.95	28.3.95
Study	24.3.95	27.3.95	27.3.95
Study	31.3.95	3.4.95	4.4.95
Study	31.3.95	11.4.95	12.4.95
Study	3.4.95	11.4.95	12.4.95
Study	5.5.95	22.5.95	(1)
Study	10.7.95	(2)	2.8.95
Report	21.8.95	28.8.95	15.9.95

The inspections were performed in compliance with C.I.T. Quality Assurance Unit procedures and the principles of Good Laboratory Practice Regulations.

(*) The dates mentioned correspond to the dates of signature of audit reports by Study Director and Management.
(1) Not signed.
(2) Signed but not dated.

L. Valette Talbi Date: 12.12.95
Doctor of Biochemistry
Head of Quality Assurance Unit
and Scientific Archives

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(to be completed by the Sponsor)

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SUMMARY

The objective of this study was to evaluate the potential toxicity of the test substance, PT 810® (TGIC), when administered by dietary admixture to Sprague-Dawley rats for 13 weeks.
The possible toxic effects on fertility and embryonic and pup development were also determined in this study.
The test substance is a cross linking agent for polyester powder coatings.

Methods

- TOXICITY STUDY IN MALES (principal groups)
Three groups of 10 male Sprague-Dawley rats received the test substance, PT 810® (TGIC), daily by dietary admixture at 10, 30 or 100 ppm for 13 weeks. These concentrations were determined on the basis of the results of a 19-day range-finding study performed at concentrations of 10, 40, 160 and 640 ppm. At 640 ppm, numerous signs of toxicity were noted. At 160 ppm, the principal findings were lower body weight gain and food consumption associated to histopathological findings on lymphoid or genital tissues.
At 40 or 10 ppm, the principal findings (lower body weight gain and/or food consumption) were attributed to palatability problems. Consequently for the present study, the high concentration was chosen between 40 and 160 ppm i.e. 100 ppm. 10 ppm was kept as low dose-level and 30 ppm as intermediate dose-level (approximation of the geometric mean between 10 and 100 ppm).
An additional group of 10 males received the untreated diet (A04C 2.5 ground diet) and acted as a control group.
The males were checked daily for clinical signs and mortality. Body weight was recorded once a week and food consumption twice a week (except during the mating period).
Ophthalmological examinations were performed before the treatment period in all males and on week 13 in males of control and high dose-level groups. Haematological and blood biochemical investigations and urinalysis were performed on week 13.
At the end of the treatment period, the males were killed. Selected organs were weighed.
On the day of sacrifice, a sampling of sperm was performed in order to determine the concentration and viability of spermatozoa.
All males were submitted to a full macroscopic examination and a microscopic examination was performed on all tissue specimens for all males from the control and high dose-level groups; macroscopic lesions and target tissues only were examined for the males of the intermediate and low dose-level groups.

- FERTILITY STUDY (satellite groups)
Four groups of 20 female Sprague-Dawley rats received the untreated diet (A04C 2.5 ground diet) and were included in the study on week 10.
After 64 days of dosing, the males were placed with the females of the same group (one male with two females) until mating occurred (a maximum of two times: seven days per male).
The females were checked daily for clinical signs and mortality.
On day 19 of pregnancy, the females of each group were allocated equally to two subgroups (hysterectomy subgroup and delivery subgroup).
Body weight was recorded for the mated females, on days 0, 6, 9, 12, 15 and 20 of pregnancy, and in addition, for females allowed to deliver on days 1, 7, 14, 21 post partum. The food consumption was recorded on the same times.

Hysterectomy groups
On day 20 of pregnancy, the females allocated to the subgroup hysterectomy were killed by asphyxiation using carbon dioxide, and fetuses removed by Caesarean section.
The ovaries and uterus of the females were examined to determine number and/or distribution of corpora lutea, live and dead fetuses, early and late resorptions and implantation sites.
The sex of each fetus was determined and they were weighed and submitted to an external examination to check for the presence of malformations.

Delivery groups
The delivery group females were allowed to deliver normally and rear their progeny until weaning. The day of parturition was recorded for each female of this group. Neither females nor pups received TGIC.
Each pup was identified on day one post-partum.
The progeny (F1 generation) was examined during the lactation period in order to determine the total litter size and number of pups of each sex. The litters were examined daily for clinical signs for the number of live, dead and cannibalized pups and for eventual abnormalities. The weight of each live pup was measured on days 1, 4, 7, 14 and 21 post-partum.
In order to determine the pup development and pup behaviour, the following characteristics were recorded; pinna unfolding, hair growth and surface righting reflex (on day 5 post-partum), cliff avoidance (on day 11 post-partum), tooth eruption (on day 13 post-partum) and eye opening, auricular canal opening and air righting reflex (on day 17 post-partum).
Between day 22 and 25 post-partum, the females and pups were killed.
All females and pups were submitted to a macroscopic examination of main thoracic and abdominal organs. The number of implantation sites were noted in females that delivered.
No tissues were preserved.

Results

- TOXICITY STUDY IN MALES

Clinical examinations

. No treatment-related clinical signs and no mortalities occurred during the treatment period.
. Slightly lower body weight gain was noted over the first 6 weeks of dosing in males given 100 ppm (-16%). Thereafter, the body weight gain of this group was similar to that of controls.
The body weight gain of males given 10 or 30 ppm was similar to that of controls.
. No statistically significant differences from controls were observed in food consumption for any male treatment groups.
. The achieved dosages were on average to 0.72, 2.08 or 7.32 mg/kg/day in the males given 10, 30 or 100 ppm, respectively. The achieved dosages decreased throughout of the treatment period (in mean -60% between weeks 1 and 13).
. The efficiency of food utilization was similar in all treated groups.
. No treatment-related ophthalmological findings were observed in control and high dose groups.

Laboratory investigations

. Slightly lower leucocyte and lymphocyte counts were noted in 2/10 males given 100 ppm.
. No relevant differences from controls were noted in blood biochemistry and urinalysis parameters.

Pathology
. No treatment-related differences from controls were recorded in the organ weights.
. Reddish colouration of mesenteric lymph nodes in association with haemosiderosis and congestion were found in some individuals given 100 ppm.

Analysis of sperm
. Slightly lower number of spermatozoa was observed with a dose-related frequence in all treated groups.
. The mean spermatozoa viability was similar in male treatment groups when compared to the control group.

- FERTILITY STUDY

Maternal data
. No clinical signs and no unscheduled mortalities occurred during the study period.
. No abortions occurred in any female.
. The body weight gain and the food consumption of females was similar in all groups during the pregnancy and lactation periods.
. No relevant macroscopic findings were made at necropsy.

Reproductive data in males
. The mating index of males was 100% in all groups.
. No treatment-related infertility was recorded in any male group.

Litter data in hysterectomy subgroup
. The gestation index was 100% in all groups.
. The mean number of corpora lutea and implantation sites and therefore the pre-implantation loss were similar in all groups.
. The rate of resorption and the post-implantation loss were similar in all groups.
. No dead fetuses were noted in any group.
. The mean number, the mean fetal body weight and the sex-ratio of live fetuses were similar in all groups.
. No external anomalies or malformations were observed in fetuses of any group.

Litter data in delivery subgroup

- Litter data
. At birth, the mean number of live born pups was similar in all groups.
. The viability index of pups on day 4 post-partum was similar in all groups.
. The viability index of pups on day 21 post-partum (or lactation index) was considered to be similar in all groups.
. The mean body weight of pups was similar in all groups, over the period of study (Day 1 to Day 21 post-partum).
. No toxicologically significant clinical observations were noted for pups in any groups.

- Pup development
. The physical development of pups as assessed by: pinna unfolding, hair growth, tooth eruption, eye opening and auditory canal opening, was similar in all groups.
. The reflex development of pups as assessed by: surface righting, cliff avoidance and air righting, was similar in all groups.

CONCLUSION

The administration of the test substance, PT 810® (TGIC), by dietary admixture for 13 weeks to male rats was well-tolerated at dose-levels of 10 or 30 ppm (0.72 or 2.08 mg/kg/day).
Slightly lower body weight gain was recorded over the first six weeks of dosing at 100 ppm (7.32 mg/kg/day).
A slight but dose-related decrease in the mean number of spermatozoa was observed in TGIC treated male groups.
No treatment-related infertility was noted in males and no influence on embryonic and pup development were observed after mating with untreated females.
Consequently, on the basis of these study results, 30 ppm is considered as the No Observable Effect Level. At 100 ppm, the lower number of spermatozoa did not impair the fertility of the males and therefore this dose-level can be considered as the No Observable Adverse Effect Level.

1. INTRODUCTION

This study was performed at the request of Ciba-Geigy Ltd/Polymers Division, Basel, Switzerland and Nissan Chemical Industries Ltd, Japan.
The objective of this study was to evaluate the potential toxicity of the test substance, PT 810® (TGIC), when administered by dietary admixture to Sprague-Dawley rats for 13 weeks.
Moreover possible toxic effects on fertility and embryonic and pup development were determined in this study.
The rat was chosen because it is a rodent species commonly requested by regulatory authorities and the Sprague-Dawley strain was selected due to the background data available from previous studies performed at our laboratory.
The test substance is a cross linking agent in polyester powder coatings.
The oral route was used since it is a possible route of exposure in humans.
The dose-levels were determined by the Sponsor, following the results of a 19-day range-finding study (CIT/Study No. 12289 TSR).
This study was designed in accordance with the following guidelines:
. O.E.C.D. guideline No. 408, 12th May 1981,
. U.S.A./E.P.A./T.S.C.A. Federal Register, Vol 50 No. 188, 27th September 1985.

2. MATERIALS AND METHODS

2.1. TEST SUBSTANCE

2.1.1 Identification and analysis of the test substance

The test substance, PT 810®(TGIC), used in the study was supplied by Ciba-Geigy Limited/Polymers Division/Basel.
Documentation supplied by the Sponsor identified the test substance as follows:
. Denomination
- protocol: PT 810® (TGIC)
- labelling: ARALDITE PT 810 PASTILLEN

. Batch number
- protocol: 407923.48 (40792348: data at reception)
- labelling: 40792348

. description: white pastilles
. quantity and container: two cardboard boxes each containing 25 kg of pastilles in a plastic bag.
. date of receipt: 11.5.94.
. storage conditions: at +4°C, away from light.

Data relating to the characterization of the test substance are documented in an analytical certificate provided by the Sponsor and presented in appendix 1.

A sample of the test substance received at C.I.T. for the study was taken for confirmation of identity by infra-red spectroscopy, once before the beginning of the study and once at the end of the *in vivo* study. The analytical method provided by the Sponsor was used.
This analysis was performed by Laboratoires Simon France (92110 Clichy, France) in compliance with the Good Laboratory Practice Regulations specified in page 3 and under the responsibility of C.I.T.

2.1.2 Vehicle for dietary admixture

The vehicle used was A04C 2.5 ground diet, batch Nos. 40927 and 41114 (U.A.R., 91360 Villemoisson-sur-Orge, France).

2.1.3 Preparation

The test substance was mixed with a small quantity of diet in a Braün mixer for 5 minutes and blended with a further small quantity of diet using a mortar and pestle; this premix was transferred into a Lödige FM 50 mixer (A.T.R., 75015 Paris) with the required total quantity of diet in order to achieve the concentration of 640 ppm, and mixed for 15 minutes.
All the concentrations required (10 ppm, 30 ppm and 100 ppm) were prepared by direct dilution of the preparation at 640 ppm.
The preparations were made once a week, and were stored at +4°C and away from humidity, in double closed, opaque plastic bags. The inner bag contained the dietary admixture and the outer bag contained silica gel.

2.1.4 Chemical analysis of the preparations

Homogeneity and stability

The homogeneity and stability were checked as part of a previous study (CIT/Study No. 12289 TSR) over a concentration range between 10 and 640 ppm. The results of these analyses are presented in this report but the raw data are stored with study 12289 TSR documentation.

Concentrations

On weeks 1, 4, 5, 8, 9 and 12, each preparation (control group included) was checked in duplicate on day of preparation for achieved concentration of the test substance.
On week 9, since no test substance was found in the 100 ppm dietary admixture, a new preparation at this concentration has been prepared and checked. At the same time, the diet of untreated animals was also checked.
The analytical procedure is presented in appendix 3.

2.2. TEST SYSTEM

2.2.1 Animals

One hundred and thirty-three Sprague-Dawley rats (45 males and 88 females) of the Crl CD (SD) BR strain were supplied by Charles River France (76410 Saint-Aubin-lès-Elbeuf, France) and received at C.I.T. on 22.12.94 (males) or 23.2.95 (females).
Upon their arrival the animals were given a clinical examination to ensure that they were in good clinical condition.
At least 8-day acclimatization period to the conditions of the study preceded the beginning of the treatment period for the males or the beginning of the mating period for the females.
Before the beginning of the treatment period for the males or the beginning of the mating period for the females, the respective required number of animals was selected according to body weight and clinical condition and allocated by sex to groups, according to a computerized stratified procedure so that the average body weight of each group is similar.
The remaining animals were killed later.
Each animal was then identified by ear tattoo.

At the beginning of the treatment period, the males were approximately 6 weeks old and had a mean body weight of 204 g (between 191 and 224 g).

2.2.2 Environmental conditions

Upon their arrival at C.I.T., the animals were housed in a protected zone.
The animal room was disinfected before the arrival of the animals and cleaned regularly thereafter. Microbiological analyses of the air and the surfaces of the walls and floor of the animal room are performed regularly (Laboratoire Départemental d'Analyses d'Evreux, 27000 Evreux, France).
The environmental conditions in the animal room were set as follows:
. temperature: 21 ± 2°C
. relative humidity: 50 ± 20%
. light/dark cycle: 12h/12h (07:00 - 19:00)
. ventilation: about 12 cycles/hour of filtered, non-recycled air.

The temperature and relative humidity were recorded continuously and records retained in C.I.T. study archives. The housing conditions (temperature, relative humidity and ventilation) were checked monthly.
The males were housed in polycarbonate cages (43.0 x 21.5 x 20.0 cm) containing autoclaved sawdust and each cage contained 2 rats of the same sex and group. The females were housed individually in polycarbonate cages (43.0 x 21.5 x 20.0 cm) containing autoclaved sawdust (SICSA, 94142 Alfortville, France). Bacteriological analysis of the sawdust and detection of possible contaminants (pesticides, heavy metals) are performed periodically (Laboratoire Départemental d'Analyses d'Evreux, 27000 Evreux, France; Laboratoire Municipal et Régional de Rouen, 76000 Rouen, France).
Bottles, cages, sawdust and feeders were changed at least once a week.
The cages were not randomized in the room but placed in numerical order vertically on the racks. Fortnightly, all the racks were moved around clockwise, rack by rack (except during the mating period).

There were no deviations in temperature and relative humidity which could have influenced the outcome of the study.

2.2.3 Food and water

The animals had free access to A04 C 2.5 ground diet (batch Nos. 40728 and 41213, U.A.R., 91360 Villemoisson-sur-Orge, France) distributed at least once a week for all animals during the acclimatization period and for females throughout the study and twice a week in males during the treatment period, except as noted in "Laboratory investigations". The diet formula is presented in appendix 4.
Each batch of food was analysed (composition, contaminants) by the supplier. Results are archived at C.I.T.
The animals had free access to bottles containing tap water filtered using a 0.22 micron filter (Millipore S.A., 78140 Vélizy, France).
Bacteriological and chemical analyses of water and detection of possible contaminants (pesticides, heavy metals and nitrosamines) are made periodically (Laboratoire Départemental d'Analyses d'Evreux, 27000 Evreux, France; Laboratoire Municipal et Régional de Rouen, 76000 Rouen, France; Centre de Nutrition Humaine, 54000 Nancy, France). Results are archived at C.I.T.

There were no known contaminants in the diet, water or sawdust at levels likely to have influenced the outcome of the study.

2.3. TREATMENT

Rationale for dose-level selection

The concentrations to be used in this study were determined on the basis of the results of a 19-day range-finding study: *CIT/Study No. 12289 TSR* performed at concentrations of 10, 40, 160 and 640 ppm.

In this preliminary study numerous signs of poor clinical condition (including: piloerection, round back, body weight loss) correlated with haematological and macroscopic findings were noted at 640 ppm.

At 160 ppm, the principal observations were lower body weight gain and food consumption, large mesenteric lymph nodes, small prostate (1/6 males) and seminal vesicles.

At 40 ppm, lower body weight gain and food consumption in males and a small prostate (1/6) was noted in males.

At 10 ppm, slightly lower body weight gain was recorded in males.

Consequently, for the present study 100 ppm was selected as the high dose-level and the low dose-level of 10 ppm is expected to be the No Observable Adverse Effect Level. The intermediate dose-level of 30 ppm represents an approximation of the geometric mean between 10 and 100 ppm.

2.3.1 Concentrations and groups

The groups, concentrations and animal numbers are detailed in the following table:

Group	Animals per group	Concentration (ppm)	Animal numbers
Males (principal group)			
1	10	0	L26031 to L26040
2	10	10	L26041 to L26050
3	10	30	L26051 to L26060
4	10	100	L26061 to L26070
Females (satellite group)			
1	20	0	L26101 to L26120
2	20	0	L26121 to L26140
3	20	0	L26141 to L26160
4	20	0	L26161 to L26180

2.3.2 Administration

The test substance mixed with the diet was supplied *ad libitum* to males of groups 2, 3 or 4 only for a period of 94 days.

The concentration of the test substance in the diet was constant throughout the study.

The males of the 100 ppm group were not treated with the test substance on day 57 of the study because no test substance was found in the 100 ppm dietary admixture distributed on the morning of 24.2.95 (first day of week 9). The concentration of the new preparation distributed on 25.2.95 to the males, in the morning was checked and showed a good concordance between nominal (100 ppm) and obtained concentration (102 ppm).

Since only one day opf treatment was affected by this error, it was judged not to have jeopardised the scientific integrity of the study.

Control males and all females were received the untreated diet *ad libitum*.

During the night of the mating period, males and females were placed in cages with feeders containing untreated diet. Each morning, males and females were returned to their original cages.

2.4. MATING

After 64 days of treatment, the males were placed with the females from the same group (1 male, 2 females) overnight. The female was placed with the same male until mating occurred or 7 days had elapsed.
If no evidence of mating was observed after 7 days, the female was placed with another male (of the same treatment group, that had already successfully mated) until mating occurred or a further 7-day period has elapsed.
Each morning, a vaginal lavage was performed in order to detect the presence of spermatozoa.
Day 0 of pregnancy was defined as the day spermatozoa was found.

2.5. CLINICAL EXAMINATIONS

2.5.1 Clinical signs

Clinical signs, including evidence of abortion, were observed for each animal at least once a day, at the same approximate daily time.

2.5.2 Mortality

All animals were checked at least twice a day for mortality and signs of morbidity, except for weekends and public holidays when they were checked once a day.

2.5.3 Body weight

Body weight was recorded for each male, once before allocation of the animals into groups, on the first day of treatment, then once a week until the end of the study.
Body weight was recorded for each female once before mating (for allocation of the animals into groups) then on days 0, 6, 9, 12, 15 and 20 of pregnancy and days 1, 7, 14 and 21 post-partum (delivery subgroup).

2.5.4 Food consumption

The quantity of food consumed by the males of each cage was recorded twice a week (over a 3 or 4-day period) until the end of the study, except during the mating period (due to cohabitation of the animals).
As the males of the 100 ppm group were not treated with the test substance on day 57 of the study, the measure of food consumption was performed over a 2-day period, (between 25.2.95 and 27.2.95 instead of 24.2.95 and 27.2.95), at one occasion, on week 9.

In addition, the quantity of food consumed by each mated female was recorded at the following intervals: days 0-6, 6-9, 9-12, 12-15 and 15-20 of pregnancy, and days 1-7, 7-14 and 14-21 post-partum (delivery subgroup).

Food intake per animal and per day was calculated using the amount of food given and left in each feeder and divided by 2 for the males only. In the summary tables, n corresponds to the number of cages.

2.5.5 Achieved dosages

The achieved intake of test substance in terms of mg/kg/day was calculated on a weekly basis for each treated group of males using mean body weight, mean food consumption and the nominal concentrations in the diet:

$$D = C \times \frac{FC}{BW}$$

D : achieved dosage (mg/kg/day)
C : nominal concentration (ppm)
FC: mean food consumption (g/animal/day)
BW: mean body weight (g)

2.5.6 Efficiency of food utilization

Efficiency of food utilization was estimated by calculation of food conversion ratios.
The food conversion ratio was calculated on a weekly basis for each treated group of males, using body weight and food consumption means.

$$FCR = \frac{FC}{BWG}$$

FCR = food conversion ratio
FC = mean food consumption (g/animal/week)
BWG = mean body weight gain (g/animal/week)

2.5.7 Ophthalmological examinations

Ophthalmological examinations were performed on all groups of males once before the beginning of the treatment period and on all males of control and high dose-level groups on week 13.
These examinations included corneal reflexes and the examination of the appendages, optic media and fundus by indirect ophthalmoscopy (All Pupil, Keeler, Windsor Berks, England).
Prior to examination, the pupils of the animals were dilated using Mydriaticum ® (Merck Sharp & Dohme-Chibret, 75008 Paris, France).

2.6. ALLOCATION OF THE FEMALES IN SUBGROUPS FOR HYSTERECTOMIES AND DELIVERY

The females were allocated equally to two subgroups (hysterectomy subgroup and delivery subgroup) on day 19 of pregnancy of the first mated females (23.3.95).

Hysterectomy subgroup

Group 1	Group 2	Group 3	Group 4
L26101	L26122	L26141	L26161
L26103	L26124	L26143	L26163
L26105	L26126	L26145	L26165
L26107	L26128	L26147	L26167
L26109	L26131	L26149	L26169
L26111	L26132	L26152	L26171
L26113	L26133	L26154	L26173
L26115	L26135	L26156	L26176
L26117	L26137	L26158	L26178
L26119	L26139	L26160	L26180

Delivery subgroup

Group 1	Group 2	Group 3	Group 4
L26102	L26121	L26142	L26162
L26104	L26123	L26144	L26164
L26106	L26125	L26146	L26166
L26108	L26127	L26148	L26168
L26110	L26129	L26150	L26170
L26112	L26130	L26151	L26172
L26114	L26134	L26153	L26174
L26116	L26136	L26155	L26175
L26118	L26138	L26157	L26177
L26120	L26140	L26159	L26179

2.7. EXAMINATION OF FEMALES OF HYSTERECTOMY SUBGROUP

2.7.1 Hysterectomies

On day 20 of pregnancy, the females allocated to the hysterectomy subgroup were killed by asphyxiation using carbon dioxide and fetuses removed by Caesarean section.
The ovaries and uterus of the females were examined to determine:

- number of corpora lutea,
- number and distribution of live and dead fetuses,
- number and distribution of early and late resorptions,
- number of implantation sites.

In apparently non-pregnant females, presence of implantation sites was checked using the Salewski staining technique.

2.7.2 Examination of fetuses

2.7.2.1 Body weight

Each live fetus was weighed.

2.7.2.2 Sex of fetuses

The sex of each fetus was determined at the time of Caesarian sections.

2.7.2.3 External examination

Each fetus was submitted to an external examination to check for the presence of anomalies or malformations. All fetuses were discarded therafter.

2.8. EXAMINATION OF FEMALES OF DELIVERY SUBGROUP

2.8.1 Parturition

The other females were allowed to deliver normally and rear their progeny until weaning.
The day of parturition was recorded.

2.8.2 Examination of progeny (f1 generation) during the lactation period

2.8.2.1 Litter size

The total litter size and number of pups of each sex was recorded as soon as possible after birth.
Each pup was identified by individual tattoo on day 1 post-partum.
The litters were examined daily in order to note the number of live, dead and cannibalized pups.
Any gross abnormality in pups was noted.

2.8.2.2 Body weight

The weight of each live pup was measured on days 1, 4, 7, 14 and 21 post-partum.

2.8.2.3 Clinical signs

Each pup was examined daily for possible clinical signs.

2.8.2.4 Pup development

The number of pups in each litter exhibiting the following characteristics was recorded:
. pinna unfolding: on day 5 post-partum,
. hair growth: on day 5 post-partum,
. tooth eruption: on day 13 post-partum,
. eye opening: on day 17 post-partum
. auricular canal opening: on day 17 post-partum,
. surface righting reflex: on day 5 post-partum,
. cliff avoidance: on day 11 post-partum,
. air righting reflex: on day 17 post-partum.

2.9. LABORATORY EXAMINATIONS

Blood samples were taken from the orbital sinus of the males under light ether anaesthesia. The samples were collected into tubes containing the appropriate anticoagulant (see below).
For urine and blood collection, the males were deprived of food and placed in diuresis cages over an overnight period of at least 14 hours. The urine was collected into a tube containing thymol crystals.
At the scheduled necropsy (week 14), bone marrow samples were taken from the femoral bone of all males.

2.9.1 Haematology (principal groups)

The following parameters were determined in all males on week 13.

Parameter	Apparatus/Method	Unit
Blood collected on EDTA		
Erythrocytes (RBC)	Bayer Diagnostics H1 (1) Haematology Analyzer/laser	T/l
Haemoglobin (HB)	Bayer Diagnostics H1 Haematology Analyzer/Drabkin	g/dl
Mean Cell Volume (MCV)	Bayer Diagnostics H1 Haematology Analyzer/laser	fl
Packed Cell Volume (PCV)	Bayer Diagnostics H1 Haematology Analyzer/calculated	l/l
Mean Cell Haemoglobin Concentration (MCHC)	Bayer Diagnostics H1 Haematology Analyzer/calculated/laser	g/dl
Mean Cell Haemoglobin (MCH)	Bayer Diagnostics H1 Haematology Analyzer/calculated	pg
Thrombocytes (PLAT)	Bayer Diagnostics H1 Haematology Analyzer/laser	G/l
Leucocytes (WBC)	Bayer Diagnostics H1 Haematology Analyzer/ peroxidase cytochemistry/laser morphometry	G/l

(1) Bayer Diagnostics (95331 Domont, France)

Parameter	Apparatus/Method	Units
Differential White Cell Count with cell morphology	Bayer Diagnostics H1 Haematology Analyzer/ peroxidase cytochemistry/laser morphometry (if reject,a microscopic control was determined after May Grünwald staining) (2) (a)	
. neutrophils (N)		% and G/l
. eosinophils (E)		% and G/l
. basophils (B)		% and G/l
. lymphocytes (L)		% and G/l
. monocytes (M)		% and G/l
Blood collected on sodium citrate		
Prothrombin Time (PT)	ACL 300 Thromboplastin (IL France) (3)	s
Activated Partial Thromboplastin Time (APTT)	ACL 300 Ellagic acid (IL France)	s
Fibrinogen (FIB)	ACL 300 Thromboplastin (IL France)	g/l
Bone marrow sample		
Myelogram (b)	Microscopic/ May Grünwald staining (3)	%

(a) Blood smears were prepared for all sampled animals.
(b) In the absence of abnormalities in the haemogram, the myelogram was not determined.
(2) Merck Clévenot (77500 Chelles, France)
(3) IL France (75562 Paris, France)

2.9.2 Blood biochemistry

The following parameters were determined in all males on week 13.

Parameter	Apparatus/Method	Unit
Blood collected on lithium heparinate		
Sodium (Na^+)	Hitachi 717 Selective electrode (Boehringer) (1)	mmol/l
Potassium (K^+)	Hitachi 717 Selective electrode (Boehringer)	mmol/l
Chloride (Cl^-)	Hitachi 717 Selective electrode (Boehringer)	mmol/l
Calcium (Ca^{++})	Hitachi 717 Ortho cresol-phthalein (Boehringer)	mmol/l

(1) Boehringer (38242 Meylan, France)

Parameter	Apparatus/Method	Unit
Inorganic phosphorus (I.PHOS)	Hitachi 717 Phosphomolybdic reaction (Boehringer)	mmol/l
Glucose (GLUC)	Hitachi 717 GOD-PAP (Boehringer)	mmol/l
Urea (UREA)	Hitachi 717 Urease UV(Boehringer)	mmol/l
Creatinine (CREAT)	Hitachi 717 Jaffe without deproteinisation (Boehringer)	µmol/l
Total Bilirubin (TOT.BIL)	Hitachi 717 Jendrassik (Boehringer)	µmol/l
Total Proteins (PROT)	Hitachi 717 Biuret (Boehringer)	g/l
Albumin (ALB)	Hitachi 717 Bromocresol green (Boehringer)	g/l
Albumin/globulin ratio (A/G)	Hitachi 717 Calculated	1
Cholesterol (CHOL)	Hitachi 717 CHOD-PAP (Boehringer)	mmol/l
Triglycerides (TRIG)	Hitachi 717 GPO-PAP (Boehringer)	mmol/l
Alkaline phosphatase (ALP)	Hitachi 717 DGKC Standard/30°C (Boehringer)	IU/l
Aspartate aminotransferase (ASAT)	Hitachi 717 IFCC Standard/30°C (Boehringer)	IU/l
Alanine aminotransferase (ALAT)	Hitachi 717 IFCC Standard/30°C(Boehringer)	IU/l

2.9.3 Urinalysis

The following parameters were determined in all males on week 13.

Parameter	Apparatus/Method	Unit
Quantitative parameters		
Volume (VOLUME)		ml
pH (pH)	10-Multistix SG test strips (Ames) (1)	
Specific gravity (SP.GRAV)	10-Multistix SG test strips (Ames) Refractometer (2) when superior to 1025	x1000
Semi-quantitative parameters		
Proteins (PROT)	10-Multistix SG test strips (Ames)	(a)
Glucose (GLUC)	10-Multistix SG test strips (Ames)	(a)
Ketones (CETO)	10-Multistix SG test strips (Ames)	(a)
Bilirubin (BILI)	10-Multistix SG test strips (Ames)	(a)
Nitrites (NITR)	10-Multistix SG test strips (Ames)	(a)
Blood (BLOOD)	10-Multistix SG test strips (Ames)	(a)
Urobilinogen (UROB)	10-Multistix SG test strips (Ames)	U%(=Ehrlich unit/100ml)
Cytology	microscopic	
. leucocytes (WBC)		(a)
. erythrocytes (RBC)		(a)
. cylinders (CYLIN)		(a)
. magnesium ammonium phosphate crystals (AMM.PH.)		(a)
. calcium phosphate crystals (CAL.PH)		(a)
. calcium oxalate crystals (CAL.OX.)		(a)
. cells (CELLS)		(a)
Qualitative parameters		
Appearance (APP)		(a)
Colour (COLOUR)		(a)

(a) see key or grading of cell frequency to appendix with individual values.
(1) Ames, Miles (75755 Paris, France)
(2) Aventec (92170 Vanves, France)

2.10. DETERMINATION OF SPERM CONCENTRATION

On the day of final sacrifice, all male rats were anaesthetized by intramuscular injection of 0.5 ml per animal of a mixture of Imalgène 500®/V-Tranquil® (10/1.5, v/v).
The testis and epididymide were exposed through a scrotal incision.
Samples of sperm were obtained from the caput epididymides by puncture with a micropipette.
Sperm was then diluted and one aliquot was put on a calibrated slide (Malassez cell) and observed under microscope in order to determine the sperm concentration. The results were expressed as a number of spermatozoa per mm^3 of sperm.
Viability of spermatozoa was also determined after eosin and nigrosin staining, and counting of about 100 spermatozoa which were classified as dead or alive. Results were expressed as a percentage of live spermatozoa.

2.11. PATHOLOGY

2.11.1 Sacrifice

On completion of the treatment period for the males, after at least 14 hours fasting, all surviving males were asphyxiated using carbon dioxide and killed by exsanguination.
Unmated females of the satellite groups, were killed, by asphyxiation using carbon dioxide, after the mating period (except two females, L26151 and L26129, which were kept in the delivery subgroup; (these two females did not show evidence of mating by vaginal lavage but were suspected to be pregnant).
Females that delivered were killed by asphyxiation using carbon dioxide between day 22 and day 25 post-partum.
Females that did not deliver were killed after day 25 post-coitum.
Pups were killed by asphyxiation using carbon dioxide between day 22 and day 25 post-partum.

2.11.2 Organ weights (principal groups)

In all males killed at the end of the treatment period, the body weight was recorded before necropsy and the following organs were weighed wet as soon as possible after dissection:

adrenals	kidneys	mandibular lymph nodes	spleen
brain	liver	prostate	testes
heart	mesenteric lymph nodes	seminal vesicles	thymus

Paired organs were weighed separately, except seminal vesicles which were weighed together.

2.11.3 Macroscopic examination

A complete macroscopic examination was performed on all males (principal groups).
All gross observations were recorded individually.

In the satellite groups, a macroscopic examination of the main thoracic and abdominal organs was made on females killed on day 20 of pregnancy, unmated females, delivered females killed between day 22 and day 25 post-partum and in females that did not deliver on day 25 post-coitum.
The number of corpora lutea (except for the females that delivered) and implantation sites were noted, whenever possible.
In pups (including any that died during lactation), a macroscopic examination of the main thoracic and abdominal organs was made.

2.11.4 Preservation of tissues

For all males of the principal groups, all the macroscopic lesions and the following tissues were preserved in 10% buffered formalin (except for the eyes and pituitary gland which were fixed in formol-sublimate for the males killed at the end of the treatment period).

adrenals	liver	skin*
aorta	lungs with bronchi	spinal cord* (cervical, thoracic and lumbar)
brain including medulla/ pons, cerebellar and cerebral cortex	lymph nodes (mandibular and mesenteric)	spleen
caecum	oesophagus	sternum with bone marrow
colon	pancreas	stomach with forestomach
duodenum	pituitary gland	testes and epididymides
eyes with Harderian glands*	prostate	thymus
femoral bone with articulation*(1)	rectum	thyroids with parathyroids
heart	salivary glands (sublingual and submaxillary)	tongue*
ileum	sciatic nerve	trachea
jejunum	seminal vesicle	urinary bladder
kidneys	skeletal muscle*	

The tissues marked by * were preserved in fixative for possible future microscopic examination.
(1) Bone marrow samples will be taken from the femoral bone of all males.

2.11.5 Microscopic examination (principal groups)

All tissues required for microscopic examination were embedded in paraffin wax, sectioned at approximately 4 microns in thickness and stained with hematoxylin-eosin.
Microscopic examination was performed on:

- all macroscopic lesions and tissues listed above (except those marked by *) in males of the control and high dose-level groups killed at the end of the treatment period,
- all macroscopic lesions and lungs, liver, kidneys, prostate, seminal vesicles, testes and epididymides and lymph nodes (mandibular and mesenteric) of all the males of the low and intermediate dose-level groups.

2.12. STATISTICAL ANALYSIS

2.12.1 Principal groups

The following sequence was used for the statistical analysis of body weight, food consumption, haematology, blood biochemistry, urinalysis and organ weight data:
The normality of the distribution of the values in each group was checked by Kolmogorov-Smirnov's test (1948).
If the distribution was normal, the homogeneity of variances between the groups was assessed by Bartlett's test (1937) (more than 2 groups) or Fisher's test (1934) (2 groups).
If no significant heterogeneity of the variances was established, the comparison between treated and control groups was performed by Dunnett's test (1955).
If the variances were heterogeneous, the comparison between treated and control groups was performed by Dunn's test (1964) (more than 2 groups) or by Mann Whitney's test (1947) (2 groups).
If the distribution of values in the groups was not normal, the analysis was repeated after logarithmic transformation of the values (except for organ weights).
If this logarithmic transformation failed to normalise the distribution of the values, comparison of treated and control groups was performed by Dunn's test using original values.

2.12.2 Satellite groups

2.12.2.1 Expression of data

Data are expressed as group mean values ± standard deviation (maternal body weight and food consumption, fetal body weight, corpora lutea, implantation sites, fetuses, resorptions) or as percentage (pre- and post-implantation loss, mating, fertility, gestation, live birth and viability indices, fetal and pup findings).

The data were calculated as follows:

$$\text{Pre-implantation loss:} \quad \frac{\text{Number of corpora lutea - Number of implantation sites}}{\text{Number of corpora lutea}} \times 100$$

$$\text{Post-implantation loss:} \quad \frac{\text{Number of implantation sites - Number of live fetuses}}{\text{Number of implantation sites}} \times 100$$

$$\text{Male mating index:} \quad \frac{\text{Number of males able to mate}}{\text{Number of paired males}} \times 100$$

$$\text{Male fertility index:} \quad \frac{\text{Number of pregnant females}}{\text{Number of males table to mate}} \times 100$$

$$\text{Gestation index:} \quad \frac{\text{Number of females with live born pups}}{\text{Number of pregnant females}} \times 100$$

$$\text{Live birth index:} \quad \frac{\text{Number of live born pups}}{\text{Number of delivered pups}} \times 100$$

$$\text{Viability index: on day 4 post-partum} \quad \frac{\text{Number of live pups on day 4 post-partum}}{\text{Number of live born pups}} \times 100$$

$$\text{Viability index: on day 21 post-partum} \quad \frac{\text{Number of live pups on day 21 post-partum}}{\text{Number of live pups on day 4 post-partum}} \times 100$$

Fetal findings were analysed as follows:

$$\frac{\text{Number of fetuses with a particular observation}}{\text{Total number of fetuses examined}} \times 100$$

In addition, the total number of litters within each group containing fetuses with a particular observation was calculated.

2.12.2.2 Statistical analysis during pregnancy and lactation periods

Mean values were compared by one-way analysis of variance and Dunnett's test.
Percentage values were compared by Fischer's exact probability test.

2.13. ARCHIVES

The study documentation and materials:
. protocol and amendments
. raw data,
. correspondence,
. final report and possible amendments,
. tissues in preservative, blocks and histological slides,
. haematological slides,
. samples of test substance and untreated diet,

are stored on the premises of C.I.T., 27005 Miserey, Evreux, France for 10 years after the end of the *in vivo* study. At the end of this period, the study archives will be returned to the Sponsor.

2.14. CHRONOLOGY OF THE STUDY

The chronology of the study is summarized as follows:

Procedure	Date	Day
Protocol approval by:		
. Study Director	26.12.94	
. Study Monitor	29.12.94	
Arrival of the males	22.12.94	-8
Preidentification and body weight	23.12.94	-7
Randomization and identification of the males	26.12.94	-4
Ophthalmological examinations (males)	28.12.94	-2
First day of treatment for the males	30.12.94	1
Week 8-9		
Arrival of the females	23.2.95	56
Preidentification	24.2.95	57
Randomization	27.2.95	60
Identification of the females	28.2 or 1.3.95	60 or 61
Week 10-11		
Mating		
. first females	4.3.95	65
. last females	10.3.95	71
Week 13		
Sacrifice for hysterectomy		
. first females	24.3.95	85
. last females	27.3.95	88
Males		
Ophthalmological examinations	28.3.95	89
Haematology, blood biochemistry and urinalysis	29.3.95	90
Week 13-14		
Delivery		
. first females	25.3.95	86
. last females	1.4.95	93
Day of necropsy for the males	3.4.95	95
Week 15-16-17		
Sacrifice of the females (group delivery)		
. first females	7.4.95	99
. last females	24.4.95	116

3. RESULTS OF TOXICITY STUDY

3.1. CHEMICAL ANALYSIS

3.1.1 Confirmation of identity of the test substance (appendix 2)

The infra-red spectra obtained with the two samples of PT 810® (TGIC), batch No. 407923.48 before and after the *in vivo* study were in accordance with the reference spectrum given by the Sponsor.

3.1.2 Chemical analysis of the preparations

Homogeneity and stability

Results of analyses performed in a previous study (CIT/Study No. 12289 TSR) revealed a satisfactory homogeneity for the lowest concentration (10 ppm).

At the highest dose, homogeneous results were obtained although the measured value exceeded the theoretical value by 15%.

Results of the cumulated stability of dietary mixtures at the same concentration was checked. Each preparation was kept in closed bags for four days at +4°C and away from humidity, followed by three and five days storage in open feeders in animal room conditions. Analyses indicated satisfactory stability for dietary mixtures held under these conditions.

Concentration

Throughout the study, reasonable concordance was found between measured and nominal concentrations of test material in the dietary mixtures.

During week 4, the concentration of the 30 ppm mixture was found to be 18% greater than theoretical. When a check was performed in week 5, a similar increase (18%) was measured for the 10 ppm mixture.

On week 8, the concentration of the 100 ppm mixture was 20% lower than theoretical; and in week 9, the concentration of the 10 ppm mixture was 16% lower than theoretical. In addition on week 9, no test substance was quantified in 100 ppm group nor in 0 ppm group. A new preparation of 100 ppm group was made and revealed a good concordance between measured and nominal concentrations.

Detailed results are presented in appendix 3.

3.2. CLINICAL EXAMINATIONS

3.2.1 Clinical signs (table 1, appendix 5)

No treatment-related clinical signs were observed.

3.2.2 Mortality (appendix 6)

No mortalities occurred during the treatment period.

3.2.3 Body weight (figure 1, table 2, appendix 7)

Slightly lower mean body weight gain was noted over the first 6 weeks of dosing in males given 100 ppm (+244 g vs. +289 g in controls, -16%).

Thereafter, the mean body weight gain of this group was similar to that of controls (+94 g vs. +92 g in controls over the last 7 weeks of treatment).

In the 10 and 30 ppm groups, the mean body weight gain was similar to that of controls over the 13-week treatment period (+369 g and +377 g vs. +381 g in controls, -3% and -1% respectively).

3.2.4 Food consumption (table 3, appendix 8)

No statistically significant differences from controls were observed for any male treatment groups.
Slighly lower mean food consumption, which did not achieve statistical significance, was noted over the first 2 weeks of treatment in males given 100 ppm (in mean -8%).

3.2.5 Achieved dosages (table 4)

The achieved dosages decreased throughout the treatment period as follows:
. 10 ppm group: 1.16 mg/kg/day on week 1 to 0.50 mg/kg/day on week 13 (- 57%),
. 30 ppm group: 3.40 mg/kg/day on week 1 to 1.37 mg/kg/day on week 13 (- 60%),
. 100 ppm group: 11.7 mg/kg/day on week 1 to 4.60 mg/kg/day on week 13 (- 61%).
The achieved dosages were on average to 0.72, 2.08 or 7.32 mg/kg/day in the males given 10, 30 or 100 ppm, respectively.

3.2.6 Efficiency of food utilization (table 5)

The efficiency of food utilization was similar in all treated groups.

3.2.7 Ophthalmological examinations (appendix 9)

No relevant findings were observed in control and high dose groups at the end of the treatment period.

3.3. LABORATORY INVESTIGATIONS

3.3.1 Haematology (table 6, appendix 10)

When compared to the control values, slightly lower mean leucocyte count (-20%) attributed to slightly lower mean lymphocyte count (-24%) was noted in males given 100 ppm. This difference from controls was principally due to 2/10 males (L26061 and L26066) with individual values below the lower limit of the control range (WBC: 6.47 G/l and 6.68 G/l vs. 7.4 G/l in controls, lymphocytes: 5.22 G/l and 5.46 G/l vs. 6.10 G/l in controls). The relationship of the differences in the 2 males given 100 ppm to treatment cannot be ruled out.

The slight differences from controls noted among the other haematological parameters (including: prothrombin time and activated partial thromboplastin time) were also considered not to be of toxicological significance since they were minor, often not dose-related and the individual values were within or close to the normal range of our historical background data.

3.3.2 Blood biochemistry (table 7, appendix 11)

The slight differences from controls noted among the blood biochemical parameters (including: chloride, glucose, total bilirubin and protein levels) were considered not to be of toxicological significance since they were minor, often not dose-related and the individual values were within or close to the normal range of our historical background data.

3.3.3 Urinalysis (table 8, appendix 12)

No relevant qualitative and quantitative differences from controls were noted in the urinary parameters.

3.4. PATHOLOGY

3.4.1 Organ weights (table 9, appendix 13)

Some differences in organ weight between individuals within the same group and between groups were noted. However, there were no differences of major order that were considered to be treatment-related.

3.4.2 Macroscopic examination (table 10, appendix 14)

Reddish colouration of the mesenteric lymph nodes was found in 2 males given 100 ppm. This was considered to be treatment-related.
All the other findings encountered are all commonly recorded changes in the rat of this strain and age and are spontaneous in nature such as dilated pelvis, angular surface of the kidneys and greyish/whitish foci on the liver. None was considered to be treatment-related.

3.4.3 Microscopic examination (table 11, appendix 14)

Haemosiderosis and/or congestion were noted in the mesenteric lymph nodes of 4 animals given 100 ppm. As these findings were not recorded in controls and in animals given the lower dose-levels, they were considered to be treatment-related.
All the other microscopic findings observed were those which are commonly recorded changes in the rat of this strain and age. Moreover, their incidence, severity and morphological characteristics were comparatively similar in both control and treated animals.
Consequently, they were considered not to be of toxicological importance.

3.5. ANALYSIS OF SPERM (table 12, appendix 15)

3.5.1 Numeration

The mean number of spermatozoa, expressed per mm^3 of sperm, was slightly lower in males given 10 ppm (-5%, 1/10 males lower than the lowest value in the control group range, L26049), 30 ppm (-13%, 2/10 males lower than the lowest value in the control group range, L26058 and L26060) or 100 ppm (-23%, 4/10 males lower than the lowest value in the control group range, L26063, L26065, L26067 and L26070).
Although no effect was noted on viability (see § 3.5.2) and fertility in concerned males (see § 4.2.2), it cannot be excluded that the slightly lower number of spermatozoa noted in all treated groups could be related to treatment.

3.5.2 Viability

The mean spermatozoa viability, expressed in %, was similar in all male treatment groups when compared to the control group (- 10%, +14% and - 5% respectively). No relevant differences from controls were noted in treated males for which the number of spermatozoa was low (see § 3.5.1).

4. RESULTS OF FERTILITY STUDY

4.1. MATERNAL DATA (figures 2 and 3, tables 13 to 21, appendices 7 and 16 to 22)

4.1.1 Clinical signs

No clinical signs were observed during the study.

4.1.2 Mortality

No unscheduled mortalities occurred during the study period.

4.1.3 Abortions

No abortions occurred in any female.

4.1.4 Body weight

The mean body weight gain of females was similar in all groups during the pregnancy and lactation periods.

4.1.5 Food consumption

The mean food consumption of females was similar in all groups during the pregnancy and lactation periods.

4.1.6 Maternal necropsy observations

No relevant macroscopic findings were made at necropsy.

4.2. REPRODUCTIVE DATA IN MALES (table 22, appendix 23)

4.2.1 Mating

The mating index of males was 100% in all groups.

4.2.2 Fertility

The fertility index in males was 100% in the 10 and 30 ppm groups.
In the control group, it was 90% as one male (L26031) was able to mate two female partners but neither female subsequently became pregnant.
In the 100 ppm group, it was 90% as one male (L26068) was not able to mate one of its two female partners (L26175). Mating with the other female (L26175) did not result in pregnancy.
These two males were considered not to be fertile.
As the fertility index was similar in the control and high dose groups, this infertility was not attributed to the treatment with the test substance.

4.3. LITTER DATA IN FEMALES OF HYSTERECTOMY SUBGROUP (tables 23 and 24, appendices 24 to 27)

4.3.1 Corpora lutea and implantation sites

The mean number of corpora lutea and implantation sites and therefore the pre-implantation loss was similar in all groups.

4.3.2 Post-implantation loss

. resorptions

The rate of resorption was similar in all groups (2.9%, 0.0%, 4.1% and 6.4 % in groups 1, 2, 3 and 4 respectively).

. dead fetuses
No dead fetuses were noted in any group.

. total post-implantation loss
The post-implantation loss was similar in all groups (2.9%, 0.0%, 4.1% and 6.4 % in groups 1, 2, 3 and 4 respectively).

4.3.3 Live fetuses

. mean number
The mean number of live fetuses was similar in all groups (14.7, 16.0, 15.4 and 14.6 in groups 1, 2, 3 and 4 respectively).

. body weight
The mean fetal body weight was similar in all groups (3.48 g, 3.53 g, 3.61 g and 3.68 g in groups 1, 2, 3 and 4 respectively).

. sex-ratio
The sex-ratio was similar in all groups.

4.3.4 Fetal external observations

No external anomalies or malformations were observed in fetuses of any group.

4.4. LITTER DATA IN DELIVERY SUBGROUP (tables 25 to 27, appendices 28 to 34)

4.4.1 Litter data

4.4.1.1 Data at birth

The gestation index was 100% in all groups.
The mean number of live born pups was similar in all groups (14.0, 14.8, 13.6 and 15.3 in groups 1, 2, 3 and 4 respectively).

4.4.1.2 Pup viability

The viability index on day 4 post-partum, expressed in %, was similar in all groups (100, 98.6, 98.5 and 96.7 in groups 1, 2, 3 and 4 respectively).
The viability index on day 21 post-partum (or lactation index), expressed in %, was considered to be similar in all groups (98.4, 100, 99.3 and 99.2 in groups 1, 2, 3 and 4 respectively).

4.4.1.3 Pup body weight

The mean body weight of pups was similar in all groups over the period of study (Day 1 to Day 21 post-partum).

4.4.1.4 Clinical signs

The principal observations were:
. necrosed left or right hindlimb in one pup of 1/9 females (L26116) of the group 1, 3 pups of 1/10 females (L26159) of the group 3, in one pup of 1/10 females (L26177) of the group 4, generally from day 6 post-partum,
. left or right hindlimb damaged in 2 pups of 1/10 females (L26134) of the group 2, one pup of 1/10 females of the group 3 (L26144), from day 7 to 21/22 post-partum,
. haematoma on abdomen in one pup of 1/10 females (L26155) of the group 3, or on back in one pup of 1/10 females (L26177) of the group 4,
. swelling navel in one pup of 1/10 females (L26159) of the group 3, from day 7 to 21 post-partum,

All these observations were present at a low incidence and were considered not to be of toxicological significance.

4.4.2 Pup development

4.4.2.1 Pup physical development

The physical development of pups as assessed by the following parameters: pinna unfolding, hair growth, tooth eruption, eye opening and auditory canal opening, was similar in all groups.

4.4.2.2 Pup reflex development

The reflex development of pups as assessed by the following parameters: surface righting, cliff avoidance and air righting, was similar in all groups.

5. CONCLUSION

The administration of the test substance, PT 810® (TGIC), by dietary admixture for 13 weeks to male rats was well-tolerated at dose-levels of 10 or 30 ppm (0.72 or 2.08 mg/kg/day).
Slightly lower body weight gain was recorded over the first six weeks of dosing at 100 ppm (7.32 mg/kg/day).
A slight but dose-related decrease in the mean number of spermatozoa was observed in TGIC treated male groups.
No treatment-related infertility was noted in males and no influence on embryonic and pup development were observed after mating with untreated females.
Consequently, on the basis of these study results, 30 ppm is considered as the No Observable Effect Level. At 100 ppm, the lower number of spermatozoa did not impair the fertility of the males and therefore this dose-level can be considered as the No Observable Adverse Effect Level.

6. REFERENCES

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Figure 1

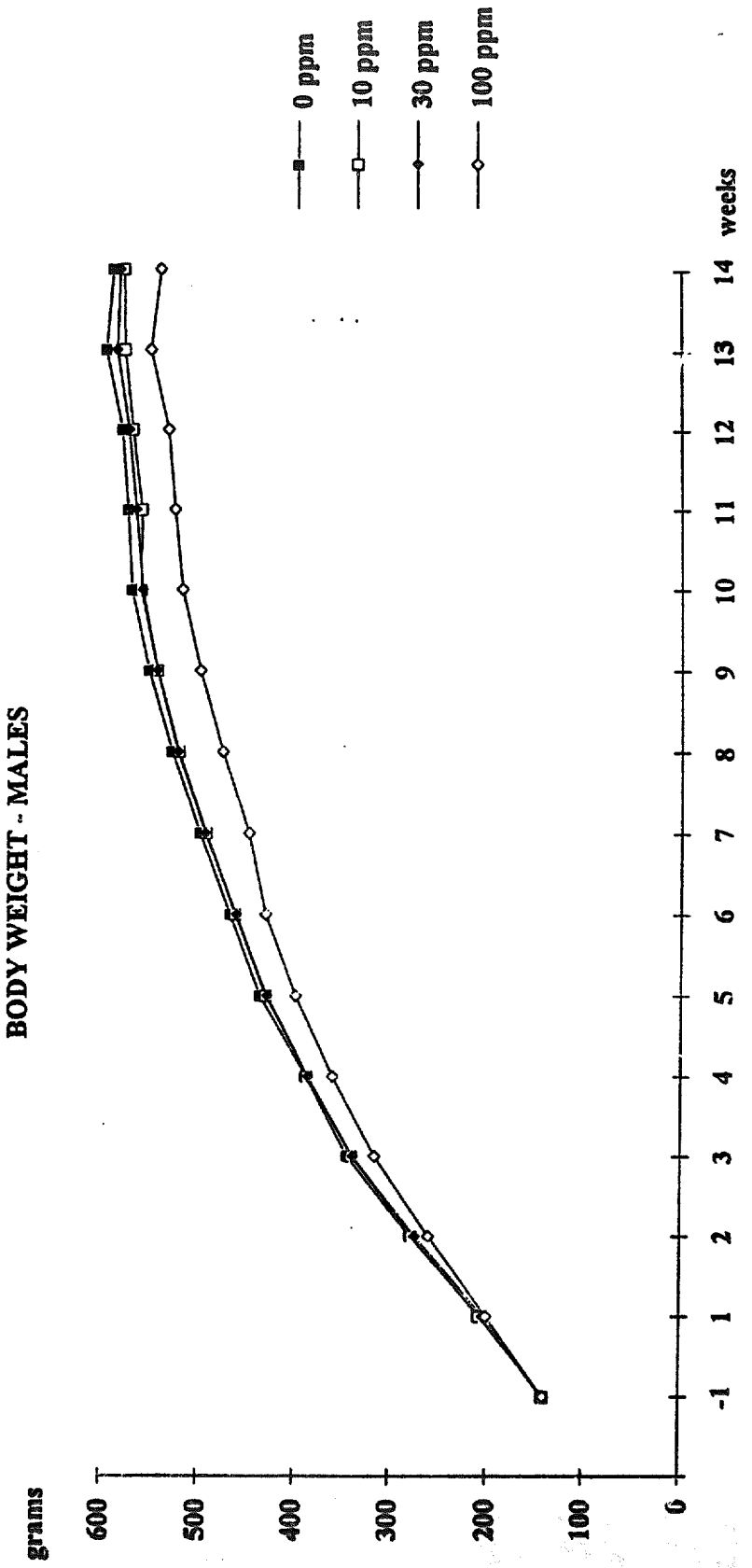

BODY WEIGHT - MALES
0 ppm
10 ppm
30 ppm
100 ppm
grams
600
500
400
300
200
100
0
-1
1
2
3
4
5
6
7
8
9
10
11
12
13
14
weeks

Figure 2

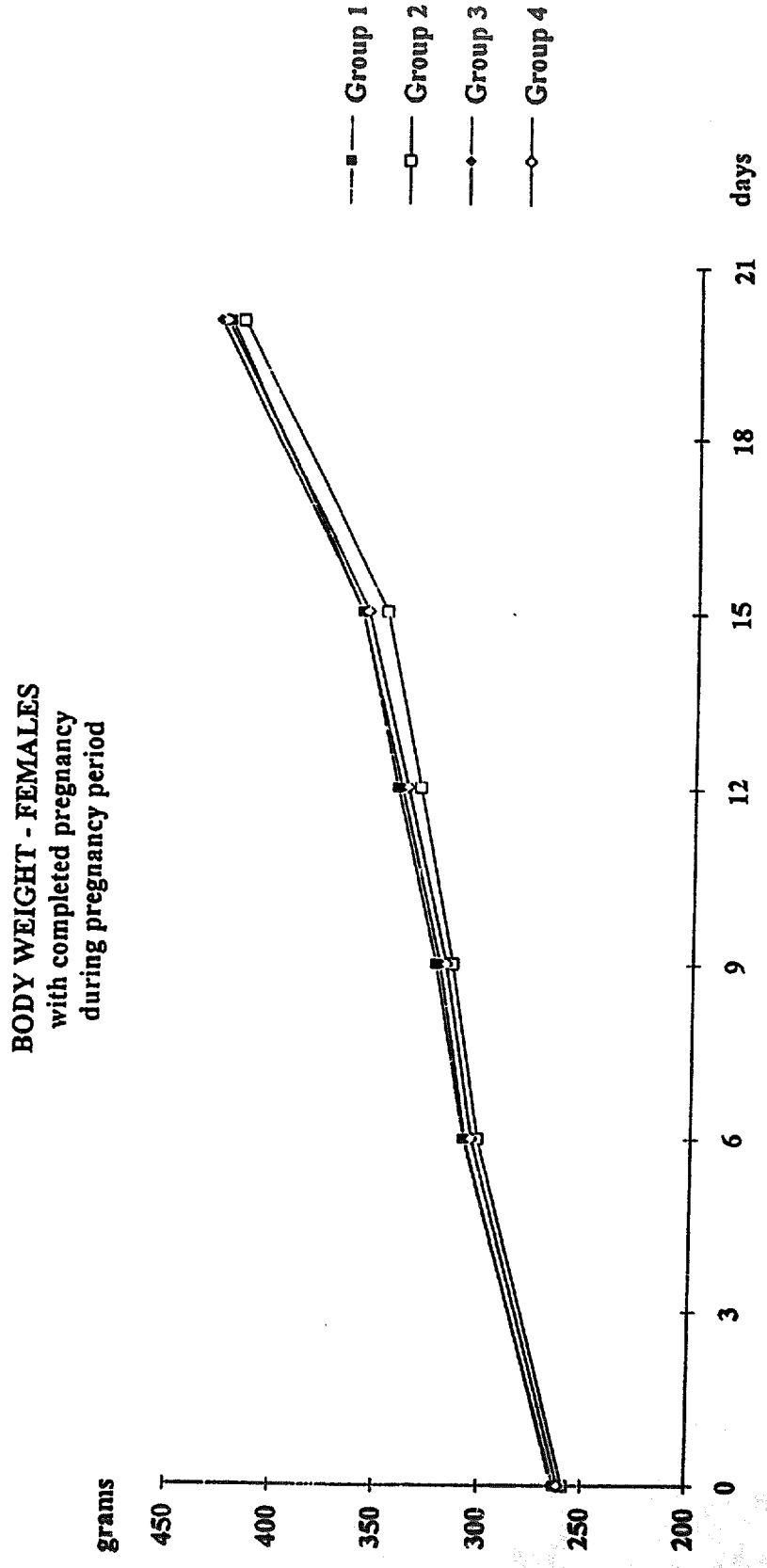
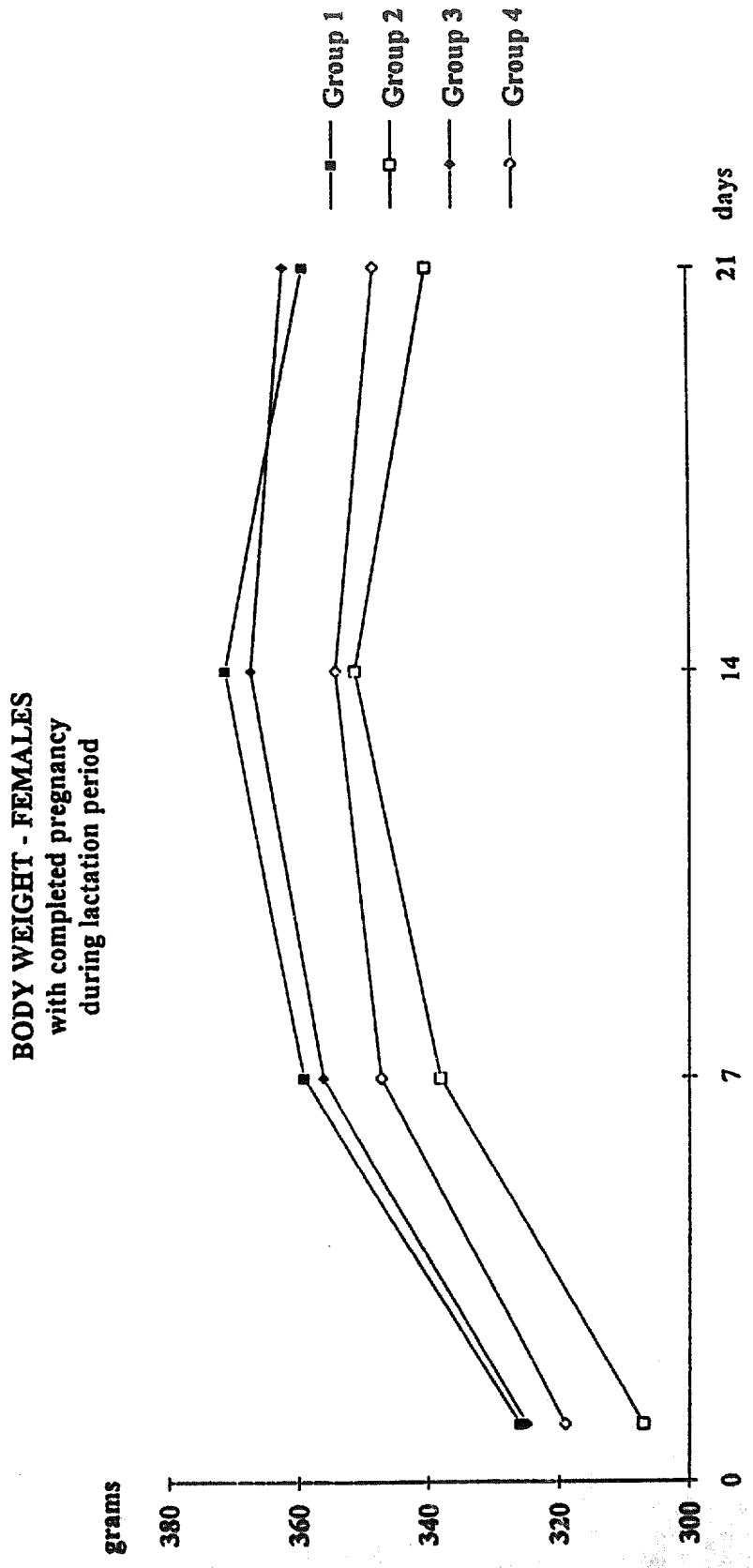

BODY WEIGHT - FEMALES
with completed pregnancy
during pregnancy period
Group 1
Group 2
Group 3
Group 4
grams
450
400
350
300
250
200
0
3
6
9
12
15
18
21
days

Figure 3


BODY WEIGHT - FEMALES
with completed pregnancy
during lactation period
grams
380
360
340
320
300
0
7
14
21
days
Group 1
Group 2
Group 3
Group 4

CLINICAL SIGNS (SUMMARY TABLE)

Table: 1

Sex: male
Period: Week 1 to 14

Conc.: ppm	0		10		30		100	
Observations	No	%	No	%	No	%	No	%
Area of hair loss on head	0	0	0	0	1	10	0	0

BODY WEIGHT
(mean values - g)

Table: 2

Sex: Male

Conc. ppm / Week		0	10	30	100	
-1	M (1)	141	140	140	140	
	SD	4.2	4.2	3.8	4.0	
	n	10	10	10	10	
1	M (1)	206	207	203	200	
	SD	9.5	6.7	8.3	7.3	
	n	10	10	10	10	
2	M (1)	278	276	273	259	*
	SD	16.4	11.8	10.9	14.8	
	n	10	10	10	10	
3	M (1)	343	338	337	315	**
	SD	24.4	17.9	15.7	21.7	
	n	10	10	10	10	
4	M (1)	386	385	384	358	*
	SD	23.7	23.5	14.9	25.0	
	n	10	10	10	10	
5	M (1)	433	427	426	397	*
	SD	32.0	28.5	17.5	32.8	
	n	10	10	10	10	
6	M (1)	464	459	458	427	*
	SD	37.7	29.9	20.0	33.5	
	n	10	10	10	10	
7	M (1)	495	489	490	444	**
	SD	41.7	32.7	22.9	35.8	
	n	10	10	10	10	
8	M (1)	524	517	518	472	**
	SD	44.8	34.9	25.5	36.9	
	n	10	10	10	10	
9	M (1)	548	539	539	495	*
	SD	47.7	38.7	25.8	39.9	
	n	10	10	10	10	

Significance of the difference between treated and control groups
* P<0.05
** P<0.01
(1) : Dunnett test
(2) : Mann-Whitney test
(3) : Dunn test

Sample distribution-relative tests

(B) Bartlett test P<0.01
(F) Fisher test P<0.01
(K) Kolmogorov-Smirnov test P<0.01
(L) Logarithmic transformation
- Statistics excluded group

BODY WEIGHT
(mean values - g)

Table: 2 (continued)

Sex: Male

Conc. ppm / Week		0	10	30	100	
10	M (1)	566	557	555	514	*
	SD	53.4	40.0	24.9	39.8	
	n	10	10	10	10	
11	M (1)	571	556	562	522	*
	SD	50.7	39.1	26.2	40.5	
	n	10	10	10	10	
12	M (1)	577	566	570	529	*
	SD	50.0	45.0	25.1	39.7	
	n	10	10	10	10	
13	M (1)	594	575	583	548	
	SD	56.0	49.7	27.1	39.8	
	n	10	10	10	10	
14	M (1)	587	576	580	538	*
	SD	55.5	49.8	28.0	39.2	
	n	10	10	10	10	

Significance of the difference between treated and control groups
* P<0.05
** P<0.01
(1) : Dunnett test
(2) : Mann-Whitney test
(3) : Dunn test

Sample distribution-relative tests

(B) Bartlett test P<0.01
(F) Fisher test P<0.01
(K) Kolmogorov-Smirnov test P<0.01
(L) Logarithmic transformation
- Statistics excluded group

FOOD CONSUMPTION
(mean values - g/animal/day)

Table: 3

Sex: Male

Conc. ppm / Day		0	10	30	100
1	M (1)	27.4	27.4	26.1	26.8
	SD	2.26	1.46	1.36	1.57
	n	5	5	5	5
4	M (1)	29.5	28.7	27.8	26.9
	SD	2.37	1.54	1.57	3.80
	n	5	5	5	5
8	M (1)	31.7	31.2	30.0	28.5
	SD	3.13	1.60	2.35	2.91
	n	5	5	5	5
11	M (1)	31.3	31.5	30.1	28.4
	SD	3.17	1.08	2.23	1.74
	n	5	5	5	5
15	M (1)	32.0	32.6	30.5	32.3
	SD	3.19	1.12	1.64	4.10
	n	5	5	5	5
18	M (1)	30.1	30.2	29.5	28.6
	SD	1.70	1.50	1.09	2.39
	n	5	5	5	5
22	M (1)	31.5	30.6	30.1	29.8
	SD	3.60	1.84	2.03	2.22
	n	5	5	5	5
25	M (1)	31.4	30.7	30.2	30.6
	SD	2.75	1.82	1.34	3.19
	n	5	5	5	5
29	M (1)	32.5	33.6	32.1	31.5
	SD	3.27	1.82	1.81	1.59
	n	5	5	5	5
32	M (1)	30.7	31.3	30.1	30.8
	SD	2.62	1.77	1.87	2.27
	n	5	5	5	5

Significance of the difference between treated and control groups
* P<0.05
** P<0.01
(1) : Dunnett test
(2) : Mann-Whitney test
(3) : Dunn test

Sample distribution-relative tests

(B) Bartlett test P<0.01
(F) Fisher test P<0.01
(K) Kolmogorov-Smirnov test P<0.01
(L) Logarithmic transformation
- Statistics excluded group

FOOD CONSUMPTION
(mean values - g/animal/day)

Table: 3 (continued)

Sex: Male

Conc. ppm / Day		0	10	30	100
36	M (3)	30.8	32.1	32.3	29.1
	SD	1.01	1.74	1.94	8.23
	n (B)	5	5	5	5
39	M (1)	32.3	32.1	31.9	29.0
	SD	3.98	2.61	2.26	3.74
	n	5	5	5	5
43	M (1)	32.9	33.3	32.4	31.6
	SD	3.07	2.70	2.75	3.19
	n	5	5	5	5
46	M (1)	30.6	31.5	30.1	30.2
	SD	2.34	2.42	2.10	2.59
	n	5	5	5	5
50	M (1)	33.2	33.0	33.1	32.2
	SD	3.93	2.75	2.69	2.22
	n	5	5	5	5
53	M (1)	31.6	32.3	30.3	30.4
	SD	2.53	2.21	1.48	2.35
	n	5	5	5	5
57	M (3)	33.6	34.0	31.9	-
	SD	2.97	4.15	1.33	-
	n	3	5	4	0
58	M	-	-	-	32.3
	SD	-	-	-	2.78
	n	0	0	0	5
60	M (1)	30.1	28.6	28.3	28.5
	SD	2.15	2.77	1.79	1.34
	n	5	5	5	5
71	M (3)	29.6	30.8	29.1	30.3
	SD	2.08	3.60	1.78	4.93
	n	5	4	4	4

Significance of the difference between treated and control groups
* P<0.05
** P<0.01
(1) : Dunnett test
(2) : Mann-Whitney test
(3) : Dunn test

Sample distribution-relative tests

(B) Bartlett test P<0.01
(F) Fisher test P<0.01
(K) Kolmogorov-Smirnov test P<0.01
(L) Logarithmic transformation
- Statistics excluded group

FOOD CONSUMPTION
(mean values - g/animal/day)

Table: 3 (continued)

Sex: Male

Conc. ppm Day		0	10	30	100
74	M (3)	28.5	28.4	28.3	29.3
	SD	1.89	2.99	0.55	6.18
	n	5	4	4	4
78	M (1)	30.8	31.0	29.1	29.2
	SD	2.84	2.57	1.93	4.48
	n	5	5	5	5
81	M (1)	30.2	30.6	29.4	29.3
	SD	3.15	2.32	1.02	1.99
	n	5	5	5	5
85	M (1)	30.5	33.2	30.5	28.5
	SD	4.72	3.19	1.8[illegible]	1.34
	n	5	5	5	5
88	M (1)	23.2	24.0	22.8	21.5
	SD	1.75	2.05	1.60	1.52
	n	5	5	5	5

Significance of the difference between treated and control groups
* P<0.05
** P<0.01
(1) : Dunnett test
(2) : Mann-Whitney test
(3) : Dunn test

Sample distribution-relative tests

(B) Bartlett test P<0.01
(F) Fisher test P<0.01
(K) Kolmogorov-Smirnov test P<0.01
(L) Logarithmic transformation
- Statistics excluded group

Table: 4

ACHIEVED DOSAGES
(mg/kg/day)

Week of treatment	Male		
	Concentration (ppm)		
	10	30	100
1	1.16	3.40	11.7
2	1.02	2.96	9.91
3	0.87	2.50	9.04
4	0.75	2.23	8.00
5	0.73	2.11	7.56
6	0.68	2.03	6.67
7	0.64	1.86	6.75
8	0.62	1.80	6.47
9	0.57	1.65	6.03
10	*	*	*
11	0.53	1.52	5.67
12	0.54	1.52	5.43
13	0.50	1.37	4.60
Mean	0.72	2.08	7.32
SD	0.21	0.62	2.05

*: mating period

Table: 5

EFFICIENCY OF FOOD UTILIZATION

(grams of food/unit of body weight gain)

Week of treatment	Male			
	Concentration (ppm)			
	0	10	30	100
1	2.7	2.8	2.7	3.2
2	3.4	3.5	3.3	3.6
3	5.1	4.7	4.5	5.0
4	4.6	5.1	5.0	5.4
5	7.3	7.1	6.8	7.3
6	7.2	7.5	7.0	12.0
7	7.8	8.1	7.8	7.7
8	9.5	10.4	10.6	9.5
9	12.2	12.2	13.2	11.2
10	*	*	*	*
11	34.5	20.7	25.1	29.8
12	12.7	24.0	15.8	10.8
13	-	-	-	-
week 1-4	3.7	3.9	3.7	4.1
week 5-9	8.5	8.7	8.5	9.1
week 11-13	38.9	31.2	32.9	36.9
week 1-13	6.9	7.2	7.2	7.9

*: mating period
week 1-13= week (1-9,11-13)

-: sign used when the body weight gain during the concerned week is ≤ 1 gram

$$FCR_{x\text{-}y\ (\text{cumulated values})} = \frac{\Sigma_x^y FC}{\Sigma_x^y BWG}$$

x = first week of considered period
y = last week of considered period
FCR = food conversion ratio over the period from week x to week y
FC = mean food consumption (g/animal/week)
BWG = mean body weight gain (g/animal/week)

HAEMATOLOGY
(mean values)

Table: 6

Sex: Male
Time: Week 13

Conc. ppm		0	10	30	100
WBC	M (1)	11.05	9.34	10.63	8.82 *
G/l	SD	1.726	1.485	2.954	1.680
	n	10	10	9	10
RBC	M (1)	8.92	8.81	8.85	8.74
T/l	SD	0.186	0.313	0.243	0.300
	n	10	10	9	10
HB	M (1)	15.6	15.5	15.7	15.5
g/dl	SD	0.70	0.55	0.47	0.56
	n	10	10	9	10
PCV	M (1)	0.46	0.46	0.46	0.45
l/l	SD	0.020	0.018	0.016	0.022
	n	10	10	9	10
MCV	M (1)	51.7	52.0	51.6	51.8
fl	SD	2.03	1.80	1.75	1.15
	n	10	10	9	10
MCH	M (1)	17.5	17.7	17.7	17.7
pg	SD	0.62	0.71	0.49	0.44
	n	10	10	9	10
MCHC	M (1)	33.8	33.9	34.4	34.2
g/dl	SD	0.53	0.46	0.39	0.54
	n	10	10	9	10
PLAT	M (1)	1119	1046	1076	1051
G/l	SD	97.9	162.9	109.3	144.6
	n	10	10	9	10

Significance of the difference between treated and control groups
* P<0.05
** P<0.01
(1) : Dunnett test
(2) : Mann-Whitney test
(3) : Dunn test

Sample distribution-relative tests

(B) Bartlett test P<0.01
(F) Fisher test P<0.01
(K) Kolmogorov-Smirnov test P<0.01
(L) Logarithmic transformation
- Statistics excluded group

HAEMATOLOGY
(mean values)

Table: 6 (continued)

Sex: Male
Time: Week 13

Conc. ppm		0	10	30	100
N	M (1)	13.6	16.0	18.1	17.1
%	SD	4.14	5.10	5.35	3.91
	n	10	10	9	10
E	M (1)	1.6	1.7	1.9	1.8
%	SD	0.73	0.35	0.71	0.47
	n	10	10	9	10
B	M (1)	0.2	0.2	0.2	0.2
%	SD	0.05	0.06	0.09	0.06
	n	10	10	9	10
L	M (1)	81.7	79.4	76.7	78.0
%	SD	4.50	5.15	5.20	3.59
	n	10	10	9	10
M	M (1)	2.9	2.7	3.0	2.9
%	SD	0.34	0.41	0.69	0.76
	n	10	10	9	10
N	M (1)	1.51	1.49	1.93	1.54
G/l	SD	0.557	0.557	0.776	0.569
	n	10	10	9	10
E	M (1)	0.18	0.16	0.20	0.16
G/l	SD	0.071	0.031	0.073	0.060
	n	10	10	9	10
B	M (1)	0.02	0.02	0.02	0.02
G/l	SD	0.007	0.007	0.014	0.007
	n	10	10	9	10
L	M (1)	9.03	7.42	8.16	6.85 *
G/l	SD	1.542	1.282	2.410	1.154
	n	10	10	9	10
M	M (1)	0.32	0.26	0.31	0.26
G/l	SD	0.069	0.062	0.097	0.081
	n	10	10	9	10

Significance of the difference between treated and control groups
* P<0.05
** P<0.01
(1) : Dunnett test
(2) : Mann-Whitney test
(3) : Dunn test

Sample distribution-relative tests

(B) Bartlett test P<0.01
(F) Fisher test P<0.01
(K) Kolmogorov-Smirnov test P<0.01
(L) Logarithmic transformation
\- Statistics excluded group

HAEMATOLOGY
(mean values)

Table: 6 (continued)

Sex: Male
Time: Week 13

Conc. ppm		0	10	30	100
PT	M (1)	19.7	18.8	17.5	20.6
s	SD	2.60	1.57	1.70	2.32
	n	10	10	10	10
APTT	M (1)	29.0	26.1	22.9 *	27.8
s	SD	5.74	3.55	5.94	6.55
	n	10	10	10	10
FIB	M (1)	3.67	3.41	3.60	3.65
g/l	SD	0.395	0.625	0.384	0.331
	n	10	10	10	10

Significance of the difference between treated and control groups
* P<0.05
** P<0.01
(1) : Dunnett test
(2) : Mann-Whitney test
(3) : Dunn test

Sample distribution-relative tests

(B) Bartlett test P<0.01
(F) Fisher test P<0.01
(K) Kolmogorov-Smirnov test P<0.01
(L) Logarithmic transformation
- Statistics excluded group

BLOOD BIOCHEMISTRY
(mean values)

Table: 7

Sex: Male
Time: Week 13

Conc. ppm		0	10	30	100
Na+ mmol/l	M (1)	143.0	143.2	142.7	144.1
	SD	1.16	0.97	1.82	1.29
	n	10	10	10	10
K+ mmol/l	M (1)	3.64	3.60	4.01	3.96
	SD	0.228	0.312	0.587	0.373
	n	10	10	10	10
Cl- mmol/l	M (1)	102.4	102.3	102.3	104.1 *
	SD	1.53	1.48	1.76	1.41
	n	10	10	10	10
Ca++ mmol/l	M (1)	2.62	2.59	2.65	2.56
	SD	0.100	0.051	0.081	0.068
	n	10	10	10	10
I.PHOS mmol/l	M (1)	2.04	1.98	2.01	2.00
	SD	0.095	0.167	0.192	0.081
	n	10	10	10	10
GLUC mmol/l	M (1)	7.88	8.09	8.11	8.25
	SD	0.497	0.624	0.889	0.961
	n	10	10	10	10
UREA mmol/l	M (1)	4.3	4.7	4.6	4.6
	SD	0.59	0.46	0.53	0.64
	n	10	10	10	10
CREAT µmol/l	M (1)	49	53	51	51
	SD	2.8	4.1	5.0	3.3
	n	10	10	10	10

Significance of the difference between treated and control groups
* P<0.05
** P<0.01
(1) : Dunnett test
(2) : Mann-Whitney test
(3) : Dunn test

Sample distribution-relative tests

(B) Bartlett test P<0.01
(F) Fisher test P<0.01
(K) Kolmogorov-Smirnov test P<0.01
(L) Logarithmic transformation
- Statistics excluded group

BLOOD BIOCHEMISTRY
(mean values)

Table: 7 (continued)

Sex: Male
Time: Week 13

Conc. ppm		0	10	30	100
PROT	M (1)	70	72	74 *	71
g/l	SD	2.4	3.4	4.1	2.6
	n	10	10	10	10
ALB	M (1)	32	33	33	33
g/l	SD	1.0	0.8	1.4	1.3
	n	10	10	10	10
A/G	M (1)	0.86	0.87	0.79 *	0.88
1	SD	0.044	0.058	0.061	0.065
	n	10	10	10	10
TOT.BIL.	M (1)	2	2	1	1 *
µmol/l	SD	0.6	0.5	0.5	0.4
	n	10	10	10	10
CHOL	M (1)	1.5	1.7	1.9	1.5
mmol/l	SD	0.31	0.48	0.62	0.22
	n	10	10	10	10
TRIG	M (1)	0.60	0.86	0.88	0.71
mmol/l	SD	0.187	0.365	0.337	0.400
	n	10	10	10	10
ALP	M (1)	124	137	136	134
IU/l	SD	20.7	14.7	30.8	40.8
	n	10	10	10	10
ASAT	M (1)	68	53 *	56	58
IU/l	SD	17.3	7.5	15.3	7.3
	n	10	10	10	10
ALAT	M (1)	15	16	14	14
IU/l	SD	3.6	4.9	4.0	4.1
	n	10	10	10	10

Significance of the difference between treated and control groups
* P<0.05
** P<0.01
(1) : Dunnett test
(2) : Mann-Whitney test
(3) : Dunn test

Sample distribution-relative tests

(B) Bartlett test P<0.01
(F) Fisher test P<0.01
(K) Kolmogorov-Smirnov test P<0.01
(L) Logarithmic transformation
- Statistics excluded group

URINALYSIS
(mean values)

Table: 8

Sex: Male
Time: Week 13

Conc. ppm		0	10	30	100
VOLUME	M (1)	13	13	12	14
ml	SD	4.2	5.2	4.1	1.6
	n	10	10	10	10
SP.GRAV	M (1)	1017	1019	1018	1018
-	SD	3.5	3.4	4.8	4.3
	n	10	10	10	10
pH	M (1)	7.1	7.1	6.9	7.1
-	SD	0.52	0.60	0.62	0.70
	n	10	10	10	10

Significance of the difference between treated and control groups
* P<0.05
** P<0.01
(1) : Dunnett test
(2) : Mann-Whitney test
(3) : Dunn test

Sample distribution-relative tests

(B) Bartlett test P<0.01
(F) Fisher test P<0.01
(K) Kolmogorov-Smirnov test P<0.01
(L) Logarithmic transformation
- Statistics excluded group

Table: 9

SUMMARY TABLE OF BODY/ORGAN WEIGHTS AND STATISTICS
STATUS AT NECROPSY: KO
SEX: MALE

ORGAN	DOSE GROUP: / NO.ANIMALS:	1 10	2 10	3 10	4 10
FINAL BODY WEIGHT	n:	10	10	10	10
MEAN WEIGHT (G)	:	563.9	549.3	557.4	512.4*
STD. DEVIATION	:	54.07	47.93	28.19	34.28
HEART	n:	10	10	10	10
MEAN WEIGHT (G)	:	1.71	1.71	1.69	1.59
STD. DEVIATION	:	0.101	0.190	0.138	0.229
MEAN % BODY	:	0.305	0.313	0.303	0.310
STD. DEVIATION	:	0.023	0.041	0.029	0.036
BRAIN	n:	10	10	10	10
MEAN WEIGHT (G)	:	2.27	2.24	2.19	2.17*
STD. DEVIATION	:	0.052	0.092	0.094	0.076
MEAN % BODY	:	0.406	0.411	0.393	0.424
STD. DEVIATION	:	0.033	0.038	0.025	0.024
LIVER	n:	10	10	10	10
MEAN WEIGHT (G)	:	14.41	13.97	14.78	13.93
STD. DEVIATION	:	1.62	1.31	1.83	1.28
MEAN % BODY	:	2.55	2.55	2.65	2.72
STD. DEVIATION	:	0.121	0.175	0.236	0.231
MESENT. LYMPH NODE	n:	10	10	10	10
MEAN WEIGHT (G)	:	0.307	0.267	0.266	0.260
STD. DEVIATION	:	0.083	0.089	0.078	0.064
MEAN % BODY	:	0.055	0.049	0.048	0.051
STD. DEVIATION	:	0.018	0.017	0.015	0.014
ADRENAL GLANDS	n:	10	10	10	10
MEAN WEIGHT (G)	:	0.063	0.058	0.057	0.055
STD. DEVIATION	:	0.011	0.008	0.008	0.008
MEAN % BODY	:	0.011	0.011	0.010	0.011
STD. DEVIATION	:	0.002	0.001	0.001	0.002

Table: 9 (continued)

SUMMARY TABLE OF BODY/ORGAN WEIGHTS AND STATISTICS
STATUS AT NECROPSY: K0
SEX: MALE

ORGAN	DOSE GROUP: / NO.ANIMALS:	1 10	2 10	3 10	4 10
SPLEEN	n:	10	10	10	10
MEAN WEIGHT (G)	:	0.894	0.850	0.927	0.889
STD. DEVIATION	:	0.113	0.071	0.160	0.136
MEAN % BODY	:	0.159	0.156	0.167	0.174
STD. DEVIATION	:	0.014	0.016	0.029	0.028
THYMUS	n:	10	10	10	10
MEAN WEIGHT (G)	:	0.373	0.372	0.320	0.319
STD. DEVIATION	:	0.074	0.082	0.072	0.093
MEAN % BODY	:	0.066	0.067	0.058	0.062
STD. DEVIATION	:	0.012	0.011	0.014	0.014
PROSTATE	n:	10	10	10	10
MEAN WEIGHT (G)	:	1.45	1.45	1.56	1.44
STD. DEVIATION	:	0.216	0.297	0.222	0.225
MEAN % BODY	:	0.258	0.266	0.280	0.280
STD. DEVIATION	:	0.042	0.063	0.037	0.035
KIDNEYS	n:	10	10	10	10
MEAN WEIGHT (G)	:	3.56	3.34	3.44	3.36
STD. DEVIATION	:	0.251	0.154	0.276	0.346
MEAN % BODY	:	0.634	0.613	0.618	0.657
STD. DEVIATION	:	0.051	0.057	0.050	0.062
TESTES	n:	10	10	10	10
MEAN WEIGHT (G)	:	3.84	3.66	3.72	3.66
STD. DEVIATION	:	0.324	0.277	0.264	0.303
MEAN % BODY	:	0.687	0.671	0.668	0.718
STD. DEVIATION	:	0.093	0.081	0.048	0.083
MANDIBULAR L.N R & L	n:	10	10	10	10
MEAN WEIGHT (G)	:	0.195	0.193	0.228	0.206
STD. DEVIATION	:	0.047	0.054	0.052	0.054
MEAN % BODY	:	0.035	0.036	0.041	0.041
STD. DEVIATION	:	0.009	0.012	0.010	0.011

*/**):DUNNETT'S TEST BASED ON POOLED VARIANCES AT 5% (*) OR 1% (**) LEVEL
Assigned control group(s) : 1,

Table: 9 (continued)

SUMMARY TABLE OF BODY/ORGAN WEIGHTS AND STATISTICS
STATUS AT NECROPSY: K0
SEX: MALE

ORGAN	DOSE GROUP: / NO.ANIMALS:	1 10	2 10	3 10	4 10
R & L SEM.VESICLES	n:	10	10	10	10
MEAN WEIGHT (G)	:	1.88	1.83	1.92	1.83
STD. DEVIATION	:	0.264	0.254	0.230	0.304
MEAN % BODY	:	0.338	0.337	0.346	0.359
STD. DEVIATION	:	0.063	0.070	0.045	0.070

No statistically significant weight differences noted between treated groups and controls

END OF REPORT SECTION

11099 / PDS PATHDATA SYSTEM TM

Table: 10

NUMBER OF ANIMALS WITH NECROPSY FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0 MALE

ORGAN/FINDING	DOSE GROUP:	1	2	3	4
	NO.ANIMALS:	10	10	10	10
LIVER					
- FOCI GREYISH/WHITISH		1		1	
MESENT. LYMPH NODE					
- REDDISH COLOR					2
MANDIBUL. LYMPH NODE					
- FOCI REDDISH/PURPLISH		1		1	
- REDDISH COLOR			2		1
ADRENAL GLANDS					
- FOCI REDDISH/PURPLISH		1			
SPLEEN					
- FOCI GREYISH/WHITISH				1	
KIDNEYS					
- DILATED PELVIS		4			2
- IRREGULAR SURFACE		1			
TESTES					
- HAEMATOMA			1		
ADIPOSE TISSUE					
- NODULES YELLOWISH		1			
OTHER GEN. COMMENTS					
- CLINICAL OBSERVATIONS NOT SEEN AT NECROPSY				1	

11099 / PDS PATHDATA SYSTEM TM

Table: 11

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO
THE DOSE LEVEL IN PPM: 1=0; 2=10; 3=30; 4=100

	SEX :					MALE
	DOSE GROUP:	1	2	3	4	
ORGAN/FINDING	NO.ANIMALS:	10	10	10	10	
HEART	NO. EXAM.:	10			10	
- MYOCARDIOPATHY.		2			2	
LIVER	NO. EXAM.:	10	10	10	10	
- MONO.CELL AGGREGAT.		7	7	5	7	
- MICROGRANULOMA(S)			1	2		
- FOC.COAG.H.CEL.NECR.				1		
- TENSION LIPIDOSIS.		1	2	2		
- ARTERITIS.				1		
- STEATOSIS.		1	1	1		
MESENT. LYMPH NODE	NO. EXAM.:	10	10	10	10	
- HISTIOCYTOSIS.		7	6	6	8	
- CEROID LADEN MACROPH		6	6	9	7	
- HAEMOSID.LADEN MACRO					4	
- CONGESTION.					3	
MANDIBUL. LYMPH NODE	NO. EXAM.:	10	10	10	10	
- HAEMORRHAGE.		4	3	3	5	
- REACTIVE LYMPH NODE		6	8	8	8	
- PLASMOCYTOSIS		7	8	8	6	
- HISTIOCYTOSIS.			1	1	1	
- POSTREACT.ACID.INCL.		2				
THYROID GLANDS	NO. EXAM.:	10			10	
- ECTOPIC THYMUS		1				
- DEVELOPMENTAL CYST		3			6	
PANCREAS	NO. EXAM.:	10			10	
- INTERS.MON.CELL AGG.		1			1	
- LIPOMATOSIS.		2			2	
SPLEEN	NO. EXAM.:	10		1	10	
- CAPSULAR THICKENING.				1		
URINARY BLADDER	NO. EXAM.:	10			10	
- SUBACUTE CYSTITIS.					1	

11099 / PDS PATHDATA SYSTEM TM

Table: 11 (continued)

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0
THE DOSE LEVEL IN PPM: 1=0; 2=10; 3=30; 4=100

	SEX :					MALE
	DOSE GROUP:	1	2	3	4	
ORGAN/FINDING	NO.ANIMALS:	10	10	10	10	
PROSTATE	NO. EXAM.:	10	10	10	10	
- INTERS.INFL.CEL.AGGR		1				
- INTERS.MONO.CEL.AGGR			3	1		
- SUBACUTE PROSTATITIS			2	2	1	
KIDNEYS	NO. EXAM.:	10	10	10	10	
- DILATED PELVIS		4			3	
- TUBULAR BASOPHILIA		1	3	2		
- CHR.TUB.INTERS.NEPH.		1		1	1	
- LEUCOHISTIOCYT.PYEL.				1		
- SUBACUTE PYELITIS.					1	
- HYPERPL.PELV.EPITHE.		1			1	
LUNGS	NO. EXAM.:	10	10	10	10	
- CHR.INTERS.PNEUMON.				1		
- ALVEOLAR HAEMORRHAGE		1	1			
TESTES	NO. EXAM.:	10	10	10	10	
- HAEMORRHAGE.			1			
ADIPOSE TISSUE	NO. EXAM.:	1				
- FAT NECROSIS.		1				

Table: 12

SUMMARY OF ANALYSIS OF SPERM

Concentration (ppm)	0	10	30	100
Number of spermatozoa/mm³ of sperm				
Mean	362000	343600	313200	278400
Standard-deviation	54724	78778	89682	73520
N	10	10	10	10
Viability (%)				
Mean	83	75	95	79
Standard-deviation	8	12	5	9
N	9	9	10	8

Table: 13

SUMMARY INCIDENCE OF CLINICAL OBSERVATIONS DURING GESTATION

Groups	1	2	3	4
+FINAL SACRIFICE	10	10	10	10
+SP DECISION OF SACRIFICE (no delivery)	1	0	0	1
+NO REMARKABLE OBSERVATIONS	20	19	19	19

Table: 14

SUMMARY INCIDENCE OF CLINICAL OBSERVATIONS DURING LACTATION

Groups	1	2	3	4
	9	10	10	8
+FINAL SACRIFICE				
	0	1	0	0
+AREA OF HAIR LOSS ON FORELIMB				
	9	9	10	8
+NO REMARKABLE OBSERVATIONS				

Table: 15

MEAN BODY WEIGHT OF FEMALES WITH COMPLETED PREGNANCY (grams)

	Groups	1	2	3	4
DAY 0	MEAN	263 d	259	264	261
	S.D.	12	10	10	9
	N	18	18	18	16
DAY 6	MEAN	309 d	302	308	305
	S.D.	16	14	14	13
	N	18	18	18	16
DAY 9	MEAN	323 d	315	321	318
	S.D.	18	15	15	15
	N	18	18	18	16
DAY 12	MEAN	342 d	331	340	337
	S.D.	21	14	18	16
	N	18	18	18	16
DAY 15	MEAN	360 d	348	360	357
	S.D.	23	17	18	16
	N	18	18	18	16
DAY 20	MEAN	425 d	419	430	427
	S.D.	29	21	21	20
	N	18	18	18	16

Statistical key: d=ANOVA + Dunnett-test

Table: 16

MEAN BODY WEIGHT CHANGES OF FEMALES WITH COMPLETED PREGNANCY (grams)

	Groups	1	2	3	4
DAYS 0 TO 6	MEAN	46 d	43	44	44
	S.D.	8	5	7	7
	N	18	18	18	16
mean percent change	MEAN%	17.4	16.5	16.6	17.0
DAYS 6 TO 9	MEAN	15 d	13	13	14
	S.D.	6	4	7	3
	N	18	18	18	16
mean percent change	MEAN%	4.7	4.3	4.2	4.5
DAYS 9 TO 12	MEAN	18 d	16	20	19
	S.D.	6	5	6	5
	N	18	18	18	16
mean percent change	MEAN%	5.6	5.0	6.2	6.0
DAYS 12 TO 15	MEAN	18 d	18	19	19
	S.D.	6	5	4	4
	N	18	18	18	16
mean percent change	MEAN%	5.4	5.3	5.7	5.8
DAYS 15 TO 20	MEAN	65 d	70	70	70
	S.D.	15	9	7	7
	N	18	18	18	16
mean percent change	MEAN%	18.1	20.2	19.6	19.6
DAYS 0 TO 20	MEAN	162 d	159	166	166
	S.D.	23	14	17	14
	N	18	18	18	16
mean percent change	MEAN%	61.7	61.4	63.0	63.8

Statistical key: d=ANOVA + Dunnett-test

Table: 17

MEAN BODY WEIGHTS OF FEMALES DURING LACTATION (grams)

	Groups	1	2	3	4
DAY 1	MEAN	326 d	307	325	319
	S.D.	23	18	24	10
	N	9	10	10	8
DAY 7	MEAN	359 d	338	356	347
	S.D.	24	20	16	11
	N	9	10	10	8
DAY 14	MEAN	371 d	351	367	354
	S.D.	29	23	17	19
	N	9	10	10	8
DAY 21	MEAN	359 d	340	362	348
	S.D.	24	17	15	15
	N	9	10	10	8

Statistical Key: d=ANOVA + Dunnett-test

Table: 18

MEAN BODY WEIGHT CHANGES OF FEMALES DURING LACTATION (grams)

	Groups	1	2	3	4
DAYS 1 TO 7	MEAN	33 d	31	32	29
	S.D.	7	24	21	9
	N	9	10	10	8
mean percent change	MEAN%	10.0	10.4	10.1	9.0
DAYS 7 TO 14	MEAN	12 d	13	10	7
	S.D.	14	33	23	28
	N	9	10	10	8
mean percent change	MEAN%	6.7	7.4	6.6	5.6
DAYS 14 TO 21	MEAN	-12 d	-11	-5	-7
	S.D.	12	14	9	27
	N	9	10	10	8
mean percent change	MEAN%	3.4	3.9	4.0	3.2
DAYS 1 TO 21	MEAN	33 d	33	37	29
	S.D.	19	20	28	17
	N	9	10	10	8
mean percent change	MEAN%	5.1	5.7	5.9	4.7

Table: 19

MEAN FOOD CONSUMPTION OF FEMALES WITH COMPLETED PREGNANCY (grams/animal/day)

	Groups	1	2	3	4
DAYS 0 TO 6	MEAN	27 d	26	26	27
	S.D.	3	3	3	2
	N	18	18	18	16
DAYS 6 TO 9	MEAN	30 d	29	29	30
	S.D.	3	4	3	3
	N	18	18	18	16
DAYS 9 TO 12	MEAN	29 d	27	28	28
	S.D.	4	3	3	4
	N	18	18	18	16
DAYS 12 TO 15	MEAN	30 d	29	29	29
	S.D.	6	4	4	5
	N	18	17	18	16
DAYS 15 TO 20	MEAN	30 d	28	30	30
	S.D.	6	4	3	2
	N	18	18	18	16

Statistical key: d-ANOVA + Dunnett-test

Table: 20

MEAN FOOD CONSUMPTION OF FEMALES DURING LACTATION (grams/animal/day)

	Groups	1	2	3	4
DAYS 1 TO 7	MEAN	46 d	46	46	52
	S.D.	4	12	6	5
	N	9	10	9	8
DAYS 7 TO 14	MEAN	72 d	71	70	73
	S.D.	6	6	4	6
	N	9	10	10	8
DAYS 14 TO 21	MEAN	91 d	96	87	91
	S.D.	6	13	4	3
	N	9	10	10	8

Statistical key: d=ANOVA + Dunnett-test

Table: 21

SUMMARY OF NECROPSY OBSERVATIONS

Groups		1	2	3	4
DAMS	N	20	20	20	20
SKIN	N	0	1	0	0
ALOPECIA OF FORELIMBS	N	0 f	1	0	0
	%	0.0	5.0	0.0	0.0
KIDNEY	N	0	0	0	1
DILATED RENAL PELVIS	N	0 f	0	0	1
	%	0.0	0.0	0.0	5.0
UTERUS	N	0	0	0	2
SEROUS LIQUID IN UTERINE HORN	N	0 f	0	0	2
	%	0.0	0.0	0.0	10.0
NO REMARKABLE OBSERVATIONS		20	19	20	17

Statistical key: f=Fishers exact test

Table: 22

SUMMARY OF REPRODUCTIVE DATA IN MALES

Concentration (ppm)			0	10	30	100
Males on study			10	10	10	10
Paired males			10	10	10	10
Males able to mate	N	f	10	10	10	10
Mating index	%		100.0	100.0	100.0	100.0
at least one pregnant female partner	N	f	9	10	10	9
Male fertility index	%		90.0	100.0	100.0	90.0

f=Fishers exact test

Table: 23

SUMMARY OF MATERNAL AND FETAL DATA

Groups		1	2	3	4
Pregnant Females Alive at Term	N	9	9	9	8
with Total Resorptions	N	0	0	0	0
with all Dead Fetuses	N	0	0	0	0
with Live Fetuses	N	9	9	9	8
Corpora Lutea	TOTAL	165	162	162	142
No. per animal	MEAN	18.3 d	18.0	18.0	17.8
	S.D.	1.8	1.5	2.6	1.9
Implantation Sites	TOTAL	136	144	145	125
No. per animal	MEAN	15.1 d	16.0	16.1	15.6
	S.D.	3.6	1.5	1.5	1.2
Preimplantation Loss	TOTAL	29 f	18	17	17
	%	17.6	11.1	10.5	12.0
Fetuses	N	131	144	139	117
No. per animal	MEAN	14.6 d	16.0	15.4	14.6
	S.D.	3.4	1.5	2.1	1.1
Alive	%	100.0	100.0	100.0	100.0
Dead	%	0.0	0.0	0.0	0.0
Live Fetuses	N	131 f	144*	139	117
% of implantation sites		96.3	100.0	95.9	93.6
No. per animal	MEAN	14.6 d	16.0	15.4	14.6
	S.D.	3.4	1.5	2.1	1.1
Dead Fetuses	N	0 f	0	0	0
% of implantation sites		0.0	0.0	0.0	0.0
No. per animal	MEAN	0.0	0.0	0.0	0.0
	S.D.	0.0	0.0	0.0	0.0

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test * = p<0.05

Table: 23 (continued)

SUMMARY OF MATERNAL AND FETAL DATA

Groups		1	2	3	4
Resorptions: early+late	N	5 f	0*	6	8
% of implantation sites		3.7	0.0	4.1	6.4
No. per animal	MEAN	0.6 d	0.0	0.7	1.0
	S.D.	0.7	0.0	0.9	0.8
Resorptions: early	N	5 f	0*	4	8
% of implantation sites		3.7	0.0	2.8	6.4
No. per animal	MEAN	0.6 d	0.0	0.4	1.0
	S.D.	0.7	0.0	0.7	0.8
Resorptions: late	N	0 f	0	2	0
% of implantation sites		0.0	0.0	1.4	0.0
No. per animal	MEAN	0.0 d	0.0	0.2	0.0
	S.D.	0.0	0.0	0.4	0.0
Postimplantation Loss	TOTAL	5 f	0*	6	8
% of implantation sites		3.7	0.0	4.1	6.4
No. per animal	MEAN	0.6 d	0.0	0.7	1.0
	S.D.	0.7	0.0	0.9	0.8
Male Fetuses	N	69 f	70	75	58
	%	52.7	48.6	54.0	49.6
Female Fetuses	N	62 f	74	64	59
	%	47.3	51.4	46.0	50.4
Fetal Body Weight (g)	MEAN	3.48 d	3.35	3.61	3.68
	S.D.	0.23	0.20	0.14	0.21
Male Fetuses	MEAN	3.60 d	3.64	3.65	3.79
	S.D.	0.26	0.18	0.16	0.24
Female Fetuses	MEAN	3.35 d	3.48	3.55	3.60
	S.D.	0.25	0.20	0.12	0.22

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test * = $p<0.05$

Table: 24

SUMMARY OF FETAL EXTERNAL ANOMALIES

Groups		1	2	3	4
Litters Evaluated	N	9	9	9	8
Fetuses Evaluated	N	131	144	139	117
Live	N	131	144	139	117
Dead	N	0	0	0	0
TOTAL FETAL EXTERNAL ANOMALIES					
Fetal Incidence	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
Litter Incidence	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0

Statistical key: f-Fishers exact test

Table: 24 (continued)

SUMMARY OF FETAL EXTERNAL MALFORMATIONS

	Groups	1	2	3	4
Litters Evaluated	N	9	9	9	8
Fetuses Evaluated	N	131	144	139	117
Live	N	131	144	139	117
Dead	N	0	0	0	0
TOTAL FETAL EXTERNAL MALFORMATIONS					
Fetal Incidence	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
Litter Incidence	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0

Statistical key: f=Fishers exact test

Table: 25

SUMMARY OF REPRODUCTIVE AND LITTER DATA

Groups		1	2	3	4
Females on Study	N	10	10	10	10
Females Mated	N	10 f	10	10	9
Mating Index	%	100.0	100.0	100.0	90.0
Females Pregnant	N	9 f	10	10	8
Female Fertility Index	%	90.0	100.0	100.0	88.9
Females with Liveborn	N	9 f	10	10	8
Gestation Index	%	100.0	100.0	100.0	100.0
Females Surviving Delivery	N	9 f	10	10	8
Duration of Gestation	MEAN	21.4 d	21.7	21.4	21.4
	S.D.	0.5	0.5	0.5	0.5
with Stillborn Pups	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
with all Stillborn	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
with Entire Liveborn Litter Dying and/or Missing, Cannibalized, Culled					
days 0-4	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
days 0-21	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test

Table: 25 (continued)

SUMMARY OF REPRODUCTIVE AND LITTER DATA

Groups		1	2	3	4
Litters with Liveborn Pups	N	9	10	10	8
Pups Delivered (total)	N	126	148	136	122
	MEAN	14.0 d	14.8	13.6	15.3
	S.D.	2.4	2.8	2.8	2.8
Liveborn	N	126 f	148	136	122
Live Birth Index	%	100.0	100.0	100.0	100.0
Stillborn	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
Uncertain	N	0	0	0	0
Culled (total)	N	0	0	0	0
Cannibalized	N	0	0	0	2
Missing	N	0	0	0	0
Died	N	2	2	3	3
Liveborn, not culled prior to day 21	N	126	148	136	122
Pups Dying, Missing, and/or Cannibalized					
day 0	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
days 1-4	N	0 f	2	2	4
	%	0.0	1.4	1.5	3.3
days 5-7	N	2 f	0	0	1
	%	1.6	0.0	0.0	0.8
days 8-14	N	0 f	0	1	0
	%	0.0	0.0	0.7	0.0
days 15-21	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
Pups Surviving 4 days	N	126 f	146	134	118
Viability Index	%	100.0	98.6	98.5	96.7
Pups Surviving 21 days	N	124 f	146	133	117
Lactation Index	%	98.4	100.0	99.3	99.2
Implantation Sites	N	135	166	155	135
per Litter	MEAN	15.0 d	16.6	15.5	16.9
	S.D.	2.4	2.6	2.5	2.9

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test

Table: 25 (continued)

SUMMARY OF REPRODUCTIVE AND LITTER DATA

Groups		1	2	3	4
Live Pups/Litter					
day 1	MEAN	14.0 d	14.6	13.5	15.0
	S.D.	2.4	2.5	2.7	2.8
day 4	MEAN	14.0 d	14.6	13.4	14.8
	S.D.	2.4	2.5	2.8	2.9
day 7	MEAN	13.8 d	14.6	13.4	14.6
	S.D.	2.4	2.5	2.8	2.6
day 14	MEAN	13.8 d	14.6	13.3	14.6
	S.D.	2.4	2.5	2.8	2.6
day 21	MEAN	13.8 d	14.6	13.3	14.6
	S.D.	2.4	2.5	2.8	2.6
Pup Weight/Litter (grams)					
day 1	MEAN	6.6 d	6.5	6.7	6.7
	S.D.	0.5	0.7	0.6	0.8
day 4	MEAN	9.5 d	9.0	9.5	9.0
	S.D.	1.0	1.0	1.2	1.1
day 7	MEAN	13.8 d	12.9	13.8	13.0
	S.D.	1.6	1.0	2.1	1.9
day 14	MEAN	26.5 d	24.9	26.8	25.8
	S.D.	3.2	2.5	3.7	3.8
day 21	MEAN	42.0 d	39.6	41.8	40.7
	S.D.	5.6	4.5	6.2	7.1
Sex Ratio - Male Pups:Total Pups					
day 0	N	58 f	75	60	67
	%	46.0	50.7	44.1	54.9
day 21	N	58 f	74	59	66
	%	46.8	50.7	44.4	56.4

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test

Table: 26

SUMMARY OF PUP DEVELOPMENT (% of pups reaching criteria on a definite day)

Groups		1	2	3	4
PINNA UNFOLDING day 5					
Number of pups tested	N	125	/146	134	120
Number of pups exhibiting positive response	N	125 f	146	134	120
	%	100.0	100.0	100.0	100.0
HAIR GROWTH day 5					
Number of pups tested	N	125	146	134	120
Number of pups exhibiting positive response	N	125 f	146	134	120
	%	100.0	100.0	100.0	100.0
TOOTH ERUPTION day 13					
Number of pups tested	N	124	146	133	117
Number of pups exhibiting positive response	N	124 f	146	133	116
	%	100.0	100.0	100.0	99.1
EYE OPENING day 17					
Number of pups tested	N	124	146	133	117
Number of pups exhibiting positive response	N	124 f	146	133	117
	%	100.0	100.0	100.0	100.0
MIDITORY CANAL OPENING day 17					
Number of pups tested	N	124	146	133	117
Number of pups exhibiting positive response	N	124 f	146	133	117
	%	100.0	100.0	100.0	100.0
SURFACE RIGHTING day 5					
Number of pups tested	N	125	146	134	120
Number of pups exhibiting positive response	N	123 f	146	134	116
	%	98.4	100.0	100.0	96.7
CLIFF AVOIDANCE day 11					
Number of pups tested	N	124	146	133	117
Number of pups exhibiting positive response	N	124 f	143	133	116
	%	100.0	97.9	100.0	99.1
AIR RIGHTING day 17					
Number of pups tested	N	124	146	133	117
Number of pups exhibiting positive response	N	124 f	146	133	117
	%	100.0	100.0	100.0	100.0

Statistical key: f-Fishers exact test

Table: 27

SUMMARY OF MACROSCOPIC POSTMORTEM OBSERVATIONS OF DEAD PUPS

Groups		1	2	3	4
Litters Evaluated	N	2	1	3	2
Pups Evaluated	N	2	2	3	3
GROSS EXAM					
Litter Incidence	N	0	1	0	0
Pup Incidence	N	0	1	0	0
AUTOLYSIS					
Pup Incidence	N	0 f	1	0	0
	%	0.0	50.0	0.0	0.0
Litter Incidence	N	0 f	1	0	0
	%	0.0	100.0	0.0	0.0
ABDOMINAL CAVITY					
Litter Incidence	N	1	1	2	1
Pup Incidence	N	1	1	2	1
AUTOLYSIS OF ABDOMINAL CAVITY					
Pup Incidence	N	1 f	1	2	1
	%	50.0	50.0	66.7	33.3
Litter Incidence	N	1 f	1	2	1
	%	50.0	100.0	66.7	50.0
TOTAL PUPS DEAD OBSERVATIONS					
Pup Incidence	N	1 f	2	2	1
	%	50.0	100.0	66.7	33.3
Litter Incidence	N	1 f	1	2	1
	%	50.0	100.0	66.7	50.0

Statistical key: f=Fishers exact test

Table: 27 (continued)

SUMMARY OF MACROSCOPIC POSTMORTEM OBSERVATIONS OF SACRIFICED AFTER WEANING PUPS

Groups		1	2	3	4
Litters Evaluated	N	9	10	10	8
Pups Evaluated	N	124	146	133	117
SPLEEN					
Litter Incidence	N	1	0	0	0
Pup Incidence	N	1	0	0	0
Pup Incidence	N	1 f	0	0	0
	%	0.8	0.0	0.0	0.0
Litter Incidence	N	1 f	0	0	0
	%	11.1	0.0	0.0	0.0
SPLEEN : ENLARGED					
Pup Incidence	N	1 f	0	0	0
	%	0.8	0.0	0.0	0.0
Litter Incidence	N	1 f	0	0	0
	%	11.1	0.0	0.0	0.0
LIVER					
Litter Incidence	N	0	0	0	1
Pup Incidence	N	0	0	0	1
LIVER: GREYISH COLOR.					
Pup Incidence	N	0 f	0	0	1
	%	0.0	0.0	0.0	0.9
Litter Incidence	N	0 f	0	0	1
	%	0.0	0.0	0.0	12.5
KIDNEY					
Litter Incidence	N	1	0	0	0
Pup Incidence	N	3	0	0	0

Statistical key: f-Fishers exact test

Table: 27 (continued)

SUMMARY OF MACROSCOPIC POSTMORTEM OBSERVATIONS OF SACRIFICED AFTER WEANING PUPS

Groups		1	2	3	4
Litters Evaluated	N	9	10	10	8
Pups Evaluated	N	124	146	133	117
DILATED RENAL PELVIS					
Pup Incidence	N	3 f	0	0	0
	%	2.4	0.0	0.0	0.0
Litter Incidence	N	1 f	0	0	0
	%	11.1	0.0	0.0	0.0
LIMBS					
Litter Incidence	N	1	0	1	1
Pup Incidence	N	1	0	1	1
HINDLIMB: NECROSED					
Pup Incidence	N	0 f	0	1	1
	%	0.0	0.0	0.8	0.9
Litter Incidence	N	0 f	0	1	1
	%	0.0	0.0	10.0	12.5
HINDLIMB: CUTANEOUS LESIONS					
Pup Incidence	N	1 f	0	0	0
	%	0.8	0.0	0.0	0.0
Litter Incidence	N	1 f	0	0	0
	%	11.1	0.0	0.0	0.0
TAIL					
Litter Incidence	N	1	0	0	0
Pup Incidence	N	1	0	0	0
TAIL: NECROSIS, SHORTENED.					
Pup Incidence	N	1 f	0	0	0
	%	0.8	0.0	0.0	0.0
Litter Incidence	N	1 f	0	0	0
	%	11.1	0.0	0.0	0.0

Statistical key: f-Fishers exact test

Table: 27 (continued)

SUMMARY OF MACROSCOPIC POSTMORTEM OBSERVATIONS OF SACRIFICED AFTER WEANING PUPS

Groups		1	2	3	4
TOTAL PUPS SACRIFICED A OBSERVATIONS					
Pup Incidence	N	6 f	0**	1	2
	%	4.8	0.0	0.8	1.7
Litter Incidence	N	3 f	0	1	2
	%	33.3	0.0	10.0	25.0

Statistical key: f=Fishers exact test ** = p<0.01

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