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VIA CERTIFIED MAIL

December 20, 1995

8EHQ-0196-13519

Document Processing Center (TS-790)
Office of Pollution Prevention and Toxics
Attention: Section 8(e) Coordinator
U.S. Environmental Protection Agency
401 M Street, SW
Washington, D.C. 20460

ORIGINAL

Contains No CBI

Re: Follow-Up to 8(e) Notification Dated September 22, 1995

Submission of Final Report - Sodium Bromide: Developmental
Toxicity Study in Rats



8EHQ-95-13519

SP001 01/17/96

Dear Section 8(e) Coordinator:

As a follow-up to our 8(e) notification dated September 22, 1995,
enclosed are three copies of the laboratory final report
entitled: Sodium Bromide-Developmental Toxicity Study in Rats.

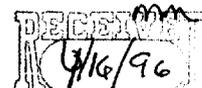
Should you have any questions please contact me at the above
telephone.

Sincerely,

Ruben Westin



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SODIUM BROMIDE
DEVELOPMENTAL TOXICITY (EMBRYOFOETAL TOXICITY AND
TERATOGENIC POTENTIAL) STUDY IN RATS
(GAVAGE ADMINISTRATION)

Data requirement	US EPA Subdivision F Guideline 83-3 OECD Guideline for testing of chemicals No. 414
HRC project identity	DSB 90
Study completed on	20 November 1995

Sponsor

Bromine Compounds Ltd.,
Health, Safety and Environmental Division,
P.O. Box 180,
Beer-Sheva 84101,
ISRAEL.

Testing facility

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Study Director

D.P. Myers

DSB 90/950921

RESERVED FOR SPECIAL REGULATORY/COUNTRY REQUIREMENTS

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid.

Good Laboratory Practice, The United Kingdom Compliance Programme, Department of Health & Social Security 1986 and subsequent revision, Department of Health 1989.

EC Council Directive, 87/18 EEC of 18 December 1986, (No. L 15/29).

Good Laboratory Practice in the testing of Chemicals OECD, ISBN 92-64-12367-9, Paris 1982, subsequently republished OECD Environment Monograph No. 45, 1992.

United States Environmental Protection Agency, (FIFRA), Title 40 Code of Federal Regulations Part 160, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August 1989.

Japan Ministry of Agriculture, Forestry and Fisheries, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984.

A. P. Myers

David P. Myers, B.Sc., Ph.D.,
Study Director,
Huntingdon Research Centre Ltd.

20 November 1995

Date

T. Cohen

T. Weiss-Cohen,
Head, Toxicology and Regulatory Affairs,
Bromine Compounds Ltd.,
Sponsor.

28 November 1995

Date

R. Westin

Mr. R. Westin,
Ameribrom Inc.,
Submitter.

8 December 1995

Date

DSB 90/950921

**RESERVED FOR A FLAGGING STATEMENT REQUIRED FOR
SUBMISSION UNDER US EPA FIFRA**

QUALITY ASSURANCE STATEMENT

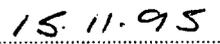
This report has been audited by the Huntingdon Research Centre Quality Assurance Department. The methods, practices and procedures reported herein are an accurate description of those employed at HRC during the course of the study. Observations and results presented in this final report form a true and accurate representation of the raw data generated during the conduct of the study at HRC.

Inspections were made by the Quality Assurance Department of various phases of the study as conducted at HRC and described in this report. The dates on which the inspections were made and the dates on which 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	-	16 December 94
Pre-experimental Period	-	-
Experimental Period	6 and 7 February 95 20 February 95	8 February 95 20 February 95
Date of reporting audit findings to the Study Director and HRC Management		3 August 95



 Caroline Sheets,
 Audit Team Supervisor,
 Department of Quality Assurance,
 Huntingdon Research Centre Ltd.



 Date

RESPONSIBLE PERSONNEL

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I. S. Dawe

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SUMMARY

This report describes the study performed to assess the effects of the biocide, sodium bromide, on the pregnant rat and her unborn offspring. Dosages of 0 (Control), 100, 300 and 1000 mg/kg/day were administered daily by intragastric intubation, to groups of 25 time-mated female rats per group from Day 6 to Day 15 *post coitum* inclusive. On Day 20 *post coitum*, females were sacrificed and subjected to *post mortem* examination, litter values determined and foetuses subsequently sexed and examined for visceral or skeletal changes.

The following comments in relation to principal findings during the study are made in summary:

Treatment at 1000 mg/kg/day was associated with:

Unsteady gait, feet falling through the cage grid floor during ambulation, poorly coordinated movements, reduced bodytone and hair loss.

One mortality.

Lower bodyweight gains during Days 6 to 12 and 16 to 20 of pregnancy.

Increased food consumption during Days 6 to 9 and 14 to 15.

Lower food consumption during Days 18 to 19.

There were no obvious adverse effects on any of the litter parameters recorded.

Detailed examination of foetal morphology revealed higher incidences of foetuses/litters showing absent left kidney, absent left ureter, absent/narrow left uterine horn, distorted ribs, shortened/absent 13th ribs, irregular ossification of the thoracic vertebral centra, reduced and/or unossified sternbrae and, reduced ossification of one or more cranial centres, than in controls.

Treatment at 300 mg/kg/day was associated with:

Lower bodyweight gains during Days 16 to 20 of pregnancy.

There were no obvious adverse effects on any of the litter parameters recorded.

Detailed examination of foetal morphology revealed a higher incidence of foetuses showing reduced ossification of various components of the skeleton compared with controls.

Treatment at 100 mg/kg/day was not associated with any observable maternal response or adverse effects on *in utero* development of the conceptus.

Conclusion

Within the context of this study, it is concluded that the no effect level for the parent female and *in utero* development of the conceptus is 100 mg/kg/day.

INTRODUCTION

This report describes the study performed to assess the effect of sodium bromide, a biocide, upon the pregnant rat and her unborn offspring, when administered by intragastric intubation to the parent female from Day 6 through to Day 15 of pregnancy inclusive.

The study was designed and conducted taking into consideration the objectives of the following guidelines:

- OECD: Guideline for testing of chemicals No. 414, adopted May 1981.
- EEC: Council recommendation (83/571/EEC) Annex 1 - Repeated Dose Toxicity -Official Journal of the European Communities L332, 20 - 22 October 1983.
- USA EPA (FIFRA): Pesticide Assessment Guidelines, subdivision F Guideline series 83 - 3 published in Fed Reg 47, No. 100, 1982.
- JMAFF: Requirements for Safety Evaluation of Agricultural Chemicals published in NohSan No. 4200, Ministry of Agriculture, Forestry and Fisheries 28 January 1985.

The dosages employed, 0 (Control), 100, 300 and 1000 mg/kg/day were selected by the Sponsor based on the results of a range-finding developmental toxicity study in rats (HRC Report No.: DSB 80/942582). In this study, treatment at 1000 mg/kg/day, the limit dose, was associated with poorly coordinated movements, reduced bodytone, increased bodyweight gains during Days 6 to 8 of pregnancy, lower bodyweight gains during Days 8 to 12 and 18 to 20 of pregnancy and increased food consumption. Treatment at 250 or 500 mg/kg/day, was associated with poorly coordinated movements and lower bodyweight gains during Days 18 to 20 of pregnancy. There were no obvious adverse effects on litter parameters or macroscopic external foetal structure at any dosage investigated.

Oral administration was selected by the Sponsor as an acceptable route of administration for a developmental toxicity study and, allows for accurate quantification and administration of the dose. The rat was selected as the test species as it is a universally accepted species in reproductive toxicology studies. In addition, Huntingdon Research Centre has background control data for reproductive performance in developmental toxicity studies for the strain used.

RELEVANT STUDY DATES

Protocol approved by:

Study Director	2 December 1994
HRC Management	2 December 1994
Sponsor	11 December 1994

Arrival of animals at HRC:

Batch A	31 January 1995
Batch B	1 February 1995

Commencement of treatment
(Day 6 of pregnancy):

Batch A	6 February 1995
Batch B	7 February 1995

Sacrifice (Day 20 of pregnancy):

Batch A	20 February 1995
Batch B	21 February 1995

TEST SUBSTANCE

Identity:	Sodium bromide, technical grade
Chemical name:	Sodium bromide
Action:	Biocide
Supplier:	Sponsor
Date of receipt:	1 March 1994
Expiry date:	February 1996
Batch no.:	940045
Purity:	99.84 %
Appearance:	White crystalline solid
Storage conditions:	Room temperature in the light
Stability of formulations:	At least 7 days
Storage conditions for formulations:	refrigerator (ca 4°C)

EXPERIMENTAL PROCEDURE

PARENT ANIMALS

A total of 108 sexually mature (8 - 10 weeks old) Specific Pathogen Free female rats (CrI: CD® BR VAF/Plus strain) which were time-mated to identified males of the same strain, were obtained from Charles River UK Limited, Manston Road, Margate, Kent. The first batch (A) consisted of 64 animals (weight range on arrival 201 - 259 g) followed by a second batch (B) consisting of 44 animals (weight range on arrival 196 - 239 g) mated one day later. The day of mating, as judged by the appearance of sperm in the vaginal smear or by the presence of a vaginal plug, was considered as Day 0 of pregnancy. Ten additional animals were obtained for health check purposes.

On arrival all animals were examined for abnormalities and for signs of overt ill health. Those designated as health check animals were killed within 24 hours after arrival at HRC and subjected to routine macroscopic examination. Lungs, liver, kidneys, spleen and heart were preserved in fixative, but not processed further.

Macroscopic examination of the health check animals revealed enlarged cervical lymph nodes in all 10 animals. Subsequent microscopic examination revealed minimal lymphoid proliferation in lymph node tissue of all animals, accompanied in seven cases by minimal plasmacytosis, and also, in one instance by a unilateral, minimal focus of necrosis and inflammation. No changes considered to be related to the presence of infectious disease was apparent in any animal.

The remaining animals were weighed on arrival and 60 animals from Batch A (weight range 208 - 250 g) and 40 animals from Batch B (weight range 196 - 235 g) were assigned to four groups by computerised stratified randomisation to give approximately equal initial group mean bodyweights within each batch. (Adjustments were made to the group allocation in order to ensure an acceptable distribution of the males to which females were mated). Following allocation, the animals were earmarked to give individual identification. Prior to the commencement of treatment, the health status of the animals was reviewed by a Veterinary Officer and considered to be satisfactory for the study.

The four animals in each batch that were excess to requirements were not dosed and were discarded on the day that treatment commenced for the allocated Batch B animals.

GENERAL ANIMAL MANAGEMENT AND ACCOMMODATION

The study was housed in a barriered building, E28, Rooms 4a and 4b.

Animal room controls for temperature and relative humidity were set at 21°C and 55% respectively. Recorded values were within the ranges $21 \pm 1^\circ\text{C}$ and $54 \pm 14\%$ respectively. Permanent weekly recordings of these parameters were made by a Cambridge recorder and these are archived with all other raw data for this study. Lighting was controlled to give 12 hours light (8 am to 8 pm) and 12 hours dark per 24 hours.

The animals were housed individually in suspended stainless steel cages (Biotech®) equipped with solid sides and wire grid front, back, floor and top (26 cm high; 36 cm wide; 53 cm from front to back). The cages constituting each treatment group were dispersed within the batteries so that possible environmental influences arising from their spatial distribution would be equilibrated as far as possible for all treatments.

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

All animals were given free access to Special Diet Services Laboratory Animal Diet No. 1 and to tap water via water bottles.

There was no information available to the Study Director to indicate that any non-nutrient substance likely to influence the effect of the test substance could reasonably have been expected to be present in the diet or water, both of which were routinely subjected to regular chemical analyses, results of which are lodged in HRC Archives.

The experimental design was as follows:

Group/ colour code	Treatment sodium bromide (mg/kg/day)#	Concentration sodium bromide (mg/ml)	Vehicle	Dose volume (ml/100 g)	No. of rats ♀		Animal numbers	
					A	B	A	B
1: White	Control	0	Distilled H ₂ O	1.0	15	10	1-15	16- 25
2: Yellow	100	10	Distilled H ₂ O	1.0	15	10	26-40	41- 50
3: Green	300	30	Distilled H ₂ O	1.0	15	10	51-65	66- 75
4: Red	1000	100	Distilled H ₂ O	1.0	15	10	76-90	91-100

Material as supplied

METHOD AND FREQUENCY OF DOSE PREPARATION

An appropriate amount of the test substance was dissolved in the vehicle by shaking and/or stirring in order to achieve the highest required concentration. Lower concentrations were prepared by serial dilution of the highest concentration. Fresh formulations were prepared weekly.

FORMULATION SAMPLING AND ANALYSIS

Prior to the commencement of the study, the proposed formulation procedure was checked by chemical analysis to confirm that the method was acceptable and that the chemical stability of the formulations were satisfactory under the conditions of the study. The method of analysis had been agreed with the Sponsor. The results obtained for analytical method validation and chemical stability of sodium bromide in aqueous formulations at concentrations of 1 mg/ml and 100 mg/ml are presented in the Analytical Chemistry Report contained within HRC Report No.: DSB 80/942582.

Samples were taken from each concentration of the dosage preparation formulated for use on the first and last days of treatment for analysis of achieved concentration. Analyses were performed by the Department of Analytical Chemistry at HRC. Full details of methods and results are presented in the **FORMULATION ANALYSIS REPORT**.

TEST SUBSTANCE CHARACTERISATION

The test substance was characterised (with reference to appearance, identification and assay) prior to the commencement of the study. Full details of methods and results are presented in the **ANALYTICAL CHEMISTRY REPORT** contained within HRC Report No.: DSB 80/942582.

TREATMENT

Treatment by gavage commenced on Day 6 of pregnancy and continued daily up to and including Day 15 of pregnancy. Dosing commenced at 11.10 am \pm 35 minutes on each day of treatment.

Dosage volumes were calculated for individual animals on Day 6 of pregnancy and adjusted according to bodyweight on Days 8, 10, 12 and 14.

OBSERVATIONS AND MEASUREMENTS

Dated and signed records of all activities relating to the day by day running and maintenance of the study within the animal unit as well as to the group observations and examinations outlined in this procedure, were recorded in the Study Daybook. In addition, observations relating to individual animals made throughout the study were recorded.

The following observations were made during the study:

Parent animals

Clinical signs - All animals were regularly handled and observed daily for obvious changes or signs of reaction to treatment.

Mortalities - The one animal that was killed for humane reasons was weighed and subjected to *post mortem* examination.

Bodyweights - All animals were weighed initially (=Day 0 of pregnancy) and on Days 3, 6, 8, 10, 12, 14, 16, 18 and 20.

Food consumption - Food consumption was measured from weighday to weighday commencing on Day 3 of pregnancy.

Water consumption - Daily visual inspection of the level of water in water bottles did not indicate any obvious intergroup differences in water intake. Therefore, water intake was not gravimetrically measured.

Litter data and foetal examinations

On Day 20 of presumed pregnancy the animals were killed by CO₂ asphyxiation, dissected and examined for congenital abnormalities and macroscopic pathological changes in maternal organs. Abnormal tissues were preserved at the discretion of the *post mortem* pathologist. The ovaries and uteri were examined immediately to determine:

Gravid uterine weight

Number of corpora lutea

Number and distribution of live young

Number and distribution of embryofoetal deaths

Individual foetal weight from which the litter weight was calculated

Foetal abnormalities

Embryofoetal deaths were classified as:

Early: only placenta visible at termination.

Late: both placental and embryonic remnants visible at termination.

Uteri or individual uterine horns without visible implantations were examined for evidence of implantation using a modified Salewski (Salewski 1964) technique.

Live young were examined externally and weighed. Approximately half the foetuses in each litter were preserved in Bouin's solution for subsequent free-hand section to discover visceral abnormalities by the Wilson technique (Wilson 1965); the remainder were fixed in 74 OP industrial methylated spirit for subsequent macroscopic examination, evisceration, clearing and alizarin staining by the modified Dawson technique (Dawson 1926) for skeletal examination. Young showing suspected abnormalities were processed further by the more appropriate technique for clarification of initial observations. Where considered appropriate by the foetal pathologist, foetal tissues were processed for microscopic examination. The Sponsor requested photographs of 3 foetuses with absent left kidney and ureter from the 1000 mg/kg/day group, these were sent to the Sponsor and copies retained in the HRC Archive.

All foetuses were sexed by gonadal inspection following preservation.

Foetuses were uniquely identified to allow correlation of initial with subsequent findings.

Structural changes are presented as:

Malformations: rare and/or probably lethal, eg exencephaly, anury

Anomalies: minor differences from 'normal' that are detected relatively frequently either by free-hand sectioning, eg increased renal pelvic dilatation, or at skeletal examination, eg bipartite centrum.

Variants: alternative structures occurring regularly in the control population are classified as variants eg unossified sternbra(e).

ASSESSMENT OF RESULTS

Individual litter values

In assessing litter parameters, pre-implantation loss was calculated as a percentage from the formula:

$$\frac{(\text{No. of corpora lutea} - \text{no. of implantations})}{\text{No. of corpora lutea}} \times 100$$

Post implantation loss was similarly calculated from the formula:

$$\frac{(\text{No. of implantations} - \text{no. of live young})}{\text{No. of implantations}} \times 100$$

Litter weight and mean foetal weight were calculated from individual foetal weight.

Sex ratios at Day 20 were calculated as a percentage from the formula:

$$\frac{(\text{No. of live males})}{\text{Total no. of live young}} \times 100$$

Group values

Parent females - Group mean values for bodyweights, bodyweight change and food consumption were calculated from individual values using all data from animals with live young at Day 20.

Litter data - Group mean values calculated from individual litter values are presented to include data from all animals with live young at Day 20. All derived values (eg means, percentages) were calculated within the litter and the group value derived as a mean of the individual litter values.

STATISTICAL ANALYSIS

Significance tests, employing analysis of variance followed by an intergroup comparison with the control, were performed on the following parameters and results are presented in relevant tables of this report:

bodyweight change, mean food consumption, litter data, sex ratio and foetal abnormalities and variants.

Depending on the heterogeneity of variance between treatment groups, parametric tests, analysis of variance (Snedecor and Cochran 1967) followed by Williams' test (Williams 1981/2) or non-parametric tests, Kruskal-Wallis (Hollander and Wolfe 1973) followed by Shirley's test (Shirley 1977) were used to analyse these data, as appropriate.

For litter data and foetal changes the basic sample unit was the litter and, due to the preponderance of non-normal distributions, non-parametric analyses were routinely used.

Analysis of foetal abnormalities was performed using a trend test on the number of litters affected, followed by a one-tail 2 sample permutation test (Edgington 1980; Franck 1986).

All significant (ie $p \leq 0.05$) intergroup differences from the control are reported only where supported by a significant analysis of variance ($p \leq 0.05$).

Where 75% or more of the values for a given variable were the same, a Fisher's exact test (Fisher 1950) was used.

LOCATION OF STUDY RECORDS

All specimens, raw data and study-related documents generated during the course of the study at HRC, together with a copy of the final report, are lodged in the Huntingdon Research Centre Ltd Archive.

Such specimens and records will be retained for a minimum period of five years from the date of issue of the final report. At the end of the five-year retention period the Client will be contacted and advice sought on the future requirements. Under no circumstances will any item be discarded without the Client's knowledge.

PROCEDURES

The procedures used during this study were those documented in the relevant HRC Procedure Manuals.

DEVIATIONS FROM PROTOCOL

There were no deviations from protocol considered to have affected the integrity of the study.

Animal weight range on arrival at HRC (196 - 259 g) was outside the range of 200 - 220 g specified in the protocol.

RESULTS

ANALYTICAL CHEMISTRY

Data are presented in the **FORMULATION ANALYSIS REPORT**.

The mean achieved concentrations of sodium bromide in formulations prepared on the first and last days of treatment were within 8% of nominal concentrations.

PARENT FEMALES

Clinical signs and mortality (Tables 1 and 2, Appendix 1)

Treatment at 1000 mg/kg/day was associated with unsteady gait in all animals; this sign was first apparent following administration of the second dose on Day 7 *post coitum*. Thereafter, all animals showed this sign at daily examination prior to dosing on Days 8 to 11 inclusive and, after dosing on Days 8 to 15. From Day 11, although most animals showed unsteady gait prior to dosing on Days 12 and 13, the incidence was lower on Days 14 and 15, with only 12/24 animals showing this sign prior to dosing on Day 15. Although the final dose was administered on Day 15 *post coitum*, unsteady gait was apparent for 8/24 animals on Day 16 and 2/24 animals on Day 17.

As the treatment period progressed, additional abnormalities of movement became apparent: all animals showed feet falling through the cage grid floor during ambulation on at least one occasion, and 23 animals showed poorly coordinated movements on at least one occasion. Both of these signs were first apparent after dosing on Day 9 *post coitum*. In both cases, there was a striking difference in the incidence of affected animals prior to dosing as opposed to after dosing: although only 1 or 2 animals showed these signs prior to dosing up to 24 animals showed feet falling through the cage grid floor during ambulation after dosing, and up to 16 animals showed poorly coordinated movements. It was noted that the incidence of poorly coordinated movements was highest towards the end of the treatment period (Days 13 to 15). Following administration of the final dose on Day 15, these signs were not apparent on Days 16 to 20.

Treatment at 1000 mg/kg/day was also associated with reduced bodytone in all animals; this sign was first apparent following administration of the second dose on Day 7 *post coitum*. During Days 8 to 15, there was a clear difference in the incidence of affected animals prior to dosing as opposed to after dosing. This difference was most pronounced towards the end of the treatment period (Days 13 to 15), when only 1 to 4 animals were affected prior to dosing in contrast to 18 to 24 animals after dosing. Although the final dose was administered on Day 15, reduced bodytone was apparent for 3/24 animals on Day 16 and 1/24 animals on Day 17.

Treatment at 1000 mg/kg/day was also associated with hair loss; 22/24 animals showed hair loss compared with 0/25 controls. In all cases, the hair loss was first noted between Day 14 and Day 19 *post coitum* and in 7 animals was apparent on Day 20 *post coitum*.

Occasional instances of increased lacrimation, brown staining of fur, periorbital staining and wet staining around the urogenital region were also observed at 1000 mg/kg/day.

No clinical signs considered to be attributable to treatment were observed at 100 or 300 mg/kg/day.

There was one mortality on the study and this was considered to be related to treatment. Animal no. 99, receiving 1000 mg/kg/day, was sacrificed for humane reasons prior to dosing on Day 11 of pregnancy. Prior to sacrifice, reduced bodytone, unsteady gait, red periorbital staining, brown staining of fur, poorly coordinated movements, increased lachrymation, wet urogenital staining and bodyweight loss were evident. *Post mortem* examination failed to establish any obvious cause for the physical condition. This is clear evidence that this animal showed a more severe response to treatment compared with the rest of the animals treated at 1000 mg/kg/day, all of which survived to termination: a loss of 19 g of bodyweight was recorded during Days 6 to 8 of pregnancy. This was double the greatest loss (9 g) recorded among animals surviving to termination. In addition, poorly coordinated movements were observed earlier for animal no. 99 (after dosing on Day 9) than in animals surviving to termination (first observed after dosing on Day 10).

Bodyweight change (Figure 1, Table 3, Appendix 2)

At 1000 mg/kg/day, mean bodyweight gain during the first six days of treatment was significantly lower than in controls. Thereafter, mean bodyweight gains during Days 12 to 16 were comparable to the controls. However, bodyweight gains during Days 16 to 20 was significantly lower than in controls.

At 300 mg/kg/day, bodyweight gain throughout Days 6 to 16 was comparable to the controls. However as at 1000 mg/kg/day, bodyweight gain during Days 16 to 20 was significantly lower than in controls.

At 100 mg/kg/day, there were no obvious adverse effects on bodyweight gain.

Food consumption and efficiency of food utilisation (Tables 4 and 5, Appendix 3)

At 1000 mg/kg/day, food consumption was higher than in controls during the first four days of treatment (differences attained statistical significance for Days 8 and 9), despite the fact that bodyweight gains were significantly lower than in controls during this period. This was reflected in higher food conversion ratios during this period, indicative of impaired efficiency of food utilisation. Food consumption at this dosage was noticeably higher than in controls during Days 14 to 15 and lower during Days 18 to 19.

At 100 or 300 mg/kg/day, there were no adverse effects on food consumption or food utilisation.

Macroscopic pathology (Appendix 1)

Other than the previously mentioned increased incidence of hair loss in the 1000 mg/kg/day group compared with controls, the incidence of findings noted at macroscopic *post mortem* examination did not indicate any obvious adverse effect of treatment.

LITTER PARAMETERS (Tables 1 and 6, Appendices 4 and 5)

One female receiving 100 mg/kg/day showed total litter loss *in utero* (total resorption). In view of the absence of similar findings at higher dosages this finding is considered to be co-incident and unrelated to treatment.

The following assessment is based on the 23, 21, 24 and 22 females with live young at Day 20 sacrifice in Groups 1 to 4 respectively.

There were no obvious adverse effects of treatment on any of the litter parameters, recorded during the study.

DETAILED EXAMINATION OF FOETAL MORPHOLOGY**Skeletal and visceral malformations** (Tables 7 and 8, Appendices 6 and 7)

There were 5/324, 0/295, 5/315 and 16/289 malformed foetuses (3/23, 0/21, 4/24 and 8/22 litters affected) in Groups 1 to 4 respectively.

The higher incidence of litters and foetuses showing malformations at 1000 mg/kg/day was considered to be treatment-related. These malformations were principally visceral malformation affecting the uro-genital system (*ie*, absent left kidney and/or ureter, absent or narrow left uterine horn), and thoracic skeletal malformations manifest as abnormalities of the ribs. No similar malformations were observed in the controls.

At 300 or 100 mg/kg/day, the type and incidence of malformations did not indicate any adverse effect of treatment.

Skeletal anomalies and variants (Tables 7, 10 and 11, Appendices 6 to 8)

At 1000 mg/kg/day, the incidence and distribution within litter of foetuses with skeletal anomalies was significantly different from that of Controls. Minimally distorted ribs were seen in 8 foetuses in 7 litters (another 5 foetuses in 4 litters showed more severe rib abnormalities and were classified as malformed), and there was an increased incidence of foetuses/litters showing irregular ossification of the thoracic vertebral centra, and shortened/absent 13th ribs. The latter finding being corroborated by a complete absence of foetuses showing supernumerary ribs, a highly unusual incidence. There was also a statistically significant increase in the percentage of foetuses with unossified and/or reduced sternbrae. In addition to these thoracic changes, the incidence of foetuses/litters with reduced ossification of the cranial centres was higher than in controls.

At 300 mg/kg/day, the incidence and distribution within litters of foetuses with skeletal anomalies was also statistically significantly different from that of the controls. The difference was principally due to an increased incidence of foetuses with reduced ossification. In addition, there was a slightly higher percentage incidence of foetuses with variant sternbrae, principally due to an increase in unossified sternbrae. It was noted that only one foetus showed supernumerary ribs. In view of the effects observed at 1000 mg/kg/day, these differences are considered to be related to treatment.

At 100 mg/kg/day the type, incidence and distribution of skeletal anomalies and the percentage incidence of supernumerary ribs and variant sternabrae did not indicate any obvious adverse effects of treatment.

Visceral anomalies (Tables 7 and 9, Appendices 6 and 7)

The type, distribution and incidence of visceral anomalies did not indicate any obvious adverse effects of treatment.

DISCUSSION

Treatment of the parent female at 1000 mg/kg/day was associated with clear signs of maternal toxicity principally manifest as a lower rate of bodyweight gain during Days 6 to 12 of pregnancy, abnormalities of gait, reduced bodytone and poorly coordinated movements. Detailed examination of foetal morphology revealed a higher incidence of foetuses/litters showing absent left kidney, absent left ureter, absent/narrow left uterine horn, distorted ribs, shortening/absence of 13th ribs, irregular ossification of the thoracic vertebral centra, reduced and/or unossified sternbrae and, reduced ossification of one or more cranial centres, than in the controls group. Although it was noted that seven foetuses in one litter had no left kidney and ureter (three of these foetuses also had an absent or narrow left uterine horn), these abnormalities were also apparent for one foetus in each of two further litters. In addition, one foetus in another litter had a small left kidney, absent left ureter and a markedly narrow left uterine horn. Therefore, an association with treatment is considered likely, since the litter and not the foetus is the principal unit of assessment. It is noteworthy that there was no obvious reduction in mean foetal weight. Some of the observed skeletal abnormalities may reflect effects on maternal bodyweight gain and food consumption. In contrast, the defects observed in the urogenital system are extremely rare in our experience and are considered more likely to reflect a selective effect on embryofoetal development than a secondary effect resulting from toxicity to the parent female.

At 300 mg/kg/day, no adverse effects on the parent female were observed during the treatment period. However, following the withdrawal of treatment, bodyweight gain was statistically significantly lower than in controls. This effect was also recorded at 1000 mg/kg/day on the present study and, on the preliminary study (DSB/80). This is circumstantial evidence that the lower rate of bodyweight gain following the withdrawal of treatment reflects an earlier effect during the dosing period, which has not been detected within the context of this screening study. Detailed examination of foetal morphology revealed a higher incidence of foetuses showing reduced ossification of various components of the skeleton compared with controls. It is noteworthy that there was no obvious reduction in mean foetal weight.

At 100 mg/kg/day, there was no observable maternal response to treatment and no obvious adverse effects on morphological development of the conceptus.

CONCLUSION

Within the context of this study, it is concluded that the no effect level for the parent female and *in utero* development of the conceptus is 100 mg/kg/day.

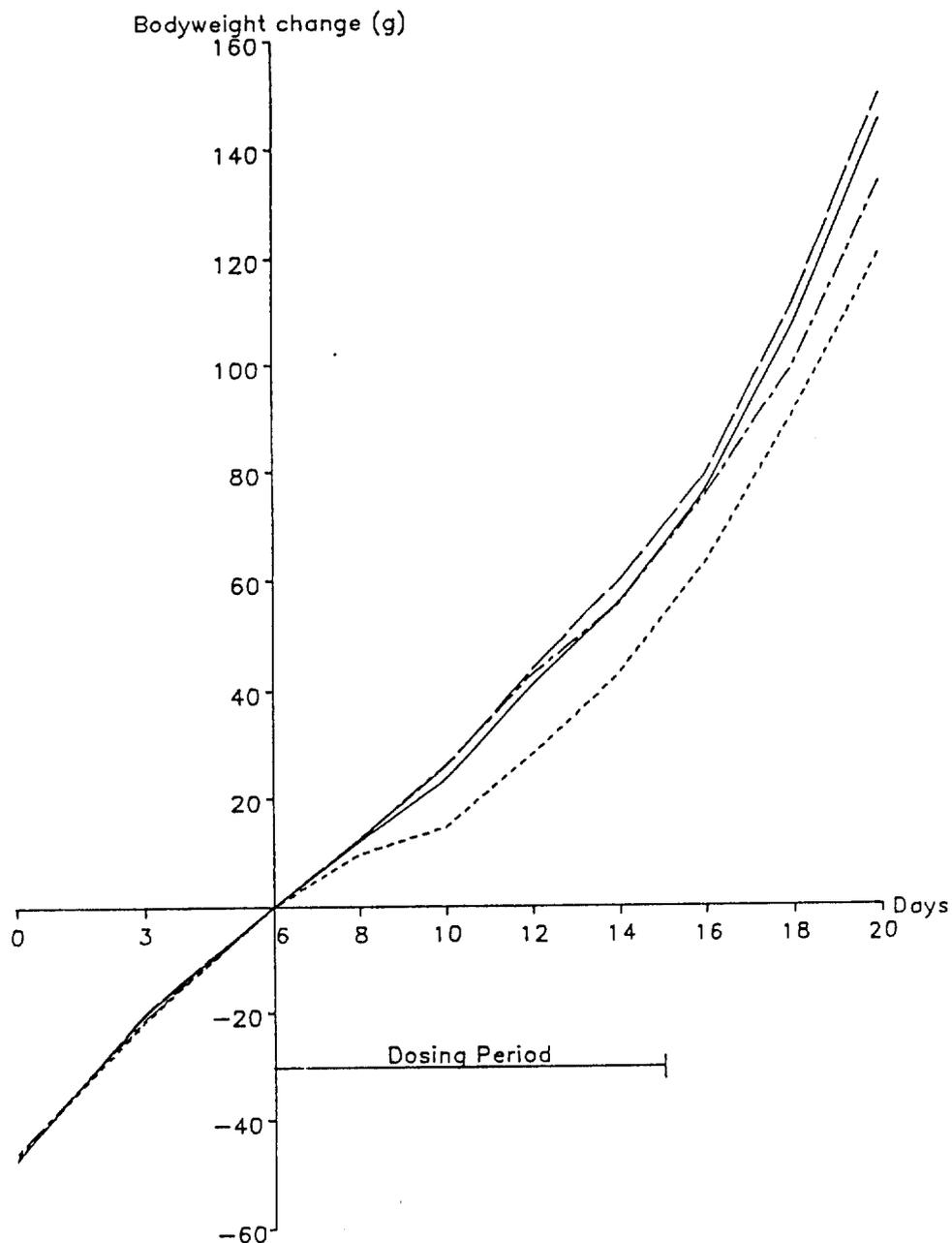
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FIGURE 1

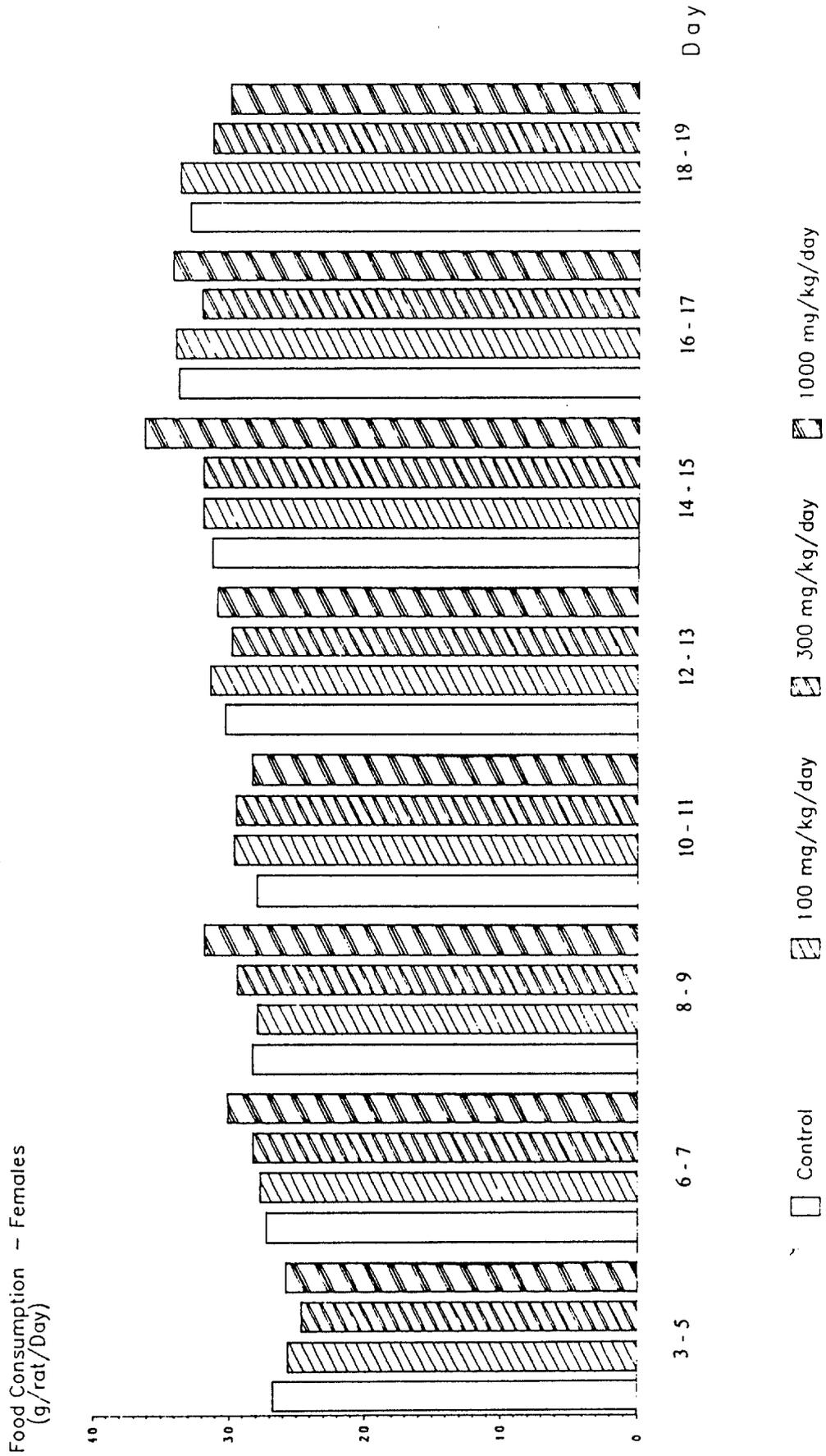
Bodyweight change during pregnancy - dams with live young - group mean values

- Control
- 100 mg/kg/day
- 300 mg/kg/day
- 1000 mg/kg/day



Bodyweight changes calculated from Day 6 of pregnancy

FIGURE 2
Food consumption during pregnancy - group mean values



Treatment period: Days 6 to 15 of pregnancy inclusive

TABLE 1
Summary of adult performance

Category	No. of animals in group/dosage (mg/kg/day)			
	1 Control	2 100	3 300	4 1000
Mated	25	25	25	25
Killed (Day 11 of pregnancy)	0	0	0	1
Non-pregnant	2	3	1	2
Total litter loss <i>in utero</i>	0	1	0	0
With live young at Day 20	23	21	24	22

TABLE 2

Daily incidence of principal clinical signs at 1000 mg/kg/day

Day <i>post coitum</i>	Number of animals showing:			
	Unsteady gait	Feet falling through cage grid floor during ambulation	Poorly coordinated movements	Reduced bodytone
	(25)	(25)	(25)	(25)
6 pre-dose post-dose				
7 pre-dose post-dose	25			2
8 pre-dose post-dose	25 25			3 19
9 pre-dose post-dose	25 25	5	1	6 20
10 pre-dose post-dose	25 25	13	6	10 24
11 pre-dose post-dose*	25 24	2 10	1 3	6 16
12 pre-dose post-dose	20 24	2 18	1 4	9 18
13 pre-dose post-dose	23 24	1 19		3 24
14 pre-dose post-dose	17 24	14	10	4 21
15 pre-dose post-dose	12 24	22	16	1 18
16	8			3
17	2			1
18				
19				
20				

Treatment period: Days 6 to 15 inclusive

() Number of animals examined

* Since animal no. 99 was killed prior to dosing on Day 11, the number of animals examined after Day 11 pre-dose was 24

No instances of unsteady gait, feet falling through the cage grid floor during ambulation, poorly coordinated movements or reduce bodytone were apparent among control animals or, animals treated at 100 or 300 mg/kg/day

TABLE 3

Bodyweights and bodyweight change during pregnancy - dams with live young
- group mean values (g)

Group/ dosage (mg/kg/day)	No. of animals	Bodyweight (g) at Day of pregnancy									
		0	3	6	8	10	12	14	16	18	20
1 Control	23	222.7	249.9	269.7	281.8	293.4	310.9	325.5	346.0	376.5	414.9
2 100	21	221.1	248.4	268.0	280.5	294.1	312.0	327.9	347.4	379.1	417.9
3 300	24	224.2	249.0	269.8	282.5	296.2	312.8	325.5	345.3	369.3	403.7
4 1000	22	223.4	248.5	269.7	279.6	284.5	298.1	312.9	333.0	360.1	390.7

Group/ dosage (mg/kg/day)	No. of animals	Bodyweight change (g) from Day 6 of pregnancy									
		0	3	6	8	10	12	14	16	18	20
1 Control	23	-47.0	-19.8	0.0	12.1	23.7	41.2	55.8	76.3	106.8	145.2
2 100	21	-46.8	-19.5	0.0	12.6	26.2	44.0	59.9	79.4	111.1	149.9
3 300	24	-45.6	-20.8	0.0	12.8	26.5	43.0	55.7	75.6	99.5	133.9
4 1000	22	-46.3	-21.2	0.0	9.9	14.9	28.4	43.2	63.3	90.4	121.0

Group/ dosage (mg/kg/day)	No. of animals	Bodyweight change (g) during Days of pregnancy			
		0 - 6	6 - 12	12 - 16	16 - 20
1 Control	23	47.0	41.2	35.1	68.8
2 100	21	46.8	44.0	35.4	70.5
3 300	24	45.6	43.0	32.5	58.3
4 1000	22	46.3	28.4	34.9	57.7

Treatment period: Days 6 to 15 of pregnancy inclusive

Statistical analysis of bodyweight change:

* $p \leq 0.05$, ** $p \leq 0.01$

TABLE 4

Food consumption during pregnancy - dams with live young - group mean values (g/rat/day)

Days of pregnancy	Group/dosage (mg/kg/day)			
	1 Control	2 100	3 300	4 1000
	(23)	(21)	(24)	(22)
3 - 5	27	26	25	26
6 - 7	27	28	28	30
8 - 9	28	28	29	32*
10 - 11	28	30	29	28
12 - 13	30	31	30	31
14 - 15	31	32	32	36**
16 - 17	34	34	32	34
18 - 19	33	34	31	30**

Treatment period: Days 6 to 15 of pregnancy inclusive

() Number of dams with live young

Statistical analysis of food consumption:

* $p \leq 0.05$, ** $p \leq 0.01$

TABLE 5

Food conversion ratios during pregnancy - dams with live young - group mean values

Days of pregnancy	Group/dosage (mg/kg/day)			
	1 Control	2 100	3 300	4 1000
	(23)	(21)	(24)	(22)
3 - 5	4.0	3.9	3.6	3.6
6 - 7	4.5	4.4	4.4	6.1
8 - 9	4.9	4.1	4.3	12.8
10 - 11	3.2	3.3	3.6	4.1
12 - 13	4.1	3.9	4.7	4.2
14 - 15	3.0	3.3	3.2	3.6
16 - 17	2.2	2.1	2.7	2.5
18 - 19	1.7	1.7	1.8	2.0

Treatment period: Days 6 to 15 of pregnancy inclusive

() Number of dams with live young

Food conversion ratio = food consumption/bodyweight gain

TABLE 6

Litter data - group values

Group Dosage (mg/kg/day)		1 Control	2 100	3 300	4 1000
Dams with live young					
No. of litters		23	21	24	22
Group mean values					
No. of corpora lutea		16.2	15.4	15.3	15.7
No. of implantations		14.9	14.6	14.0	14.1
No. of <i>in utero</i> deaths:					
- early		0.6	0.6	0.7	0.8
- late	F	0.2	0.0	0.1	0.2
- early and late		0.8	0.6	0.8	1.0
No. of live young		14.1	14.0	13.1	13.1
Litter weight (g)		53.69	55.00	50.52	49.00
Foetal weight (g)		3.81	3.92	3.84	3.75
Gravid uterine weight (g)		81.48	81.88	75.35	74.46
Sex ratio (% males)		48.0	54.0	47.5	48.0
Litter incidence#	'n'				
<i>in utero</i> deaths: early					
	0	13	12	13	10
	1	7	6	6	8
	2	2	3	4	3
	3	1		1	1
<i>in utero</i> deaths: late					
	0	19	21	21	19
	1	4		3	2
	2				1
<i>in utero</i> deaths: early and late					
	0	10	12	11	9
	1	10	6	8	7
	2	2	3	4	4
	3				2
	4	1		1	

No. of litters with 'n' *in utero* deathsStatistical analysis of mean values: Not significant ($p \geq 0.05$)

F Fisher's exact test

TABLE 7

Foetal abnormalities - prevalence and distribution in litters

Category	No. of affected fetuses/litter (n)	Group/dosage (mg/kg/day)			
		11	2	3	4
		Control	100	300	1000
		No. of litters with 'n' fetuses affected			
Number of litters examined		23	21	24	22
Malformation	0	20	21	20	14
	1	2		3	5
	2			1	2
	3	1			
	7				1
Visceral anomaly	0	11	10	16	14
	1	11	6	7	3
	2	1	4	1	3
	3		1		1
	4				1
Skeletal anomaly	0	12	12	*	**
	1	6	4	4	3
	2	2	3	6	5
	3	1	2	6	3
	4	2		1	5
	5			1	
	6				2

Statistical significance * $p \leq 0.05$, ** $p \leq 0.01$

Mean % fetuses affected per litter

Malformations	1.7	0.0	1.7	5.3
Visceral anomalies	7.8	11.4	5.7	13.2
Skeletal anomalies	13.9	11.9	29.1	40.6

TABLE 8

Skeletal and visceral malformations - incidence summary

Group	Foetuses				Litters			
	1	2	3	4	1	2	3	4
Dosage (mg/kg/day)	Control	100	300	1000	Control	100	300	1000
No. examined	324	295	315	289	23	21	24	22
No. affected	5	0	5	16	3	0	4	8
REGION/Description	Incidence							
CRANIAL								
Anophthalmia	-	-	1	1	-	-	1	1
THORACIC								
Distorted/minimally distorted/ossification irregularities ribs	-	-	-	5	-	-	-	4
Multiple sternebral/costal cartilage abnormalities	-	-	1	-	-	-	1	-
Duplicated inferior vena cava	1	-	-	-	1	-	-	-
LUMBAR/ABDOMINAL								
Umbilical hernia	-	-	1	-	-	-	1	-
Absent kidney	-	-	-	9 ^{b,j}	-	-	-	3
Absent ureter	-	-	-	10 ^{a,j}	-	-	-	4
Small kidney	-	-	-	1 ^a	-	-	-	1
Absent uterine horn	-	-	-	2 ^{f,g}	-	-	-	2
Narrow uterine horn	-	-	-	5 ^{a-e}	-	-	-	3
Small indeterminate gonad	-	-	-	2 ^{b,c}	-	-	-	2
SACROCAUDAL								
Interrupted vertebral column	1	-	-	-	1	-	-	-
OTHER								
Squat foetus syndrome	3	-	2	-	1	-	1	-

Superscripts indicate findings common to one foetus

TABLE 9

Visceral anomalies - incidence summary

Group	Foetuses				Litters			
	1	2	3	4	1	2	3	4
Dosage (mg/kg/day)	Control	100	300	1000	Control	100	300	1000
No. examined #	160	146	156	136	23	21	24	22
No. affected #	13	17	9	16	12	11	8	8
REGION/Description	Incidence*							
Subcutaneous haemorrhage:								
cranium	-	-	-	1	-	-	-	1
trunk	1	-	1	-	1	-	1	-
CRANIAL								
Haemorrhages affecting:								
brain	3	5	-	2	3	5	-	2
eyes	-	-	1	-	-	-	1	-
CERVICAL								
Small thyroid	-	-	-	1	-	-	-	1
Displaced oesophagus	-	-	-	1	-	-	-	1
THORACIC								
Interventricular septal defect (small)	2	2	3	-	2	2	3	-
Partially duplicated inferior vena cava	1	-	-	-	1	-	-	-
LUMBAR/ABDOMINAL								
Liver: abnormal lobation	2	3	3	4	2	3	3	4
haemorrhage within lobe	1	1	-	2	1	1	-	1
Intra-abdominal haemorrhage	1	2	-	3	1	2	-	2
Dilated renal pelvis/ureter	1	3	1	2	1	2	1	2
Displaced testis(es)	1	2	-	4	1	2	-	4
Dilated umbilical vein, minimal	-	-	-	1	-	-	-	1

* Individual foetuses may occur in more than one category

Excludes malformed foetuses

TABLE 10

Skeletal anomalies - incidence summary

Group	Foetuses				Litters			
	1	2	3	4	1	2	3	4
Dosage (mg/kg/day)	Control	100	300	1000	Control	100	300	1000
No. examined #	159	149	154	137	23	21	24	22
No. affected #	21	16	43	54	11	9	18	18
REGION/Description	Incidence*							
Reduced ossification of:								
one or more cranial centres	7	5	22	28	4	3	13	13
cervical vertebral arches	1	1	-	-	1	1	-	-
sacrocaudal vertebral arches	6	5	19	8	4	4	10	6
one or more centres pelvic girdle	2	2	7	1	2	2	7	1
digital centres	1	1	6	1	1	1	5	1
CRANIAL								
Sutural bone(s)	1	-	4	2	1	-	4	2
CERVICAL								
Cervical rib(s)	4	-	2	3	2	-	2	2
THORACIC								
Irregular ossification vertebral centra	5	6	6	12	3	5	5	8
Distorted rib(s), minimal	-	-	-	8	-	-	-	7
Shortened/absent 13 th rib(s)	-	1	1	8	-	1	1	6
Misshapen sternebra(e)	-	1	1	-	-	1	1	-
LUMBAR/ABDOMINAL								
Irregular ossification vertebral elements	1	-	-	-	1	-	-	-
OTHER								
One less thoracolumbar vertebra	-	-	1	-	-	-	1	-
OBSERVATIONS AT AUTOPSY / EVISCERATION								
Minimally protruding tongue	-	-	1	-	-	-	1	-
Abnormal lobation liver	-	2	-	1	-	2	-	1

* Individual foetuses may occur in more than one category

Excludes malformed foetuses

TABLE 11
Skeletal variants - group values

Group/ dosage (mg/kg/day)	Foetuses examined#	Foetuses with													
		13 Ribs		14 Ribs		Normal sternbrae		Unossified sternbrae		Reduced sternbrae		Asym./Bip. sternbrae		Total variant sternbrae	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1♀ Control	159	F 146	92.5	13	7.5	F 94	58.6	45	27.9	26	17.9	1	0.4	65	41.4
2♀ 100	149	130	88.1	19	11.9	78	51.9	40	27.1	38	25.9	5	3.7	71	48.1
3♀ 300	154	153	99.4	1	0.6	64	42.9	62	40.0	37	23.0	5	3.0	90	57.1
4♀ 1000	137	* 137	100.0	0	0.0	** 27	20.3	89	63.3	51	38.8	5	2.8	110	79.7

Excludes malformed foetuses
 Statistical analysis of % affected: * $p \leq 0.05$, ** $p \leq 0.01$
 F Fisher's exact test applied