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Ciba Specialty Chemicals Corporation
USA
Additives

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February 1, 1999

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Office of Pollution Prevention and Toxics
Environmental Protection Agency
401 M Street, SW
Washington, DC 20460-0001

8EHQ-99-14366

Subject: TSCA 8(e) Notice - Irganox 1076 (TK 10044)

Dear Section 8(e) Coordinator:

This letter and the enclosed report do not contain Confidential Business Information.

In Accordance with EPA's March 16, 1978, Policy Statement on Section 8(e) reporting under the Toxic Substances Control Act (TSCA) and the EPA's June, 1991, TSCA Section 8(e) Reporting guide, Ciba Specialty Chemicals Corporation wishes to bring to the attention of the Environmental Protection Agency results observed in a rat toxicology study with Irganox 1076 (octadecyl 3,5-Di-*tert*-butyl-4-hydroxyhydrocinnamate; CAS 2082-79-3) entitled "Effect of TK 10044 on Reproductive Functions of Two Generations in the Rat" (study enclosed). In this study, the most notable finding was histological evidence of minimal centrolobular hepatocyte enlargement in F1 adults exposed to the highest dietary treatment (5000 ppm TK 10044). Changes in certain organ weights were found in other treatment groups but were observed in the absence of any histological abnormalities. In addition, there was a significant decrease in total litter size at birth and an increase in post partum pup mortality at 5000 ppm; this effect was, for the most part, confined to the F0 generation. However, mean litter weight and mean litter size during lactation were significantly reduced in both generations at 5000 ppm. No adverse effects were found on mating performance, pregnancy rate and duration of gestation.

We are currently conducting an audit of the toxicology studies for our most widely used products and as part of this evaluation reviewed this study. It was determined the results warrant reporting under TCSA 8(e).

This information is also being provided to EPA's Office of Enforcement and Compliance Assurance.

Respectfully,

Ciba Specialty Chemicals Corporation

Naeem H. Mady

Naeem Mady, Director
Regulatory Compliance

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Contains No CBI

99 MAR 24 AM 8:00

Enclosure

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Value beyond chemistry

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Received by:

- 8. Jan. 1986

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CBG 337/831043
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PRODUCT SAFETY
AND ECOLOGY

EFFECT OF TK 10 044 ON REPRODUCTIVE FUNCTIONS OF TWO GENERATIONS IN THE RAT

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Addressee:

Professor R. Hess,
Ciba-Geigy Limited,
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SWITZERLAND.

3 January 1986.

Authors:

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Huntingdon Research Centre Ltd.,
Huntingdon,
Cambridgeshire,
ENGLAND.

A 06

CBG 337/831043
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We the undersigned, hereby declare that the work was performed under our supervision according to the procedures herein described, and that this report provides a correct and faithful record of the results obtained.

James A. Edwards, B.Sc.,
Study Director,
Department of Reproductive Toxicology

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Department of Reproductive Toxicology

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A 07

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

HRC Report No. CBG 337/831043
Sponsors Test No. 81 0673

To the best of my knowledge and belief the study described in this report was conducted in compliance with the following Good Laboratory Practice Standards:

United States Food and Drug Administration, Title 21 Code of Federal Regulations Part 58, Federal Register 22 December 1978, and subsequent Amendment, Federal Register 11 April 1980.

Japan Ministry of Health and Welfare, Notification No. 313 Pharmaceutical Affairs Bureau, 31 March 1982.

Organization for Economic Co-operation and Development, ISBN 92-64-12367-9, Paris 1982.

James A. Edwards, B.Sc.,
Study Director
Huntingdon Research Centre

8 Feb 1985
Date

A 08

QUALITY ASSURANCE AUDIT STATEMENT

HRC REPORT No. CBG 337/831043
Sponsors Test No. 81 0673

This report has been audited by HRC Quality Assurance Unit. It is considered to be an accurate description of the procedures and practices employed during the course of the study and an accurate presentation of the findings.

Kenneth W.G. Shillam, B.Sc., Ph.D., F.I.Biol.
Director, Quality Assurance

29.8.85

Audit notes

Short reports and some parts of other reports are audited completely. Large amounts of data are verified by means of sampling methods as described in British Standards Institution BS 6000, 6001 (1972) and US Military Standard 105D, the Acceptable Quality Level (the maximum percentage errors considered satisfactory as a process average) being 0.4.

Any computerised presentations which are the outcome of verified entry direct from the raw data and which are secure against manual alteration are not normally audited.

The findings of any investigations made by the sponsor and which are included in HRC reports are audited using the sponsor's report to HRC.

A 09

HRC

DATES OF QAU STUDY INSPECTIONS

HRC Report No. CBG 337/831043
Sponsors Test No. 81 0673

Inspections were made by the Quality Assurance Unit of the various phases of the study described in this Report and, in the case of repetitive continuing operations, at the intervals required by the U.S. FDA Good Laboratory Practice Regulations. The dates on which inspections were made and the dates on which the findings were reported to the Study Director and to HRC Management are given below:

Phase of Study	Date of Inspection*	Date of Reporting
Protocol Review	-	26.10.82
Pre-experimental Period	10.11.82	2.12.82
Experimental Period	18.11.82	2.12.82
"	30. 1.83	3. 2.83
"	15. 2.83	25. 2.83
"	7. 3.83	17. 3.83
"	15. 4.83	28. 4.83
"	22. 6.83	28. 6.83
"	5. 7.83	13. 7.83
"	25. 7.83	3. 8.83
"	18. 8.83	26. 8.83
"		
"		
"		
"		
"		
"		
Post experimental Period		
Final Report Audit	-	4.4.84/28.8.85

K.W.G. Shillam 28.5.85
Director,
Quality Assurance Unit

*Week beginning. This has been adopted to avoid the presentation of excessive dates in instances of an inspection being conducted over several days. It should also be noted that although inspection of each repetitive procedure was made at the required intervals, not all the various procedures may have been inspected on the same calendar occasion.

Dates on which any 'process-based' inspections were made while the study was in progress are not included in the above listing.

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SUMMARY

1. Study design

In this assessment of the effect of the plastics stabiliser TK 10 044 on growth and reproductive performance of the rat, dietary inclusions of 0, 500, 1500 and 5000 ppm of the test material were fed continuously to animals of both sexes over two generations.

2. Dietary analysis and achieved intake

Initial analyses of the test material in the diet showed satisfactory stability and homogeneity within the range of intended dietary inclusion (500 to 5000 ppm). Subsequent analysis during the study consistently showed recoveries close to the intended levels of inclusion.

As is normally anticipated when using fixed dietary concentrations, calculated intakes of the test material (mg/kg bodyweight/day) were highest at the start of each generation and subsequently slowly declined. This pattern reflected a gradual decrease in food intake in proportion to bodyweight as the animals matured.

3. 5000 ppm

At 5000 ppm there were no obvious signs of reaction to treatment among adults. There was a total of four mortalities. One F0 male and one F1 female both showed lymphosarcoma, but there was no obvious indication of a treatment relationship. A third death was associated with gastro-intestinal changes and a fourth with dental malocclusion.

Findings at 5000 ppm among adults of both generations (F0 and F1) that did appear treatment-related were as follows:

(a) Slight reductions in food consumption, weekly weight gain and weight gain of females during pregnancy.

(b) Statistically significant increases in liver weight and reductions in spleen weight. Histological examination of livers from F1 adult animals showed minimal centrilobular hepatocyte enlargement.

Although significantly lower brain weight was recorded among F1 adults, the reduction in adjusted mean weights was slight (approximately 3.5%) and F0 adults were unaffected. Histology examination of spleens and brain sections from F1 adult animals showed no treatment-related abnormality.

Mating performance, pregnancy rate and the duration of gestation were unaffected by treatment.

Among litter data at 5000 ppm inferior values in comparison with control animals occurred as follows:

(a) In the F0 generation initial litter size was reduced. Post partum pup loss was increased and pup weight gain reduced.

(b) In the F1 generation initial litter size was again reduced. There was no adverse effect on pup loss. Pup weight gain was slightly lower than among control animals despite the lower litter size and hence reduced intra-litter competition.

Organ weight analyses of selected F1 and F2 weanlings showed significantly increased liver weights and reduced spleen weights; histological examination of these tissues from F2 weanlings showed no treatment-related changes. Slightly lower brain weights (by 5.1% among males and 2.8% among females) were recorded among F1 weanlings with differences attaining statistical significance; histological examination, however, showed no abnormality and brain weights of F2 weanlings were unaffected.

Macroscopic examination of offspring showed no obvious increase in the incidence of deformities.

4. <u>1500 ppm</u>

Among adults at 1500 ppm there were no obvious signs of reaction and a single mortality appeared unrelated to treatment. Food consumption and weekly bodyweight gain were not significantly affected by treatment and weight gain of females during pregnancy, although slightly lower than among control animals in the F0 generation, was unaffected in the F1 generation.

Mating performance, pregnancy rate and the duration of gestation were unaffected by treatment.

Organ weight analyses of F0 and F1 adults showed statistically significant reductions in spleen weight. Liver weight was not adversely affected. Histological examination of livers from F1 adults (performed in view of findings at 5000 ppm) showed no treatment-related changes.

Among litter data, initial litter size, although below the control value in the F0 generation, was unaffected in the F1 generation, and there was no obvious effect on pup loss or pup weight gain in either generation. Overall, there was no clear indication of an adverse effect of treatment on litter data.

Among selected F1 and F2 weanlings statistically significant increases in liver weight and reductions in spleen weight were again recorded but deviation from controls was less than at 5000 ppm. Slight but statistically significant reductions in brain weight were also recorded, but only among F1 weanlings.

5. 500 ppm

At 500 ppm, there was no effect on adults other than for statistically significant reductions in spleen weight among F0 males and F1 females. There was no treatment-related effect on reproductive performance of adults or on litter data.

Among weanlings statistically significant organ weight differences were observed but were confined to higher liver weights, lower spleen weight among F2 females only, and slightly lower brain weight among F1 males only.

Conclusion

In this investigation at 5000 ppm, the highest dietary inclusion of TK 10 044 employed, reaction to treatment was indicated by differences among adult observations during the study, litter data and, at termination, organ weight differences among adults and weanlings. Histological examinations showed centrilobular hepatocyte enlargement in the livers of F1 adults.

At 500 and 1500 ppm reaction to treatment among adults and offspring was confined to statistically significant organ weight differences. These differences however occurred in the apparent absence of histological change.

Mating performance, pregnancy rate and the duration of gestation at all three dietary concentrations were unaffected by treatment.

B 02

This report describes the experiment performed to assess the effects of the plastics stabiliser TK 10 044 upon growth and reproductive performance of the rat over two generations of continuous treatment.

The dietary concentrations used were selected by the Sponsor.

The material, a white powder, batch no. EN 10097.24 was received in these laboratories on 10 September 1982.

The study was performed between 27 October 1982 when the animals arrived and 7 September 1983 when the last animals were sacrificed.

The Sponsor's test number was 810673.

Animal management and accommodation

Four-week old (± 1 day) Specific Pathogen Free male and unrelated female rats of the Crl:COBS® CD® (SD) BR strain were obtained from Charles River UK Ltd., Manston Road, Margate, Kent. On arrival they were weighed and examined for abnormalities and overt signs of ill health. After an acclimatisation period of 8 days they were weighed and assigned to four groups by computerised stratified randomisation to give approximately equal initial group mean bodyweights. Following allocation, the animals were earmarked to give individual identification and treatment commenced after a further 7 days.

Temperature and relative humidity were recorded daily and, over the course of the study, were generally 22°C ± 3°C and 50% ± 20%≠ respectively. Natural lighting in the room was supplemented by artificial light between 8 am and 8 pm with 13 air changes per hour in the room.

During the pre-mating period, the rats were housed four to a cage in suspended galvanised metal cages (Bowman R), equipped with solid sides and back and wire mesh front, floor and top. Cages of males were interspersed amongst those holding females to promote the development of regular oestrous cycles.

During the mating period for each generation, the rats were housed on the basis of one male to one female in plastic breeding cages (North Kent Plastics, RM-2 type).

At the end of the mating period, for each generation, the males were returned to their original cages and the females were retained in their individual breeding cages for the birth and rearing of young, during which time suitable nesting material was provided. (See Procedure Appendix i).

All animals were given free access to diet throughout the study. Spratt's Laboratory Diet No. 2 (low fat) was used both for control animals and as a base diet into which the test compound was mixed for administration to the test groups.

There was no information available to the Study Director to indicate that any non-nutrient substance likely to influence the effect of the test compound could reasonably be expected to be present in the diet or drinking water.

Analyses were made on all batches of diet used to establish levels of basic nutrients and of specified substances and micro-organisms likely to be present in feed components and which, if in excess of specified amounts, might have an undesirable effect on the test system. All batches of diet conformed with the acceptable standards agreed by the Study Director and Director, HRC Quality Assurance Unit. (See Procedure Appendix ii).

≠ The temperature and humidity ranges quoted above and occasional values falling outside this range, were not considered to affect the integrity of the study

Results of the routine physical and chemical analyses of water at source (sampling point, Sapley reservoir) as conducted quarterly by the supplier, Anglian Water Authority, are made available to HRC. Additionally, levels of specified substances known to be present from time to time in the local water, and which if in excess of the maxima recommended for (human) drinking water, might have an undesirable effect on the test system, are determined in the tap water at approximately 6-monthly intervals. A list of the principal determinands is given in Procedure Appendix iii.

The analytical data have been lodged in HRC Archives.

Treatment

The experimental design was as follows:

Group: colour code	Dietary inclusion (ppm)≠ TK 10 044	F0 generation				F1 generation			
		Number of allocated rats		Animal numbers		Number of rats		Animal numbers	
		♂	♀	♂	♀	♂	♀	♂	♀
1:white	Control	28	28	1- 28	113-140	24	24	225-248	321-344
2:yellow	500	28	28	29- 56	141-168	24	24	249-272	345-368
3:green	1500	28	28	57- 84	169-196	24	24	273-296	369-392
4:red	5000	28	28	85-112	197-224	24	24	297-320	393-416

≠ Material as supplied - commercial grade

Throughout the study, each cage was identified by a label coloured according to the group and recording the study schedule number, animal number(s), details of treatment and the name of the Study Supervisor.

Diet preparation

An appropriate quantity of material as supplied was weighed and ground directly into a weighed amount of sieved Spratt's Laboratory Diet No. 2 and stirred by hand to give a premix of suitable strength. The dietary concentrations required were obtained from this premix by direct dilution with further quantities of diet, homogeneity being achieved by mixing for 7 minutes in a rotary double-cone blender. The diets were then stored until use in heat-sealed opaque polythene bags.

Fresh batches of each concentration of diet were prepared weekly throughout the study. Diet remaining in the food hoppers at the end of each week was discarded.

The concentration of dietary inclusion remained constant for each test group throughout.

Dietary analysis (Addendum 1)

Analyses were performed for each generation at the start of the pre-mating treatment period, at the start of mating and at the end of gestation/start of lactation. A final sample was also taken at the end of the study.

Further details of analytical procedures and results are presented in Addendum 1.

Administration

Treatment of the F0 males and females by dietary inclusion began when they were six weeks of age, and continued until all F1 litters had been weaned. Direct treatment of the F1 males and females was considered to commence when they were four weeks (± 4 days) of age, and continued until all F2 litters had been weaned.

Chronological sequence of study (Figure 1)

1. Animals of the F0 generation were approximately 6 weeks of age at the commencement of treatment. They were maintained on their respective diets for 10 weeks prior to mating.

2. The animals were then paired on the basis of 1 male to 1 female for a period of 20 days. Vaginal smears were taken daily throughout the mating period.

3. The dams were allowed to rear their young to Day 21 post partum, when 24 male and 24 female pups per group were retained to form the basis of the F1 generation.

4. Following selection of pups for the F1 generation, a further male and female pup per litter were selected for organ weight analysis and preservation of selected tissues. Remaining pups were sacrificed, examined macroscopically and discarded.

5. Females that failed to produce a litter at the first mating were remated with proven males for a further period of 20 days. Any resultant litters were sacrificed on Day 8 post partum.

6. With the exception of the occasional animals which were involved in the remate, F0 parents were sacrificed following weaning of the F1 litters. Organ weight analysis and preservation of selected tissues was performed on all F0 parents.

7. After selection, F1 animals were rehoused four to a cage, uniquely identified by earmark and reared on their respective diets for 12 weeks prior to mating.

8. The animals were then paired on the basis of 1 male to 1 female for a period of 20 days (brother and sister pairings were avoided). Vaginal smears were taken daily throughout the mating period.

9. The dams were allowed to rear their young to Day 21 post partum when 1 male and 1 female pup per litter were selected for organ weight analysis and preservation of selected tissues. Remaining pups were sacrificed, examined macroscopically and discarded.

10. Females that failed to produce a litter at the first mating were remated with proven males for a further period of 20 days. Any resultant litters were sacrificed on Day 8 post partum.

11. With the exception of the occasional animals which were involved in the remate, F1 parents were sacrificed following weaning of the F2 litters. Organ weight analysis and preservation of selected tissues was performed on all F1 parents.

Observations

Observations performed for both generations were as follows:

Parent animals

(a) Signs and mortalities

All animals were regularly handled and examined for obvious changes or signs of reaction to treatment.

All animals that died or were killed for reasons of animal welfare were weighed and subjected to post mortem examination.

(b) Food consumption

The food consumed by each cage of rats was recorded weekly during the pre-mating phase of each generation and intake of the test material (as mg/kg/day) was calculated using the following formula:

$$\text{Achieved intake (mg/kg/day)} = \frac{\text{Group mean weekly food consumption (g)} \times \text{ppm}}{\text{Mid-week group mean bodyweight (g)} \times 7}$$

Food conversion ratios were calculated for the pre-mating period of each generation, using the following formula:

$$\text{Food conversion ratio} = \frac{\text{Group mean food consumed (g/rat/week)}}{\text{Group mean weekly bodyweight gain (g)}}$$

(c) Water intake

The water consumed by each cage of rats was measured daily during the first two and last two weeks of the pre-mating phase of each generation.

(d) Bodyweight change

The weight of each animal of the F0 generation was determined initially (i.e. week -1) and subsequently at weekly intervals. All pups from the main mating phase of each generation (F1 and F2) were weighed at birth, and on Days 4, 8, 12 and 21 post partum. Pups derived at remating were weighed at birth and on Days 4 and 8. Animals retained for the F1 generation were weighed weekly from selection.

During the mating period, all females were additionally weighed on alternate days throughout. The occurrence of a positive indication of mating (i.e. sperm or plug) was considered as Day 0 of pregnancy and thereafter females were also weighed on presumed Days 0, 7, 14, 17 and 20 of gestation. Weights of pregnant animals without positive indication of mating were obtained by retrospective calculation from birth (assuming a 22-day gestation period.

Dams that littered were weighed as soon as possible after parturition and on Days 7, 14 and 21 post partum.

(e) Pregnancy rate

Pregnancy rate was determined as the percentage of paired females that became pregnant.

(f) Mating performance

Vaginal smears were taken during the mating period to enable the number of animals that mated on specific days to be determined, this information was used:

(i) to detect whether pregnancy was interrupted after mating
(ii) to detect marked anomalies of the oestrous cycle
(iii) to determine the median pre-coital time for the group.

(g) Gestation period

The duration of gestation was taken as the time between the day of successful mating and parturition.

Litter data

F0 generation

F1 litters

As soon as possible after parturition, which was observed wherever possible, the young were counted, individually identified within the litter by toe amputation, sexed, weighed and examined for external abnormalities. Keeping nest disturbance to a minimum all litters were examined daily for dead and/or abnormal young. Pups from the main mate were also weighed on Days 4, 8, 12 and 21 post partum; pups from the remated females were weighed on Days 4 and 8.

If possible, dead young, except those excessively cannibalised, were autopsied.

On, or shortly after Day 21 post partum, 24 male and 24 female pups per group were selected to form the basis of the F1 generation. The litters chosen were those weaned as close as possible to the overall median weaning date. Where possible, 1 male and 1 female pup were taken from each of 24 selected litters.

Within each litter, young of each sex were retained by reference to a computerised random number system. Where less than 24 litters were within a reasonable range of weaning dates, or where all pups in a litter were of one sex, the complement was restored by taking a second pup from an appropriate litter. Records of lineage were maintained (Appendix 9).

Following selection of pups for the F1 generation, a further 1 male and 1 female pup per litter were selected for organ weight analysis with preservation of selected tissues (see 'Terminal studies'). Within each litter, young of each sex were chosen by reference to a computerised random number system. All pups selected for organ weight analysis were sacrificed on Day 23 post partum.

Remaining F1 pups were sacrificed and examined externally and internally for abnormalities. Sex of the pups was confirmed by gonadal inspection. Any tissue showing macroscopic abnormality was preserved in buffered neutral 10% formalin.

F0 females were sacrificed 3 weeks after weaning of their litter. F0 males were retained until after the majority of F1 litters had been weaned, then sacrificed and examined externally and internally for abnormalities. Selected organs were weighed and tissues preserved from all F0 adults. Uteri of those females still failing to produce young after remating were immersed in a 10% solution of ammonium sulphide to reveal evidence of implantation (Salewski, E. 1964). The uterus and ovaries were quickly washed with tap water and preserved in buffered neutral 10% formalin.

F1 generation

F2 litters

As soon as possible after parturition, which was observed wherever possible, the young were counted, individually identified within the litter by toe amputation, sexed, weighed and examined for external abnormalities. Keeping nest disturbance to a minimum all litters were examined daily for dead and/or abnormal young. Pups from the main mate were also weighed on Days 4, 8, 12 and 21 post partum; pups from the remated females were weighed on Days 4 and 8.

If possible, dead young, except those excessively cannibalised, were autopsied.

On, or shortly after Day 21 post partum, 1 male and 1 female pup per litter were selected for organ weight analysis and preservation of selected tissues. Within each litter, young of each sex were chosen by reference to a computerised random number system. All pups selected for organ weight analysis were sacrificed on Day 23 post partum. (see 'Terminal studies').

Remaining F2 pups were sacrificed and examined externally and internally for abnormalities. Sex of the pups was confirmed by gonadal inspection. Any tissue showing macroscopic abnormality was preserved in buffered neutral 10% formalin.

F1 parent females were sacrificed 3 weeks after weaning of their litter. F1 parent males were retained until after the F2 litters had been weaned, then sacrificed and examined externally and internally for abnormalities. Selected organs were weighed and tissues preserved from all F1 adults. Uteri of those females still failing to produce evidence of pregnancy following remating were immersed in a 10% solution of ammonium sulphide to reveal evidence of implantation (Salewski, E. 1964). The uterus and ovaries were quickly washed with tap water and preserved in buffered neutral 10% formalin.

Terminal studies

The following was performed for all F0 and F1 adult animals and on selected weanlings from F0 and F1 litters at terminal sacrifice.

Sample tissues, as indicated below, were preserved in buffered neutral 10% formalin (except eyes, preserved in Davidson's fluid). Capital letters indicate organs routinely weighed:

ADRENALS
aorta
bone (femur)
bone marrow (sternum)
BRAIN
cranial vault (for lachrymal glands, teeth, nasal turbinates, inner ear)
caecum
colon
duodenum
eyes
HEART
ileum
jejunum
KIDNEYS
LIVER
LUNGS
lymph nodes (cervical/ mesenteric)
mammary gland
oesophagus
OVARIES
pancreas
pituitary
PROSTATE WITH SEMINAL VESICLE (adults only)
salivary gland
sciatic nerve
Skeletal muscle
skin
spinal column (vertebral column)
SPLEEN
stomach
TESTES WITH EPIDIDYMIDES
THYMUS (if present)
thyroids
tongue
trachea (with larynx, pharynx)
urinary bladder
uterus with vagina

Any other tissue showing macroscopic abnormality.

Selected organs were weighed prior to preservation, where appropriate organs were weighed as a pair. When one of a pair of organs was abnormal, both organs were weighed individually.

The testes, prostate and seminal vesicles of adult males from either generation that failed to induce pregnancy were examined histologically. Tissues for histological examination were routinely processed in 56°C MP paraffin wax, sectioned at 5 µm and stained with haematoxylin and eosin.

Following organ weight analysis, tissues (liver, spleen and brain) from certain animals were examined histologically, see Addendum 3. Where histological changes were observed at the high dietary concentration, 5000 ppm, examinations were extended to lower concentrations.

Assessment of results

Within each generation group mean values for litter parameters were calculated from observations at birth, and on Days 4, 8, 12 and 21 post partum in two ways (see below). Litter data for remated females was not included into group mean calculations:

Mean A: includes all valid data from surviving animals that provided evidence of pregnancy including those subsequently losing the entire litter, i.e. failing to wean any young.

Mean B: includes all valid data from any animals with young surviving to weaning.

This procedure was adopted because Mean A values are affected by the loss of whole litters, which is often due to maternal neglect or effects upon the parent animal. Mean B excludes such losses and gives an indication of any general effect on all young.

For litter and mean pup weights only Mean B values were calculated.

For all values expressed as a percentage or ratio, values were first calculated within the litter and the group mean values derived as a mean of individual litter percentages.

Statistical analyses were then performed routinely on litter data, using the litter as the basic sample unit and non-parametric tests (i.e. Jonckheere and Kruskal-Wallis)[1] since litter parameters rarely follow a 'normal' distribution.

Weekly food consumption and bodyweights of adults were analysed by analysis of variance[2].

Organ weights were analysed by analysis of variance[2]. An analysis of covariance was carried out using final bodyweight as covariate when the relationship between organ weight and bodyweight was significant at the 10% level ($P<0.1$). A log ($\chi + 1$) transformation was used if significant ($P<0.01$) heterogeneity of variance was indicated by Bartlett's test[3] and if the transformed data showed less heterogeneity of variance than the untransformed data. Intergroup comparisons were carried out using the Williams' test[4].

Maintenance of records

All specimens, raw data and other documents generated at HRC during the course of this study together with a copy of this Final Report, have been lodged in the Huntingdon Research Centre Archives, Huntingdon, England.

Any such material arising from investigations made by the Sponsor or other laboratory, the findings of which are included in this Final Report, has been retained by the organisation conducting these investigations.

No data or material relating to the study will be discarded without the Sponsor's written approval.

Reference

Salewski, E. 1964 Farbemethode zum makroskopischen Nachweis von Implantationsstellen am Uterus der Ratte. Naunyn-Schmiedebergs Arch. exp. Pathol. Pharmakol. 247 : 367.

Statistical references

1. Hollander, M. & Wolfe, D.A. 1973 — Kruskal-Wallis and Jonckheere tests in Non-parametric Statistical Methods. p. 114-132. Publ. J. Wiley & Sons NY.

2. Snedecor, G.W. & Cochran, W.G. 1967 — 'Statistical Methods' 6th ed. The Iowa State University Press.

3. Bartlett, M.S. 1937 — Proc. Roy. Soc. A. 160 : 268-282.

4. Williams, D.A. 1971/2 — Williams' test for comparing the effect of increasing doses of a substance with a zero dose. Biometrics, 27 : 103-171 Biometrics, 28 : 519.

Procedure Appendix i

CBG/337

Quality assurance aspects of nesting material

The nesting material used, designated SMC sawdust grade 6, is produced by the Sawdust Marketing Co. The sawdust is principally derived from UK grown Norway Spruce, Picea abies, the addition of small amounts of UK grown Scots Pine, Pinus sylvestris is permitted. Combination with hardwood species or imported wood is not permitted. No chemical preservative is applied to timber during processing or storage. The standards of production adopted by the manufacturers have been approved by the Quality Assurance Unit.

As a precautionary measure a batch of sawdust is analysed for chemical contaminants every 3 months at a laboratory approved by HRC.

The maximum permitted levels of contaminants are:

Pentachlorophenols	2.0 ppm
Dieldrin	0.1 ppm

The certificate of analysis is made immediately available to HRC.

Composition and quality assurance aspects of diet

Spratt's LAD is a closed formula diet. The standards of production adopted by the manufacturers have been approved by the HRC Quality Assurance Unit.

Every batch of diet is analysed and only those which meet the following specification were used.

(A) Nutrients

	Typical level (%)	Maximum tolerance (%)
Crude fat	3.5	+/-15
Crude protein	21.5	+/-10
Ash	5.5	+/-15
Calcium	1.0	+/-20
Phosphorus	0.9	+/-20
Moisture	9.5	+10

(B) Contaminants

		Maximum allowable concentration
Lead	mg/kg	2.5
Cadmium	mg/kg	0.5
Arsenic	mg/kg	1.0
Mercury	mg/kg	0.1
Selenium	mg/kg	0.6
Nitrites (as sodium nitrite)	mg/kg	10
Nitrates (as sodium nitrate)	mg/kg	200
PCB's	mg/kg	0.05
Total DDT	mg/kg	0.15
Dieldrin	mg/kg	0.05
Lindane	mg/kg	0.15
Heptachlor	mg/kg	0.05
Malathion	mg/kg	5.0
Total aflatoxins (B_1, B_2, G_1, G_2)	µg/kg	5

(C) M crobial content

Maximum count/g diet (at time of manufacture)

	LAD 2
Total viable organisms	100,000
Mesophilic spores	30,000
Total coliforms	5
E. Coli Type 1	0
Salmonella spp.	0
Fungal units	1,200

Every six months a full nutrient analysis is performed to ensure that diets meet the following specifications:

		Typical evel	Maximum tolerance (%)
Sodium	%	0.3	+100/-50
Potassium	%	0.8	+100/-50
Manganese	mg/kg	70	+/-50
Iron	mg/kg	150	+/-50
Copper	mg/kg	15	+/-50
Zinc	mg/kg	60	+/-50
Fluoride	mg/kg	25	+100/-50
Vitamin A	IU/kg	11,000	+50/-20
Vitamin D_3	IU/kg	1,200*	+50/-20
Alpha Tocopherol	IU/kg	30	+150/-20
Menadione	mg/kg	2*	+100/-20
Thiamine	mg/kg	6	+100/-20
Riboflavin	mg/kg	8	+100/-20
Pyridoxine	mg/kg	10	+100/-20
Pantothenic acid	mg/kg	15	+100/-20
Nicotinic acid	mg/kg	45	+100/-20
Folic acid	mg/kg	1.5	+100/-50
Choline	mg/kg	1,200	+/-20
Cyanocobalamin	µg/kg	15*	+100/-50
Gross energy	MJ/kg	14.0	+/-10

* Levels normally assessed from pre-mix contribution

C 02

Procedure Appendix iii

CBG/337

Quality assurance aspects of drinking water

Routine quarterly analyses of water at source (Sapley Reservoir) include the following determinations with units expressed as mg/l except where stated otherwise.

Colour (Hazen)
Turbidity (Formazin)
pH
Electrical conductivity (us/cm at 25°C)
Total dissolved solids (105°C)
Temperature (°C)
Permanganate value (4 hours at 27°C - mg 0/1)
Ammonia Free and Saline (as N)
Ammonium Albuminoid (as N)
Nitrite (as N)
Nitrate (as N)
Total hardness (as $CaCO_3$)
Alkalinity (as $CaCO_3$)
Calcium
Magnesium
Sodium
Potassium
Phosphate soluble (as P)
Fluoride
Chloride
Sulphate (as SO_4)
Silicate (as SiO_2)
Iron (total)
Manganese (total)
Aluminium
Zinc
Copper
Lead
TOC
Free chlorine
Total chlorine

Others*

Cadium	
Chromium	
Cobalt	
Mercury	
Nickel	
Arsenic	
Selenium	
Anionic detergents (as Manoxol)	
Chloroform	µg/l
Bromodichloromethane	µg/l
Chlorodibromomethane	µg/l
Bromoforms	µg/l
Total haloforms	µg/l
Boron	
BHC	
Dieldrin	µg/l
Aldrin	µg/l
Endrin	µg/l
pp DDE	µg/l

* Substances listed as 'Others' are included in some analyses but not all

RESULTS

1. DIETARY INCLUSION LEVELS (Addendum 1)

Initial analysis of TK 1) 044 in diet showed satisfactory stability over the intended time period between dietary formulations, and satisfactory homogeneity at the intended high and low dietary inclusions (5000 and 500 ppm).

Subsequent analysis of diets performed at intervals during the study consistently showed recoveries close to the intended levels of inclusion at all three dietary concentrations.

2. ACHIEVED INTAKES (Tables 6A, B)

As expected, because the dietary inclusion remained constant throughout the study, the calculated intake (mg/kg/day) of test material was highest at the start of each generation when the animals were small and growing rapidly. Initial intake of F1 generation animals was higher than for F0 animals as direct dietary treatment commenced at a younger age. Dietary intakes were generally slightly higher among females than among males. These tendencies appeared common to all test groups.

Corresponding dietary intakes for animals in the F0 and F1 generations, when they were the same age, were similar.

3. ADULT ANIMALS

(a) Signs and mortalities (Table 1, Appendix 9A, B)

There were no obvious signs of reaction to treatment.

Five adult mortalities occurred, one at 1500 ppm and four at 5000 ppm. Principal observations are summarised below. There was no clear indication of a direct effect of treatment.

Principal observations	Animal number (generation and sex) at dietary inclusion	
	1500 ppm	5000 ppm
Lymphosarcoma	-	107 (F0 ♂) 409 (F1 ♀)
Gastro-intestinal changes, reduced spleen	185 (F0 ♀)	-
Gastro-intestinal changes	-	415 (F1 ♀)
Maloccluded incisors	-	319 (F1 ♂)

(b) Water consumption (Figure 2A,B, Table 2, Appendix 1A,B)

There was no clear or consistent effect on mean water consumption.

(c) Food consumption (Figure 3A,B, Table 3A,B, Appendix 2A,B)

At 5000 ppm there appeared to be a slight general reduction in mean food consumption. Cumulative values were significantly different from those of control animals in the F0 generation and to a lesser extent in the F1 generation (P values, see Tables).

There was no clear effect on mean food consumption at 500 or 1500 ppm; marginally lower values did occur but not consistently and there were no statistically significant differences ($P>0.05$).

(d) Bodyweight change (Figures 4A,B, 5A,B, Tables 4A,B, 7A,B, Appendices 3A,B, 4A,B)

At 5000 ppm mean weekly bodyweight gain of males and females in both generations tended to be slightly lower than among control animals during the first part of the treatment period. Subsequently, towards the end of each generation there was a tendency among males for parity with control animals to be regained. Occasional statistically significant differences from control animals were recorded (P values, see Tables).

Weight gain of females at 5000 ppm during gestation tended to be slightly lower than among control animals, in both generations.

At 500 and 1500 ppm there were no consistent or obvious dosage-related differences among mean weekly bodyweights. At both concentrations, mean weight gain of F0 females during gestation was slightly lower than among control animals; in the F1 generation, weight gain during gestation was similar to that of control animals.

(e) Food conversion ratio (Table 5A,B)

Among overall group mean food conversion ratios there were no marked or obvious treatment-related differences in either generation.

Weekly values for food conversion ratio tended to slightly increase with increasing age in all groups; this tendency was occasionally disturbed usually by transient variation in weekly weight gain.

(f) Mating performance and pregnancy rate (Tables 8,9, Appendix 5A,B)

Mating performance, as judged by the median pre-coital time and vaginal smear records, and pregnancy rate, as indicated by the proportion of females that became pregnant, were similar for all groups in both generations.

(g) Gestation period (Table 10, Appendix 5A,B)

The duration of gestation was similar for all groups in both generations.

(h) Terminal autopsy (Appendix 9A,B)

Among F0 generation males at 5000 ppm there was a slight increase in the incidence of animals showing macroscopically enlarged livers (5/28 compared with 0/28 among control animals). No other macroscopic changes attributable to treatment were observed at terminal autopsy of F0 or F1 generation adults (for organ weight data see Results, section 5).

4. LITTER DATA

(a) Total litter loss (Tables 1, 11A,B, Appendix 6A,B)

At 5000 ppm in the F0 generation the incidence of total litter losses (4/27) was slightly higher than among control litters (0/26). Although the difference was not significant (P>0.05) the possibility of a treatment relationship was supported by slightly increased pup mortality among F0 dams rearing some young to weaning (see section 4b). In the F1 generation there were no total litter losses among surviving dams at 5000 ppm compared with one (1/20) in the control group.

There was no obvious effect on the incidence of total litter losses at 500 or 1500 ppm; a low incidence (1/24) and 2/24 respectively) occurred among F0 dams and there were none among F1 dams.

(b) Litter size and pup mortality (Tables 11A,B, 12A,B; Appendices 6A,B, 7A,B, Addendum 2)

At 5000 ppm mean total litter size at birth in the F0 generation was significantly lower than among control animals (P<0.05), and in the F1 generation although not significantly affected (P>0.05) was both below the concurrent control value and slightly below the the recent laboratory control range.

Post partum pup mortality at 5000 ppm in the F0 generation appeared slightly but significantly higher than among control animals even with the exclusion of dams showing total litter loss (mean B values, P<0.05). The majority of these losses occurred by Day 4 post partum; there was no indication that losses occurred selectively among pups of one sex. In the F1 generation there was no obvious effect on pup mortality.

Reflecting the higher pup mortality in the F0 generation and lower initial litter size in both generations, litter size during lactation at 5000 ppm was significantly reduced in the F0 generation (P<0.01 to P<0.001) and to a lesser extent in the F1 generation (P<0.05)

At 500 and 1500 ppm, mean total litter sizes at birth in the F0 generation were lower than the control value to a similar extent as at 5000 ppm. In the F1 generation mean litter size at birth at 1500 ppm was similar to the control value and at 500 ppm slightly lower. In the absence of a consistent pattern of differences across the two generations and taking into account the extent of variation in litter size among control groups in recent studies, there was no clear indication of a treatment-related effect on initial litter size at these two dietary inclusions in either generation.

Pup mortality at 500 and 1500 ppm was not obviously affected in either generation. The significantly lower values for viable litter size occurring at birth and during lactation in the F0 generation at these two dietary concentrations (P<0.05) mainly reflected differences in initial total litter size at birth rather than an obvious adverse effect on pup mortality.

(c) Litter and mean pup weight (Tables 11A,B, Appendix 6A,B)

At all three dietary concentrations there was no obvious effect on mean pup weight at birth in either generation.

Subsequently during lactation at 5000 ppm in the F0 generation there was a tendency for lower pup weight gain with significantly lower values occurring at Days 12 and 21 post partum. In the F1 generation at 5000 ppm there was no significant effect on pup weight although marginally lower values were recorded despite the lower litter size and hence reduced intra-litter competition.

Reflecting these differences and differences in litter size, mean litter weight during lactation at 5000 ppm was significantly reduced in both generations (P<0.05 to P<0.001).

During lactation at 500 and 1500 ppm there was no clear effect on mean pup weight in either generation. Significantly lower values for litter weight at both concentrations in the F0 generation (P<0.05 to P<0.01) mainly reflected initial differences in total litter size at birth rather than an effect on mean pup weight.

(d) Terminal autopsy (Appendix 9A,B)

No macroscopic morphological changes obviously attributable to treatment were observed at autopsy of F0 or F1 offspring.

Offspring of F1 dams that showed total litter loss often showed stomachs with reduced milk content at or prior to death; this observation however may readily have been maternally mediated and provided no direct indication of abnormality among the offspring.

5. ORGAN WEIGHT ANALYSIS (Tables 13, 14A-H, Appendices 8A-D)

Values given in square brackets indicate percentage difference between adjusted mean organ weight and corresponding control value.

(a) Brain

At 5000 ppm slightly lower mean brain weights in comparison with corresponding control values were observed among F1 weanling males [-5.1%] and females [-2.8%] and F1 adult males [-3.4%] and females [-3.6%], with differences attaining statistical significance ($P<0.05$ to $P<0.01$). Among F0 adults and F2 weanlings however, mean values appeared unaffected by treatment and there were no significant differences ($P>0.05$).

At 1500 ppm slightly but significantly lower ($P<0.05$) brain weights were recorded for F1 weanling males [-2.4%] and females [-3.2%]. There was no apparent effect on values for F0 or F1 adults or on values for F2 weanlings.

At 500 ppm F1 weanling males showed slightly [-3.0%] but significantly lower brain weights; there were no other significant differences ($P>0.05$).

(b) Liver

At 5000 ppm male and female adults and weanlings of both generations showed significantly higher liver weights [+9.9 to +26.6%] in comparison with control values ($P<0.01$).

At 500 and 1500 ppm significantly higher liver weights [+9.5 to +14.8%] occurred among weanlings of both generations ($P<0.05$ to $P<0.01$). Among adults of both generations mean liver weights appeared unaffected by treatment.

(c) Spleen

At 5000 and 1500 ppm, with the exception of F0 females, adults and weanlings of both generations showed significantly lower spleen weights [-6.7 to -23.3%] in comparison with control animals ($P<0.05$ to $P<0.01$).

At 500 ppm F0 adult males, F1 adult females and F2 weanling females showed significantly lower spleen weights [-9.6 to 10.3%] ($P<0.05$); occasional other mean values were slightly lower than among control animals but were not statistically significant ($P>0.05$).

(d) Lungs, thymus and kidneys

Among values for lungs, thymus and kidneys occasional statistically significant differences occurred ($P<0.05$ to $P<0.01$). These differences showed no consistent pattern through the sequential generations and there was no obvious indication of a treatment relationship.

(e) Heart, adrenals and gonads

At all dietary concentrations there were no obvious or statistically significant ($P>0.05$) effects on heart, adrenal or gonad weights throughout the study.

6. HISTOLOGICAL EXAMINATIONS

(a) Brain (Addendum 3, Parts 1 and 2)

Microscopic examination was confined to the brain from male and female F1 generation weanling and adult rats from the control and 5000 ppm treatment group.

The microscopic observations are listed individually in the Addendum. The following comment is given in summary:

No microscopic abnormality was detected in any of the brain sections examined.

(b) Liver and spleen (Addendum 3, Parts 3 and 4)

(i) F1 generation, adults

Microscopic examination was confined to liver and spleen from male and female adult rats from the control and 5000 ppm treatment groups and the liver only from rats in the 1500 ppm treatment group of the F1 generation.

The microscopic observations are listed individually in the Addendum. The following comments are given in summary:

Liver

Minimal centrilobular hepatocyte enlargement was observed in 7/24 male and 11/19 female rats in the 5000 ppm treatment group compared to the controls. This change was considered to be treatment-related.

Similar changes were not observed in any rat examined from the 1500 ppm treatment group.

Changes seen which were considered to be of no toxicological significance included:

Minimal centrilobular hepatocyte vacuolation in a proportion of male rats in all groups examined including controls.

Moderate generalised hepatocyte vacuolation in rat 401♀ (5000 ppm).

Spleen

No microscopic abnormality was detected in any spleen examined.

(ii) F2 generation, weanlings

Microscopic examination was confined to liver and spleen from male and female weanling rats from the control and 5000 ppm treatment group of the F2 generation.

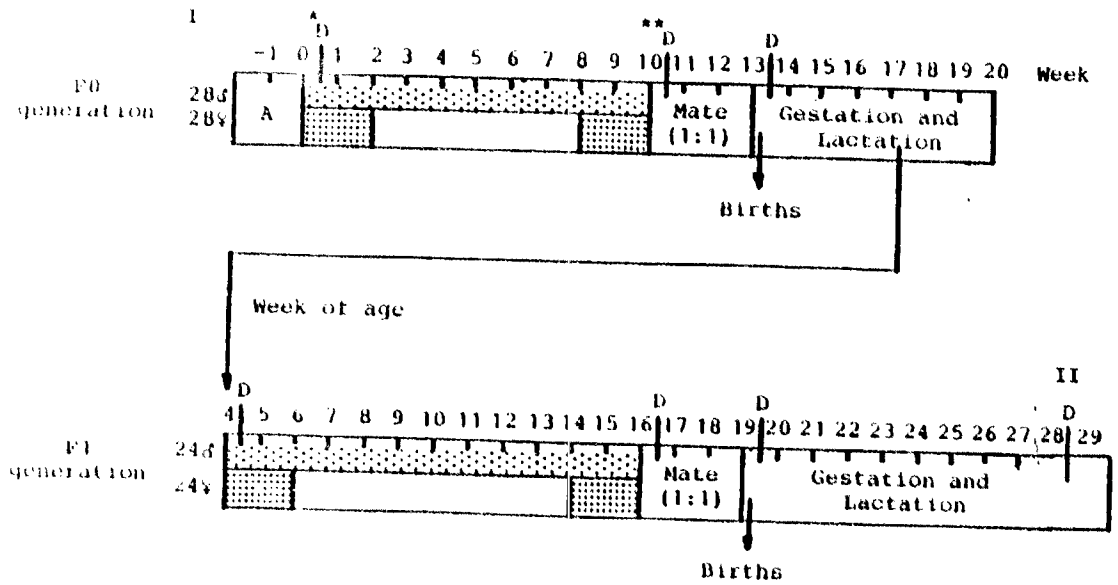
The microscopic observations are listed individually in the Addendum. The following comment is given in summary:

No microscopic abnormality was detected in any liver or spleen examined.

FIGURE 1

Diagrammatic representation of the sequence of the study

For each group:



Select 24♂, 24♀ for F1 generation. Macropathology all adults and excess pups. Organ weights and tissue preservation all adults and 1♂, 1♀ pup per litter.

Macropathology all adults and pups. Organ weights and tissue preservation all adults and 1♂, 1♀ pup per litter.

I Animals arrived: 27 October 1982

A Acclimatisation

* Commencement of treatment - approx. 6 weeks of age

** Approximately 16 weeks of age

Food consumption recorded

Water consumption recorded

II Last animals killed: 7 September 1983

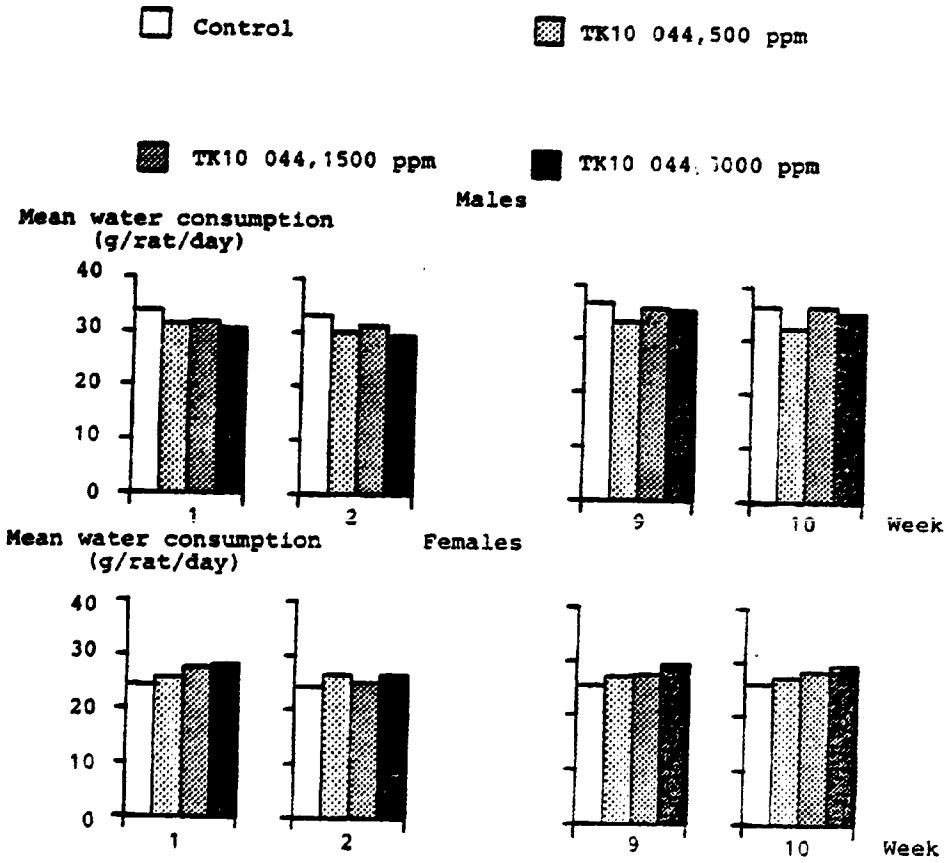
D Dietary analysis

C 11

FIGURE 2a

CBG/337

Group mean water consumption - FO generation



C 12

FIGURE 2b

CBG/337

Group mean water consumption - F1 generation

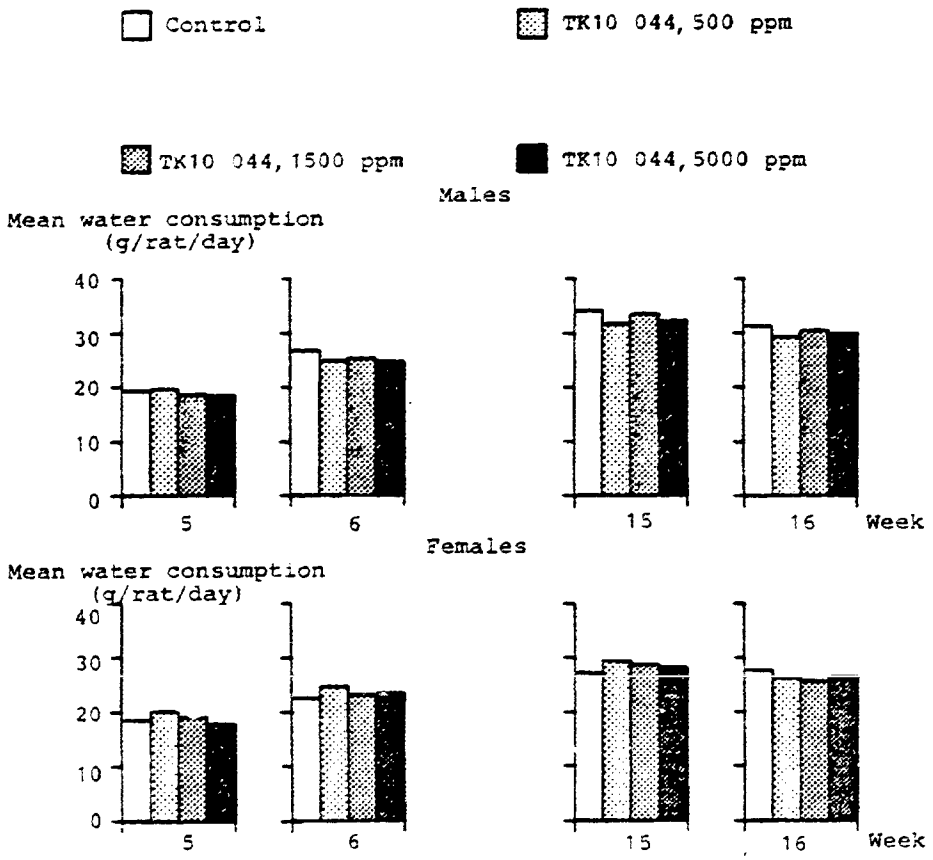
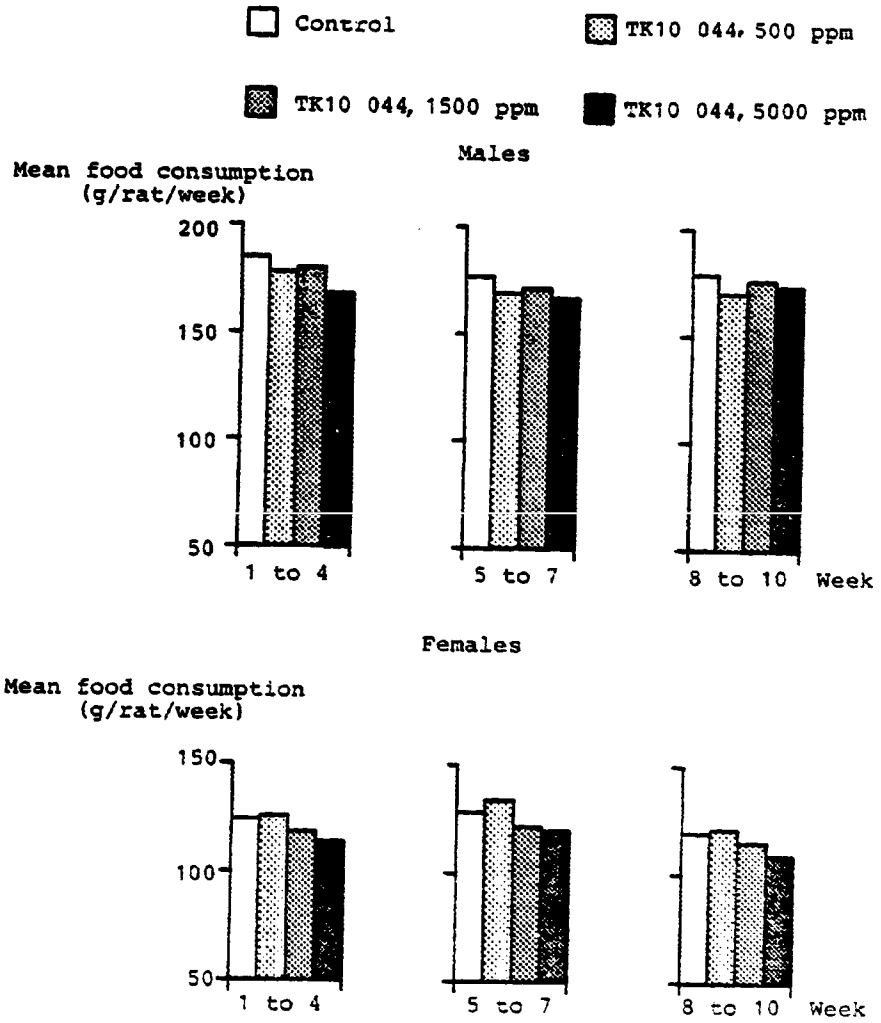


FIGURE 3a

CBG/337

Group mean food consumption - F0 generation



C 14

FIGURE 3b

CBG/337

Group mean food consumption - F1 generation

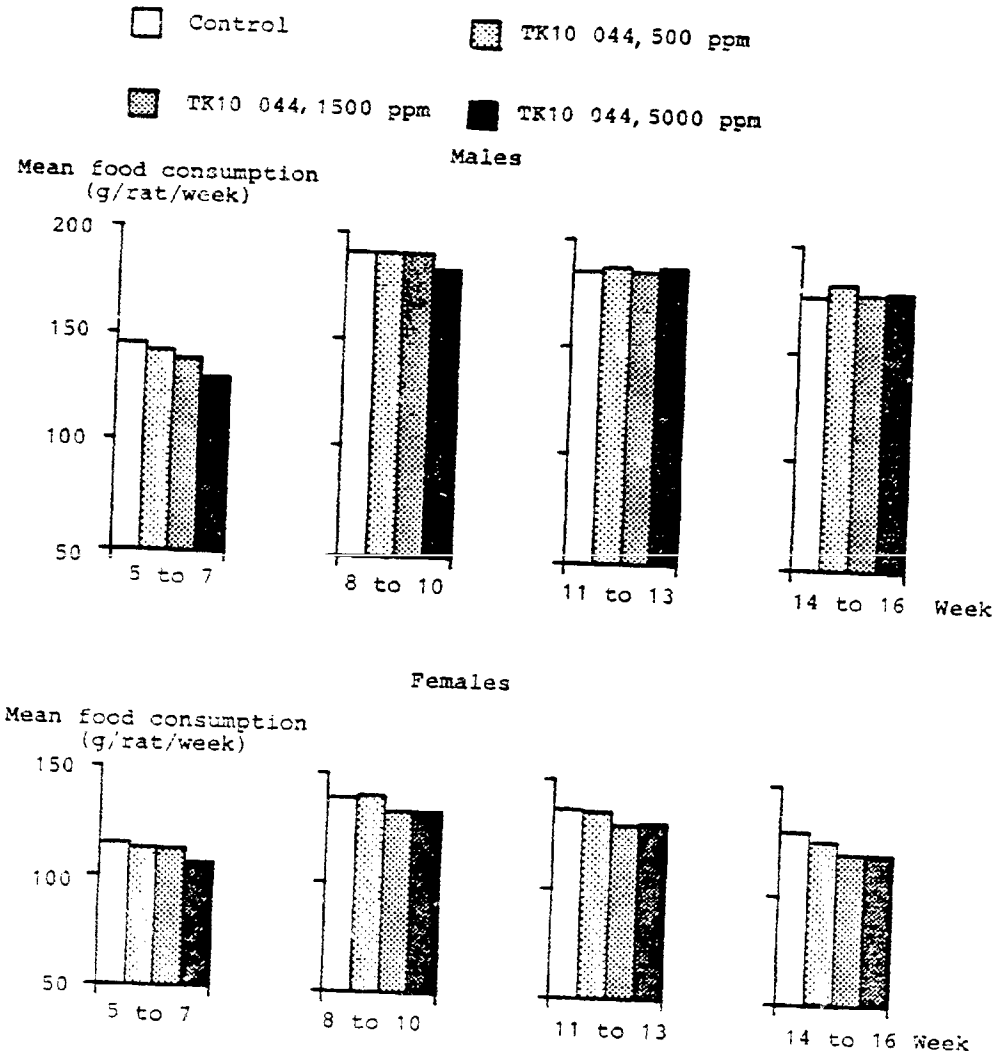


FIGURE 4a

Group mean bodyweights - F0 generation

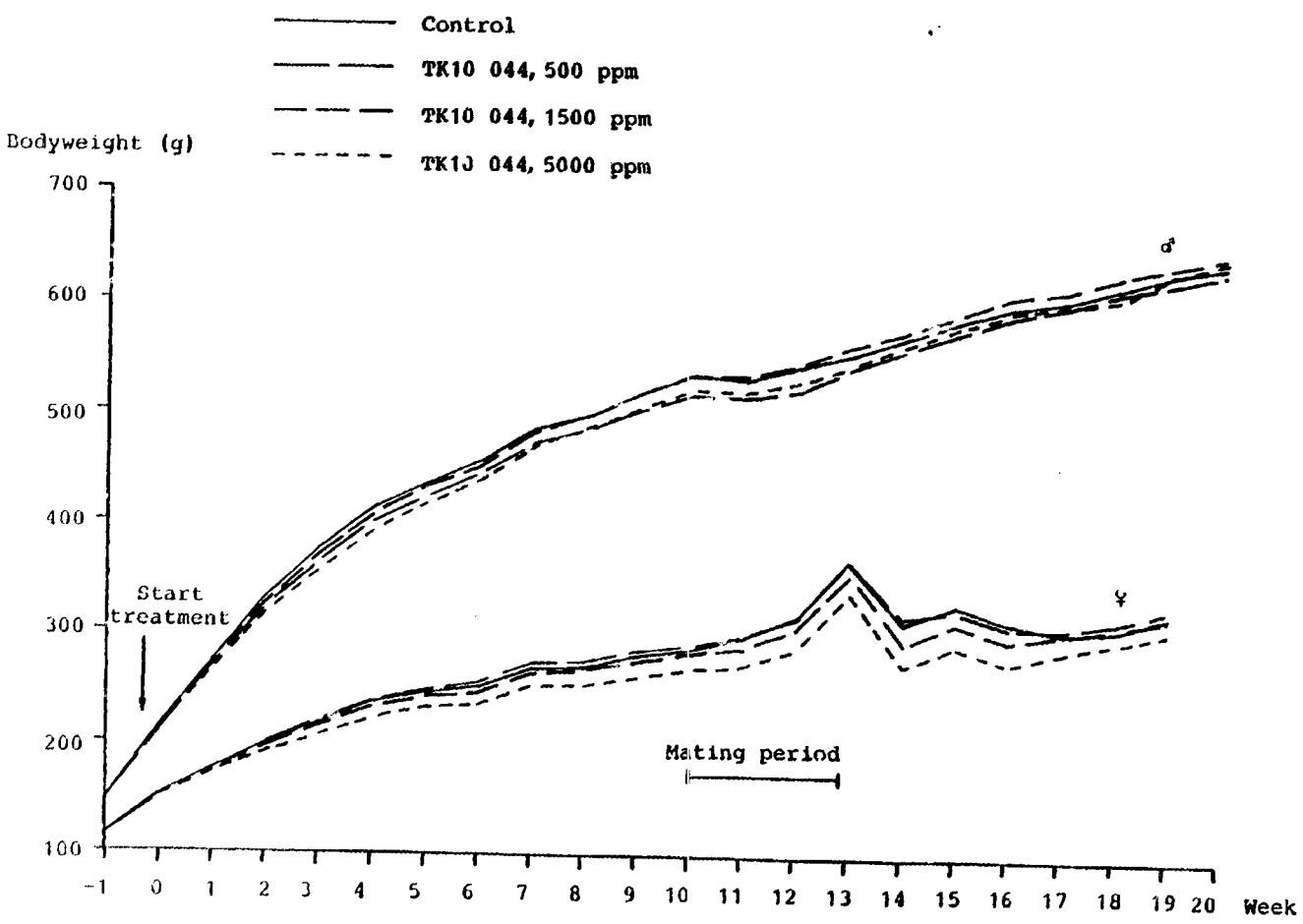
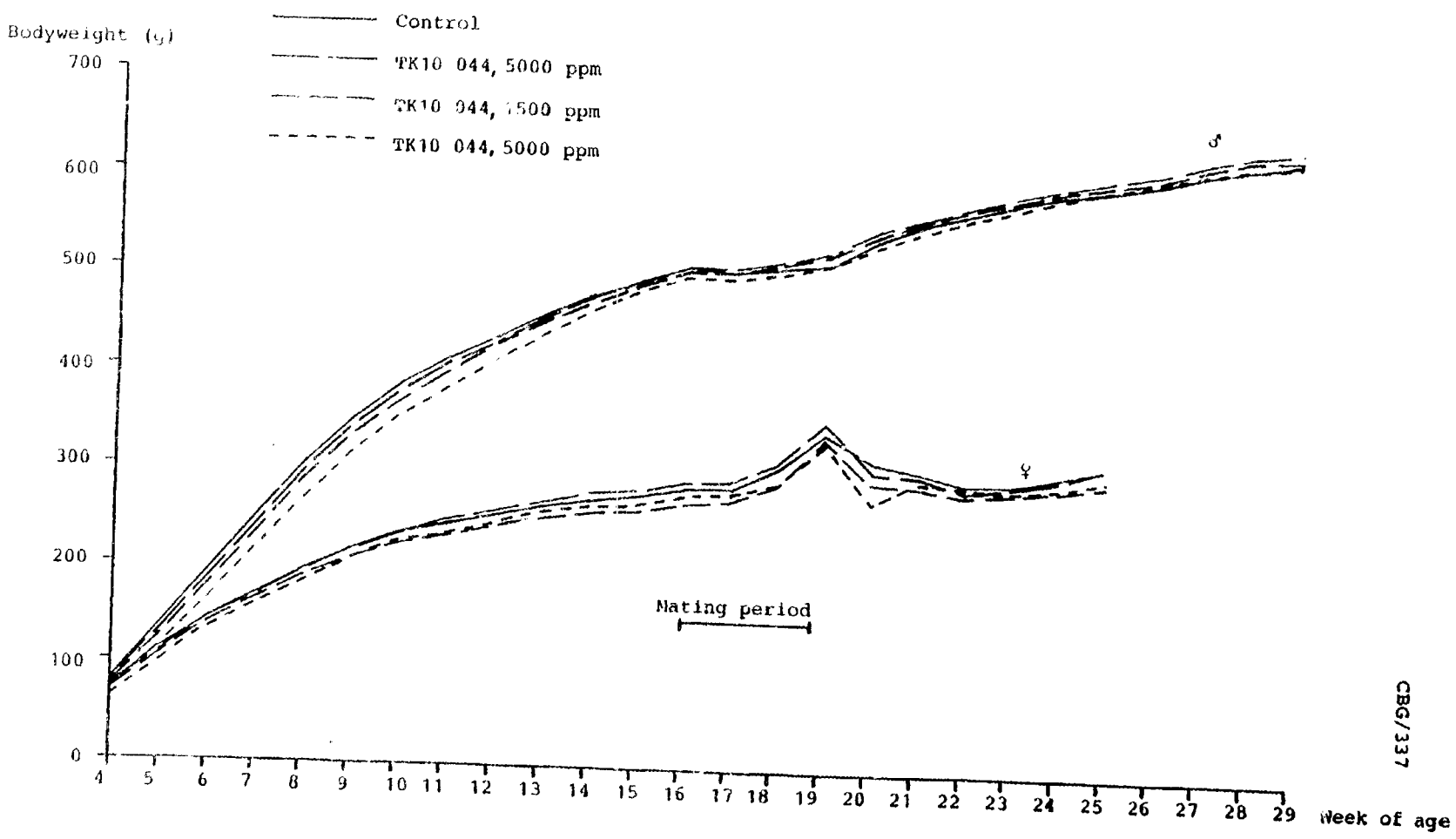


FIGURE 4b

Group mean weekly bodyweights - F1 generation



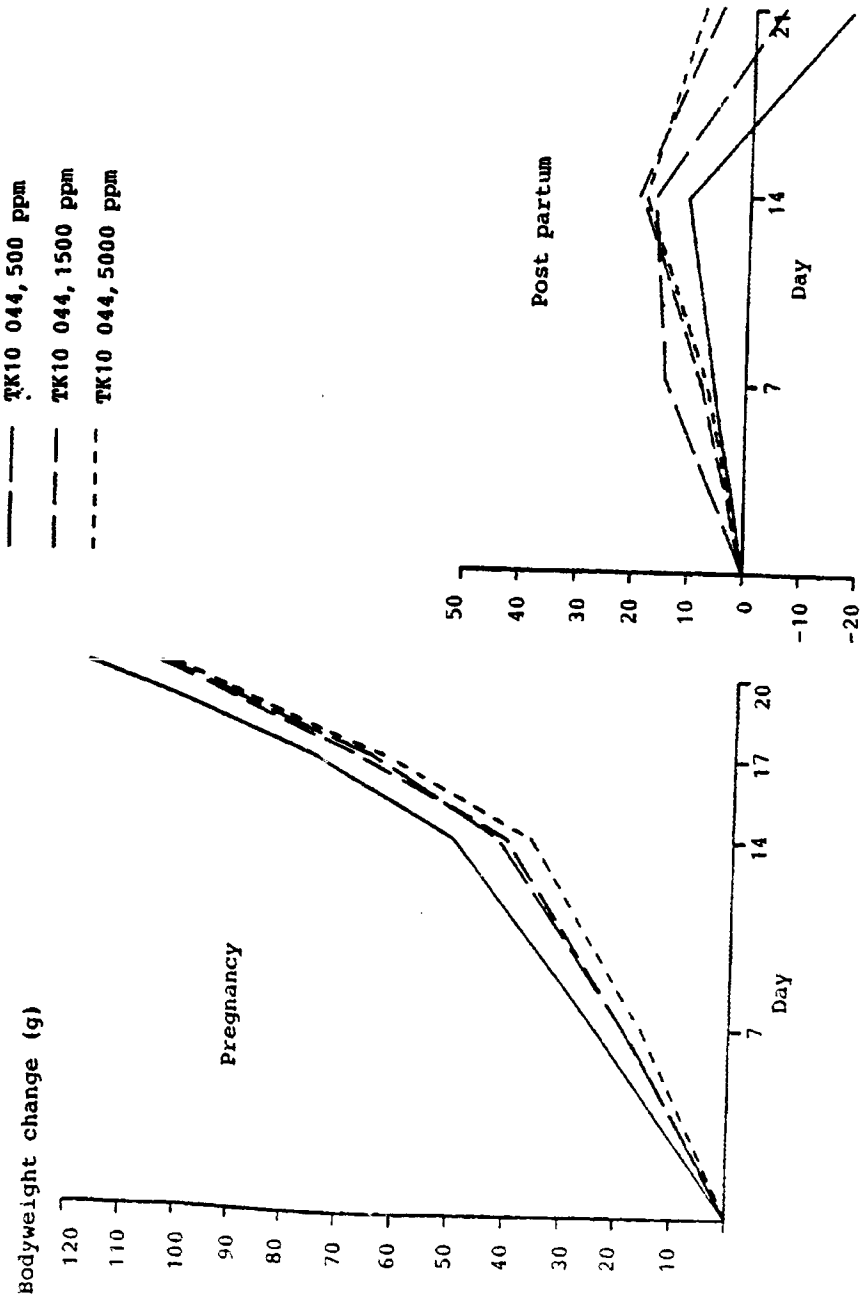
CBG/337

D 03

CBG/337

FIGURE 5a

Group mean bodyweight change of dams - F0 generation

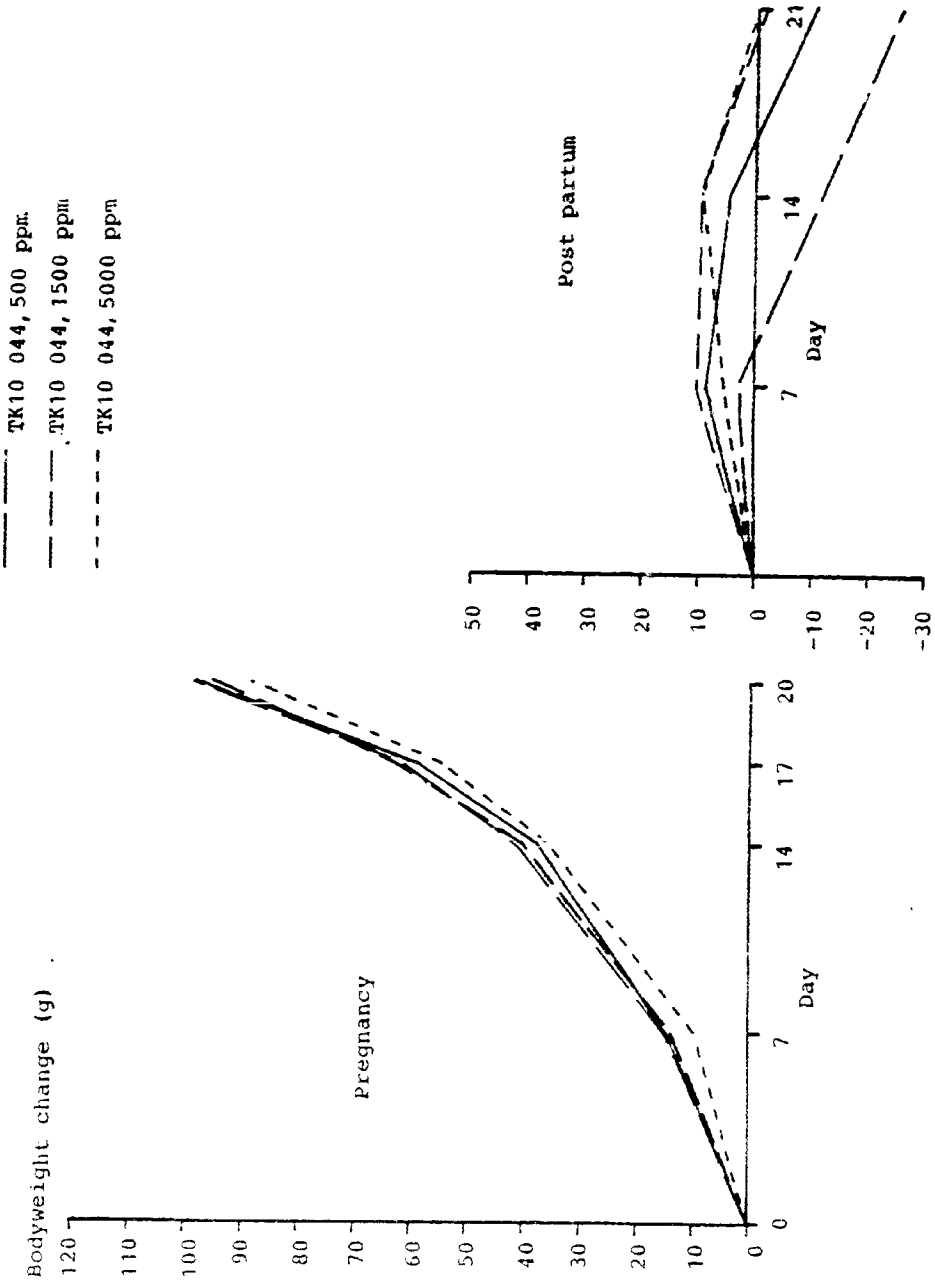


D 04

CBG/337

FIGURE 5b

Group mean bodyweight change of dams - F1 generation



D 05

TABLE 1

CBG/337

Summary of adult performance

Group:	1	2	3	4
Compound:	Control		TK10 044	
Dietary inclusion (ppm):	-	500	1500	5000

Category	Number of animals: F0 generation 1	2	3	4	F1 generation 1	2	3	4
Group size: males	28	28	28	28	24	24	24	24
females	28	28	28	28	24	24	24	24
Mortalities: males	-	-	-	1	-	-	-	1
females (post partum only)	-	-	1	-	-	-	-	2
Pairs failing to mate sucessfully	2	4	3	1	4	3	3	3
Total litter loss post partum	-	1	2≠	4	1	-	-	-≠
With viable young at Day 21 post partum	26	23	22	23	19	21	21	19
Remate of apparently non-pregnant females with proven males:								
Without viable young	-	1	1	-	2	1	-	1
With viable young (birth)	2	3	2	1	2	2	3	2

≠ Excludes 1 dam that showed total litter loss, but subsequently died.

TABLE 2

CBG/337

Group mean water consumption

Group:	1	2	3	4
Compound:	Control		TK 10 044	
Dietary inclusion (ppm):	-	500	1500	5000

Generation	Week	Mean water consumption (g/rat/day) for group:							
		1♂	2♂	3♂	4♂	1♀	2♀	3♀	4♀
F0	1	33.9	31.4	31.8	30.9	24.5	25.8	26.9	28.1
	2	33.3	30.0	31.1	29.5	24.1	26.3	24.9	26.7
	9	36.9	33.2	35.9	35.3	25.6	27.2	27.7	29.7
	10	36.1	32.3	36.1	35.1	26.0	27.1	28.2	29.4
F1	5	19.3	19.8	18.9	18.8	18.6	20.1	19.1	17.8
	6	26.9	25.0	25.2	24.9	22.3	24.7	23.2	23.4
	15	34.2	31.9	33.7	32.3	27.2	29.2	28.8	28.4
	16	31.2	29.6	30.3	29.9	27.7	26.0	25.8	26.1

Individual data are presented in Appendix 1

TABLE 3A

CBG/337

Group mean food consumption - F0 generation

Group:	1	2	3	4
Compound:	Control		TK 10 044	
Dietary inclusion (ppm):	-	500	1500	5000

Week	Mean food consumption (g/rat/week) for group:							
	1♂	2♂	3♂	4♂	1♀	2♀	3♀	4♀
1	168	163	165	150	108	110	125	98
2	186	177	183	171***	126	128	122	116*
3	191	184	186	173***	127	132	120	119*
4	193	186	187	178***	133	133	125	122**
5	166	153	156	151**	129	130	122	125*
6	184	179	183	178**	131	136	123	121*
7	181	174	177	172**	123	135	120	114*
8	178	170	173	174*	123	122	118	109**
9	174	168	172	169*	111	113	110	104*
10	183	173	182	175*	122	127	116	116*
Cumulative total	1805	1726	1764	1690	1234	1266	1181	1143
% of control	-	96	98	94	-	103	96	93

LSD from controls (cumulative, baseline week 1) significant at:
P<0.05 *
P<0.01 **
P<0.001 ***
Individual data are presented in Appendix 3A

TABLE 3B

CBG/337

Group mean food consumption - F1 generation

Group:	1	2	3	4
Compound:	Control		TK 10 044	
Dietary inclusion (ppm):	-	500	1500	5000

Week	Mean food consumption (g/rat/week) for group:							
	1♂	2♂	3♂	4♂	1♀	2♀	3♀	4♀
5	113	110	104	95	98	[illegible]	96	87
6	150	146	143	134**	119	115	118	114
7	173	171	166	159*	128	126	125	122
8	187	185	184	175*	135	136	130	129
9	191	189	193	185*	140	144	138	135
10	192	195	193	189*	141	140	132	134
11	192	193	194	195	140	144	134	137
12	186	186	184	185	141	134	129	130
13	178	183	177	180	129	129	125	123
14	182	188	181	182	130	130	124	125
15	176	184	180	178	133	124	118	115
16	170	175	168	173	126	120	114	116
Cumulative total	2090	2104	2066	2030	1560	1539	1483	1466
% of Control	-	101	99	97	-	99	95	94

LSD from controls (cumulative, baseline week 5) significant at:
P<0.05 *
P<0.01 **
Individual data are presented in Appendix 3B

TABLE 4A

CBG/337

Group mean bodyweights - F0 generation

Group:	1	2	3	4
Compound:	Control		TK 10 044	
Dietary inclusion (ppm):	-	500	1500	5000

Week	Mean bodyweights (g) for group:							
	1♂	2♂	3♂	4♂	1♀	2♀	3♀	4♀
-1	145	145	145	146	114	114	114	115
0 T	215	211	214	212	152	151	151	150
1	275	272	274	268	177	176	176	172
2	332	323	327	317	200	200	197	191
3	376	364	370	357	218	220	215	207
4	412	400	408	393	237	229	231	222
5	436	424	432	418	247	248	242	231
6	456	444	450	438	251	254	245	233
7	486	471	482	470	269	274	265	253
8	498	484	496	485	270	275	268	254
9	519	502	518	504	280	285	276	261
10 M	535	518	536	522	288	292	285	270
11 M	532	515	534	520	298	299	288	272
12 M	541	523	544	530	319	318	307	289
13	555	542	562	545	369	365	356	340
14	570	559	574	564	312	317	294	271
15	586	577	592	581	329	323	313	292
16	599	590	609	596	316	310	299	277
17	606	601	615	599	306	308	302	289
18	618	614	628	608	311	315	309	298
19 S	630	623	637	630	320	328	319	306
20	638	632	647	643	366	402	362	323

Statistical analysis of weight gains (Anovar) relative to control group

Time period (weeks)	Males			Females		
	2	3	4	2	3	4
0 to 3	NS	NS	P<0.001	NS	NS	P<0.001
0 to 10	NS	NS	NS	NS	NS	P<0.01
0 to 20	NS	NS	NS	-	-	-
3 to 10	NS	NS	NS	NS	NS	NS
3 to 20	NS	NS	NS	-	-	-
10 to 20	NS	NS	P<0.05	-	-	-

NS Non-significant (P>0.05)
T Commencement of treatment (=time 0 at beginning of week 1 of treatment)
M Mating
S Reduced group size related to sacrifice of dams
Individual data are presented in Appendix 4A

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TABLE 4B

CBG/337

Group mean bodyweights - F1 generation

Group:	1	2	3	4
Compound:	Control		TK 10 044	
Dietary inclusion (ppm):	-	500	1500	5000

Week	Mean bodyweights (g) for group:							
	1♂	2♂	3♂	4♂	1♀	2♀	3♀	4♀
4≠	83	81	74	68	73	74	69	62
5	135	133	124	113	113	112	109	100
6	193	186	178	164	144	144	140	134
7	250	243	233	218	171	169	164	161
8	304	296	287	268	195	194	187	184
9	350	342	332	315	216	216	209	209
10	386	379	369	354	234	233	224	225
11	413	407	398	383	243	246	232	234
12	436	430	427	412	252	257	242	245
13	461	456	450	438	263	267	251	257
14	479	477	470	462	270	277	258	265
15	495	493	487	479	276	282	261	268
16 M	511	511	505	498	285	291	270	278
17 M	503	507	505	496	286	292	273	278
18 M	511	515	513	503	306	311	291	294
19	515	527	522	514	343	354	336	333
20	541	550	544	534	316	306	295	276
21	557	563	560	550	307	302	291	297
22	571	576	572	564	296	289	285	291
23	581	588	582	575	297	295	287	289
24	591	598	593	587	305	304	292	295
25	598	607	601	598	315	315	298	303
26 S	605	615	609	606	353	368	326	324
27	616	627	622	617	416	382	356	328
28	626	638	632	626	379	370	340	298
29	632	642	635	631	404	383		286

Statistical analysis of weight gains (Anovar) relative to control group

Time period (weeks)	Males			Females		
	2	3	4	2	3	4
4 to 8	NS	NS	P<0.01	NS	NS	NS
4 to 16	NS	NS	NS	NS	NS	NS
4 to 24	NS	NS	NS	NS	NS	NS
8 to 16	NS	NS	P<0.05	NS	NS	NS
8 to 24	NS	NS	NS	NS	NS	NS
16 to 24	NS	NS	NS	NS	NS	NS

NS Non-significant (P>0.05)
≠ Nominal 4 weeks of age (age range 24-32 days)
M Mating
S Reduced group size related to sacrifice of dams
Individual data are presented in Appendix 4B

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TABLE 5A

CBG/337

Group mean food conversion ratios - F0 generation

Group:	1	2	3	4
Compound:	Control		TK 10 044	
Dietary inclusion (ppm):	-	500	1500	5000

Week	Mean food conversion ratio for group:							
	1♂	2♂	3♂	4♂	1♀	2♀	3♀	4♀
1	2.8	2.7	2.8	2.7	4.4	4.5	4.1	4.6
2	3.3	3.5	3.4	3.5	5.4	5.4	5.8	6.0
3	4.3	4.4	4.4	4.3	6.9	6.6	6.5	7.4
4	5.4	5.2	5.0	5.0	7.3	14.5	7.9	8.3
5	7.0	6.4	6.3	6.0	12.5	6.7	11.0	14.2
6	9.0	9.0	10.3	9.0	33.4	22.9	39.4	53.0
7	6.0	6.3	5.6	5.3	6.8	6.7	6.1	5.7
8	15.8	13.2	12.6	12.2	90.5	162.6	36.6	89.6
9	8.1	9.4	7.8	8.7	11.7	11.4	13.7	14.4
10	11.5	11.1	9.8	9.5	15.4	17.4	13.2	13.5
Overall mean 1 - 10	5.6	5.6	5.5	5.5	9.1	8.9	8.8	9.5

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TABLE 5B

CBG/337

Group mean food conversion ratios - F1 generation

Group:	1	2	3	4
Compound:	Control		TK 10 044	
Dietary inclusion (ppm):	-	500	1500	5000

Week	Mean food conversion ratio for group:							
	1♂	2♂	3♂	4♂	1♀	2♀	3♀	4♀
5	2.2	2.1	2.1	2.1	2.5	2.5	2.4	2.3
6	2.6	2.7	2.6	2.6	3.8	3.6	3.8	3.4
7	3.0	3.0	3.0	3.0	4.8	5.1	5.3	4.6
8	3.4	3.5	3.4	3.5	5.7	5.5	5.6	5.5
9	4.2	4.1	4.2	3.9	6.5	6.4	6.4	5.4
10	5.2	5.4	5.3	4.9	7.9	8.2	8.8	8.2
11	7.3	6.9	6.6	6.7	15.2	11.7	16.5	15.5
12	7.8	7.9	6.5	6.3	16.6	12.2	12.4	12.0
13	7.3	7.0	7.7	7.1	11.1	12.0	14.3	10.3
14	10.0	9.0	8.7	7.5	19.1	13.5	17.7	15.3
15	11.3	11.4	10.8	10.7	21.5	24.5	49.0	38.9
16	10.6	9.8	9.2	9.0	14.6	12.7	12.2	11.9
Overall mean 5 - 16	4.9	4.9	4.8	4.7	7.4	7.1	7.4	6.8

TABLE 6A

CBG/337

Group mean intakes - F0 generation

Group:	1	2	3	4
Compound:	Control		TK 10 044	
Dietary inclusion (ppm):	-	500	1500	5000

Week	Mean intake (mg/kg/day) for group					
	2♂	3♂	4♂	2♀	3♀	4♀
1	48.22	145.00	447.29	48.06	138.28	435.09
2	42.61	130.16	416.42	48.73	140.22	456.45
3	38.31	114.31	367.33	44.82	124.63	426.05
4	34.68	103.21	339.08	42.42	120.24	405.51
5	26.58	79.72	266.59	39.02	110.82	395.13
6	29.42	88.63	296.38	38.63	107.75	373.04
7	27.14	81.22	270.55	36.44	100.96	334.89
8	25.38	75.88	260.18	31.72	94.45	306.25
9	24.30	72.81	243.57	28.84	86.60	287.40
10	24.22	73.91	243.79	31.34	88.64	310.56
Overall mean 1 - 10	32.09	96.48	315.12	39.00	111.26	373.04

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TABLE 6B CBG/337

Group mean intakes - F1 generation

Group:	1	2	3	4
Compound:	Control		TK 10 044	
Dietary inclusion (ppm):	-	500	1500	5000

Weeks	Mean intake (mg/kg/day) for group					
	2♂	3♂	4♂	2♀	3♀	4♀
5	73.52	225.54	743.60	73.83	231.36	761.32
6	65.24	202.03	687.37	64.33	203.30	692.06
7	57.10	172.80	593.55	57.78	175.98	592.12
8	49.07	151.53	513.66	53.65	158.58	534.03
9	42.38	133.43	452.40	50.27	148.94	492.01
10	38.72	117.89	404.34	44.58	130.58	441.80
11	35.10	108.32	379.16	42.96	125.73	426.00
12	31.76	95.35	333.18	38.06	116.14	386.74
13	29.45	86.71	301.76	35.29	108.75	350.20
14	28.74	84.52	288.92	34.24	104.16	342.96
15	27.03	80.56	271.09	31.64	97.79	308.96
16	24.87	72.52	253.48	29.80	92.42	304.49
Overall mean 5 - 16	41.92	127.60	435.21	46.37	141.14	469.39

TABLE 7A

Group mean bodyweights and bodyweight changes of dams - F0 generation

Group:	1	2	3	4
Compound:	Control		TK 10 044	
Dietary inclusion (ppm):	-	500	1500	5000

Group	Bodyweight (g) at day										
	of pregnancy						post partum				
	No. of females	0	7	14	17	20	No. of females	0/1	7	14	21
1	A=B=26	286.1	309.8	335.5	362.0	403.5	A=B=26	318.2	323.6	329.4	300.6
2	A=B=24	286.5	306.5	329.2	352.1	389.9	A= 24 B= 23	304.3 304.5	318.0 318.5	321.0 321.1	299.9 298.7
3	A=B=24	277.0	295.8	318.1	344.3	381.0	A= 24 B= 22	293.6 299.3	299.7 307.3	313.7 319.1	302.2 304.8
4	A=B=27	265.6	281.6	301.9	328.8	366.7	A= 27 B= 23	277.1 279.3	283.6 289.5	295.6 298.5	285.6 284.9
	Bodyweight change (g) from Day 0 of pregnancy						Bodyweight change (g) from Day 0/1 post partum				
1	A=B=26	0	23.7	49.4	75.9	117.4	A=B=26	0	5.4	11.2	-17.6
2	A=B=24	0	20.0	42.7	65.6	103.4	A= 24 B= 23	0 0	13.7 14.1	16.7 16.7	- 4.4 - 5.7
3	A=B=24	0	18.8	41.3	67.4	104.2	A= 24 B= 22	0 0	6.1 8.0	20.1 19.8	8.6 5.5
4	A=B=27	0	16.0	36.3	63.2	101.1	A= 27 B= 23	0 0	6.5 10.2	18.5 19.2	8.5 5.6

Individual data are presented in Appendix 4A

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TABLE 7B

Group mean bodyweights and bodyweight changes of dams - F1 generation

Group:	1	2	3	4
Compound:	Control		TK 10 044	
Dietary inclusion (ppm):	-	500	1500	5000

Group	Bodyweight (g) at day										
	of pregnancy						post partum				
	No. of females	0	7	14	17	20	No. of females	0/1	7	14	21
1	A=B=20	272.0	286.3	309.1	330.9	370.1	A= 20 B= 19	294.5 295.2	303.7 303.7	300.1 299.9	284.4 283.3
2	A=B=21	280.0	291.5	321.4	342.4	378.6	A=B=21	304.5	307.6	293.0	278.8
3	A=B=21	264.1	278.1	304.5	326.8	361.1	A=B=21	279.5	290.3	289.3	277.4
4	A=B=19	275.6	285.4	311.1	330.0	362.9	A=B=19	285.9	291.3	295.8	285.8
	Bodyweight change (g) from Day 0 of pregnancy						Bodyweight change (g) from Day 0/1 post partum				
1	A=B=20	0	14.3	37.1	58.9	98.1	A= 20 B= 19	0 0	9.2 8.5	5.6 4.7	-10.1 -11.9
2	A=B=21	0	14.5	41.4	62.4	98.6	A=B=21	0	3.1	-11.5	-25.7
3	A=B=21	0	14.0	40.4	62.7	97.0	A=B=21	0	11.0	10.0	- 1.9
4	A=B=19	0	9.8	35.5	54.4	87.3	A=B=19	0	5.4	9.9	- 0.1

Individual data are presented in Appendix 4B

TABLE 8

Mating performance of pregnant dams

Group:	1	2	3	4
Compound:	Control		TK 10 044	
Dietary inclusion (ppm):	-	500	1500	5000

Group	No. of females paired	No. of rats successfully mating at 'n' days after pairing																					Median precoital time
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	With viable young	
F0 Generation																							
1	28	4	5	7	9																	26	3.0
2	28	8	6[c]	4[a]	4	1			1										1			24	2.0
3	28	4[c]	6[c]	10[b]	4															1		25	3.0
4	28	6[c]	5[a]	9[ca]	5		1													1		27	3.0
F1 Generation																							
1	24	4	5	5	2	1						2[c]										20	3.0
2	24	5	6	3[a]	6								1			1						21	2.0
3	24	9	3	1	5	1			1[a]					1								21	2.0
4	24	16[a]	2[b]	5[b]	2																1[a]	21	2.0

a Includes 1 dam with no indication of mating - day obtained retrospectively
b Includes 1 dam subsequently dead/sacrificed post partum
c Includes 1 dam subsequently showing total litter loss post partum
Individual data are presented in Appendix 5

TABLE 9

Determination of pregnancy

Group:	1	2	3	4
Compound:	Control		TK 10 044	
Dietary inclusion (ppm):	-	500	1500	5000

Group	No. of females paired	No. of females with following types of vaginal smear observed on day of mating leading to pregnancy					Non-pregnant			Pregnancy rate %
		Positive smear (sperm or plug)	Imprecise smears		No indication of mating	Total	With sperm or plug	No indication of mating	Total	
			mating from oestrus smear	anoestrus smear (animal mating 1st or last day of pairing)						
F0 Generation										
1	28	23	3	-	-	26	1	1	2	92.9
2	28	23[c]	-	-	1	24	4	-	4	85.7
3	28	23[bcc]	-	-	1	25	2	1	3	89.3
4	28	25[cccc]	1	-	1	27	1	-	1	96.4
F1 Generation										
1	24	19[c]	1	-	-	20	4	-	4	83.3
2	24	20	-	-	1	21	2	1	3	87.5
3	24	20	-	-	1	21	2	1	3	87.5
4	24	19[bb]	-	2	-	21	2	1	3	87.5

b Includes 1 dam subsequently dead/sacrificed post partum
c Includes 1 dam subsequently showing total litter loss post partum
Individual data are presented in Appendix 5

E 05

TABLE 10

CBG/337

Gestation periods

Group:	1	2	3	4
Compound:	Control		TK10 044	
Dietary inclusion (ppm):	-	500	1500	5000

Group	No. of females paired	No. of rats with gestation period of (days)					Total	Mean duration (days)
		21	22	23	24	Not positively determined		
F0 Generation								
1	28	5	20	1	-	-	26	21.9
2	28	2	15[c]	6	-	1	24	22.2
3	28	-	18[bc]	7[c]	-	-	25	22.3
4	28	1	15[cc]	10[cc]	-	1	27	22.3
F1 Generation								
1	24	6	12[c]	2	-	-	20	21.8
2	24	5	12	3	-	1	21	21.9
3	24	3	14	3	-	1	21	22.0
4	24	-	15[bb]	5	1	-	21	22.3

b Includes 1 dam subsequently dead/sacrificed post partum
c Includes 1 dam subsequently showing total litter loss post partum
Individual data are presented in Appendix 5

TABLE 11A

Group mean litter data - F0 generation

Group:	1	2	3	4
Compound:	Control		TK 10 044	
Dietary inclusion (ppm):	-	500	1500	5000

Group	No. of animals		At birth					At Day 4				At Day 8			
	Mated	Pregnant	Litter size			Litter wt. (g)	Mean pup wt. (g)	Litter size	Cumulative loss %	Litter wt. (g)	Mean pup wt. (g)	Litter size	Cumulative loss %	Litter wt. (g)	Mean pup wt. (g)
			Total	Live	Loss %										
1	28	A=B= 26	13.2	13.1	1.0	76.5	5.9	12.5	5.2	108.7	8.8	12.4	6.3	176.4	14.5
2	28	A = 24 B = 23	11.8 11.7	11.6 11.5*	2.0 2.0	- 68.1*	- 6.0	10.7* 11.0	8.6 5.6	- 101.2	- 9.3	10.5* 10.9	10.4 6.5	- 161.5	- 15.1
3	27≠	A = 24 B = 22	11.8* 11.8	11.4* 11.4*	3.8 4.1	- 66.0**	- 5.9	10.8* 11.0*	8.5 7.0	- 94.2**	- 8.8	10.0* 10.9	16.0 8.3	- 152.2*	- 14.4
4	28	A = 27 B = 23	11.8* 11.6**	10.7*** 11.0**	9.4" 5.2(*)	- 61.0***	- 5.7	9.0*** 10.2***	22.1** 11.5*	- 86.5***	- 8.6	8.6*** 10.1***	25.5* 12.5	- 134.0***	- 13.5
Kruskal-Wallis H stat.		A B	NS P<0.05	P<0.01 P<0.05	NS NS	- P<0.001	- NS	P<0.001 P<0.01	P<0.01 NS	- P<0.001	- NS	P<0.001 P<0.01	P 0.05 NS	- P<0.001	- NS
Jonckheere J stat.		A B	P<0.05 P<0.01	P<0.001 P<0.01	P<0.05 NS	- P<0.001	- NS	P<0.001 P<0.001	P<0.01 P<0.05	- P<0.001	- NS	P<0.001 P<0.001	P<0.01 NS	- P<0.001	- NS

Intergroup differences from controls statistically significant at: Kruskal-Wallis test:

* P<0.05
** P<0.01
*** P<0.001
( ) Intergroup significance unsupported by significant H or J statistic

NS Not significant (P>0.05)
≠ Excludes one female that died post partum after showing total litter loss
Individual data are presented in Appendix 7

TABLE 11A

(continued)

Group	No. of animals		At Day 12				At Day 21			
	Mated	Pregnant	Litter size	Cumulative loss %	Litter wt. (g)	Mean pup wt. (g)	Litter size	Cumulative loss %	Litter wt. (g)	Mean pup wt. (g)
1	28	A=B= 26	12.2	7.3	252.4	21.1	12.2	7.3	464.6	38.8
2	28	A = 24 B = 23	10.4 10.9	10.7 6.8	- 231.2	- 21.8	10.4 10.9	10.7 6.8	- 426.9	- 40.4
3	27	A = 24 B = 22	9.9* 10.8	16.6 9.0	- 216.4*	- 20.7	9.8* 10.7	17.4 9.9	- 385.5	- 37.5
4	28	A = 27 B = 23	8.5*** 10.0***	26.1* 13.3	- 189.5***	- 19.2*	8.4*** 9.9***	27.1** 14.4*	- 321.9***	- 33.2**
Kruskal-Wallis H stat.		A B	P<0.01 P<0.01	P<0.05 NS	- P<0.001	- NS	P<0.001 P<0.01	P<0.05 NS	- P<0.001	- P<0.01
Jonckheere J stat.		A B	P<0.001 P<0.001	P<0.01 NS	- P<0.001	- P<0.05	P<0.001 P<0.001	P<0.01 P<0.05	- P<0.001	- P<0.01

Intergroup differences from controls statistically significant at: Kruskal-Wallis test:
* P<0.05
** P<0.01
*** P<0.001

NS Not significant (P>0.05)
Individual data are presented in Appendix 7

TABLE 11B

Group mean litter data - F1 generation

Group:	1	2	3	4
Compound:	Control		TK 10 044	
Dietary inclusion (ppm):	-	500	1500	5000

Group	No. of animals		At birth					At Day 4				At Day 8			
	Mated	Pregnant	Litter size			Litter wt. (g)	Mean pup wt. (g)	Litter size	Cumulative loss %	Litter wt. (g)	Mean pup wt. (g)	Litter size	Cumulative loss %	Litter wt. (g)	Mean pup wt. (g)
			Total	Live	Loss %										
1	24	A = 20	12.3	12.1	1.1	-	-	11.2	8.1	-	-	11.2	8.1	-	-
		B = 19	12.2	12.1	1.1	67.2	5.7	11.8	3.3	104.7	9.0	11.8	3.3	170.6	14.7
2	24	A=B= 21	11.5	11.2	2.7	65.1	5.9	11.0	4.0	100.0	9.3	11.0	4.4	160.8	15.0
3	24	A=B= 21	12.1	11.7	3.4	66.4	5.7	11.3	6.3	102.5	9.2	11.2	6.6	161.4	14.6
4	22≠	A=B= 19	10.4	10.2	2.2	56.9*	5.8	9.5*	7.6	82.9**	8.9	9.5*	7.6	131.6***	14.2
Kruskal-Wallis H stat.		B	NS	NS	NS	NS	NS	NS	NS	P<0.05	NS	NS	NS	P<0.01	NS
Jonckheere J stat.		B	NS	NS	NS	P<0.05	NS	P<0.05	NS	P<0.01	NS	P<0.05	NS	P<0.001	NS

Intergroup differences from controls statistically significant at: Kruskal-Wallis test:
* P<0.05
** P<0.01
*** P<0.001

NS Not significant (P>0.05)
≠ Excludes 2 females, dead post partum, one of which showed total litter loss prior to death
Individual data are presented in Appendix 7

TABLE 11B

(continued)

Group	No. of animals		At Day 12				At Day 21			
	Mated	Pregnant	Litter size	Cumulative loss %	Litter wt. (g)	Mean pup wt. (g)	Litter size	Cumulative loss %	Litter wt. (g)	Mean pup wt. (g)
1	24	A = 20 B = 19	11.1 11.7	8.9 4.1	- 241.4	- 21.1	11.1 11.6	9.3 4.5	- 452.7	- 39.8
2	24	A=B= 21	11.0	4.4	229.1	21.5	10.9	4.7	430.7	40.7
3	24	A=B= 21	11.1	7.4	230.4	21.0	11.0	8.0	429.5	39.6
4	22	A=B= 19	9.4*	8.0	187.3***	20.4	9.4*	8.0	348.3***	38.2
Kruskal-Wallis H stat.		B	NS	NS	P<0.01	NS	NS	NS	P<0.001	NS
Jonckheere J stat.		B	P<0.05	NS	P<0.001	NS	P<0.05	NS	P<0.001	NS

Intergroup differences from controls statistically significant at: Kruskal-Wallis test:
* P<0.05
*** P<0.001

NS Not significant (P>0.05)
Individual data are presented in Appendix 7

TABLE 12A

Group mean sex ratios of young - F0 generation

Group:	1	2	3	4
Compound:	Control		TK 10 044	
Dietary inclusion (ppm):	-	500	1500	5000

Group	Number of litters	Mean litter size at birth				Mean litter size at weaning			
		Male/	Female/	Total	Mean sex ratio (% male)	Male	Female	Total	Mean sex ratio (% male)
1	A=B=26	6.7	6.5	13.2	49.6	6.3	5.9	12.2	51.0
2	A= 24	5.9	5.8	11.8	49.2	-	-	10.4	-
	B= 23	5.9	5.7	11.7	49.2	5.6	5.3	10.9	49.3
3	A= 24	5.5	6.2	11.8	47.0	-	-	9.8	-
	B= 22	5.4	6.2	11.8	47.6	4.9	5.8	10.7	45.5
4	A= 27	5.5	6.3	11.7	46.3	-	-	8.4	-
	B= 23	5.4	6.1	11.6	45.2	4.6	5.3	9.9	46.7

No statistically significant differences, proportion of males in the litter, Kruskal-Wallis test ($P>0.05$)

/ Excludes occasional dead pups, impossible to sex

Individual data are presented in Appendix 7A

TABLE 12B

Group mean sex ratios of young - F1 generation

Group:	1	2	3	4
Compound:	Control		TK 10 044	
Dietary inclusion (ppm):	-	500	1500	5000

Group	Number of litters	Mean litter size at birth				Mean litter size at weaning			
		Male≠	Female≠	Total	Mean sex ratio (% male)	Male	Female	Total	Mean sex ratio (% male)
1	A= 20	6.7	5.6	12.3	54.8	-	-	11.1	-
	B= 19	6.7	5.5	12.2	55.3	6.4	5.3	11.6	54.7
2	A=B=21	5.8	5.7	11.5	51.6	5.5	5.4	10.9	51.3
3	A=B=21	5.7	6.4	12.0	46.4	5.1	5.9	11.0	46.6
4	A=B=19	4.9	5.4	10.4	47.7	4.5	4.9	9.4	47.0

No statistically significant differences, proportion of males in the litter, Kruskal-Wallis test (P>0.05)

≠ Excludes occasional dead pups, impossible to sex

Individual data are presented in Appendix 7B

TABLE 13

CBG/337

Summary of organ weights showing statistical significance

Group:	1	2	3	4
Compound:	Control		TK 10 044	
Dietary inclusion (ppm):	-	500	1500	5000

Organ	Sex	Group and significance level≠			
		F0 generation		F1 generation	
		Adults	Offspring	Adults	Offspring
Brain	♂	-	2, 3: L* 4: L**	4: L**	-
	♀	-	3, 4: L*	4: L*	-
Heart	♂,♀	-	-	-	-
Lungs	♂	4: L*	-	-	-
	♀	-	-	-	-
Liver	♂	4: H**	2: H* 3, 4: H**	4: H**	2, 3, 4: H**
	♀	4: H**	2, 3, 4: H**	4: H**	3, 4: H**
Spleen	♂	2, 3: L* 4: L**	3: L* 4: L**	4: L** 4: L**	3, 4: L** 3, 4: L**
	♀	-	3, 4: L**	2, 3: L* 4: L**	2, 3: L* 4: L**
Thymus	♂	-	-	-	-
	♀	-	-	2, 3: H* 4: H**	4: L*
Kidney	♂	4: H*	-	-	-
	♀	-	-	-	-
Adrenal	♂,♀	-	-	-	-
Gonad	♂,♀	-	-	-	-
Prostate and seminal vesicle	♂	-		-	

≠ Statistically significant differences from control at Williams' test
* P<0.05
** P<0.01
H Higher than control values
L Lower than control values

For further details, see following Tables 14A-H

TABLE 14A

Group mean organ weights - F0 adult males

Group:	1	2	3	4
Compound:	Control		TK10 044	
Dietary inclusion (ppm):	-	500	1500	5000

Group	No. of Animals	Bodyweight (g)	Organ weight (g) Brain	Heart	Lungs	Liver	Spleen	Thymus	Prostate Seminal ≠ vesicles	Kidney	Adrenal	Testes
		Absolute values										
1	28	641.30	2.083	1.688	1.949	25.483	0.8601	0.241	3.237	4.430	0.0581	4.612
2	28	637.48	2.021	1.626	1.875	24.285	0.7679	0.234	3.106	4.453	0.0546	4.614
3	28	648.71	2.070	1.672	1.866	25.995	0.8099	0.218	3.268	4.656	0.0556	4.747
4	27	645.60	2.080	1.700	1.788	28.226	0.7595	0.245	3.354	4.755	0.0561	4.732
		Adjusted values										
1	28		2.083	1.688	1.951	25.561	0.8619	0.242	-	4.436	-	4.614
2	28		2.022	1.635	1.879	24.514	0.7731*	0.235	-	4.471	-	4.625
3	28		2.070	1.661	1.860	25.777	0.8050*	0.217	-	4.639	-	4.741
4	27		2.079	1.691	1.786*	28.132**	0.7573**	0.245	-	4.748*	-	4.725
Method of analysis			W	LW	LW	W	W	W	W	W	W	W

W Parametric analysis of variance followed by Williams' test
LW Parametric analysis of variance on log (x+1) transformed data followed by Williams' test
Difference from Control statistically significant: * P<0.05, ** P<0.01
≠ Weights of fixed tissues

TABLE 14B

Group mean organ weights - F0 adult females

Group:	1	2	3	4
Compound:	Control		TK10 044	
Dietary inclusion (ppm):	-	500	1500	5000

Group	No. of Animals	Bodyweight (g)	Organ weight (g) Brain	Heart	Lungs	Liver	Spleen	Thymus	Kidney	Adrenal	Ovaries
		Absolute values									
1	26	316.86	1.870	1.071	1.361	13.117	0.4912	0.235	2.417	0.0706	0.0884
2	24	319.85	1.850	1.197	1.310	13.752	0.4966	0.267	2.341	0.0653	0.0861
3	24	309.14	1.871	1.105	1.382	13.395	0.4783	0.248	2.400	0.0652	0.0803
4	27	300.69	1.847	1.124	1.285	14.157	0.4856	0.268	2.249	0.0677	0.0922
		Adjusted values									
1	26		1.866	1.062	1.343	12.851	0.4872	0.231	2.394	-	-
2	24		1.844	1.185	1.286	13.341	0.4905	0.262	2.305	-	-
3	24		1.873	1.107	1.388	13.506	0.4799	0.249	2.410	-	-
4	27		1.855	1.139	1.319	14.679**	0.4933	0.276	2.295	-	-
Method of analysis			W	LW	W	W	W	LW	W	W	W

W Parametric analysis of variance followed by Williams' test
LW Parametric analysis of variance on log (x+1) transformed data followed by Williams' test
Difference from Control statistically significant: * P<0.05, ** P<0.01

TABLE 14C

Group mean organ weights - F1 weanling males

Group:	1	2	3	4
Compound:	Control		TK10 044	
Dietary inclusion (ppm):	-	500	1500	5000

Group	No. of Animals	Bodyweight (g)	Organ weight (g) Brain	Heart	Lungs	Liver	Spleen	Thymus	Kidney	Adrenal	Testes
		Absolute values									
1	25	46.73	1.416	0.266	0.505	2.462	0.210	0.187	0.680	0.0192	0.300
2	22	47.69	1.381	0.284	0.556	2.731	0.220	0.200	0.708	0.0199	0.296
3	21	44.92	1.370	0.261	0.470	2.649	0.180	0.179	0.666	0.0188	0.278
4	21	40.15	1.298	0.224	0.460	2.542	0.140	0.152	0.580	0.0172	0.242
		Adjusted values									
1	25		1.404	0.257	0.492	2.349	0.201	0.175	0.658	0.0187	0.288
2	22		1.362*	0.269	0.534	2.557*	0.206	0.182	0.672	0.0191	0.279
3	21		1.370*	0.261	0.472	2.653**	0.180*	0.178	0.666	0.0188	0.278
4	21		1.332**	0.250	0.500	2.854**	0.165**	0.182	0.644	0.0186	0.273
Method of analysis			W	W	W	W	W	LW	W	W	W

W Parametric analysis of variance followed by Williams' test
LW Parametric analysis of variance on log (x+1) transformed data followed by Williams' test
Difference from Control statistically significant: * P<0.05, ** P<0.01

TABLE 14D

Group mean organ weights - F1 weanling females

Group:	1	2	3	4
Compound:	Control		TK10 044	
Dietary inclusion (ppm):	-	500	1500	5000

Group	No. of Animals	Bodyweight (g)	Organ weight (g) Brain	Heart	Lungs	Liver	Spleen	Thymus	Kidney	Adrenal	Ovaries
		Absolute values									
1	26	44.72	1.356	0.260	0.480	2.413	0.212	0.200	0.690	0.0195	0.0213
2	23	44.41	1.346	0.266	0.536	2.630	0.194	0.206	0.696	0.0196	0.0237
3	22	42.06	1.297	0.245	0.452	2.473	0.161	0.187	0.657	0.0169	0.0183
4	23	38.42	1.275	0.223	0.432	2.585	0.137	0.175	0.582	0.0166	0.0187
		Adjusted values									
1	26		1.341	0.248	0.461	2.288	0.202	0.185	0.657	0.0189	0.0205
2	23		1.333	0.257	0.519	2.524**	0.185	0.195	0.671	0.0190	0.0230
3	22		1.300*	0.248	0.454	2.507**	0.163*	0.188	0.662	0.0171	0.0185
4	23		1.303*	0.243	0.466	2.808**	0.155*	0.198	0.635	0.0178	0.0202
Method of analysis			W	W	W	W	LW	LW	W	W	W

W Parametric analysis of variance followed by Williams' test
LW Parametric analysis of variance on log (x+1) transformed data followed by Williams' test
Difference from Control statistically significant: * P<0.05, ** P<0.01

TABLE 14E

Group mean organ weights - F1 adult males

Group:	1	2	3	4
Compound:	Control		TK10 044	
Dietary inclusion (ppm):	-	500	1500	5000

Group	No. of Animals	Bodyweight (g)	Brain	Heart	Lungs	Liver	Spleen	Thymus	Prostate Seminal vesicles	Kidney	Adrenal	Testes
			Organ weight (g)									
		Absolute values										
1	24	626.90	2.111	1.688	1.863	23.280	0.801	0.201	2.997	4.179	0.0549	4.621
2	24	634.67	2.054	1.657	1.854	22.158	0.747	0.208	2.604	4.087	0.0515	4.482
3	24	628.52	2.068	1.636	1.835	22.360	0.751	0.225	2.868	4.192	0.0530	4.634
4	23	626.94	2.041	1.649	1.854	25.678	0.666	0.224	2.836	4.201	0.0523	4.488
		Adjusted values										
1	24		2.113	1.692	1.865	23.368	0.802	0.201	3.000	4.191	0.0550	4.624
2	24		2.051	1.648	1.849	21.958	0.743	0.207	2.597	4.062	0.0514	4.474
3	24		2.068	1.637	1.835	22.388	0.751	0.226	2.869	4.196	0.0530	4.635
4	23		2.042**	1.652	1.856	25.765**	0.668**	0.224	2.839	4.212	0.0524	4.491
Method of analysis			W	W	LW	W	W	W	W	W	W	W

W Parametric analysis of variance followed by Williams' test
LW Parametric analysis of variance on log (x+1) transformed data followed by Williams' test
Difference from Control statistically significant: * P<0.05, ** P<0.01

TABLE 14F

Group mean organ weights - F1 adult females

Group:	1	2	3	4
Compound:	Control		TK10 044	
Dietary inclusion (ppm):	-	500	1500	5000

Group	No. of Animals	Bodyweight (g)	Organ weight (g) Brain	Heart	Lungs	Liver	Spleen	Thymus	Kidney	Adrenal	Ovaries
		Absolute values									
1	20	303.40	1.908	1.097	1.343	13.114	0.534	0.207	2.383	0.0663	0.1251
2	21	312.54	1.889	1.088	1.344	13.295	0.490	0.260	2.385	0.0627	0.0887
3	21	294.37	1.864	1.038	1.296	12.478	0.468	0.242	2.288	0.0664	0.0871
4	19	310.40	1.846	1.076	1.385	14.742	0.449	0.276	2.325	0.0669	0.0920
		Adjusted values									
1	20		1.909	1.001	1.344	13.192	0.536	0.208	2.393	-	0.1095
2	21		1.882	1.072	1.333	12.945	0.483*	0.257*	2.342	-	0.0849
3	21		1.873	1.062	1.312	12.979	0.478*	0.247*	2.350	-	0.0834
4	19		1.841*	1.065	1.378	14.492**	0.444**	0.274**	2.294	-	0.0880
Method of analysis			W	W	LW	W	W	W	W	W	LW

W Parametric analysis of variance followed by Williams' test
LW Parametric analysis of variance on log (x+1) transformed data followed by Williams' test
Difference from Control statistically significant: * P<0.05, ** P<0.01

TABLE 14G

Group mean organ weights - F2 weanling males

Group:	1	2	3	4
Compound:	Control		TK10 044	
Dietary inclusion (ppm):	-	500	1500	5000

Group	No. of Animals	Bodyweight (g)	Organ weight (g) Brain	Heart	Lungs	Liver	Spleen	Thymus	Kidney	Adrenal	Testes
		Absolute values									
1	19	46.72	1.427	0.271	0.493	2.442	0.216	0.202	0.698	0.0190	0.309
2	20	48.47	1.429	0.279	0.473	2.799	0.218	0.216	0.740	0.0193	0.307
3	21	47.14	1.407	0.270	0.488	2.839	0.183	0.194	0.720	0.0176	0.286
4	19	47.06	1.430	0.266	0.472	3.124	0.178	0.191	0.713	0.0202	0.310
		Adjusted values									
1	19		1.433	0.274	0.500	2.485	0.219	0.205	0.707	0.0192	0.314
2	20		1.420	0.273	0.460	2.723**	0.212	0.210	0.724	0.0190	0.298
3	21		1.409	0.271	0.491	2.853**	0.184**	0.196	0.723	0.0177	0.287
4	19		1.433	0.267	0.475	3.145**	0.179**	0.192	0.717	0.0203	0.312
Method of analysis			W	W	W	W	W	W	W	W	W

W Parametric analysis of variance followed by Williams' test
LW Parametric analysis of variance on log (x+1) transformed data followed by Williams' test
Difference from Control statistically significant: * P<0.05, ** P<0.01

TABLE 14H

Group mean organ weights - F2 weanlings females

Group:	1	2	3	4
Compound:	Control		TK10 044	
Dietary inclusion (ppm):	-	500	1500	5000

Group	No. of Animals	Bodyweight (g)	Organ weight (g) Brain	Heart	Lungs	Liver	Spleen	Thymus	Kidney	Adrenal	Ovaries
		Absolute values									
1	19	45.09	1.368	0.262	0.478	2.484	0.207	0.205	0.673	0.0194	0.0210
2	21	46.34	1.382	0.267	0.473	2.575	0.195	0.207	0.703	0.0193	0.0235
3	21	44.42	1.382	0.258	0.448	2.673	0.180	0.196	0.675	0.0177	0.0219
4	19	44.56	1.363	0.255	0.485	3.084	0.171	0.179	0.686	0.0179	0.0214
		Adjusted values									
1	19		1.369	0.262	0.485	2.486	0.208	0.205	0.673	0.0194	0.0210
2	21		1.373	0.260	0.457	2.491	0.188*	0.200	0.686	0.0190	0.0232
3	21		1.388	0.263	0.454	2.721**	0.184*	0.200	0.685	0.0179	0.0221
4	19		1.367	0.258	0.489	3.122**	0.174**	0.182*	0.694	0.0180	0.0215
Method of analysis			W	W	W	W	W	W	W	W	W

W Parametric analysis of variance followed by Williams' test
LW Parametric analysis of variance on log (x+1) transformed data followed by Williams' test
Difference from Control statistically significant: * P<0.05, ** P<0.01