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Chemical Category	AMINES, C10-16-ALKYLDIMETHYL, N-OXIDES		

A 03

OFFICE OF TOXIC SUBSTANCES
CODING FORM FOR GLOBAL INDEXING

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Procter & Gamble

The Procter & Gamble Company
Ivorydale Technical Center
5299 Spring Grove Avenue, Cincinnati, Ohio 4521



89990000043

November 9, 1998



8EHQ-98-14208

Document Processing Center (TS-790)
Attention: Section 8(e) Coordinator
Office of Toxic Substances
U.S. Environmental Protection Agency
401 M Street, S. W.
Washington, D. C. 20460

Contains No CBI

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This submission of information is made as required by TSCA Section 8(e) regulations and guidance.

The enclosed information does not, however, scientifically support the conclusion that the test material poses a substantial risk of injury to people or the environment.

This provides information for Amines, C10-16-alkyldimethyl, N-oxides, CASRN 70592-80-2 and follows our earlier submissions (June 17 and August 3, 1998) regarding a developmental toxicity study for the material. Our earlier submissions outlined preliminary findings regarding the test substance from a dose-setting study and preliminary tables from this definitive, larger scale developmental toxicity study. This submission includes additional information contained within the draft final report for the study (attached) which we have recently received from the contract laboratory.

The draft study report includes results of skeletal examination of fetuses. No malformations (*irreversible fetal alterations*) were noted in the study which were attributed to the test substance, indicative of a lack of teratogenicity. Evidence of delayed ossification was noted in both Group III and IV, both doses where significant maternal toxicity was observed. Fetal variations (*alterations defined by the laboratory as common findings in this species and strain and reversible delays or accelerations in development*) noted in litters from Group IV included increased incidences of bifid thoracic vertebrae centra, and incompletely ossified sternal centra, pubes, caudal vertebrae, and metacarpals. The increase in fetal variations noted in Group IV were considered to reflect delays in skeletal ossification, related to the significantly reduced ($p \leq 0.01$) fetal body weights in this group. Consistent with the maternal toxicity observed, some delayed ossification was also noted in Group III, with increased incidences of bifid thoracic vertebrae centra (8/21 or 38% of litters; historical control range: 0-29.6%).

No fetal alterations were noted in Group II (25mg/kg BW/d) litter parameters. Total fetal occurrence of bifid thoracic vertebrae centra (6/194 fetuses; 3.1%) was statistically increased in Group II over controls (1/176), however this was well within the laboratory historical control range for this finding (0 - 4.5%). This finding was not attributed to the test substance, due to the fetal variation frequency well within the historical control range for this finding (0 - 4.5%) and the finding not being supported by an increase in the more relevant parameter (percentage of litters with this variation).

In summary, all observations are within the spectrum of effects expected for the test substance, under the exposure conditions of this study. No clear evidence of teratogenicity is apparent, even at maternally toxic doses and nothing in the study alters the safety profile of the test substance.

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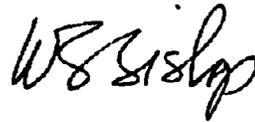
A 05

We have handled and will continue to handle this material with appropriate caution in our work environment in keeping with our standard procedures for handling all chemical substances. We will communicate appropriate hazard information for the test substance by both labels and MSDS.

If you wish further information, please contact me.

Very truly yours,

THE PROCTER AND GAMBLE COMPANY

A handwritten signature in black ink, appearing to read "W. E. Bishop". The signature is written in a cursive style with a large initial "W" and "E".

W. E. Bishop, Ph. D.
Manager
Regulatory & Government Affairs
The Procter & Gamble Company
Telephone: 513/627-6145

Material Identity (SI0801.01)

Name: Amines, C10-16-alkyldimethyl, N-oxides, CASRN 70592-80-2

Weight %	31.9%
Peroxide Value	0.16 weight %
Free Amine	0.1 weight %
Color	17 (APHA)
pH	7.2

Study Title

ORAL (GAVAGE) DEVELOPMENTAL TOXICITY STUDY
OF SI0801.01 IN RATS

SPONSOR'S STUDY NUMBER: SIBTS97.042

Data Requirement

U.S. Environmental Protection Agency
Pesticide Assessment Guidelines
Subdivision F, 83-3

U.S. Environmental Protection Agency
Toxic Substances Control Act Test Guidelines
Health Effects Testing Guidelines - 798.4900

Author

Raymond G. York, Ph.D., DABT
(Study Director)

Study Completed On

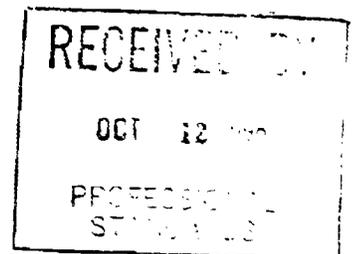
October 7, 1996
(Audited Draft Final Report)

Performing Laboratory

Argus Research Laboratories, Inc.
905 Sheehy Drive, Building A
Horsham, Pennsylvania 19044-1297

Laboratory Project ID

Argus Research Laboratories, Inc., Protocol Number: 916-025



Confidentiality Page
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GOOD LABORATORY PRACTICE STATEMENT

This study was conducted according to U.S. Environmental Protection Agency (EPA FIFRA/TSCA) "Good Laboratory Practice Standards; Final Rule" (40 CFR Part 160/792). Any areas of noncompliance are documented in the study record. No deviations existed that significantly affected the validity of the study.

Submitter:

The Procter and Gamble Company Date

Study Director:

Raymond G. York, Ph.D., DABT Date
Argus Research Laboratories, Inc.

Sponsor:

The Procter and Gamble Company Date

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**PROTOCOL 916-025: ORAL (GAVAGE) DEVELOPMENTAL TOXICITY
STUDY OF SI0801.01 IN RATS
SPONSOR'S STUDY NUMBER: SIBTS97.042**

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**TITLE: ORAL (GAVAGE) DEVELOPMENTAL TOXICITY STUDY OF
SI0801.01 IN RATS**

**ARGUS RESEARCH LABORATORIES, INC.,
PROTOCOL NUMBER: 916-025
SPONSOR'S STUDY NUMBER: SIBTS97.042**

I. SUMMARY AND CONCLUSION

A. Methods

One-hundred CrI:CD@BR VAF/Plus® presumed pregnant female rats were randomly assigned to four dosage groups (Groups I through IV), 25 rats per group. The test substance, SI0801.01, was administered orally (via gavage) once daily to these female rats on days 6 through 19 of presumed gestation (DGs 6 through 19), at dosages of 0 (Vehicle), 25, 100 and 200 mg/kg/day. The dosage volume was 5 mL/kg, adjusted daily on the basis of the individual body weights recorded before intubation. The rats were intubated at approximately the same time each day.

The rats were observed for viability at least twice a day and for general appearance weekly during acclimation and on DG 0. The rats were also examined for clinical observations of effects of the test substance, abortions, premature deliveries and deaths immediately before and approximately 60 minutes after dosage and on the day of sacrifice (DG 20). Body weights were recorded weekly during acclimation, on DG 0, daily during the dosage period and on DG 20. Feed consumption values were recorded on DGs 0, 6, 9, 12, 15, 18 and 20.

All surviving rats were sacrificed on DG 20, and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Caesarean-sectioning and subsequent fetal observations were conducted without knowledge of dosage group in order to minimize bias. The number of corpora lutea in each ovary was

-
- a. Detailed descriptions of all procedures used in the conduct of this study are provided in the appropriate sections of this report and in APPENDIX C (PROTOCOL).

recorded. The uterus of each rat was excised and examined for pregnancy, number and distribution of implantations, live and dead fetuses and early and late resorptions. The gravid uterus was weighed. Each fetus was removed from the uterus and subsequently weighed and examined for sex and gross external alterations. Approximately one-half of the fetuses in each litter were examined for soft tissue alterations using a variation of the microdissection technique of Staples. The remaining fetuses in each litter were examined for skeletal alterations.

B. Results

Two rats in the 200 mg/kg/day dosage group died; one of these deaths was attributed to the test substance, the other was the result of an intubation error. All other rats survived until scheduled sacrifice.

Excessive salivation, rales, urine-stained abdominal fur, brown or red perioral substance, labored breathing and gasping occurred in 100 mg/kg/day dosage group rats and in significantly increased numbers of 200 mg/kg/day dosage group rats. Additionally, chromorhinorrhea occurred in one and two rats in these two respective dosage groups. Observations of brown or red perivaginal substance, emaciation, brown perianal or perinasal substance, dehydration, ungroomed coat and soft or liquid feces occurred in one or two rats in the 200 mg/kg/day dosage group. All necropsy observations were considered unrelated to the test substance.

Rats in the 100 and 200 mg/kg/day dosage groups had significantly reduced body weight gains for the entire dosage period (calculated as days 6 to 20 of gestation) and body weight gains were significantly reduced in the 200 mg/kg/day dosage group for the entire gestation period (DGs 0 to 20). Body weights were significantly reduced in the 200 mg/kg/day dosage group on DGs 11 through 20. The gravid uterine weight and the corrected DG 20 maternal body weight (DG 20 body weight minus the gravid uterine weight) were significantly reduced in the 200 mg/kg/day dosage group.

Absolute (g/day) feed consumption values for the entire dosage period (calculated as DGs 6 to 20) and the entire gestation period (DGs 0 to 20) were significantly reduced in the 200 mg/kg/day dosage group. Relative (g/kg/day) feed consumption values for the entire dosage period were significantly reduced in the 100 and 200 mg/kg/day dosage groups, while values for the entire gestation period were significantly reduced in the 25, 100 and 200 mg/kg/day dosage groups.

Male and female fetal body weights were significantly reduced in the 200 mg/kg/day dosage group. Live litter size was decreased and the number of early resorptions was increased in the 200 mg/kg/day dosage group, but

apparently as the result of one dam in this dosage group that had a litter consisting of 16 early resorptions.

The percentages of fetuses and litters with alterations in the 200 mg/kg/day dosage group were significantly increased and reflected delays in skeletal ossification related to the significantly reduced fetal body weights in this group. These delays in ossification included significant increases in the fetal and/or litter incidences of bifid thoracic vertebrae centra, incompletely and/or not ossified 1st or 2nd sternal centra, incompletely ossified pubes and significant decreases in the numbers of ossified caudal vertebrae, sternal centers and metacarpals. Additionally, delays in ossification occurred in the 100 mg/kg/day dosage group and included a significant increase in the litter incidence of bifid thoracic vertebrae centra.

C. Conclusion

On the basis of these data, the maternal no-observable-adverse-effect-level (NOAEL) of SI0801.01 is less than 25 mg/kg/day. The 200 mg/kg/day dosage caused mortality, the 100 and 200 mg/kg/day dosages caused adverse clinical observations, reductions in body weight gain and reduced feed consumption values, and the 25 mg/kg/day dosage also caused reductions in feed consumption values. The developmental NOAEL is 25 mg/kg/day; the 200 mg/kg/day dosage caused reduced fetal body weights and delays in skeletal ossification and the 100 mg/kg/day dosage also caused delays in skeletal ossification.

Mildred S. Christian, Ph.D., Fellows, ATS Date
Executive Director of Research

Alan M. Hoberman, Ph.D., DABT Date
Director of Research

Raymond G. York, Ph.D., DABT Date
Associate Director of Research and Study Director

II. DESCRIPTION OF TEST PROCEDURES**A. Conduct of Study:****A.1. Sponsor:**

The Procter and Gamble Company, Ivorydale Technical Center, Room 3S40,
5299 Spring Grove Avenue, Cincinnati, Ohio 45217

A.2. Testing Facility:

Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham,
Pennsylvania 19044-1297

A.3. Study Number:

916-025

A.4. Sponsor's Study Number:

SIBTS97.042

A.5. Purpose of the Study:

The purpose of this study was to evaluate the developmental toxicity (embryo-fetal toxicity and teratogenic potential) of SI0801.01 administered orally via gavage to CrI:CD@BR VAF/Plus@ presumed pregnant female rats.

A.6. Study Design:

The requirements of the U.S. Environmental Protection Agency Health Effects Test and Pesticide Assessment Guidelines^(1,2) were used as the basis for study design.

A.7. Regulatory Compliance:

The study was conducted in compliance with Good Laboratory Practice (GLP) regulations of the U.S. Environmental Protection Agency (TSCA/FIFRA)^(3,4). There were no significant deviations from the GLP regulations that affected the quality or integrity of the study. Quality Assurance Unit findings derived from the inspections during the conduct of this study are documented and have been provided to the Study Director and the Testing Facility Management.

A.8. Ownership of the Study:

The Sponsor owns the study. All raw data, analyses, reports and preserved tissues are the property of the Sponsor.

A.9. Study Monitor:

Daniel S. Marsman, D.V.M., Ph.D., DABT

A.10. Study Director:

Raymond G. York, Ph.D., DABT (Associate Director of Research)

A.11. Technical Performance:

John F. Barnett, B.S. (Director of Laboratory Operations)
Margaret M. Martin (Research Associate)
Todd J. Killino, B.S. (Laboratory Technician)

A.12. Report Preparation:

Raymond G. York, Ph.D., DABT
Nancy A. Trenton, B.S. (Study Coordinator)
Denise P. Gasiorowski (Data Management Specialist)
Georgia Y. Burnett, A.A.S. (Administrative Assistant)

A.13. Report Review:

Alan M. Hoberman, Ph.D., DABT (Director of Research)
Mildred S. Christian, Ph.D., Fellow, ATS (Executive Director of Research)

A.14. Date Protocol Signed:

5 June 1998

A.15. Dates of Technical Performance:

Rat Arrival Date	09 JUN 98
Cchabitation Peric	15 JUN 98 PM - 20 JUN 98 AM
Day 0 of Presumed Gestation (DG 0)	16 JUN 98 - 20 JUN 98
Dosage Period (DGs 6 through 19)	22 JUN 98 - 08 JUL 98
Caesarean-Sectioning Period (DG 20)	06 JUL 98 - 09 JUL 98

A.16. Records Maintained:

The original report, raw data and reserve samples of the bulk test substance and vehicle are retained in the archives of Argus Research Laboratories, Inc. Any preserved tissues are retained in the archives of the Testing Facility for twenty years after mailing of the draft final report, after which time the Sponsor will be contacted to determine the disposition of these materials. Prepared formulations were discarded at the Testing Facility. Unused bulk test substance will be returned to the Sponsor.

B. Test Substance Information:**B.1. Description:**

SI0801.01 - light, straw colored liquid

B.2. Lot Number:

.01

B.3. Date Received and Storage Conditions:

The test substance was received on 7 April 1998, and stored at room temperature.

B.4. Special Handling Instructions:

Standard safety precautions (use of protective clothing, gloves, dust-mist respirator and safety goggles or safety glasses and a face-shield) were taken when handling the test substance and test substance solutions.

B.5. Analysis of Activity:

Information regarding the identity, composition, strength and purity of the test substance is on file with the Sponsor.

C. Vehicle Information:**C.1. Description:**

Sterile Water for Injection, USP

C.2. Lot Number:

C379321

C.3. Date Received and Storage Conditions:

The vehicle was received on 12 February 1998 from Baxter Healthcare Corporation, Deerfield, Illinois, and stored at room temperature.

C.4. Analysis of Purity:

Neither the Sponsor nor the Study Director was aware of any potential contaminants likely to be present in the vehicle that would interfere with the results of this study.

D. Test Substance Preparation:

Solutions of the test substance were prepared weekly at the Testing Facility. Dosage calculations were adjusted for the 32% (w/v) concentration of the test substance. Prepared formulations were stored at room temperature and stirred continuously (magnetic stir plate with stir bar) during dosage administration.

D.1. Sample Information:

Sample Type	Size	Date Retained	Storage/Shipping Conditions	Shipped To	Date Shipped
Concentration (all levels)	2 mL ^a	22 JUN 98 ^b 06 JUL 98 ^c 08 JUL 98 ^d	Room temperature	Sponsor	22 JUN 98 06 JUL 98
Bulk Test Substance Reserve	25 mL	22 JUN 98	Room temperature	Testing Facility Archives	09 JUL 98
Vehicle Reserve	5 mL	22 JUN 98	Room temperature	Testing Facility Archives	09 JUL 98

- Duplicate samples were taken from each concentration on the first and last days of preparation. One set of samples was shipped for analysis; the remaining samples were retained at the Testing Facility as backups.
- First day of preparation.
- Last day of preparation.
- Duplicate samples were taken from each concentration prepared on 29 June 1998 with R.O. deionized water. All samples are retained at the Testing Facility.

D.2. Analytical Results:

Documentation of the homogeneity of the test substance in the vehicle over the range of concentrations used in this study is on file with the Sponsor. Stability data for prepared formulations bracketing the range of concentrations in this study are on file with the Sponsor.

E. Test System:**E.1. Species:**

Rat

E.2. Strain:

Crl:CD@BR VAF/Plus® (Sprague-Dawley)

E.3. Supplier (Source):

Charles River Laboratories, Inc., Raleigh, North Carolina

E.4. Sex:

Female

E.5. Rationale for Test System:

The Crl:CD@BR VAF/Plus® (Sprague-Dawley) rat was selected as the Test System because: 1) this strain of rat has been demonstrated to be sensitive to reproductive and developmental toxins and has been widely used throughout industry for nonclinical studies of developmental toxicity (embryo-fetal toxicity/teratology); and 2) historical data and experience exist at the Testing Facility⁽⁵⁻⁷⁾.

E.6. Test System Data:

Number of Rats	142
Approximate Date of Birth	06 APR 98
Approximate Age at Arrival	65 days
Weight (g) on the Day after Arrival	175 - 226
Weight (g) at Study Assignment	218 - 262

E.7. Breeder Male Rat Data:

Number of Rats	150
Approximate Date of Birth	01 OCT 97
Approximate Age at Arrival	42 days
Weight (g) on the Day after Arrival	130 - 169
Weight (g) at Cohabitation	506 - 969

E.8. Method of Randomization:

Upon arrival, rats were assigned to individual housing on the basis of computer-generated random units. Female rats were assigned to four dosage groups (Groups I through IV), twenty-five rats per dosage group, using a computer-generated (weight-ordered) randomization procedure based on body weights recorded on DG 0.

E.9. System of Identification:

Cage tags were marked with the study number, permanent rat number, sex, test substance identification and dosage level. Each rat was individually identified with a Monel® self-piercing ear tag (Gey Band and Tag Co., Inc., No. MSPT 20101) inscribed with the rat's designated unique permanent number.

F. Husbandry:**F.1. Research Facility Registration:**

USDA Registration No. 23-R-099 under the Animal Welfare Act, 7 U.S.C. 2131 *et seq.*

F.2. Study Rooms:

The study rooms were maintained under conditions of positive airflow relative to a hallway and independently supplied with a minimum of ten changes per hour of 100% fresh air that had been passed through 99.97% HEPA filters (Airo Clean® rooms). Room temperature and humidity were monitored constantly throughout the study. Room temperature was targeted at 64°F to 79°F (18°C to 26°C); relative humidity was targeted at 30% to 70%. See APPENDIX E (ENVIRONMENTAL AND HUSBANDRY REPORTS).

F.3. Housing:

Rats were individually housed except during cohabitation period. During cohabitation, each pair of male and female rats was housed in the male rat's cage. All cage sizes and housing conditions were in compliance with the *Guide for the Care and Use of Laboratory Animals*⁽⁸⁾.

F.4. Lighting:

An automatically-controlled fluorescent light cycle was maintained at 12-hours light:12-hours dark, with each dark period beginning at 1900 hours EST.

F.5. Sanitization:

Cage pan liners were changed approximately three times each week. Cages were changed approximately every other week.

F.6. Feed:

Rats were given *ad libitum* access to Certified Rodent Diet® #5002 (Purina Nutrition International, St. Louis, Missouri) in individual feeders.

F.7. Feed Analysis:

Analyses were routinely performed by the feed supplier. No contaminants at levels exceeding the maximum concentration limits for certified feed or deviations from expected nutritional requirements were detected by these analyses. Copies of the results of the feed analyses are available in the raw data and APPENDIX E.

Neither the Study Director nor the Sponsor was aware of any agent present in the feed that would interfere with the results of this study.

F.8. Water:

Local water that had been processed by passage through a reverse osmosis membrane (R.O. water) was available to the rats *ad libitum* from an automatic watering system and/or individual water bottles. Chlorine was added to the processed water as a bacteriostat.

F.9. Water Analysis:

The processed water is analyzed twice annually for possible chemical contamination (Lancaster Laboratories, Lancaster, Pennsylvania) and monthly for possible bacterial contamination (Analytical Laboratories, Chalfont, Pennsylvania). Copies of the results of the water analyses are available in the raw data and APPENDIX E.

Neither the Study Director nor the Sponsor was aware of any agent present in the water that would interfere with the results of this study.

G. Methods:**G.1. Dosage Administration:**

Dosage Group	Dosage* (mg/kg/day)	Concentration (mg/mL)	Volume (mL/kg)	Number of Rats	Assigned Numbers
I	0 (Vehicle)	0	5	25	10501 - 10525
II	25	5	5	25	10526 - 10550
III	100	20	5	25	10551 ^b - 10575
IV	200	40	5	25	10576 - 10600

- a. Dosage levels and concentrations were adjusted for the 32% (w/v) concentration of the test substance.
- b. Rat 10551 was moribund sacrificed due to a possible intubation error on the first day of dosage administration and replaced with rat 4883.

G.2. Rationale for Dosage Selection:

Dosages were selected on the basis of a dosage-range study (Argus Research Laboratories, Inc., Protocol 916-025P), in which dosage levels of 0, 32.5, 100, 325 and 650 mg/kg/day were evaluated.

Dosages of 325 and 650 mg/kg/day were excessively toxic to the dams and conceptuses. At the 32.5 and 100 mg/kg/day dosage levels, no developmental toxicity was observed. Excess salivation was observed at the 100 mg/kg/day dosage level, and slight, non-statistically significant decreases in maternal body weight gains and feed consumption values were observed at the 32.5 and 100 mg/kg/day dosage levels.

G.3. Route of Administration:

Oral (gavage)

G.4. Rationale for Route of Administration:

The oral (gavage) route was selected for use because: 1) in comparison with the dietary route, the exact dosage can be accurately administered; and 2) it is one possible route of human exposure.

G.5. Method and Frequency of Administration:

Female rats were given the test substance once daily on days 6 through 19 of presumed gestation. Dosage volumes were adjusted daily for body weight changes recorded prior to administration and given at approximately the same time each day.

G.6. Length of Study:

Approximately 4 weeks

G.7. Method of Study Performance²:

After acclimation, 140 healthy virgin female rats were placed into cohabitation with 140 breeder male rats (one male rat per female rat in the male rat's cage). Female rats with spermatozoa observed in a smear of the vaginal contents or a copulatory plug *in situ* were considered to be at DG 0 and returned to individual housing.

The rats were observed for viability at least twice a day and for general appearance weekly during acclimation and on DG 0. The rats were also examined for clinical observations of effects of the test substance, abortions, premature deliveries and deaths immediately before and approximately 60 minutes after dosage and on the day of sacrifice (DG 20).

Body weights were recorded weekly during acclimation, on DG 0, daily during the dosage period and on DG 20. Feed consumption values were recorded on DGs 0, 6, 9, 12, 15, 18 and 20.

G.8. Gross Necropsy:

All surviving rats were sacrificed by carbon dioxide asphyxiation on DG 20, and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Caesarean-sectioning and subsequent fetal observations were conducted without knowledge of dosage group in order to minimize bias. Uteri from rats that appeared nonpregnant were stained with 10% ammonium sulfide⁽⁹⁾ to confirm the absence of implantation sites. Tissues with gross lesions were preserved in neutral buffered 10% formalin for possible future evaluation; all other tissues were discarded. Representative photographs of gross lesions are available in the raw data.

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- a. See APPENDIX D, item 1 (DEVIATIONS FROM THE PROTOCOL AND STANDARD OPERATING PROCEDURES).

The number of corpora lutea in each ovary was recorded. The uterus of each rat was excised and examined for pregnancy, number and distribution of implantations, live and dead fetuses and early and late resorptions. The gravid uterus was weighed. An early resorption was defined as one in which organogenesis was not grossly evident. A late resorption was defined as one in which the occurrence of organogenesis was grossly evident. A live fetus was defined as a term fetus that responded to stimuli. Nonresponding term fetuses are considered to be dead (there were no dead fetuses). Dead fetuses and late resorptions are differentiated by the degree of autolysis present; marked to extreme autolysis indicated that the fetus was a late resorption.

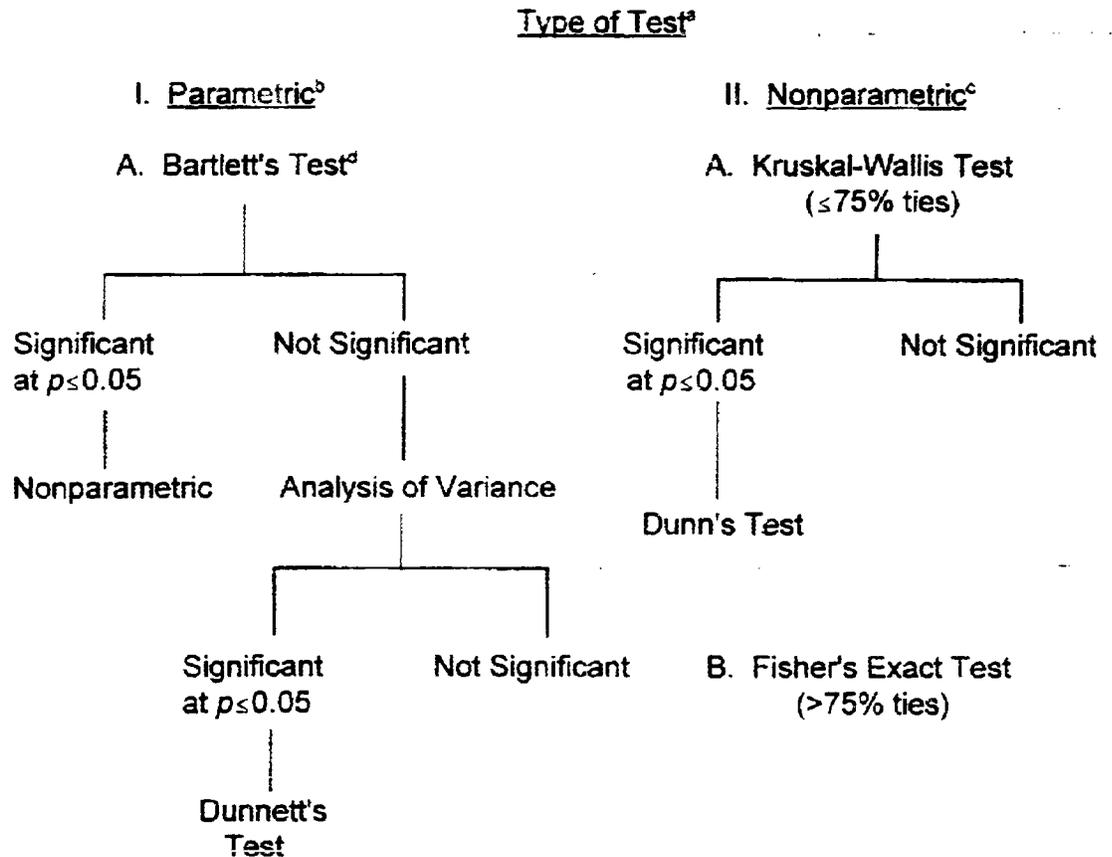
Each fetus was removed from the uterus, placed in an individual container and identified with a tag noting the study number, litter number, uterine distribution and fixative. Each fetus was subsequently weighed and examined for sex and gross external alterations. Live fetuses were sacrificed by an intraperitoneal injection of an appropriate euthanasia solution (Beuthanasia®-D Special, Schering-Plough Animal Health).

Approximately one-half of the fetuses in each litter were examined for soft tissue alterations using a variation of the microdissection technique of Staples⁽¹⁰⁾. The heads of these fetuses were fixed in Bouin's solution and subsequently examined by free-hand cross-sectioning; head sections were stored in alcohol. The decapitated carcasses were processed to be retained in glycerin with thymol added as a preservative. The remaining fetuses in each litter were eviscerated, cleared, stained with alizarin red S⁽¹¹⁾ and examined for skeletal alterations. Skeletal preparations were retained in glycerin with thymol added as a preservative. Photographs of fetal external, soft tissue and skeletal alterations are available in the raw data.

Rats that were found dead were examined on the day the observation was made. The rats were examined for gross lesions. Pregnancy status and uterine contents were recorded. Fetuses were examined to the extent possible using the same methods described for term fetuses.

G.S. Statistical Analysis:

The following schematic represents the statistical analyses of the data:



III. Test for Proportion Data

Variance Test for Homogeneity
of the Binomial Distribution

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- a. Statistically significant probabilities are reported as either $p \leq 0.05$ or $p \leq 0.01$.
 - b. Used only to analyze data with homogeneity of variance.
 - c. Proportion data are not included in this category.
 - d. Test for homogeneity of variance.

Clinical observations and other proportion data were analyzed using the Variance Test for Homogeneity of the Binomial Distribution⁽¹²⁾.

Continuous data (e.g., body weights, body weight changes, feed consumption values, organ weights and litter averages for percent male fetuses, percent resorbed conceptuses, fetal body weights and fetal anomaly data) were analyzed using Bartlett's Test of Homogeneity of Variances⁽¹³⁾ and the Analysis of Variance⁽¹⁴⁾, when appropriate [i.e., Bartlett's Test was not significant ($p > 0.05$)]. If the Analysis of Variance was significant ($p \leq 0.05$), Dunnett's Test⁽¹⁵⁾ was used to identify the statistical significance of the individual groups. If the Analysis of Variance was not appropriate [i.e., Bartlett's Test was significant ($p \leq 0.05$)], the Kruskal-Wallis Test⁽¹⁶⁾ was used, when less than or equal to 75% ties were present. In cases where the Kruskal-Wallis Test was statistically significant ($p \leq 0.05$), Dunn's Method of Multiple Comparisons⁽¹⁷⁾ was used to identify the statistical significance of the individual groups. If there were greater than 75% ties, Fisher's Exact Test⁽¹⁸⁾ was used to analyze the data.

Count data obtained at Caesarean-sectioning of the dams were evaluated using the procedures described above for the Kruskal-Wallis Test⁽¹⁶⁾.

III. RESULTS

A. Mortality, Clinical and Necropsy Observations (Summaries - Tables 1 and 2; Individual Data - Tables 14 and 15)

A.1. Mortality

Two rats in the 200 mg/kg/day dosage group died; one of these deaths was attributed to the test substance, the other was the result of an intubation error. All other rats survived until scheduled sacrifice.

Rat 10597 in the 200 mg/kg/day dosage group was found dead on day 19 of gestation (DG 19) after 13 daily dosages. This dam lost body weight after DG 9 and had reduced feed consumption values throughout the study. Adverse clinical observations included excessive salivation (DGs 8 to 9, 14 and 17 to 18), gasping (DGs 9 to 11 and 17 to 18), labored breathing (DGs 10 to 13 and 16 to 18), ungroomed coat (DGs 10 to 13), brown perianal substance (DGs 12 to 13), urine-stained abdominal fur (DGs 12 to 14 and 16 to 18), brown perivaginal substance (DGs 12 to 15), chromorhinorrhea (DG 15), rales (DG 16) and emaciation (DGs 17 to 18). No gross lesions were revealed by necropsy; all tissues appeared normal for moderate degree of autolysis. There were 17 early resorptions *in utero*. This death was attributed to effects of the test substance because similar observations occurred in surviving rats in this dosage group.

Rat 10585 in the 200 mg/kg/day dosage group was found dead on DG 18 after 12 daily dosages. This dam lost body weight after DG 13 and had reduced feed consumption values throughout the study. Adverse clinical observations included rales (DGs 10 to 11 and 13), excessive salivation (DGs 13 to 14), gasping (DGs 13 to 14), labored breathing (DGs 13 to 17), red perioral substance (DGs 15 to 17), urine-stained abdominal fur (DGs 15 to 17), dehydration (DG 16) and emaciation (DG 17). External observations at necropsy included red substance on fur of the nose, forepaws and forelimbs. Gross necropsy revealed a tear in the esophagus; all other tissues appeared normal for slight degree of autolysis. There were 14 fetuses *in utero*. The viability of the fetuses could not be determined because of the maternal death. This death was attributed to an intubation accident.

A.2. Clinical Observations

Adverse clinical observations occurred at increased incidences in the 100 and 200 mg/kg/day dosage group rats. Excessive salivation, rales, urine-stained abdominal fur, brown or red perioral substance, labored breathing and gasping occurred in 100 mg/kg/day dosage group rats and in significantly increased ($p \leq 0.01$) numbers of 200 mg/kg/day dosage group rats. Additionally,

chromorrhoea occurred in one and two rats in these two respective dosage groups. Observations of brown or red perivaginal substance, emaciation, brown perianal or perinasal substance, dehydration, ungroomed coat and soft or liquid feces occurred in one or two rats in the 200 mg/kg/day dosage group.

All other clinical observations were considered unrelated to the test substance because: 1) the incidences were not dosage-dependent; and/or 2) the observations occurred in only one or two rats. These clinical observations included one 200 mg/kg/day dosage group dam with an axillary mass and localized alopecia (limbs or underside) in one or two rats in the 0 (Vehicle), 25 and 200 mg/kg/day dosage groups.

A.3. Necropsy Observations

All necropsy observations were considered unrelated to the test substance because they were single events. These observations included slight dilation of the pelvis of the right kidney of one vehicle group dam (10505) and one 200 mg/kg/day dosage group dam (10578). Dam 10505 also had a distended urinary bladder with ten calculi and thickened and red bladder walls. One 100 mg/kg/day dosage group dam (10571) had large adrenals, four dark red areas on the fundic mucosa and numerous raised tan areas on the pyloric mucosa of the stomach, the intestines and stomach were distended with gas and the left lateral lobe of the liver was mottled. One 200 mg/kg/day dosage dam (10589) had a mass in the left axilla in-life; at necropsy, a clear tan gelatinous fluid was located subcutaneously in the area of the ventral neck, left axilla and lateral chest, the esophagus had a thickened area and the pyloric folds of the stomach were thickened. These observations were presumed to be the sequelae of a presumed intubation error. Necropsy observations in the rats that died were described previously.

B. Maternal Body Weights, Gravid Uterine Weights and Body Weight Changes (Figure 1; Summaries - Tables 3 and 4; Individual Data - Table 16)

Rats in the 100 and 200 mg/kg/day dosage groups had significantly reduced ($p \leq 0.01$) body weight gains for the entire dosage period (calculated as days 6 to 20 of gestation). Within the dosage period, body weight gains were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in these groups on DGs 18 to 20. Significant ($p \leq 0.01$) body weight loss or reductions in body weight gains also occurred in the 200 mg/kg/day dosage group on DGs 6 to 9, 9 to 12 and 15 to 18. As a result of these changes, body weight gains were significantly reduced ($p \leq 0.01$) in the 200 mg/kg/day dosage group for the entire gestation period (DGs 0 to 20) and body weights were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in this dosage group on DGs 11 through 20.

The gravid uterine weight and the corrected DG 20 maternal body weight (DG 20 body weight minus the gravid uterine weight) were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 200 mg/kg/day dosage group. When body weight changes were calculated for the entire dosage period and the entire gestation period using the corrected DG 20 body weight (DGs 6 to 20C, DGs 0 to 20C), body weight gains were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 100 and 200 mg/kg/day dosage groups.

Body weights and body weight gains were unaffected at dosages of 25 mg/kg/day of the test substance. Gravid uterine weights were unaffected by the 100 mg/kg/day dosage of the test substance.

C. Maternal Absolute (g/day) and Relative (g/kg/day) Feed Consumption Values (Summaries - Tables 5 and 6; Individual Data - Table 17)

Absolute (g/day) feed consumption values for the entire dosage period (calculated as DGs 6 to 20) and the entire gestation period (DGs 0 to 20) were significantly reduced ($p \leq 0.01$) in the 200 mg/kg/day dosage group. Absolute feed consumption values were also significantly reduced ($p \leq 0.01$) for all tabulated intervals in the 200 mg/kg/day dosage group, significantly reduced ($p \leq 0.01$) in the 100 mg/kg/day dosage group on DGs 18 to 20 and significantly reduced ($p \leq 0.05$) on DGs 15 to 18 in the 25 and 100 mg/kg/day dosage groups. Relative (g/kg/day) feed consumption values for the entire dosage period were significantly reduced ($p \leq 0.01$) in the 100 and 200 mg/kg/day dosage groups, while values for the entire gestation period were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 25, 100 and 200 mg/kg/day dosage groups. Relative feed consumption values were also significantly reduced ($p \leq 0.01$) in the 200 mg/kg/day dosage group for all tabulated intervals and significantly reduced ($p \leq 0.01$ or $p \leq 0.05$) in the 25 and 100 mg/kg/day dosage groups on DGs 12 to 15, 15 to 18 and 18 to 20.

D. Caesarean-Sectioning and Litter Observations (Summaries - Tables 7 and 8; Individual Data - Tables 18 through 21)

Caesarean-sectioning observations were based on 24, 25, 24 and 22 pregnant rats in the 0 (Vehicle), 25, 100 and 200 mg/kg/day dosage groups, respectively. Male and female fetal body weights were significantly reduced ($p \leq 0.01$) in the 200 mg/kg/day dosage group. Live litter size was decreased and the number of early resorptions was increased in the 200 mg/kg/day dosage group. These values reflect one dam in this dosage group that had a litter consisting of 16 early resorptions.

No other Caesarean-sectioning or litter parameters were affected by dosages of the test substance as high as 200 mg/kg/day. The litter averages for corpora

lutea, implantations, late resorptions, percent resorbed conceptuses (calculated excluding the completely resorbed litter in the 200 mg/kg/day dosage group), and percent live male fetuses were comparable among the four dosage groups and did not significantly differ. There were no dead fetuses.

E. Fetal Alterations (Summaries - Tables 9 through 13; Individual Data - Table 21)

Fetal alterations were defined as: 1) malformations (irreversible changes that occur at low incidences in this species and strain); or 2) variations (common findings in this species and strain and reversible delays or accelerations in development). Litter averages were calculated for specific fetal ossification sites as part of the evaluation of the degree of fetal ossification.

Fetal evaluations were based on 339, 375, 348 and 297 Caesarean-delivered live fetuses in 24, 25, 24, and 21 litters in the 0 (Vehicle), 25, 100 and 200 mg/kg/day dosage groups, respectively. Each fetus was examined for gross external alterations, approximately one half of the fetuses in each litter were examined for soft tissue alterations and the remaining fetuses were examined for skeletal alterations and the number of ossification sites.

The percentages of fetuses and litters with alterations in the 200 mg/kg/day dosage group were significantly increased ($p \leq 0.01$) and reflected delays in skeletal ossification related to the significantly reduced ($p \leq 0.01$) fetal body weights in this group. These delays in ossification included significant increases ($p \leq 0.01$) in the fetal and/or litter incidences of bifid thoracic vertebrae centra, incompletely and/or not ossified 1st or 2nd sternal centra, incompletely ossified pubes and significant decreases ($p \leq 0.01$) in the numbers of ossified caudal vertebrae, sternal centers and metacarpals. Additionally, delays in ossification occurred in the 100 mg/kg/day dosage group and included a significant increase ($p \leq 0.05$) in the litter incidence of bifid thoracic vertebrae centra.

All other gross external, soft tissue or skeletal alterations (malformations or variations) were considered unrelated to the test substance because: 1) the litter and fetal incidences were not dosage-dependent; 2) the alteration occurred in only one fetus; or 3) the incidences were within the ranges observed historically at the Testing Facility^a.

a. See APPENDIX F (HISTORICAL CONTROL DATA).

E.1. Summary of Fetal Alterations (Summary - Table 9; Individual Data - Table 21)

In Groups I through IV, litters with fetuses with alterations numbered 8 (33.3%), 11 (44.0%), 8 (33.3%) and 15 (71.4%)**, respectively. The numbers of fetuses with any alteration were 11 (3.2%), 25 (6.7%), 14 (4.0%) and 32 (10.8%)** respectively. The percentages of fetuses with any alteration were 3.6%, 6.4%, 4.5% and 11.5%* in the four respective dosage groups. All fetal alterations that occurred in this study are described in the following sections.

The significant increases in the percentages of fetuses and litters with alterations in the 200 mg/kg/day dosage group were considered to reflect delays in skeletal ossification, related to the significantly reduced ($p \leq 0.01$) fetal body weights in this group.

E.2. Fetal Gross External Alterations (Summary - Table 10; Individual Data - Table 21)

One vehicle group fetus (10510-10) had whole body edema (anasarca); this fetus also had an absent innominate artery at soft tissue examination. One 25 mg/kg/day fetus (10537-5) had a thread-like tail; at skeletal evaluation, this fetus had fused arches of the 3rd sacral vertebra and no caudal vertebrae. One 200 mg/kg/day group fetus (10595-13) had a kinked tail, the 4th and 5th digits of the left hindlimb were fused and a skin tab located to the left of its tail. At skeletal evaluation of this fetus, further evaluation of the skin tab revealed two bones (possibly a femur and fibula) fused together on the left side of the pelvis. An extra paw appeared to be attached to the underside of the left paw; also the centra of the 6th and 11th thoracic vertebrae were bifid. One 200 mg/kg/day fetus (10598-8) had micrognathia; soft tissue evaluation of this fetus revealed a small tongue.

E.3. Fetal Soft Tissue Alterations (Summary - Table 11; Individual Data - Table 21)**E.3.a. Malformations**

One 200 mg/kg/day fetus (10598-8) had a small tongue; micrognathia was identified at gross external evaluation, as previously described.

** Significantly different from the vehicle control group value ($p \leq 0.01$).

* Significantly different from the vehicle control group value ($p \leq 0.05$).

E.3.b. Variations

One control group fetus (10510-10) and one 25 mg/kg/day dosage group fetus (10548-13) had an absent innominate vessel; whole body edema (anasarca) was identified at gross external evaluation for the control fetus, as previously described and fetus 10548-13 also had the umbilical artery descending to the left of the urinary bladder.

E.4. Fetal Skeletal Alterations (Summaries - Tables 12 and 13; Individual Data - Table 21)**E.4.a. Malformations**

One control group fetus (10523-14) had fused arches of the 4th cervical vertebra; additional variations in ossification occurred in this fetus (unossified 1st sternal centra, incompletely ossified pubes).

One 25 mg/kg/day dosage group fetus (10537-5) had fused arches of the 3rd sacral vertebra; this fetus also had no caudal vertebrae (thread-like tail was noted at gross external examination).

E.4.b. Variations**E.4.b.1. Ribs**

A cervical rib was present at the 7th cervical vertebra in one 25 mg/kg/day fetus (10532-9). This fetus had no other skeletal alterations.

E.4.b.2. Vertebrae

A bifid centrum in the thoracic vertebrae occurred in 1, 6*, 5 and 9** fetuses from 1, 3, 5* and 8** litters in the 0 (Vehicle), 25, 100 and 200 mg/kg/day dosage groups, respectively. Fetus 10544-11 in the 25 mg/kg/day dosage group also had incompletely ossified 1st and 2nd sternal centra, fetus 10558-9 in the 100 mg/kg/day dosage group also had a bifid centrum of the 1st lumbar vertebra and fetus 10580-10 in the 200 mg/kg/day dosage group also had incompletely ossified pubes.

* Significantly different from the vehicle control group value ($p \leq 0.05$).

** Significantly different from the vehicle control group value ($p \leq 0.01$).

An incompletely ossified arch in the 6th lumbar vertebra occurred in one 100 mg/kg/day dosage group fetus (10573-15); this fetus also had incompletely ossified ischia and pubes.

The significant increase ($p \leq 0.05$) in the litter incidence of bifid thoracic vertebrae centra in the 100 mg/kg/day dosage group and the significant increase ($p \leq 0.01$) in the fetal and litter incidences of this alteration in the 200 mg/kg/day dosage group were considered related to the test substance because: 1) it was dosage-dependent; 2) the litter incidence, the more relevant parameter⁽¹⁹⁾ was significant; and/or 3) the litter and/or fetal incidences were outside the ranges observed historically at the Testing Facility^a. The significant increase ($p \leq 0.05$) in the fetal incidence of bifid thoracic vertebrae centra in the 25 mg/kg/day dosage group was considered unrelated to the test substance because: 1) the litter incidence, the more relevant parameter⁽¹⁹⁾ was not significant; and 2) the litter and/or fetal incidences were within the ranges observed historically at the Testing Facility.

E.4.b.3. Sternum

Delayed sternal ossification (incompletely ossified and/or not ossified 1st and/or 2nd sternbrae) occurred in 4, 8, 3 and 11** fetuses from 4, 6, 2 and 7 litters in the 0 (Vehicle), 25, 100 and 200 mg/kg/day dosage groups, respectively. The fetal incidence of only incompletely ossified 1st sternal centra was also significantly increased ($p \leq 0.05$ or $p \leq 0.01$) in the 25 and 200 mg/kg/day dosage groups. Additional skeletal alterations in the fetuses were previously discussed or include delays in pelvic ossification.

The significant increases ($p \leq 0.01$) in the fetal incidences of incompletely and/or not ossified 1st or 2nd sternal centra and incompletely ossified 1st sternal centra in the 200 mg/kg/day dosage group were considered related to the test substance because: 1) it was dosage-dependent; and/or 2) the fetal incidence was outside the ranges observed historically at the Testing Facility. The significant increase ($p \leq 0.05$) in the fetal incidence of incompletely ossified 1st sternal centra in the 25 mg/kg/day dosage group was considered unrelated to the test substance because: 1) it was not dosage-dependent; and 2) the fetal incidence was not significantly increased when all delays in sternal ossification were summarized.

a. See APPENDIX F (HISTORICAL CONTROL DATA).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Bifid thoracic vertebrae centra, Historical Control Range: 0-9 (0-4.7%) fetuses/litter
0-8 / litter / study.

E.4.b.4. Pelvis

The pubes and/or ischia were incompletely ossified in 8, 12, 7 and 16 fetuses from 6, 4, 3 and 6 litters in the 0 (Vehicle), 25, 100 and 200 mg/kg/day dosage groups, respectively. The fetal incidence of only incompletely ossified pubes was significantly increased ($p \leq 0.01$) in the 200 mg/kg/day dosage group. Additional skeletal alterations in these fetuses were discussed previously.

The significant increase ($p \leq 0.01$) in the fetal incidence of incompletely ossified pubes in the 200 mg/kg/day dosage group was considered related to the test substance because: 1) it was dosage-dependent; and 2) the fetal incidence was outside the range observed historically at the Testing Facility.

E.4.b.5. Fetal Ossification Site Averages

The litter averages for ossified caudal vertebrae, sternal centers and metacarpals per fetus were significantly decreased ($p \leq 0.01$) in the 200 mg/kg/day dosage group and considered related to the test substance because: 1) it was dosage-dependent; and/or 2) the values were below the ranges observed historically at the Testing Facility.

Analyses of the average numbers of fetal ossification sites per fetus did not reveal any other statistically significant differences among the four dosage groups. Ossification of the hyoid, vertebrae (cervical, thoracic, lumbar and sacral), ribs, sternum (manubrium and xiphoid), forelimbs (carpals and phalanges) and hindlimbs (tarsals, metatarsals and phalanges) occurred at similar incidences in litters in all dosage groups.

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