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8EHQ-98-14279

Dear Sir or Madam:

Subject: Results of a Rat Oral Developmental Toxicity Study

In accordance with the reporting requirements of TSCA Section 8(e), Ticona, a member of the Hoechst Group, hereby submits the results of a rat oral developmental toxicity study on 1,3,5 Trioxane (CAS# 110-88-3).

This study shows that this substance causes developmental effects in laboratory animals. A copy of the study is enclosed (Report Number 97.0791 by HMR Deutschland GmbH). The study was jointly sponsored by Ticona GmbH and Ultraform GmbH.

The commercial use of this substance is as a monomer for polyacetal resins, a solid fuel, in paint stripper formulations and in special adhesive systems.

This submission contains no confidential business information.

If any further information is required, please contact Dr. Gerald Kirshenbaum, Manager, Product Safety at 908-522-7662.

Sincerely,

Richard G. Hanlon 

Richard G. Hanlon
Vice President
Environmental, Health and Safety Affairs

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Ticona - A member of the
Hoechst Group

Trioxan

Rat oral developmental toxicity (teratogenicity) study

| | |
|--------------------------------|---|
| Author: | Dr. Th. Hofmann |
| Report number: | 97.0791 |
| Report completion date: | 12 March 1998 |
| Study number: | 97.0354 |
| Artemis Number: | RR0792 |
| Origin of report: | Hoechst Marion Roussel Global Preclinical Development Germany Drug Safety 65926 Frankfurt am Main GERMANY |

CONFIDENTIALITY STATEMENT

This report is confidential. No part of the report or any information contained herein may be disclosed to any party without the written authorisation of Ultraform GmbH and Ticona GmbH

CONTENTS

| | |
|--|-----------|
| 1. SUMMARY | 5 |
| 2. STATEMENTS | 7 |
| 2.1 GLP Compliance Statement..... | 7 |
| 2.2 Quality Assurance Statement..... | 8 |
| 2.3 Signatures of the Authors | 9 |
| 3. INTRODUCTION | 10 |
| 3.1 Objective | 10 |
| 3.2 Guidelines | 11 |
| 3.3 Quality Assurance | 11 |
| 3.4 Survey..... | 12 |
| 3.5 Responsibilities | 13 |
| 4. MATERIAL AND METHODS..... | 14 |
| 4.1 Test compound | 14 |
| 4.2 Test species and animal husbandry..... | 15 |
| 4.3 Test groups | 15 |
| 4.4 Test procedure..... | 16 |
| 4.5 Observations and measurements | 16 |
| 4.6 Caesarean section and post mortem examinations | 16 |
| 4.7 Data processing and statistics | 17 |
| 5. RESULTS..... | 18 |
| 5.1 Maternal data | 18 |
| 5.1.1 Mortality and clinical observations | 18 |
| 5.1.2 Body weight and food consumption..... | 18 |
| 5.1.3 Necropsy findings | 19 |
| 5.1.4 Caesarean section data..... | 19 |
| 5.2 Foetal data | 19 |
| 5.2.1 Findings at caesarean section..... | 19 |
| 5.2.2 External, skeletal and visceral examination..... | 20 |
| 6. DISCUSSION AND CONCLUSION | 23 |
| 7. REFERENCES | 25 |

| | | |
|----------|---|-----------|
| 8 | APPENDIX..... | 26 |
| 8.1 | Figures..... | 26 |
| 8.1.1 | Body weight development..... | 26 |
| 8.1.2 | Food consumption..... | 27 |
| 8.2 | Summary tables and statistics..... | 28 |
| 8.2.1 | Clinical observations..... | 28 |
| 8.2.2 | Body weight development..... | 30 |
| 8.2.3 | Food consumption..... | 32 |
| 8.2.4 | Gestation and caesarean section data..... | 33 |
| 8.2.5 | Gravid uterus weights..... | 36 |
| 8.2.6 | Necropsy findings..... | 37 |
| 8.2.7 | External / visceral defects obtained at autopsy..... | 38 |
| 8.2.8 | Skeletal defects..... | 40 |
| 8.2.9 | External / visceral defects obtained at body cross-section examination..... | 46 |
| 8.2.10 | Morphological findings in dead fetuses..... | 48 |
| 8.2.11 | Explanation of symbols..... | 49 |
| 8.3 | Individual data..... | 50 |
| 8.3.1 | Clinical observations..... | 50 |
| 8.3.2 | Body weight development..... | 61 |
| 8.3.3 | Food consumption..... | 69 |
| 8.3.4 | Caesarean section data..... | 73 |
| 8.3.5 | Gravid uterus weights..... | 77 |
| 8.3.6 | Necropsy findings..... | 85 |
| 8.3.7 | Foetal data..... | 101 |
| 8.3.8 | Explanation of symbols..... | 272 |
| 8.4 | Additional data..... | 273 |
| 8.4.1 | List of terms used in the tables..... | 273 |
| 8.4.2 | Normal ranges of the rat strain used..... | 274 |
| 8.4.3 | Composition of diet..... | 286 |
| 8.5 | Reports included..... | 287 |
| 8.5.1 | Concentration and homogeneous distribution of the test compound..... | 287 |

1. SUMMARY

Introduction

The purpose of this study was to assess the effects of oral administration of Trioxan on pregnancy and embryofetal development of the rat. It was conducted referring to international testing guidelines.

Methods

Groups of 23 mated female Wistar rats received Trioxan as 20% aqueous solution by oral gavage once daily at the dose levels of 0, 100, 315 or 1000 mg/kg body weight from day 7 - 20 of pregnancy (day 0: day of mating, day 1: day of sperm detection) and were sacrificed on day 21 of pregnancy.

Behaviour and state of health were observed daily in all groups. Body weight and food consumption were determined throughout the study.

At necropsy the dams were examined for macroscopically visible changes. Gravid uterus weight was recorded. The uterus was opened and the number of live and dead foetuses and the number of conceptuses undergoing resorption were determined. Body weights, crown-rump lengths, sex ratios of the foetuses and placental weights were determined and external, visceral and skeletal examinations of the foetuses performed.

Results

There were no deaths during the study. No clinical signs were observed in any of the animals.

Body weight gain and food consumption were slightly decreased in the animals from the high dose group during the treatment period. Body weights and food consumption were not affected by the administration of the test compound in the animals from the low and intermediate dose group. However, body weight gain between implantation and caesarean section corrected by gravid uterus weight (corrected body weight gain) was decreased in all groups treated with Trioxan.

No compound-related effects were observed at necropsy of the animals.

Gravid uterus weights, litter size and foetal sex ratios remained unaffected by the administration of the test compound. Foetal body weights and crown-rump lengths were decreased in the high dose group, whereas placental weights were increased. Incidence of early conceptuses undergoing resorption was not altered by the administration of the test compound. Five dead foetuses were observed in five litters from the high dose group.

In the high dose group, morphological examination of the foetuses revealed two cases with aplasia of the tail accompanied by aplasia of sacral vertebral arch and sacral vertebral centres. The incidences of foetuses with displaced, fused or fragmented sternbrae, wavy or thickened ribs, bent or shortened scapulae, bent, shortened or dysplastic humerus, bent or shortened ulna and bent radius were increased. Additionally, retarded ossification was observed in numerous bones.

The fetuses from the intermediate dose group showed increased incidences of wavy or thickened ribs and retarded ossification of individual skull bones and caudal vertebral centres.

No compound-related effects were observed by morphological examination of the fetuses from the low dose group.

Conclusion

Repeated oral administration of Trioxan to pregnant rats at the dose level of 1 000 mg/kg body weight caused maternal toxicity, as shown by slightly decreased body weight and food consumption. Corrected body weight gain was markedly decreased. Five dead fetuses were observed. Two fetuses showed aplasia of the tail. The fetuses were retarded and showed increased incidences of minor defects at sternbrae, ribs, scapula and forelimb.

Corrected body weight gain was still decreased at the daily dose levels of 315 and 100 mg/kg body weight per day, thus possibly indicating some maternal toxicity.

At the dose of 315 mg/kg body weight per day, retarded ossification of the fetuses was still observed. Additionally, the incidence of wavy or thickened ribs was increased. Repeated administration of 100 mg Trioxan / kg body weight was tolerated by the conceptuses without signs of toxicity.

With regard to the present study the **No Observed Effect Level (NOEL)** is 100 mg/kg/day for embryofetal toxicity. Concerning maternal toxicity, a clear NOEL could not be established, since a compound related effect on corrected body weight gain can not be excluded.

2. STATEMENTS

2.1 GLP Compliance Statement

Trioxan - Rat oral developmental toxicity (teratogenicity) study

To the best of my knowledge and belief, this study was conducted in compliance with Good Laboratory Practice regulations. No unforeseen circumstances were observed which might have affected the quality or integrity of the study.

Study Director:

Th. Hofmann, 12-Mar-98
(Dr. Th. Hofmann)

Head of Department:

Dr. D. Mayer, 1998
(Dr. D. Mayer)

2.3 Signatures of the Authors

Trioxan - Rat oral developmental toxicity (teratogenicity) study

Study Director: Th. Hofmann 12-Mar-98
(Dr. Th. Hofmann)

Reproduction Toxicology: Ch. Baeder 12 March 1998
(Dr. Ch. Baeder)

Head of Department: D. Mayer 11.11.98
(Dr. D. Mayer)

Dr. TH

3. INTRODUCTION

3.1 Objective

The present study was conducted in order to determine the effects of Trioxan on embryonic and foetal development when administered orally once daily to mated female Wistar rats from day 7– 20 of pregnancy. The study should provide a rational basis for risk assessment in man.

In a previous study, Sitarek et al. (1988) examined the embryofoetal toxicity of Trioxan in Wistar rats when administered during embryogenesis and foetogenesis. They reported malformations of brain, kidneys and/or skeletal system and a significant increase of foetuses with delayed ossifications at dose levels of 0.77 g/kg and 1.55 g/kg. However, this study was poorly documented and considered to be invalid for the characterisation of the embryotoxic and foetotoxic profile of the test compound, which is necessary for risk assessment in man.

Rationale for route of exposure:

The oral route is considered to be a potential exposure route in man.

Rationale for species selection:

The rat has proved to be a suitable species for teratogenicity testing with many chemical substances and is the species of choice (rodent) according to international testing guidelines.

Rationale for dose selection:

In a dose-range-finding study groups of 4 mated Wistar rats received Trioxan as an aqueous solution at the dose levels of 500 or 1000 mg/kg per day from day 7 - 20 of pregnancy and were killed on day 21. All animals were observed daily for clinical signs whilst body weight and food consumption were recorded at regular intervals. On day 21 the uterus was opened and the number of live and dead foetuses and the number of conceptuses undergoing resorption were determined. The foetuses were examined for gross major anomalies. Fetal body weight and crown-rump length were recorded.

Two animals from the 1000 mg/kg group showed slight loss of body weight from day 7 - 10 and exhibited increased numbers of retarded (smaller than normal) foetuses at caesarean section. No compound-related effects were observed in the other animals.

Based on these results the dose levels of 0, 100, 315 and 1000 mg/kg body weight per day were selected for the present study. Testing of dose levels greater than 1000 mg/kg body weight is not necessary with regard to the Guidelines mentioned below (Limit Test).

3.2 Guidelines

The present study was conducted referring to

**OECD Guidelines for Testing of Chemicals, Section 4, Health Effects, 414
"Teratogenicity", 12 May 1981**

Guideline of EC-Commission from November 18, 1987 (88/302/EWG).

US EPA Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Humans and Domestic Animals, Series 83: Chronic and Long Term Studies, § 83-3: "Teratogenicity Study", November 1984

Japanese Ministry of Agriculture, Forestry and Fisheries. Requirements for Safety Evaluation of Agricultural Chemicals, 59 NohSan No. 4200, 28 January 1985

3.3 Quality Assurance

This study is conducted in compliance with the Principles of Good Laboratory Practice:

OECD Principles of Good Laboratory Practice, Paris, France, 1981

and in compliance with the German GLP regulations:

Grundsätze der Guten Laborpraxis (GLP), Anhang 1 zu Paragraph 19a, Absatz 1 des Chemikaliengesetzes vom 25. Juli 1994.

The following data were archived according to the standard operating procedures: test article reference sample, protocol, report, all raw data and specimens of laboratory investigations.

No unforeseen circumstances were observed which might have affected the quality or integrity of the study.

3.4 Survey

Study Number: 97.0354
Artemis Number: RR0792
Test compound: Trioxan
Sponsor: Hoechst Aktiengesellschaft, Factory Ticona
BASF Aktiengesellschaft
Type of Study: developmental toxicity (teratogenicity) study
Species/strain/sex: Rat / Wistar / female
Vehicle: deionised water
Duration of administration: day 7 - 20 of pregnancy
Start of study (day 1): June 03rd, 1997
First administration: June 09th, 1997*
Last administration: June 25th, 1997*
End of study (in life phase): June 26th, 1997
Dose levels: 0, 100, 315 and 1000 mg/kg body weight
Number of animals per group: 23 mated females per group

* The animals were allocated successively to the four groups. The groups were filled up simultaneously.

3.5 Responsibilities

| | |
|-------------------------------|---|
| Study director: | Dr. Th. Hofmann |
| Monitoring scientist: | Dr. S. Bachmann, BASF Aktiengesellschaft |
| Head of department: | Dr. D. Mayer |
| Reproduction Toxicology: | Dr. Ch. Baeder |
| Analytics: | Dr. H.- J. Pletsch |
| Statistics: | DM R. Uhl |
| Data storage: | Dr. G. Nölken |
| Quality Assurance (GLP): | S.J. Harston (Pharmacist) |
| Testing facility and archive: | Hoechst Marion Roussel Global Preclinical Development - Germany Drug Safety 65926 Frankfurt am Main GERMANY |

4. MATERIAL AND METHODS

4.1 Test compound

| | |
|--|---|
| Name: | Trioxan (as 20% aqueous solution) |
| Analytical certificate: | from February 05th, 1997 |
| Date of delivery: | 7th February 1997 |
| Appearance: | colourless liquid |
| Purity: | 99.77 % Trioxan as 20% aqueous solution |
| Expiry date: | 31st December 1999 |
| Storage: | at approximately 20 °C, protected from light |
| Vehicle: | deionised water |
| | Homogeneity and stability of the test compound in the vehicle are guaranteed (page 287). |
| Formulation: | Freshly each day. If crystals were present, they were dissolved by slight heating (maximum 40 °C in the dark). |
| Determination of the concentration of the substance in the preparations: | For each concentration, samples were taken towards the start and end of the dosing period (day 7 to 20). All samples were stored deep frozen prior to analysis. |

4.2 Test species and animal husbandry

| | |
|-------------------------|---|
| Species: | rat |
| Sex: | female |
| Strain: | Hoe: WISKf(SPF71) Wistar |
| Origin: | Hoechst AG, Kastengrund, SPF breeding colony |
| Age at start of study: | approximately 8 - 10 weeks |
| Animal maintenance: | individually in fully air-conditioned rooms in makrolon cages (type III) on soft wood granulate |
| Room temperature: | 19 - 22 °C |
| Relative humidity: | 35 - 75 % |
| Lighting time: | 12 hours daily |
| Ventilation rate: | 16 - 20 air changes per hour |
| Food: | Ssniff R-Z (V1324), ad libitum |
| Water: | tap water in plastic bottles, ad libitum |
| Animal identification: | ear tags and cage numbering |
| Acclimatisation period: | at least five days under study conditions |

4.3 Test groups

The test animals were assigned randomly (computer-generated algorithm) to the following groups:

| Group | Dosage | Animal No's |
|-----------------------|------------------------|-------------|
| 1 (control) | 0 mg/kg body weight | 701 - 723 |
| 2 (low dose) | 100 mg/kg body weight | 724 - 746 |
| 3 (intermediate dose) | 315 mg/kg body weight | 747 - 769 |
| 4 (high dose) | 1000 mg/kg body weight | 770 - 792 |

4.4 Test procedure

Mating

In the laboratory's own breeding facility, virgin female animals in the pre-oestrus or oestrus phase were mated overnight with sexually mature males in the ratio 1 male : 1 female and were caged individually after detection of sperm in vaginal smears. The day of sperm detection was defined as day 1 of gestation, and the day of mating was defined as day 0 of pregnancy. Pregnancy was confirmed at necropsy by the detection of implantation sites or normally developed corpora lutea.

Administration of the test compound

Female rats showing sperm in the vaginal smear received Trioxan at the dose levels of 0, 100, 315 or 1000 mg/kg body weight per day as a solution in deionised water orally by gavage once daily from day 7 - 20 of pregnancy. All groups received a dose volume of 5 ml/kg body weight, with adjustment of the individual volume to the most recently recorded body weight. The test compound was prepared daily, immediately before dosing.

4.5 Observations and measurements

All animals were examined before the start of the study and were shown to be in good general health condition.

The behaviour and general health condition of the animals were observed several times daily (on weekends and public holidays once daily).

Body weights were determined on days 1, 4, 7, 10, 14, 17, 19 and 21 of pregnancy, and food consumption was recorded between days 1-4, 4-7, 7-10, 10-14, 14-17, 17-19 and 19-21 of pregnancy.

4.6 Caesarean section and post mortem examinations

The animals were killed on day 21 of pregnancy and the foetuses removed by Caesarean section. All animals were examined externally and internally (thoracic and abdominal contents) for macroscopically visible changes, with emphasis on the uterus. Gravid uterus weight was determined. The live and dead foetuses in the uterus as well as the conceptuses undergoing resorption and corpora lutea were counted and examined macroscopically. The implantation sites in the uterus were counted after staining with ammonium sulphide.

The foetuses, the placentae and conceptuses undergoing resorption were removed from the uterus, weighed or measured and examined for gross external abnormalities and the crown-rump length recorded. Then the foetuses were killed by CO₂ asphyxia.

Approximately 50% of the foetuses of each litter were fixed in alcohol, necropsied, sexed and checked for anomalies of the internal organs. The carcasses were placed in a solution of potassium hydroxide for clearing and stained with alizarin red S. The skeletons were examined and checked for stage of development and abnormalities with the aid of a stereo-microscope. Foetuses found dead in the uterus at caesarean section were fixed in alcohol and examined for external anomalies.

The remaining foetuses were transferred in Bouin's solution, examined for organ anomalies referring to Wilson's slicing technique [Wilson, J.G.: Embryological considerations in teratology. In *Teratology: Principles and Techniques* (J.G. Wilson, J. Warkany, Ed.), page 251-277. University of Chicago Press, Chicago, IL (1965)] and sexed.

4.7 Data processing and statistics

All data were recorded on-line and compiled by a data processing system (ARTEMIS).

The statistical evaluation is based on the assumption of a monotone dose-response relationship. Statistical comparisons of the low dose groups with the simultaneous control group were only carried out if significant effects were detectable in the high dose group (Hothorn & Lemacher, 1991). In the univariate analysis, two-sided questions (body weight of dams, relative food consumption, crown-rump length, foetal weight and placental weight) were generally tested as follows: a two-sided comparison with the high dose group was followed by a one-sided test for the low-dose group. In case of the caesarean section data of the foetuses (crown-rump length, foetal weight and placental weight), multivariate statistics were first of all calculated and used in selecting relevant dose groups. For the individual parameters, sequential comparisons with the high dose group (Hochberg, 1988) and sequential tests at the 5% level (Hothorn & Lemacher, 1991) for the low dose were then conducted.

The t-tests and the test statistics of Wilks (Hartung & Elpelt, 1984) are based on common variance estimations for all study groups. For the Wilcoxon test (Hollander & Wolfe, 1973) the exact distribution (Streitberg & Röhmel, 1987) of the meaned ranks was calculated.

In the case of the daily food consumption of the dams, the mean consumption per 100 g body weight was always calculated between two successive measurement times and evaluated by the rank sum test after Wilcoxon. In examining the body weights of the dams, the change in weight was determined in comparison to the initial weight. The univariate evaluation was carried out using t-tests.

The caesarean section data of the foetuses were used to calculate litter mean values. Multivariate evaluation was carried out using the test statistics of Wilks. In the univariate analysis, t-tests were used.

The number of corpora lutea, implantation sites and live foetuses, and quotas of dead embryonic primordia undergoing resorption in the animals were likewise analysed using one-sided Wilcoxon tests.

The findings obtained at autopsy and at body cross-section and skeletal examination of the fetuses were evaluated separately for the fetuses and for the litters by Fisher's Exact test (Fisher, 1935) at significance levels of 5% and 1%. It was examined whether the relative frequencies of findings in the dose groups deviated from those findings in the control group.

In addition, frequencies of findings obtained at autopsy, at body cross-section and skeletal examination of the fetuses were compared with those of corresponding findings in previous control groups.

5. RESULTS

5.1 Maternal data

5.1.1 Mortality and clinical observations

There were no deaths during the study. No clinical signs were observed in any of the animals.

(Group incidence tables page 28 - 29, Individual data page 50 - 60).

5.1.2 Body weight and food consumption

Body weight gain and food consumption was slightly, but statistically significantly decreased in the animals from the high dose group from day 10 until the end of the study, the effects on body weight mainly being caused by a stagnation between days 7 and 10.

Corrected body weight gain (body weight on day 21 minus body weight on day 7 minus gravid uterus weight), was markedly decreased in the high dose group and slightly decreased in the low and intermediate dose group. Values of corrected body weight gains were 33.60 g (control group), 27.13 g (low dose group), 28.96 g (intermediate dose group) and 18.88 g (high dose group). Although there was no clear dose-dependency in the low and intermediate dose group, these findings might indicate a slight maternal toxicity even in the low dose group.

Food consumption was statistically significantly reduced in the animals from the intermediate dose group between days 7 - 10 and from day 17 - 19 of the study. However, a compound-related effect is questionable as these changes were only minor. Statistical evaluation did not reveal any difference between the control and low dose group.

(Figures page 26 - 27, Summary table and Statistics page 30 - 32, Individual data page 61 - 72).

5.1.3 Necropsy findings

No compound-related effects were observed at necropsy of the animals.

Gravid uterus weights were comparable in all groups.

(Summary tables page 36 - 37, Individual data page 77 - 100).

5.1.4 Caesarean section data

Three females from the control group and two females from each of the treatment groups did not become pregnant.

One animal from the low and high dose each showed only implantation sites at caesarean section (designated as 'abortion' in the printouts). Statistical evaluation revealed an increase in the incidence of the numbers of early and late conceptuses undergoing resorption (designated 'early intrauterine deaths' in the printouts) in the high dose group. However, there was no dose-dependency, and similar values were recorded in a recent control group. Therefore, a compound-related effect is unlikely. All resorptions were early conceptuses undergoing resorption. Two dead foetuses were observed in one litter of the intermediate dose group. As only one litter was affected, a compound-related effect is questionable. A total of five dead foetuses occurred in five litters from the high dose group, the incidence being statistically significant compared to the control group. In view of the findings observed at caesarean section and at morphological examinations of the foetuses, a compound-related effect can not be ruled out.

(Summary tables and Statistics page 33 - 35, Individual data page 73 - 76).

5.2 Foetal data

5.2.1 Findings at caesarean section

Litter size was comparable in all groups. Foetal body weight and crown-rump length were slightly and statistically significantly decreased in the high dose group. Placental weights were increased to a statistically significant degree in this group. Foetal body weight, crown-rump length and placental weights remained unaffected by the administration of the test compound in the low and intermediate dose group. Sex ratio of the foetuses was not altered by the administration of the test compound.

(Summary tables and Statistics page 33 - 35, Individual data page 101 - 271).

5.2.2 External, skeletal and visceral examination

External and visceral effects obtained at autopsy

The incidence of retarded fetuses was increased to a statistically significant degree in the fetuses from the high dose group (6/123, 8/117, 8/139, 29/131). The values were outside the historical range of the rat strain used. A compound-related effect is evident. Two fetuses from this dose group showed aplasia of the tail. As this finding was not observed in 6025 historical control fetuses, a compound-related effect cannot be ruled out.

All other findings in the fetuses from the treatment groups were within the historical range of the rat strain used or showed no dose-dependency and were not statistically significant from the control group. Therefore a compound-related effect can be ruled out.

Skeletal defects

Major defects

The above mentioned fetuses with aplasia of the tail showed aplasia of sacral vertebral arch, sacral vertebral centres and 1st and 2nd caudal vertebral centres.

One foetus from the low dose group showed fusion of the exoccipital bone with the first cervical vertebra and dysplasia of the exoccipital bone. The latter finding also occurred in a foetus from the high dose group. There was no dose-dependency, and the incidence was within the historical range of the rat strain used.

Minor defects

The incidence of longitudinally displaced, fused or fragmented sternbrae (2/123, 3/117, 1/139, 17/131), wavy and/or thickened ribs (12/123, 14/117, 56/139, 84/131), bent or shortened scapula (0/123, 0/117, 2/139, 12/131), bent, shortened or dysplastic humerus (0/123, 0/117, 1/139, 12/131) and bent or shortened radius (0/123, 0/117, 0/139, 5/131) were increased to a statistically significant degree in the fetuses from the high dose group. Two fetuses with the latter finding showed bent ulna. The incidences were above the historical range of the rat strain used. Therefore, a compound-related effect is probable. The incidence of fragmented thoracic vertebral centres (0/123, 0/117, 2/139, 4/131) was above the historical range in the high dose group and therefore may be compound-related.

The incidence of wavy and or thickened ribs was also statistically significantly increased in the intermediate dose group, the incidence being above the historical range of the rat strain used. Therefore, a compound-related effect can not be ruled out.

In all other cases, statistical evaluation did not reveal differences and the values were within the historical range of the rat strain used or showed no dose-dependency.

Variations

There were no compound-related effects. One foetus from the high dose group with aplasia of the tail showed also anlage of only 5 lumbar vertebrae. All other findings were within the historical range of the rat strain used and showed no dose-dependency.

Retardations

High dose group: Statistical evaluation revealed numerous retardations in the high dose group: Slight or non-ossification of individual skull bones (42/123, 50/117, 74/139, 80/131), weakly or non-ossified cervical vertebral arch (0/123, 0/117, 3/139, 13/131), weakly ossified lumbar vertebral arch (0/123, 0/117, 2/139, 13/131), weakly or non-ossified sacral vertebral arch (3/123, 3/117, 2/139, 14/131), ossification of less than two caudal vertebral centres (26/123, 50/117, 85/139, 98/131), weakly ossified ribs (0/123, 1/117, 3/139, 9/131), weakly ossified metacarpale 2 (0/123, 0/117, 0/139, 5/131), non-ossified metacarpale 5 (58/123, 61/117, 92/139, 86/131) and non-ossified metatarsale 5 (3/123, 2/117, 1/139, 11/131). These incidences were above the historical range of the rat strain used. Therefore, a compound-related effect is probable. Further statistically significant changes in the high dose group consisted of non- or weakly ossified sternbrae (18/123, 42/117, 45/139, 70/131) and non-ossified phalanx III of the 1st to 5th toe of the hindpaw (1/123, 7/117, 3/139, 18/131). In these cases, the values were within the historical range of the rat strain used. However, with respect to the findings mentioned above, a compound-related effect is probable. The incidences of weakly or non-ossified thoracic vertebral arch (1/123, 0/117, 2/139, 3/131), weakly or non-ossified thoracic (1/123, 0/117, 0/139, 4/131) and sacral vertebral centres (3/123, 3/117, 2/139, 10/131) and weakly ossified metacarpale 4 (0/123, 0/117, 0/139, 3/131) were above the historical range of the rat strain used.

Intermediate dose group: The incidences of slight or non-ossification of individual skull bones, ossification of less than two caudal vertebral centres and non-ossified metacarpale 5 were also statistically significantly increased in the intermediate dose group. As the incidences were above the historical range, a compound-related effect cannot be ruled out. The incidences of weakly or non-ossified cervical and thoracic vertebral arch, weakly ossified lumbar vertebral arch and weakly ossified ribs were slightly above the historical range.

The incidence of non- or weakly ossified sternbrae was statistically significantly increased. The incidence was within the normal range, and there was no dose-dependency. Therefore, a compound-related effect is unlikely.

Low dose group: Statistical evaluation showed increases in the incidence of ossification of less than two caudal vertebral centres and of non- or weakly ossified sternbrae. The incidence of the former finding was slightly above the historical range of the rat strain used. As no other findings, which could be related to treatment were observed in this dose group, a compound-related effect is not evident. The latter finding occurred without dose-dependency in the low and intermediate dose group and therefore is considered not to be treatment-related. The same applies to the statistically significantly increased incidence of non-ossified phalanx III of the 1st to 5th toe of the hindpaw.

Incidences of non- ossified metacarpals 2, 3, 4 were above the historical range in all groups, but were not statistically significant and occurred without dose-dependency. All other findings were within the historical range of the rat strain used and were not statistically significant.

External and visceral effects obtained at body cross-section examination

The incidence of retarded fetuses was increased to a statistically significant degree in the fetuses from the high dose group (0/116, 2/109, 1/125, 17/121). The values were outside the historical range of the rat strain used. A compound-related effect is evident. One foetus from the high dose group showed enlarged bladder. However, this finding is considered not to be compound-related in view of the isolated occurrence. All other findings were within the historical range of the rat strain used and were not statistically significant.

Morphological findings in dead fetuses

One oedematous foetus occurred in the intermediate and high dose group, respectively. No skeletal defects were observed in the fetuses found dead.

(Summary tables and Statistics page 38 - 48, Individual data page 101 - 271).

6. DISCUSSION AND CONCLUSION

The purpose of this study was to assess the effects of Trioxan on pregnancy and embryofetal development of the rat, when administered in dosages of 0, 100, 315 and 1 000 mg/kg body weight per day from implantation until the end of pregnancy.

Neither deaths nor clinical signs were observed in any of the animals.

Body weight gain and food consumption were slightly decreased in the animals from the high dose group during the treatment period. Body weights and food consumption were not affected by the administration of the test compound in the animals from the low and intermediate dose group. However, body weight gain after implantation corrected by gravid uterus weight (corrected body weight gain) was decreased in all groups treated with Trioxan. Mean values were 33.6 g (control group), 27.1 g (low dose group), 29.0 g (intermediate dose group) and 18.9 g (high dose group). Although there was no clear dose-dependency, the difference between the control group and the low dose group is approximately 20%. Therefore, maternal toxicity can not fully be excluded even in the low dose group.

No compound-related effects were observed at necropsy of the animals.

Gravid uterus weights, litter size and foetal sex ratios remained unaffected by the administration of the test compound. Foetal body weights and crown-rump lengths were decreased in the high dose group, whereas placental weights were increased. Incidence of early conceptuses undergoing resorption was not altered by the administration of the test compound. Five dead fetuses were observed in five litters from the high dose group.

In the high dose group, morphological examination of the fetuses revealed two cases with major defects (aplasia of the tail accompanied by aplasia of sacral vertebral arch and sacral vertebral centres). The incidences of fetuses with minor defects (displaced, fused or fragmented sternbrae, wavy or thickened ribs, bent or shortened scapulae, bent, shortened or dysplastic humerus, bent or shortened ulna and bent radius) were increased. Additionally, retarded ossification was observed in numerous bones.

The fetuses from the intermediate dose group showed minor defects consisting of increased incidences of wavy or thickened ribs. Furthermore, retarded ossification of individual skull bones and caudal vertebral centres were observed.

No compound-related effects were observed by morphological examination of the fetuses from the low dose group.

In conclusion, repeated oral administration of Trioxan to pregnant rats at the dose level of 1 000 mg/kg body weight caused maternal toxicity, as shown by slightly decreased body weight and food consumption. Corrected body weight gain was markedly decreased. Five dead foetuses were observed. Two foetuses showed aplasia of the tail. The foetuses were retarded and showed increased incidences of minor defects at sternbrae, ribs, scapula and forelimb.

Corrected body weight gain was still decreased at the daily dose levels of 315 and 100 mg/kg body weight per day, thus possibly indicating some maternal toxicity.

At the dose of 315 mg/kg body weight per day, retarded ossification of the foetuses was still observed. Additionally, the incidence of wavy or thickened ribs was increased. Repeated administration of 100 mg Trioxan / kg body weight was tolerated by the conceptuses without signs of toxicity.

With regard to the present study the **No Observed Effect Level (NOEL)** is **100 mg/kg/day** for embryofoetal toxicity. Concerning maternal toxicity, a clear NOEL could not be established, since a compound related effect on corrected body weight gain can not be excluded.

7. REFERENCES

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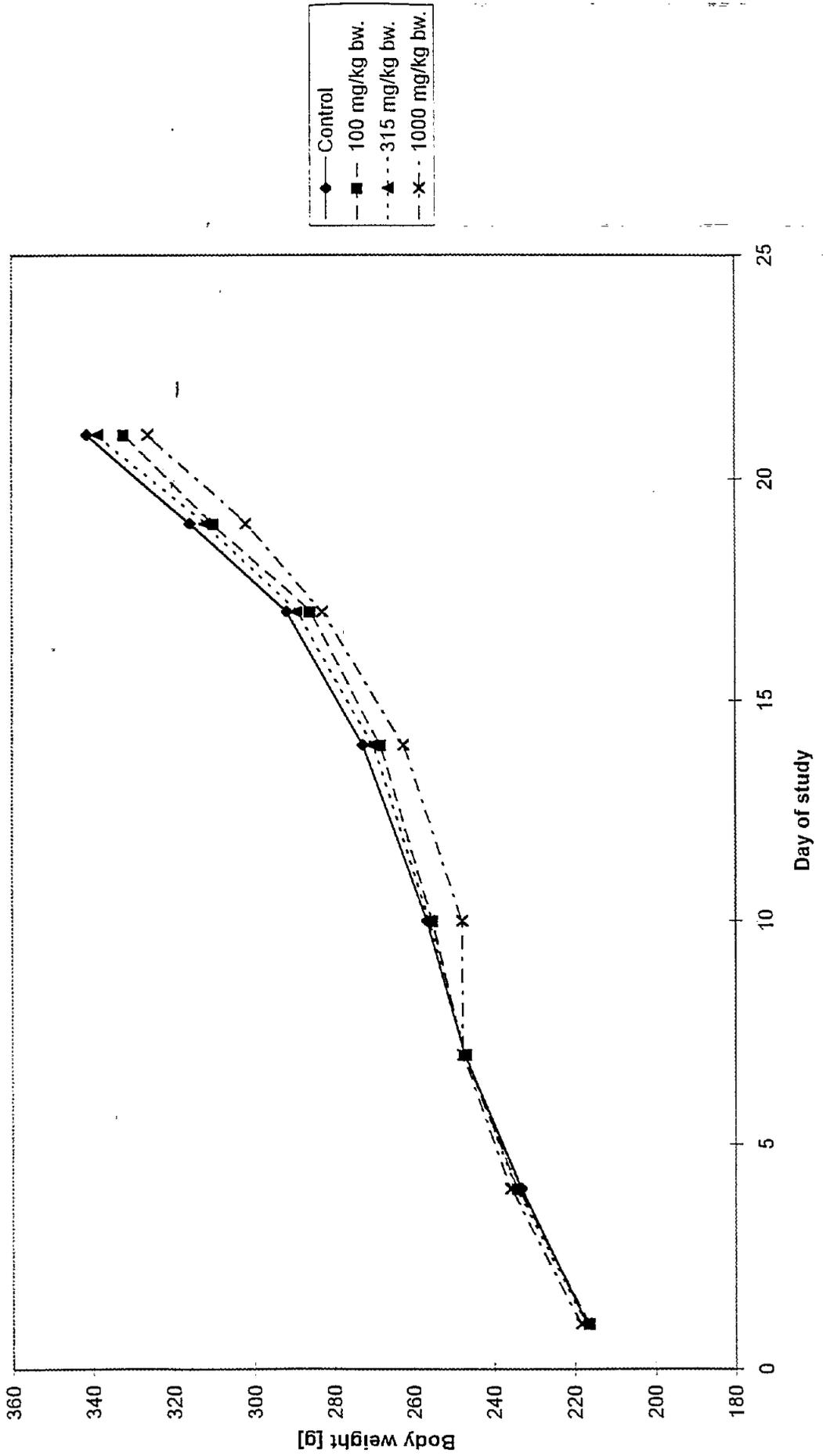
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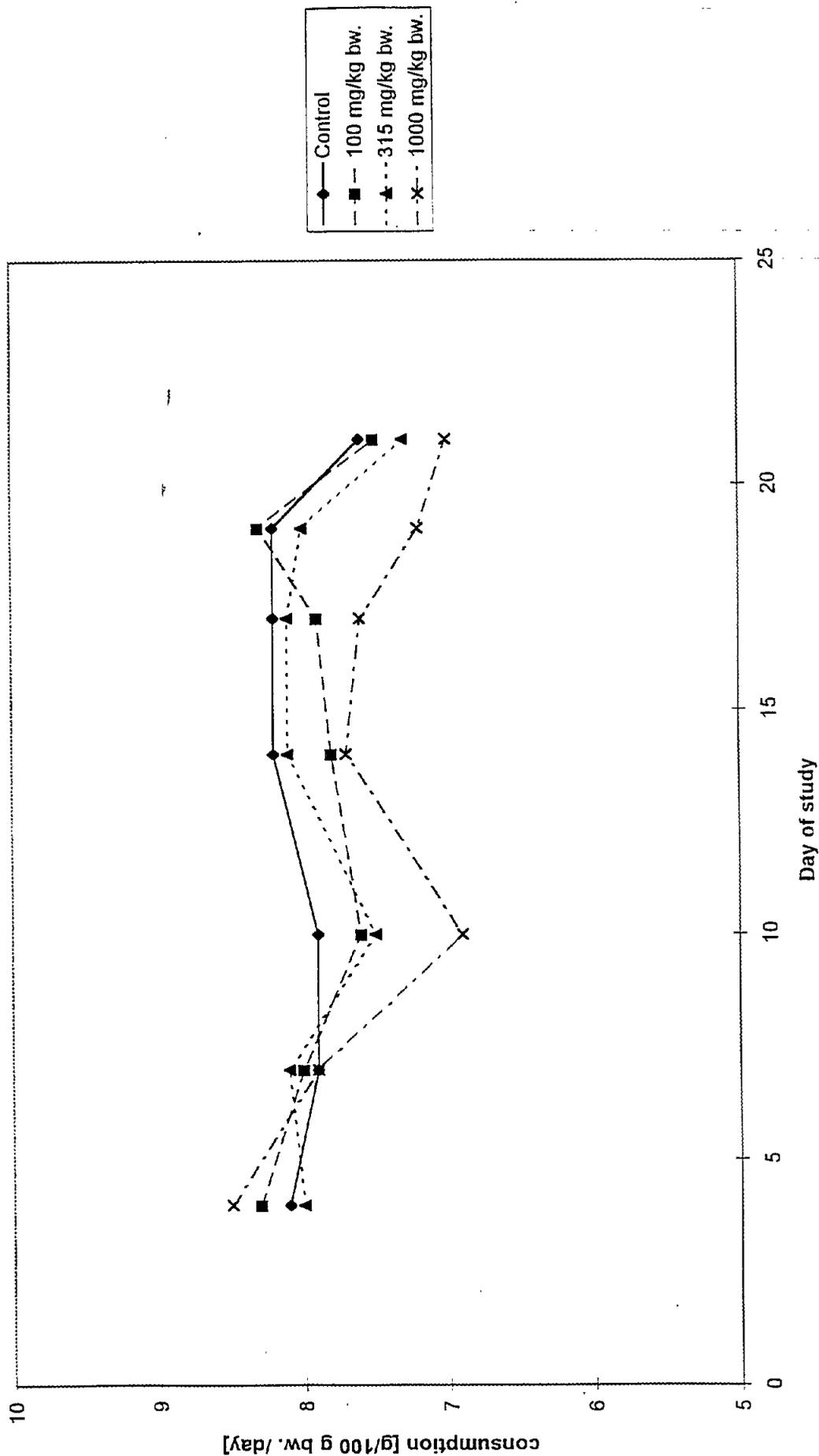
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Body weight of dams Study No. RR0792
Embryotoxicity study with Trioxan in rats



Relative food consumption of dams Study No. RR0792
Embryotoxicity study with Trioxan in rats



AC906-04/04 LAC906P/2

HOECHST MARION ROUSSEL

SECTION: PRECL.DEV.GERMANY, DRUG SAFETY

PAGE: 1

INTERGROUP COMPARISON OF CLINICAL OBSERVATIONS PER PERIOD

STUDY NO: RR0792 TITLE: EMBRYOTOXICITY STUDY IN RATS ON TRIOXAN

START DAY NO: 1 FINISH DAY NO: 21

SEX: FEMALES

GROUP 1

0 MG/KG

| DAY NUMBERS | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
|------------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| O. OF ANIMALS SURVIVED | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 0 |
| AD | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | - |
| ILLED AT TERM | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 20 |
| ILLED:NOT PREGNANT | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 3 |

GROUP 2

100 MG/KG

SEX: FEMALES

| DAY NUMBERS | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
|--------------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| O. OF ANIMALS SURVIVED | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 0 |
| AD | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | - |
| ILLED AT TERM | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 20 |
| ILLED:ONLY IMPLANT SITES | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| ILLED:NOT PREGNANT | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 2 |

HOECHST MARION ROUSSEL

SECTION: PRECL.DEV.GERMANY, DRUG SAFETY

INTERGROUP COMPARISON OF CLINICAL OBSERVATIONS PER PERIOD

PAGE: 2

STUDY NO: RR0792 TITLE: EMBRYOTOXICITY STUDY IN RATS ON TRIOXAN
 START DAY NO: 1 FINISH DAY NO: 21

| GROUP | NO. OF ANIMALS SURVIVED | DAY NUMBERS | SEX: FEMALES | | | | | | | | | | | | | | | | | | | | |
|-------|-------------------------|-------------|--------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
| 3 | | 1 2 3 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 0 |
| 3 | | 23 23 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | - |
| | KILLED AT TERM | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 21 |
| | KILLED:NOT PREGNANT | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 2 |

| GROUP | NO. OF ANIMALS SURVIVED | DAY NUMBERS | SEX: FEMALES | | | | | | | | | | | | | | | | | | | | |
|-------|---------------------------|-------------|--------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
| 4 | | 1 2 3 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 0 |
| 4 | | 23 23 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | - |
| | KILLED AT TERM | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 20 |
| | KILLED:ONLY IMPLANT.SITES | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| | KILLED:NOT PREGNANT | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 2 |

PROGRAM: RTOX2 4-1
 HOECHST MARION ROUSSEL
 PRECL. DEV. GERMANY, DRUG SAFETY
 STUDY: EMBRYOTOXICITY
 PREPARATION: TRIOXAN
 ROUTE: ORAL
 VEHICLE: DEIONIZED WATER
 STUDY NO. RR0792
 ANIMAL: WISTAR RAT

| DAY | BODY WEIGHT (G) OF DAMS DURING PREGNANCY | | | |
|-----|--|-------------------------|-------------------------|--------------------------|
| | GROUP 1 0 MG/KG | GROUP 2 100 MG/KG | GROUP 3 315 MG/KG | GROUP 4 1000 MG/KG |
| 1 | 217.0 4.4 20 (NO. OF DAMS) | 216.4 8.3 20 | 217.1 8.0 21 | 218.4 7.0 20 |
| 4 | 233.2 7.3 20 (NO. OF DAMS) | 234.1 5.0 20 | 234.5 6.8 21 | 235.9 6.3 20 |
| 7 | 247.2 8.3 20 (NO. OF DAMS) | 247.3 5.4 20 | 247.0 8.8 21 | 247.7 7.0 20 |
| 10 | 256.7 9.0 20 (NO. OF DAMS) | 255.3 8.2 19 | 256.0 10.7 21 | 247.9 b 7.3 20 |
| 14 | 272.7 11.1 20 (NO. OF DAMS) | 268.3 9.7 20 | 270.1 12.5 21 | 262.4 b 7.1 20 |
| 17 | 291.6 14.1 20 (NO. OF DAMS) | 285.7 13.1 20 | 289.1 14.6 21 | 282.7 b 8.7 20 |
| 19 | 315.8 16.4 20 (NO. OF DAMS) | 310.0 16.0 20 | 312.0 15.7 21 | 301.9 b 10.7 20 |
| 21 | 341.2 20.9 20 (NO. OF DAMS) | 332.0 18.0 20 | 338.5 20.9 21 | 326.0 b 11.6 20 |

ANIMALS NOT SURVIVING TO DAY 21 OF PREGNANCY OR HAVING AN EARLY PM ON DAY 21 OR RESORPTIONS ONLY OR NONPREGNANT ANIMALS ARE EXCLUDED FROM THE MEANS.

a : SIGNIFICANTLY HIGHER THAN CONTROL
 b : SIGNIFICANTLY LESS THAN CONTROL
 H : STATISTICAL EVALUATION NOT POSSIBLE, GROUP CONTAINS LESS THAN 5 ANIMALS
 NE : NO STATISTICAL EVALUATION
 STATISTICAL ANALYSIS BASED ON CHANGES VERSUS PRELIMINARY VALUES.

PROGRAM: RTOX2 4.1
 HOECHST MARION ROUSSEL
 PRECL. DEV. GERMANY, DRUG SAFETY
 STUDY: EMBRYOTOXICITY
 PREPARATION: TRIOXAN
 ROUTE: ORAL
 VEHICLE: DEIONIZED WATER
 STUDY NO. RR0792
 ANIMAL: WISTAR RAT

BODY WEIGHT CHANGES (G) OF DAMS DURING PREGNANCY

| DAY | MEAN S.D. (NO. OF DAMS) | GROUP 1 0 MG/KG | GROUP 2 100 MG/KG | GROUP 3 315 MG/KG | GROUP 4 1000 MG/KG |
|---------|-------------------------------|-----------------------|-------------------------|-------------------------|--------------------------|
| 1 - 4 | 16.2 4.8 20 | | 17.7 6.2 20 | 17.4 4.4 21 | 17.6 6.4 20 |
| 4 - 7 | 14.1 4.7 20 | | 13.2 4.1 20 | 12.5 4.9 21 | 11.8 4.3 20 |
| 7 - 10 | 9.5 3.9 20 | | 8.1 5.1 19 | 9.0 4.7 21 | 0.3 5.5 20 |
| 10 - 14 | 16.0 4.6 20 | | 12.8 5.0 19 | 14.1 4.7 21 | 14.5 4.5 20 |
| 14 - 17 | 18.9 6.0 20 | | 17.4 5.7 20 | 19.0 4.4 21 | 20.4 5.1 20 |
| 17 - 19 | 24.3 6.7 20 | | 24.4 6.8 20 | 22.9 5.1 21 | 19.2 5.2 20 |
| 19 - 21 | 25.4 6.3 20 | | 22.0 6.1 20 | 26.6 7.1 21 | 24.2 5.9 20 |

ANIMALS NOT SURVIVING TO DAY 21 OF PREGNANCY OR HAVING AN EARLY PM ON DAY 21 OR RESORPTIONS ONLY OR NONPREGNANT ANIMALS ARE EXCLUDED FROM THE MEANS.
 NO STATISTICAL EVALUATION

PROGRAM: RTOX2 4.1
 HOECHST MARION ROUSSEL
 PRECL.DEV.GERMANY, DRUG SAFETY
 STUDY: EMBRYOTOXICITY
 ROUTE: ORAL
 PREPARATION: TRIOXAN
 VEHICLE: DEIONIZED WATER
 STUDY NO. RR0792
 ANIMAL: WISTAR RAT

DAILY FOOD CONSUMPTION (G) / 100 G BODY WEIGHT OF DAMS DURING PREGNANCY

| DAY | GROUP 1 0 MG/KG | GROUP 2 100 MG/KG | GROUP 3 315 MG/KG | GROUP 4 1000 MG/KG |
|---------|-------------------------------|-------------------------|-------------------------|--------------------------|
| 1 - 4 | MEAN S.D. (NO. OF DAMS) | 8.3 1.2 20 | 8.0 1.1 21 | 8.5 0.9 20 |
| 4 - 7 | MEAN S.D. (NO. OF DAMS) | 7.9 1.2 20 | 8.0 0.6 21 | 7.9 0.5 20 |
| 7 - 10 | MEAN S.D. (NO. OF DAMS) | 7.9 0.8 20 | 7.6 0.8 19 | 6.9 b 0.6 20 |
| 10 - 14 | MEAN S.D. (NO. OF DAMS) | 8.2 0.4 20 | 7.8 0.7 19 | 7.7 b 0.5 20 |
| 14 - 17 | MEAN S.D. (NO. OF DAMS) | 8.2 0.4 20 | 7.9 1.1 20 | 7.6 b 0.7 20 |
| 17 - 19 | MEAN S.D. (NO. OF DAMS) | 8.4 0.6 20 | 8.3 0.7 20 | 7.2 b 0.6 20 |
| 19 - 21 | MEAN S.D. (NO. OF DAMS) | 7.6 0.7 20 | 7.5 0.7 20 | 7.0 b 0.6 20 |

ANIMALS NOT SURVIVING TO DAY 21 OF PREGNANCY OR HAVING AN EARLY PM ON DAY 21 OR RESORPTIONS ONLY OR NONPREGNANT ANIMALS ARE EXCLUDED FROM THE MEANS.

a : SIGNIFICANTLY HIGHER THAN CONTROL b : SIGNIFICANTLY LESS THAN CONTROL
 M : STATISTICAL EVALUATION NOT POSSIBLE, GROUP CONTAINS LESS THAN 5 ANIMALS

PROGRAM: RTOX2 4.1
 HOECHST MARION ROUSSEL
 PRECL. DEV. GERMANY, DRUG SAFETY
 STUDY: EMBRYOTOXICITY
 PREPARATION: TRIOXAN
 ROUTE: ORAL
 VEHICLE: DETONIZED WATER
 STUDY NO. RR0792
 ANIMAL: WISTAR RAT

SURVEY OF RESULTS DURING GESTATION AND AT CAESARIAN SECTION

| NUMBER OF | GROUP 1 | | | | GROUP 2 | | | | GROUP 3 | | | | GROUP 4 | | | |
|---|---------|--|--|--|---------|--|--|--|---------|--|--|--|---------|--|--|--|
| | MG/KG | | | | MG/KG | | | | MG/KG | | | | MG/KG | | | |
| - PREGNANCIES | 20 | | | | 21 | | | | 21 | | | | 21 | | | |
| - INTERCURRENT DEATH | 0 | | | | 0 | | | | 0 | | | | 0 | | | |
| - FEMALES WITH ABORTION | 0 | | | | 1 | | | | 0 | | | | 1 | | | |
| - FEMALES WITH PREMATURE DELIVERY | 0 | | | | 0 | | | | 0 | | | | 0 | | | |
| - FEMALES AT TERM WITH INTRAUTER. DEATHS ONLY | 0 | | | | 0 | | | | 0 | | | | 0 | | | |
| - FEMALES AT TERM WITH LIVE FOETUSES | 20 | | | | 20 | | | | 21 | | | | 20 | | | |
| - CORPORA LUTEA | 280 | | | | 287 | | | | 316 | | | | 298 | | | |
| | 14.0 | | | | 14.4 | | | | 15.0 | | | | 14.9 | | | |
| | 1.6 | | | | 1.5 | | | | 2.2 | | | | 0.9 | | | |
| - IMPLANTATIONS | 246 | | | | 245 | | | | 284 | | | | 276 | | | |
| | 12.3 | | | | 12.3 | | | | 13.5 | | | | 13.8 | | | |
| | 3.5 | | | | 3.1 | | | | 3.0 | | | | 1.9 | | | |

a : SIGNIFICANTLY HIGHER THAN CONTROL b : SIGNIFICANTLY LESS THAN CONTROL
 M : STATISTICAL EVALUATION NOT POSSIBLE, GROUP CONTAINS LESS THAN 5 ANIMALS NE : NO STATISTICAL EVALUATION

PROGRAM: RTOX2 4.1
 HOECHST MARION ROUSSEL
 PRECL.DEV.GERMANY, DRUG SAFETY
 STUDY: EMBRYOTOXICITY
 PREPARATION: TRIOXAN
 ROUTE: ORAL
 VEHICLE: DEIONIZED WATER
 STUDY NO. RR0792
 ANIMAL: Wistar Rat

SURVEY OF RESULTS DURING GESTATION AND AT CAESARIAN SECTION

| | GROUP 1 | GROUP 2 | GROUP 3 | GROUP 4 |
|-----------------------------|---------|---------|---------|---------|
| | MG/KG | MG/KG | MG/KG | MG/KG |
| PRE-IMPLANTATION LOSS % | 12.78 | 14.10 | 10.08 | 7.35 |
| NE MEAN | | | | |
| POST-IMPLANTATION LOSS % | 2.62 | 7.65 | 7.22 | 8.36 |
| NE MEAN | | | | |
| - EARLY INTRAUTERINE DEATHS | 7 | 19 | 18 | 19 |
| NE MEAN | 0.35 | 0.95 | 0.86 | 0.95 |
| S.D. | 0.67 | 1.47 | 1.35 | 1.39 |
| % OF IMPLANTATIONS | 2.62 | 7.65 | 6.48 | 6.59 a |
| MEAN | | | | |
| - LATE INTRAUTERINE DEATHS | 0 | 0 | 2 | 5 |
| NE MEAN | 0.00 | 0.00 | 0.10 | 0.25 |
| S.D. | 0.00 | 0.00 | 0.44 | 0.44 |
| % OF IMPLANTATIONS | 0.00 | 0.00 | 0.73 | 1.77 a |
| MEAN | | | | |
| - TOTAL INTRAUTERINE DEATHS | 7 | 19 | 20 | 24 |
| NE MEAN | 0.35 | 0.95 | 0.95 | 1.20 |
| S.D. | 0.67 | 1.47 | 1.36 | 1.44 |
| - LIVE FETUSES | 239 | 226 | 264 | 252 |
| TOTAL | | | | |
| MEAN | 12.0 | 11.3 | 12.6 | 12.6 |
| S.D. | 3.4 | 3.2 | 2.9 | 2.0 |

a : SIGNIFICANTLY HIGHER THAN CONTROL
 b : SIGNIFICANTLY LESS THAN CONTROL
 M : STATISTICAL EVALUATION NOT POSSIBLE, GROUP CONTAINS LESS THAN 5 ANIMALS
 NE : NO STATISTICAL EVALUATION

PROGRAM: RTOX2 4.1
 HOECHST MARION ROUSSEL
 PRECL.DEV.GERMANY, DRUG SAFETY
 STUDY: EMBRYOTOXICITY
 PREPARATION: TRIOXAN
 ROUTE: ORAL
 VEHICLE: DETONIZED WATER
 STUDY NO. RR0792
 ANIMAL: WISTAR RAT

SURVEY OF RESULTS IN LIVE FOETUSES AT CAESARIAN SECTION

| | GROUP 1 0 MG/KG | GROUP 2 100 MG/KG | GROUP 3 315 MG/KG | GROUP 4 1000 MG/KG |
|------------------------|-----------------------|-------------------------|-------------------------|--------------------------|
| NUMBER OF FOETUSES | TOTAL 239 | 226 | 264 | 252 |
| | MEAN 12.0 | 11.3 | 12.6 | 12.6 |
| | S.D. 3.4 | 3.2 | 2.9 | 2.0 |
| % OF IMPLANTATIONS | MEAN 97.38 | 92.35 | 92.78 b | 91.64 b |
| MALES (%) | NE 48.1 | 59.7 | 55.7 | 50.8 |
| BODY WEIGHT (G) | MEAN 3.3 | 3.3 | 3.2 | 2.8 b |
| | S.D. 0.3 | 0.4 | 0.3 | 0.4 |
| CROWN/RUMP LENGTH (MM) | MEAN 35.1 | 35.0 | 34.5 | 32.8 b |
| | S.D. 1.4 | 1.6 | 1.5 | 1.9 |
| PLACENTAL WEIGHT (G) | MEAN 0.46 | 0.47 | 0.49 | 0.52 a |
| | S.D. 0.07 | 0.06 | 0.09 | 0.07 |

a : SIGNIFICANTLY HIGHER THAN CONTROL
 b : SIGNIFICANTLY LESS THAN CONTROL
 M : STATISTICAL EVALUATION NOT POSSIBLE, GROUP CONTAINS LESS THAN 5 ANIMALS
 NE : NO STATISTICAL EVALUATION

PROGRAM: RTOX2 4.1
 HOECHST MARION ROUSSEL
 STUDY: EMBRYOTOXICITY
 ROUTE: ORAL

PRECL.DEV.GERMANY, DRUG SAFETY
 STUDY NO. RR0792
 ANIMAL: WISTAR RAT

PREPARATION: TRIOXAN
 VEHICLE: DEIONIZED WATER

BODY AND ORGAN WEIGHTS (G) OF DAMS (CAESARIAN SECTION)

| | GROUP 1 | GROUP 2 | GROUP 3 | GROUP 4 |
|---------------|---------|---------|---------|---------|
| | 0 | 100 | 315 | 1000 |
| | MG/KG | MG/KG | MG/KG | MG/KG |
| UTERUS | 60.40 | 57.57 | 62.57 | 59.47 |
| MEAN | 16.21 | 16.55 | 13.23 | 8.09 |
| S.D. | 20 | 20 | 21 | 20 |
| (NO. OF DAMS) | | | | |

ANIMALS NOT SURVIVING TO DAY 21 AFTER MATING OR HAVING AN EARLY PH ON DAY 21 OR RESORPTIONS ONLY OR NONPREGNANT ANIMALS ARE EXCLUDED FROM THE MEANS.
 NO STATISTICAL EVALUATION

RUN DATE: 26/06/97

PAC920-13/00 PAC920P/2

HOECHST MARION ROUSSEL

SECTION: PRECL.DEV.GERMANY, DRUG SAFETY

PAGE: 1

P A T H O L O G Y - INTERGROUP COMPARISON OF MACROSCOPIC FINDINGS INCIDENCE

STUDY NO: RR0792

TITLE: EMBRYOTOXICITY STUDY IN RATS WITH TRIOXAN

| | GROUP 1 | GROUP 2 | GROUP 3 | GROUP 4 |
|--|---------|---------|---------|---------|
| | 0 | 100 | 315 | 1000 |
| | MG/KG | MG/KG | MG/KG | MG/KG |

SEX: FEMALES

FEMALES ON STUDY
ANIMALS COMPLETED

| | | | | |
|----|----|----|----|----|
| 23 | 23 | 23 | 23 | 23 |
| 23 | 23 | 23 | 23 | 23 |

OVARY

NO. WITH FINDINGS NO HISTOLOGICAL EXAMINATIONS.....

STUDY: EMBRYOTOXICITY
 PREPARATION: TRIOXAN
 ROUTE: ORAL

VEHICLE: DEIONISED WATER

PRECL. DEV. GERMANY, DRUG SAFETY

STUDY NO. RR0792
 ANIMAL: WISTAR RAT

SUMMARY AND INTERGROUP COMPARISON OF MORPHOLOGICAL FINDINGS IN FOETUSES

FISHER'S EXACT: GROUP 1 COMPARED WITH ALL OTHER GROUPS (* P < 0.05 ** P < 0.01, ONE SIDED) PAGE: 1

0 100 315 1000
 MG/KG MG/KG MG/KG MG/KG

INCIDENCE BY FOETUS/LITTER

| CLASS | NO. | % | NO. | % | NO. | % |
|--------------------------------------|-----|--------|-----|--------|------|--------|
| EXTERNAL | 6 | (4.9) | 8 | (5.8) | 29** | (22.1) |
| RETARDED FOETUS | 5 | (25.0) | 5 | (25.0) | 11 | (55.0) |
| ABDOMINAL CAVITY | 0 | | 0 | | 1 | (0.8) |
| BLOOD IN ABDOMINAL CAVITY | 0 | | 0 | | 1 | (5.0) |
| LIVER | 1 | (0.8) | 0 | | 1 | (0.8) |
| LOBUS CAUDATUS - HAEMATOMA | 1 | (5.0) | 0 | | 1 | (5.0) |
| KIDNEY | 1 | (5.0) | 0 | | 0 | |
| REDUCED IN SIZE - LEFT | 1 | (5.0) | 0 | | 0 | |
| PELVIS DISTENDED - UNI- OR BILATERAL | 5 | (4.1) | 1 | (0.9) | 1 | (0.8) |
| | 4 | (20.0) | 1 | (5.0) | 1 | (5.0) |

EXTERNAL/VISCERAL DEFECTS OBTAINED AT AUTOPSY

| | | | | |
|-----------------------------|-----|-----|-----|-----|
| NUMBER OF FOETUSES EXAMINED | 123 | 117 | 139 | 131 |
| NUMBER OF LITERS EXAMINED | 20 | 20 | 21 | 20 |

EXTERNAL

RETARDED FOETUS

ABDOMINAL CAVITY

BLOOD IN ABDOMINAL CAVITY

LIVER

LOBUS CAUDATUS - HAEMATOMA

KIDNEY

REDUCED IN SIZE - LEFT

PELVIS DISTENDED - UNI- OR BILATERAL

PRECL. DEV. GERMANY, DRUG SAFETY

HOECHST MARION ROUSSEL

STUDY: EMBRYOTOXICITY
ROUTE: ORAL

PREPARATION: TRIOXAN
VEHICLE: DEIONISED WATER

STUDY NO. RR0792
ANIMAL: WISTAR RAT

SUMMARY AND INTERGROUP COMPARISON OF MORPHOLOGICAL FINDINGS IN FOETUSES

FISHER'S EXACT: GROUP 1 COMPARED WITH ALL OTHER GROUPS (* P < 0.05 ** P < 0.01, ONE SIDED)
PAGE: 2

| CLASS | NO. | 0 MG/KG | 100 MG/KG | 315 MG/KG | 1000 MG/KG |
|----------------------------|-----|------------|--------------|--------------|---------------|
| INCIDENCE BY FOETUS/LITTER | | | | | |
| ----- | NO. | 0 | 100 | 315 | 1000 |
| ----- | NO. | 0 | 1 | 0 | 0 |
| ----- | NO. | 0 | 1 | 0 | 0 |

EXTERNAL/VISCERAL DEFECTS OBTAINED AT AUTOPSY

| | | | | | |
|--------|-----------------------------|---|---------|---|----------|
| TESTIS | MIN | 0 | 1 | 0 | 0 |
| ----- | REDUCED IN SIZE - BILATERAL | 0 | 1 (0.9) | 0 | 0 |
| ----- | | 0 | 1 (5.0) | 0 | 0 |
| TAIL | MAJ | 0 | 0 | 0 | 2 |
| ----- | APLASIA | 0 | 0 | 0 | 2 (1.5) |
| ----- | | 0 | 0 | 0 | 2 (10.0) |

SUMMARY AND INTERGROUP COMPARISON OF MORPHOLOGICAL FINDINGS IN FOETUSES

FISHER'S EXACT: GROUP 1 COMPARED WITH ALL OTHER GROUPS (* P < 0.05 ** P < 0.01, ONE SIDED) PAGE: 3

0 MG/KG 100 MG/KG 315 MG/KG 1000 MG/KG

INCIDENCE BY FOETUS/LITTER

NO. % NO. % NO. % NO. %

SKELETAL DEFECTS

NUMBER OF FOETUSES EXAMINED 123 117 139 131

NUMBER OF LITTERS EXAMINED 20 20 21 20

SKULL

EXOCOCCIPITAL FUSED WITH 1ST MAJ 0 1 (0.9) 0 0

CERVICAL VERTEBRA - RIGHT 0 1 (5.0) 0 0

EXOCOCCIPITAL BONE - DYSPLASIA - UNILATERAL MAJ 0 1 (0.9) 0 1 (0.8)

0 1 (5.0) 0 1 (5.0)

INDIVIDUAL SKULL BONES - SLIGHT OR NON-OSSIFICATION RET 42 (34.1) 50 (42.7) 74** (53.2) 80** (61.1)

15 (75.0) 17 (85.0) 18 (85.7) 18 (90.0)

INCISOR AND JAW

LOWER INCISORS NOT VISIBLE RET 0 0 0 1 (0.8)

0 0 0 1 (5.0)

CERVICAL VERT. ARCH

DYSPLASIA 1ST - RIGHT, MIN 0 1 (0.9) 0 0

APLASIA 6TH - DORSAD - RIGHT 0 1 (5.0) 0 0

WEAKLY OR NON-OSSIFIED - UNI- OR BILATERAL RET 0 0 3 (2.2) 13** (9.9)

0 0 2 (9.5) 5* (25.0)

THORACIC VERT. ARCH

WEAKLY OR NON-OSSIFIED - UNI- OR BILATERAL RET 1 (0.8) 2 (1.4) 3 (2.3)

1 (5.0) 1 (4.8) 2 (10.0)

HOECHST MARION ROUSSEL

STUDY: EMBRYOTOXICITY
ROUTE: ORAL

PREPARATION: TRIOXAN
VEHICLE: DEIONISED WATER

STUDY NO. RR0792
ANIMAL: WISTAR RAT

SUMMARY AND INTERGROUP COMPARISON OF MORPHOLOGICAL FINDINGS IN FOETUSES

FISHER'S EXACT: GROUP 1 (* P < 0.05 ** P < 0.01, ONE SIDED) PAGE: 4

0 100 315 1000
MG/KG MG/KG MG/KG MG/KG

INCIDENCE BY FOETUS/LITTER

NO. % NO. % NO. % NO. %

SKELETAL DEFECTS

THORACIC VERT. CENTRA

WEAKLY OR NON-OSSIFIED

FRAGMENTED

LUMBAR VERTEBRAE

ANLAGE OF ONLY 5 LUMBAR VERTEBRAE

LUMBAR VERT. ARCH

WEAKLY OSSIFIED -- UNI- OR BILATERAL

LUMBAR VERT. CENTRA

WEAKLY OSSIFIED

SACRAL VERT. ARCH

APLASIA - BILATERAL

WEAKLY OR NON-OSSIFIED - UNI- OR BILATERAL

| | | | | | | |
|-----|---|--------|---|---|------|--------|
| RET | 1 | (0.8) | 0 | 0 | 4 | (3.1) |
| | 1 | (5.0) | 0 | 0 | 2 | (10.0) |
| MIN | 0 | | 0 | 2 | 4 | (3.1) |
| | 0 | | 0 | 2 | 3 | (15.0) |
| VAR | 0 | | 0 | 0 | 1 | (0.8) |
| | 0 | | 0 | 0 | 1 | (5.0) |
| RET | 0 | | 0 | 2 | 13** | (9.9) |
| | 0 | | 0 | 1 | 5* | (25.0) |
| RET | 1 | (0.8) | 0 | 0 | 1 | (0.8) |
| | 1 | (5.0) | 0 | 0 | 1 | (5.0) |
| MAJ | 0 | | 0 | 0 | 2 | (1.5) |
| | 0 | | 0 | 0 | 2 | (10.0) |
| RET | 3 | (2.4) | 3 | 2 | 14** | (10.7) |
| | 2 | (10.0) | 3 | 2 | 7 | (35.0) |

SUMMARY AND INTERGROUP COMPARISON OF MORPHOLOGICAL FINDINGS IN FOETUSES

FISHER'S EXACT: GROUP 1 COMPARED WITH ALL OTHER GROUPS (* P < 0.05 ** P < 0.01, ONE SIDED) PAGE: 5

0 315 1000
 MG/KG MG/KG MG/KG

CLASS INCIDENCE BY FOETUS/LITER

NO. % NO. % NO. %

SKELETAL DEFECTS

SACRAL VERT. CENTRA

| | | | | | |
|------------------------|-----|----------|----------|---------|----------|
| WEAKLY OR NON-OSSIFIED | RET | 3 (2.4) | 3 (2.6) | 2 (1.4) | 10 (7.6) |
| | | 2 (10.0) | 3 (15.0) | 2 (9.5) | 6 (30.0) |

APLASIA

| | | | | | |
|--|-----|---|---|---|----------|
| | MAJ | 0 | 0 | 0 | 2 (1.5) |
| | | 0 | 0 | 0 | 2 (10.0) |

CAUDAL VERT. CENTRA

| | | | | | |
|---------------------|-----|---|---|---|----------|
| APLASIA 1ST AND 2ND | MAJ | 0 | 0 | 0 | 2 (1.5) |
| | | 0 | 0 | 0 | 2 (10.0) |

OSSIFICATION OF LESS THAN 2 VERTEBRAL CENTRES

| | | | | | |
|--|-----|-----------|-------------|-------------|-------------|
| | RET | 26 (21.1) | 50** (42.7) | 85** (61.2) | 98** (74.8) |
| | | 12 (60.0) | 16 (80.0) | 20** (95.2) | 19** (95.0) |

EXTRA VERTEBRAE/EXTRA RIB

ANLAGE OF A 14TH THORACIC VERTEBRA AND AN ANALOGOUS 14TH RIB - NORMALLY LONG - BILATERAL

| | | | | | |
|--|-----|---|----------|---|---|
| | VAR | 0 | 2 (1.7) | 0 | 0 |
| | | 0 | 2 (10.0) | 0 | 0 |

STERNEBRA

LONGITUDINALLY DISPLACED, FUSED, FRAGMENTED

| | | | | | |
|--|-----|----------|----------|---------|-------------|
| | MIN | 2 (1.6) | 3 (2.6) | 1 (0.7) | 17** (13.0) |
| | | 2 (10.0) | 3 (15.0) | 1 (4.8) | 11** (55.0) |

NON-OSSIFIED OR WEAKLY OSSIFIED

| | | | | | |
|--|-----|-----------|-------------|-------------|-------------|
| | RET | 18 (14.6) | 42** (35.9) | 45** (32.4) | 70** (53.4) |
| | | 9 (45.0) | 15 (75.0) | 19** (90.5) | 19** (95.0) |