

CODING FORMS FOR SRC INDEXING

Microfiche No.			OTS0509763-16		
New Doc ID		89-941000018		Old Doc ID	
				8EHQ-1293-0576	
Date Produced		Date Received		TSCA Section	
12/14/93		12/29/93		8E	
Submitting Organization			ARCO CHEMICAL CO		
Contractor			ARGUS RESEARCH LABS INC		
Document Title			SUPPORT: LETTER FROM ARCO CHEMICAL CO TO USEPA REGARDING TERATOGENICITY STUDIES OF DERMALLY ADMINISTERED REFINERY STREAMS IN RATS WITH ATTACHMENTS DATED 122393		
Chemical Category			GAS OIL INTERMEDIATES; STRAIGHT RUN DIESEL; HEAVY VACUUM GA*		

SUPP

OFFICE OF TOXIC SUBSTANCES
CODING FORM FOR GLOBAL INDEXING

REV. 7/27/82

Microfiche No. (7) •		1		No. of Pages		2	
Doc I.D.		3		Old Doc I.D.		4	
89941000018				8EHQ-1293-0576			
Case No. (8)						5	
Date Produced (6)		6		Date Rep'd (6)		7	
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Check One:		<input type="checkbox"/> Publication		<input type="checkbox"/> Internally Generated		<input type="checkbox"/> Externally Generated	
Pub/Journal Name						9	
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Author(s)						10	
Organ. Name						11	
Dept/Div						12	
P.O. Box		13		Street No./Name		14	
City		15		State		16	
				Zip		17	
				Country		18	
MID No. (7)		19		D S B NO. (11)		20	
Contractor						21	
Doc Type				PE		22	
Doc Title						23	
Chemical Name (300 char name)		25		CAS No. (10)		24	



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William D. Leake
Vice President
Environment, Health & Safety

December 23, 1993

8EHQ-1293-0576

Be Supp.

REC'D
OFFICE OF POLLUTION
PREVENTION AND TOXICS

EPA-OTS



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Document Control Officer (TS-790)
Attention: 8(e) Coordinator
Office of Toxic Substances
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, DC 20460

DCN #: 89-94100008

Contains No CBI

Subject: Final Reports for Previously Submitted TSCA Section 8(e)
Notices on Refinery Streams Suspected of Containing Varying
Levels of Carbazoles that are Contained in Carbon Black Oil
(CAS 64741-62-4; 8EHQ-1185-0576)

PDC.N: 88860000036

Dear Sir/Madam:

The Atlantic Richfield Company (ARCO) has submitted information on the preliminary results of several studies in experimental animals to assess the developmental toxicity of refinery streams suspected of containing carbazoles. This transmittal provides you with final reports for studies on six of these materials.

These are follow-up studies initiated by ARCO in response to previous reports by Mobil and ARCO (8EHQ-1185-0576) of adverse effects on rat fetuses after dermal exposure to carbon black oil (CBO), a material which contains carbazoles.

In previous submissions to EPA, ARCO reported that preliminary results for several refinery streams tested for reproductive and developmental toxicity indicated signs of fetal toxicity for these materials. Enclosed are final reports for teratology studies on six materials.

In the studies submitted today, various refinery streams were dermally administered to groups of pregnant rats during gestational days 0 to 19 (or a shorter time period if irritation was excessive). Cesarean sections were performed on day 20 of presumed gestation and observations were made on several parameters including corpora lutea, implantation sites, live and dead fetuses, resorptions, as well as body weights and a gross external examination of all fetuses. Fetuses were divided into groups for examination of either soft tissue or skeletal alterations. The materials studied and the results are summarized below.

21048 pp.

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<u>Material Code</u>	<u>Material Name</u>	<u>CAS#</u>	<u>Previous TSCA Submissions</u>
F-193	Gas Oil Intermediates	68783-08-4	11/13/91 7/29/92
F-195	Straight Run Diesel	68410-00-4	10/22/92 1/29/93 3/23/93
F-196	Heavy Vacuum Gas Oil	64741-57-7	11/13/91 7/14/92
F-197	VDF Gas Oil	64741-57-7	11/13/91 1/29/93 3/23/93
F-199	Light Coker Gas Oil	64741-82-8	11/13/91
F-215	VDF Diesel	68410-00-4	4/8/92

For Straight Run Diesel (F-195), Light Coker Gas Oil (F-199) and VDF Diesel (F-215) the results indicate that there were no effects on embryo-fetal viability or fetal body weights or morphology at the highest doses tested. In each of these studies signs of maternal toxicity were observed.

For VDF Gas Oil (F-197) the investigators concluded that there was reduced litter size and fetal body weights and increased variations in fetal skeletal ossification in the highest dose group. The delays in ossification were considered effects of the test substance and related to the reduced fetal body weights in this dose group. The investigators concluded that the material should not be identified as a developmental toxicant since adverse effects were produced only by the highest of the three dose groups, all of which produced signs of maternal toxicity.

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For Gas Oil Intermediates (F-193) the investigators report that treatment with the test material caused reductions in litter size, increased resorptions, decreased fetal weights and reduced ossification in the mid and high dose groups. Treatment at these levels was the possible cause of increased incidences of alterations such as decreased eye size, incompletely ossified (cleft) palate, hydronephrosis, retarded ossification of the thoracic vertebrae and sternum, and umbilical hernia. No adverse effects were observed in fetuses in the low dose group. Signs of maternal toxicity were observed in all dose groups.

For Heavy Vacuum Gas Oil (F-196) the investigators concluded that treatment with this material caused reductions in litter sizes and fetal body weights, increased resorptions, retarded ossification and was the possible cause of an increased incidence of microphthalmia. Signs of maternal toxicity were observed in all treatment groups. Investigators were unable to draw conclusions regarding the relative susceptibility of the conceptuses vis a vis the dams for this material.

Our current MSD Sheets on the refinery streams tested in these studies are being reviewed with these results in mind.

Sincerely yours,

William D. Leake
By Randy Ross

William D. Leake

Enclosures

REC'D
OFFICE OF POLLUTION
PREVENTION AND TOXICS

ARGUS 1001-005

93 DEC 29 PM 2:23

Contains No CBI

Study Title

DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC
POTENTIAL) STUDY OF F-193 ADMINISTERED PERCUTANEOUSLY TO
Cr1:CD*BR VAF/Plus* PRESUMED PREGNANT RATS

Data Requirement

Toxic Substances Control Act Test Guidelines
Health Effects Testing Guidelines - 798.4900

Author

Terry Martin, D.V.M., M.S., A.B.V.T.
(Study Director)

Study Completed On

December 14, 1993
(Final Report)

Performing Laboratory

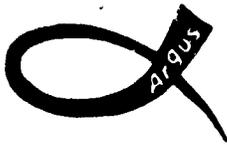
Argus Research Laboratories, Inc.
905 Sheehy Drive
Horsham, Pennsylvania 19044

Laboratory Project ID

Argus Research Laboratories, Inc., Protocol Number: 1001-005

ARGUS 1001-005

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ARGUS 1001-005

ARGUS RESEARCH LABORATORIES, INC.

905 Sheehy Drive, Bldg. A
Horsham, Pennsylvania 19044-1297
(215) 443-8710
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TITLE: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF F-193 ADMINISTERED PERCUTANEOUSLY TO Cr1:CD®BR VAF/Plus® PRESUMED PREGNANT RATS

ARGUS RESEARCH LABORATORIES, INC., PROTOCOL NUMBER: 1001-005

I. SUMMARY AND CONCLUSION

A. Methods

The purpose of this study was to evaluate the developmental toxicity (embryo-fetal toxicity and teratogenic potential) of F-193 administered percutaneously to Cr1:CD®BR VAF/Plus® (Sprague-Dawley) presumed pregnant rats. The standards of the U.S. Environmental Protection Agency (EPA TSCA)^(1,2) were used as the basis for study design and compliance with Good Laboratory Practices (GLPs).

This study was sponsored by ARCO, 515 South Flower Street, Los Angeles, California 90071. The Sponsor's Study Monitor was Mark D. Saperstein, D.Env. The study was conducted by and at Argus Research Laboratories, Inc., 905 Sheehy Drive, Horsham, Pennsylvania 19044. The Study Director was Terry Martin, D.V.M., M.S., A.B.V.T. (Senior Scientist/Staff Veterinarian).

-
- 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 AND AMENDMENT).

The first day 0 of presumed gestation occurred on June 9, 1992. The last day 20 of presumed gestation Caesarean-sectioning occurred on July 2, 1992.

Each dosage group consisted of 25 randomly assigned Crl:CD®BR VAF/Plus® (Sprague-Dawley) presumed pregnant female rats. The test substance, F-193^a, was administered percutaneously to these female rats at dosages of 0 (Vehicle, acetone), 50, 250 and 500 mg/kg/day on days 0 through 19 of presumed gestation. The dosage volume was 1 mL/kg, adjusted daily based on the individual body weights recorded immediately before application of the vehicle or the test substance.

The rats were examined daily during presumed gestation for clinical observations, abortions, premature deliveries and deaths. Body weights and feed consumption values for the rats were recorded daily during presumed gestation. During the dosage period, the rats were examined once daily for skin reactions, immediately before application of the test substance. The evaluation of skin reactions was performed without the observer's knowledge of the dosage group.

On day 20 of presumed gestation, all rats were sacrificed and necropsied. Tissues with gross lesions were retained. The numbers and distribution of implantations, early and late resorptions, live and dead fetuses and corpora lutea were recorded. Each fetus was weighed and examined for sex and external alterations. Approximately one-half of the fetuses in each litter were examined for soft tissue alterations by using an adaptation of Wilson's sectioning technique⁽³⁾. The remaining fetuses in each litter were stained with alizarin red S⁽⁴⁾ and examined for skeletal alterations.

The test substance was considered 100% pure for the purpose of dosage calculations.

B. Results

No deaths occurred during the conduct of this study.

Skin reactions related to percutaneous administration of the test substance occurred in the 50, 250 and 500 mg/kg/day dosage groups. These skin reactions included desquamation, edema, erythema and/or atonia.

Maternal body weight and body weight gains were generally reduced or significantly reduced ($P \leq 0.05$ to $P \leq 0.01$) in the 250 and 500 mg/kg/day dosage groups for the entire dosage period (calculated as days 0 to 20 of gestation). Absolute (g/day) and relative (g/kg/day) feed consumption values were significantly reduced ($P \leq 0.05$ to $P \leq 0.01$) in the 250 and 500 mg/kg/day dosage groups for the entire dosage period (calculated as days 0 to 20 of gestation). Relative feed consumption values were increased or significantly increased ($P \leq 0.05$ to $P \leq 0.01$) in the 250 and/or 500 mg/kg/day dosage groups on days 15 to 18 and 19 to 20 of gestation. These increases were interrelated with the significantly reduced ($P \leq 0.01$) body weights in these dosage groups.

Percutaneous administration of the 250 and 500 mg/kg/day dosages of F-193 caused significant reductions ($P \leq 0.01$) in the average litter sizes and live fetuses and increases or significant increases ($P \leq 0.01$) in resorptions (total and early resorptions) in these dosage groups. The numbers of dams with any resorptions was also increased in the 250 mg/kg/day dosage group and significantly increased ($P \leq 0.01$) in the 500 mg/kg/day dosage group. Reflecting these effects of the test substance, the percentage of resorbed conceptuses per litter was increased in the 250 mg/kg/day dosage group and was significantly increased ($P \leq 0.01$) in the 500 mg/kg/day dosage group. Fetal body weights (male and female) were significantly reduced ($P \leq 0.01$) in the 250 and 500 mg/kg/day dosage groups.

The 250 and 500 mg/kg/day dosages of the test substance were the possible cause of increased incidences of eye malformations [decrease in size, evident as small eye bulge(s) at gross external examination, and small eye sockets at skeletal examination] and cleft palate evident as incompletely ossified palate at skeletal examination in the fetuses. The absence of a clear dosage-dependent incidence for this malformation may be interrelated with the dosage-dependent increases in resorption (embryo-fetal deaths) in the 250 and 500 mg/kg/day dosage groups.

Other alterations possibly attributed to the test substance included increases or significant increases ($P \leq 0.01$) in the litter and/or fetal incidences of hydronephrosis and bifid thoracic vertebral centra in the 250 and 500 mg/kg/day dosage groups, and umbilical hernia and delayed sternal ossification in the 500 mg/kg/day dosage group.

Significant reductions ($P \leq 0.05$ to $P \leq 0.01$) occurred in the average number of ossified caudal vertebrae in the 250 and 500 mg/kg/day dosage groups and ossified sternal centers, metacarpals, metatarsals and hindpaw phalanges in the 500 mg/kg/day dosage group; these alterations were considered related to the test substance because: 1) the litter and fetal incidences were increased or significantly increased; 2) the incidences were significantly reduced (ossification sites); and/or 3) the incidences exceeded the ranges observed historically.

C. Conclusion

On the basis of the results of this study, the maternal no-observable-adverse-effect-level (NOAEL) for F-193 is less than 50 mg/kg/day (skin reaction occurred in the 50, 250 and 500 mg/kg/day dosage groups, and reduced body weight gains and feed consumption values occurred in the 250 and 500 mg/kg/day dosage groups). The developmental

NOAEL for F-193 was 50 mg/kg/day (reduced fetal body weights and embryo-fetal viability, and fetal gross external, soft tissue and skeletal alterations occurred in the 250 and 500 mg/kg/day dosage groups).

Mildred S. Christian 12/14/93

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

Alan M. Hoberman 12/14/93

Alan M. Hoberman, Ph.D., D.A.B.T. Date
Director of Research

Terry Martin 12/14/93

Terry Martin, D.V.M., M.S., A.B.V.T. Date
Senior Scientist and Study Director

II. DESCRIPTION OF TEST PROCEDURES

A. Methodology

1. General Experimental Design and Procedures*

Each dosage group consisted of 25 randomly assigned Cr1:CD®BR VAF/Plus® (Sprague-Dawley) presumed pregnant female rats. The test substance, F-193^b, was administered percutaneously to these female rats at dosages of 0 (Vehicle, acetone), 50, 250 and 500 mg/kg/day on days 0 through 19 of presumed gestation. The dosage volume was 1 mL/kg, adjusted daily based on the individual body weights recorded immediately before application of the vehicle or the test substance.

The rats were examined daily during presumed gestation for clinical observations, abortions, premature deliveries and deaths. Body weights were recorded weekly during the acclimation period and daily during presumed gestation. Feed consumption values for the rats were recorded daily during presumed gestation. During the dosage period, the rats were examined once daily for skin reactions, immediately before application of the test substance. The evaluation of skin reactions was performed without the observer's knowledge of the dosage group.

On day 20 of presumed gestation, all rats were sacrificed by carbon dioxide asphyxiation and necropsied. Tissues with gross lesions were retained in neutral buffered 10% formalin. The numbers and distribution of implantations, early and late resorptions, live and dead fetuses and corpora lutea were recorded. Each fetus was individually identified with a tag noting litter, uterine distribution and study

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- a. Detailed descriptions of all procedures used during the conduct of this study are provided in the appropriate sections of this report and in APPENDIX C (PROTOCOL AND AMENDMENT).
 - b. The test substance was considered 100% pure for the purpose of dosage calculations.

Coker, B.A. (Methods Writer) and Georgia Y. Burnett, A.A.S. (Word Processor). Additional personnel participating in the conduct of the study are identified in the raw data.

The report was written by Terry Martin. It was reviewed by Mila S. Martinova, M.D. (Senior Scientist), Alan M. Hoberman, Ph.D., D.A.B.T. (Director of Research) and Mildred S. Christian, Ph.D., ATS (Executive Director of Research).

Curricula vitae and training records of personnel involved in the study are on file at Argus Research Laboratories, Inc.

The standards of the U.S. Environmental Protection Agency (EPA TSCA)^(1,2) were used as the basis for study design and compliance with Good Laboratory Practices (GLPs).

3. Test Substance and Vehicle Identification

The test substance, F-193 (lot identification is on file with the Sponsor)^a, a brown to black-colored liquid, was supplied by the Sponsor. Documentation of the identity, composition, stability, strength and purity of the test substance is on file with the Sponsor. The test substance was received at the Test Facility on June 9 and June 10, 1992, and stored at room temperature, protected from light.

The vehicle, acetone, was obtained from Baxter Healthcare Corporation (Lot H845 KJDA). The vehicle was received on June 3, 1992, and was stored refrigerated (20°C to 30°C).

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- a. The test substance was considered 100% pure for the purpose of dosage calculations.

B. Animal Data

1. Test System

The CrI:CD®BR VAF/Plus® rat was selected for evaluation of the test substance because: 1) it is one mammalian species accepted for use in developmental toxicity (embryo-fetal toxicity/teratogenicity) studies; 2) this strain of rat has been demonstrated to be sensitive to developmental toxins; 3) it has been widely used throughout industry for nonclinical studies of developmental toxicity; and 4) historical data and experience exist at the Test Facility⁽⁵⁻⁷⁾.

On May 19, 1992, 140 virgin female rats from Charles River Laboratories, Inc., Portage, Michigan were received at the Test Facility. At receipt, the female rats were approximately 66 days of age (approximate birthdate: March 15, 1992) and weighed from 192 to 231 g on the day after arrival.

The breeder male rats were selected from two shipments of male rats of the same strain and source as the female rats. The following information provides the ages and weights of the populations from which the breeder male rats were selected:

	<u>Shipment 1</u>	<u>Shipment 2</u>
Date of Receipt	October 29, 1991	May 12, 1992
Number of Rats Received	110	90
Approximate Age at Receipt and Approximate Birthdate	31 days September 29, 1991	112 days January 22, 1992
Weight on Day after Receipt	60 to 96 g	375 to 445 g
Number of Rats Selected for Use	108	32
Weight on the Day Cohabitation Began	511 to 778 g	480 to 566 g

Following the acclimation period, 140 healthy virgin female rats were placed in cohabitation with 140 breeder male rats (one male rat per female rat). Female rats with spermatozoa observed in a smear of vaginal contents or a copulatory plug observed in situ were considered to be at day 0 of presumed gestation and returned to individual housing. The male rats were used only to breed the female rats, were not administered the test substance and, therefore, were not considered to be part of the Test System.

2. Experimental Design and Control of Bias

Upon arrival, the male and female rats were assigned to individual housing on the basis of a computer-generated randomization procedure. At this time, each female rat was assigned a temporary pretest number (1 through 140) that was used for identification during the acclimation period. After receipt, each male rat was individually identified with a Monel® self-piercing ear tag^a inscribed with the rat's designated unique permanent number.

A second computer-generated (weight-ordered^d) randomization procedure was used to assign 25 apparently healthy mated female rats to each of the four dosage groups, based on body weights recorded on day 0 of presumed gestation. Dosage group assignment was as follows:

<u>Dosage Group</u>	<u>Dosage F-193 (mg/kg/day)</u>	<u>Number of Rats</u>	<u>Female Rat Numbers</u>
I	0(Vehicle)	25	9201 to 9225
II	50	25	9226 to 9250
III	250	25	9251 to 9275
IV	500	25	9276 to 9300

a. Gey Band and Tag Co., Inc., No. MSPT 20101.

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3. Environmental Conditions^a

The rats were housed individually in wire-bottomed stainless-steel cages (17.5 cm x 17.5 cm x 24 cm) suspended above absorbent paper liners, except during the cohabitation period. Cages were changed approximately twice each month, and cage pan liners were changed approximately three times each week. During the cohabitation period (a maximum of five days), male and female rats were housed together (one male rat per each female rat) in the male rat's cage. Upon observation of spermatozoa in a smear of vaginal contents or a copulatory plug in situ, the presumed pregnant rat was removed from cohabitation and individually housed. Because the dams were terminated before the day natural delivery is expected to occur, nesting materials were not supplied.

The rats were housed in Room 2 (192 square feet of floor space) until the end of the cohabitation period, when the rats were moved to Room 4 (150 square feet of floor space). The rats remained in Room 4 until scheduled sacrifice. The study rooms were under conditions of positive airflow relative to a hallway and were 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 was recorded constantly throughout the study and was targeted at 70°F to 78°F. Room humidity was also recorded constantly during the study and was targeted at 40% to 70%. Deviations (small increases or decreases in room temperature and/or humidity) occurred and are cited in APPENDIX D. These deviations in temperature did not adversely affect the outcome of the study, and no other deviations in the environmental parameters occurred during the study.

a. All cage sizes and housing conditions are in compliance with the Guide for the Care and Use of Laboratory Animals, NIH Publication No. 86-23⁸¹.

An automatically-controlled fluorescent light cycle was maintained at 12 hours light:12 hours dark, with each dark period beginning at 1800 hours EST (1900 hours EDT).

Throughout the study, the rats were given Certified Rodent Chow® #5002 (Ralston Purina); feed was available ad libitum. Analyses were routinely performed by the feed supplier. No contaminants in the 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 in APPENDIX E. The rats were fed from feed lot MAR 18 92 2D for the entire study period.

Local water that had been processed by passage through a reverse osmosis membrane (R.O. water) was available to the rats ad libitum (individual sipper tubes) from an automatic watering system and/or individual water bottles. Chlorine was added to the processed water as a bacteriostat. The processed water is analyzed twice annually for possible chemical contamination (Lancaster Laboratories, Inc., Lancaster, Pennsylvania) and monthly for possible bacterial contamination (Purity-Standard Laboratories, Chalfont, Pennsylvania). The water contained approximately 0.2 to 0.6 ppm chlorine at the time of the analyses and was determined to be suitable for consumption. Copies of the results of the water analyses are available in the raw data and in APPENDIX F.

No agent present in either the feed or water was known to interfere with the results of this study.

4. Preparation of the Test Substance^a

Safety precautions (use of protective clothing, gloves, a half-face cartridge respirator or positive pressure hood and safety glasses with

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- a. Detailed preparation procedures are available in the raw data and APPENDIX C (PROTOCOL AND AMENDMENT).

face shield or safety goggles) were taken when handling or administering the bulk test substance or dosage suspensions. Suspensions of the test substance in acetone were prepared daily. The following concentrations of F-193 were prepared:

<u>Concentration^a</u> <u>(mg/mL)</u>	<u>Argus Batch Number^b</u>
0 (Vehicle)	B-1001-005-A (Month.Day.Year)
50	B-1001-005-B (Month.Day.Year)
250	B-1001-005-C (Month.Day.Year)
500	B-1001-005-D (Month.Day.Year)

Two samples (5 g each) of the bulk test substance were taken, one on the day the first set of suspensions of F-193 were prepared and one at the end of the dosage period. These samples are retained frozen at the Test Facility for possible analysis.

Duplicate 10 mL samples for analysis of test substance content were taken from each prepared concentration on the first and last days of the dosage period. These samples were retained frozen at the Test Facility for possible analysis.

Samples (1 g each) of the bulk test substance (frozen) and the vehicle (refrigerated) used in the study were reserved at the Test Facility on the first day the dosage suspensions were prepared. These samples are retained in the Test Facility archives.

5. Route of Administration and Dosage Selection

F-193 was administered to the rats percutaneously. The percutaneous route was selected for use because it is one possible route of human exposure.

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- a. The test substance was considered 100% pure for the purpose of dosage calculations.
 - b. Argus Batch Number refers to the identification number assigned to each concentration at preparation.

In this study, dosages of 0 (Vehicle), 50, 250 and 500 mg/kg/day of F-193 were tested. These dosages were selected on the basis of a pilot evaluation conducted by the Test Facility (Argus Research Laboratories, Inc., Protocol 1001-005P, see APPENDIX G).

6. Administration of the Test Substance

Before cohabitation, the back of each rat was shaved using an Oster® clipper with a size 40 surgical blade. Any regrowth of hair was shaved, when necessary, throughout the dosage period. The areas shaved extended from the shoulders to approximately 2 cm anterior to the hip joints and were approximately 5 cm wide (extended ventrolaterally from the dorsal midline approximately 2.5 cm on each side). A "map" of the shaved area was made for each rat before application of the first dosage, noting any areas of apparent hair tufts, lesions resulting from shaving or natural differences in skin colorization.

The female rats were administered appropriate dosages on days 0 through 19 of presumed gestation. The dosage volume was 1 mL/kg, adjusted daily on the basis of the individual body weights recorded immediately before application of the vehicle or the test substance. The rats were administered each daily dosage at approximately the same time each day.

On each day of the dosage period (days 0 through 19 of presumed gestation), before application of the test substance and approximately 24 hours after application of the last dosage of the test substance, the application site on each rat was evaluated for skin irritation without the observer's knowledge of the dosage group. The rat was then fitted with an Elizabethan collar to prevent oral ingestion of the test substance. The required dosage volume was applied evenly to the shaved area using a blunt-tipped glass syringe. Separate syringes were used for each dosage level and for each day of dosage. Each rat was then placed in a holding cage with the feed jar, and free access to water was supplied via an automatic watering system. After a six-hour exposure

period, the application site was rinsed^a with a cloth dipped in acetone and then dried with a clean cloth. The collar was removed, and each rat was returned to its home cage with the feed jar^a.

7. Observations

All rats were observed for viability at least twice each day during the study. The rats were also examined for general health several times during the acclimation period and daily during presumed gestation. Observations for clinical signs of test substance effect and/or viability were recorded several times each day during the dosage period (days 0 through 19 of presumed gestation). The observations were made before test substance administration and then approximately 60 minutes after each application. During the dosage period, skin reactions were evaluated once each day without the observer's knowledge of the dosage group, immediately before application of the test substance. Skin reactions were graded using the internationally accepted standards of Draize⁽⁹⁾ and the National Research Council⁽¹⁰⁾ in APPENDIX C.

Body weights of the rats were recorded weekly during the acclimation period and daily during presumed gestation. Feed consumption values were recorded daily during presumed gestation^a.

All rats were sacrificed by carbon dioxide asphyxiation on day 20 of presumed gestation, and a gross necropsy of the thoracic and abdominal viscera was performed. To confirm the pregnancy status, uteri from rats that appeared nonpregnant were examined while transilluminated and pressed between two glass plates. Tissues with gross lesions were preserved in neutral buffered 10% formalin for possible future evaluation; all other maternal tissues were discarded.

a. See APPENDIX D (DEVIATIONS FROM THE PROTOCOL).

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. 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 mechanical stimuli. Nonresponding term fetuses are considered to be dead^a. 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 individually identified with a tag noting its litter, uterine distribution and study number. Each fetus was subsequently weighed and examined for sex and gross external alterations. Live fetuses were sacrificed according to the Standard Operating Procedures of the Test Facility. Representative photographs of fetal gross alterations were taken and are available in the raw data.

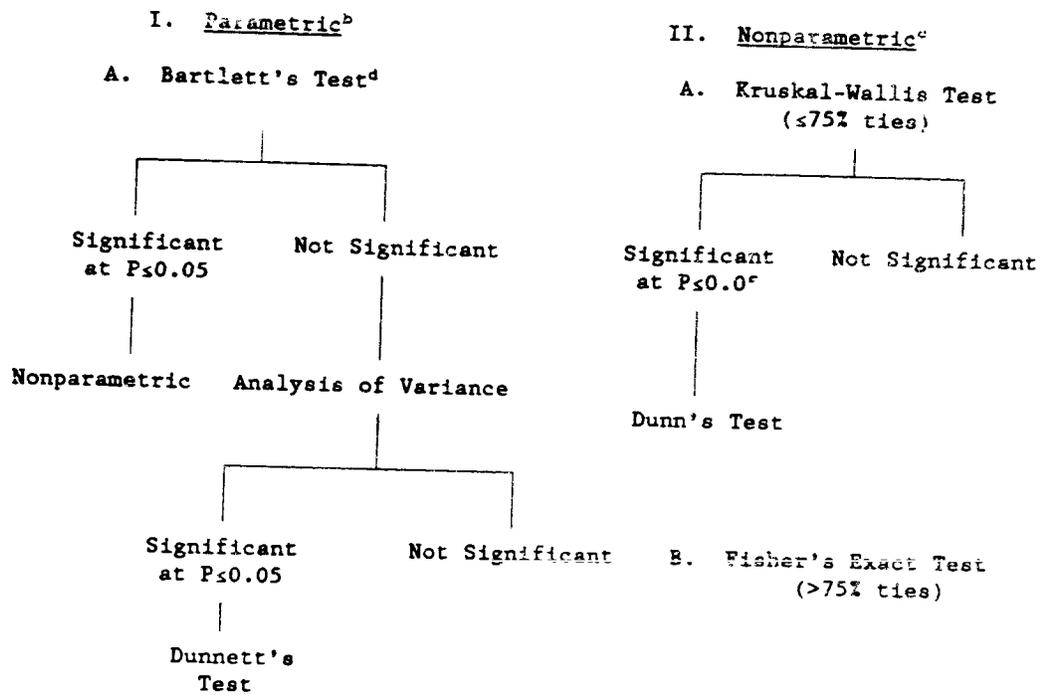
Approximately one-half of the fetuses in each litter were fixed in Bouin's solution and examined for soft tissue alterations by using an adaptation of Wilson's sectioning technique⁽³⁾. The remaining fetuses in each litter were eviscerated, cleared, stained with alizarin red S⁽⁴⁾ and examined for skeletal alterations^b.

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- a. There were no dead fetuses.
b. See APPENDIX D (DEVIATIONS FROM THE PROTOCOL).

C. Statistical Tests

The following schematic represents the statistical analyses of data:

Type of Test^a



III. Test for Proportion Data

Variance Test for Homogeneity of the Binomial Distribution

-
- a. All tests evaluated at $P \leq 0.05$ to $P \leq 0.01$.
 - 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.

Maternal and fetal incidence data were analyzed using the Variance Test for Homogeneity of the Binomial Distribution⁽¹¹⁾.

Maternal body weights, body weight changes, feed consumption values, litter averages for fetal body weights, percent male conceptuses, percent resorbed conceptuses, fetal ossification sites and percent fetal alterations were analyzed using Bartlett's Test of Homogeneity of Variances⁽¹²⁾ and the Analysis of Variance⁽¹³⁾, 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⁽¹⁴⁾ 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⁽¹⁵⁾ was used, when 75% or fewer ties were present; when more than 75% ties were present, Fisher's Exact Test⁽¹⁶⁾ was used. 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.

All other Caesarean-sectioning data were evaluated using the procedures described previously for the Kruskal-Wallis Test⁽¹⁵⁾.

D. Storage

The original report, raw data, unused bulk test substance and preserved tissues are retained in the Test Facility archives. All unused test substance suspensions were discarded at the Test Facility. All unused bulk test substance will remain at the Test Facility for use in other studies.

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III. RESULTS

A. Mortality, Skin Reactions, Clinical and Necropsy Observations (Summary - Table 1; Individual Data - Tables 13 and 14)

No deaths occurred during the conduct of this study.

Skin reactions related to percutaneous administration of the test substance occurred in the 50, 250 and 500 mg/kg/day dosage groups. These skin reactions included desquamation [(grade 1) occurred in one 50 and one 250 mg/kg/day dosage group rat], edema [(grade 1) occurred in two 50 mg/kg/day dosage group rats and one 500 mg/kg/day dosage group rat], erythema [(grade 1) occurred in two 50 mg/kg/day dosage group rats, one 250 mg/kg/day dosage group rat and two 500 mg/kg/day dosage group rats] and atonia [(grade 1) occurred in two 500 mg/kg/day dosage group rats].

All other clinical observations were unrelated to administration of F-193 because: 1) the incidences were not dosage-dependent; 2) the values were not significantly increased, as compared with the control group values; or 3) the observations commonly occur in this strain of rat. These observations included chromodacryorrhea, chromorrhinorrhea, swollen digit and localized alopecia. No gross lesions were identified at necropsy.

B. Maternal Body Weights and Body Weight Changes (Figure 1; Summaries - Tables 2 and 3; Individual Data - Table 15)

Maternal body weight gains were significantly reduced ($P \leq 0.01$) in the 250 and 500 mg/kg/day dosage groups for the entire dosage period (calculated as days 0 to 20 of gestation). Within the dosage period, body weight gains were reduced or significantly reduced ($P \leq 0.05$ to $P \leq 0.01$) in these dosage groups. Body weights were generally reduced or significantly reduced ($P \leq 0.05$ to $P \leq 0.01$) in the 250 and 500 mg/kg/day dosage groups throughout the dosage period. Body weight gains were

significantly reduced ($P \leq 0.05$) in the 50 mg/kg/day dosage group on days 19 to 20 of gestation. This reduction was not biologically important and considered unrelated to the test substance.

C. Maternal Absolute (g/day) and Relative (g/kg/day) Feed Consumption Values (Summaries - Tables 4 and 5; Individual Data - Table 16)

Absolute (g/day) and relative (g/kg/day) feed consumption values were significantly reduced ($P \leq 0.05$ to $P \leq 0.01$) in the 250 and 500 mg/kg/day dosage groups for the entire dosage period (calculated as days 0 to 20 of gestation). Within the dosage period, absolute and relative feed consumption values were generally reduced or significantly reduced ($P \leq 0.05$ to $P \leq 0.01$) in these dosage groups. Relative feed consumption values were increased or significantly increased ($P \leq 0.05$ to $P \leq 0.01$) in the 250 and/or 500 mg/kg/day dosage groups on days 15 to 18 and 19 to 20 of gestation. These increases were interrelated with the significantly reduced ($P \leq 0.01$) body weights in these dosage groups.

D. Caesarean-Sectioning and Litter Observations (Summaries - Tables 6 and 7; Individual Data - Tables 17 through 19)

Percutaneous administration of the 250 and 500 mg/kg/day dosages of F-193 caused significant reductions ($P \leq 0.01$) in the average litter sizes and live fetuses and increases or significant increases ($P \leq 0.01$) in resorption (total and early resorptions) in these dosage groups. The numbers of dams with any resorptions was also increased in the 250 mg/kg/day dosage group and significantly increased ($P \leq 0.01$) in the 500 mg/kg/day dosage group. Reflecting these effects of the test substance, the percentage of resorbed conceptuses per litter was increased in the 250 mg/kg/day dosage group and was significantly increased ($P \leq 0.01$) in the 500 mg/kg/day dosage group. Fetal body weights (male and female) were significantly reduced ($P \leq 0.01$) in the 250 and 500 mg/kg/day dosage groups.

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All other Cesarean-sectioning and litter parameters were unaffected by dosages of the test substance as high as 500 mg/kg/day. Litter averages for implantations and sex ratios did not demonstrate any significant or biologically important differences. The average number of corpora lutea was significantly reduced ($P \leq 0.01$) in the 250 mg/kg/day dosage group. This event was considered unrelated to the test substance because the incidence was not dosage-dependent. No dam resorbed all conceptuses, and the numbers of dams with viable fetuses were comparable among the four dosage groups.

E. Fetal Alterations (Summaries - Tables 8 through 12;
Individual Data - Table 29)

Fetal alterations were classified as: 1) malformations (irreversible changes which occur at low incidences in this species and strain); or 2) variations (relatively common developmental changes in this species and strain, including minor reversible delays or accelerations in development).

The 250 and 500 mg/kg/day dosages of the test substance were the possible cause of increased incidences of eye malformations [decrease in size, evident as small eye bulge(s) at gross external examination, and as small eye sockets at skeletal examination] and cleft palate evident as incompletely ossified palate at skeletal examination in the fetuses. The absence of a clear dosage-dependent incidence for this malformation may be interrelated with the dosage-dependent increases in resorptions (embryo-fetal deaths) in the 250 and 500 mg/kg/day dosage groups.

Other alterations possibly attributed to the test substance included increases or significant increases ($P \leq 0.01$) in the litter and/or fetal incidences of hydronephrosis and bifid thoracic vertebral centra in the 250 and 500 mg/kg/day dosage groups and umbilical hernia and delayed sternal ossification in the 500 mg/kg/day dosage group. Significant reductions ($P \leq 0.05$ to $P \leq 0.01$) occurred in the average number of ossified caudal vertebrae in the 250 and 500 mg/kg/day dosage groups and ossified sternal centers, metacarpals, metatarsals and hindpaw

phalanges in the 500 mg/kg/day dosage group; these alterations were considered related to the test substance because: 1) the litter and fetal incidences were increased or significantly increased; 2) the incidences were significantly reduced (ossification sites); and/or 3) the incidences exceeded the ranges observed historically^a.

All other gross external, soft tissue and skeletal alterations were considered unrelated to percutaneous administration of F-193 because: 1) the incidences were not dosage-dependent; or 2) the observations occurred as single events in the high dosage group. The significant reductions ($P \leq 0.01$) in the fetal and litter incidences of incompletely ossified pubes and ischia and unossified pubes in the 50, 250 and 500 mg/kg/day dosage groups were considered unrelated to the test substance because: 1) increases in fetal variations, rather than reductions, are expected effects of a developmental toxicant; and 2) the events were not dosage-dependent.

1. Summary of Alterations (Summary - Table 8; Individual Data - Table 20)

In the 0(Vehicle), 50, 250 and 500 mg/kg/day dosage groups, there were 11(45.8%), 9(37.5%), 10(40.0%) and 17(77.3%)** litters with fetuses with any alteration observed. The numbers of fetuses with any alteration observed were 18(5.0%), 13(3.8%), 16(5.0%) and 25(11.7%)**, and the average percentages of fetuses with any alteration per litter were 5.13, 3.88, 5.84 and 11.99, in these same respective dosage groups. The significant increases ($P \leq 0.01$) in these observations in the 500 mg/kg/day dosage group were considered related to the test substance because: 1) they occurred in the highest dosage group; and 2) they correspond to the significant increases ($P \leq 0.01$) in the soft tissue and skeletal alterations in this dosage group.

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

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All fetal alterations are described in the following information.

2. Fetal Gross External Alterations (Summary - Table 9;
Individual Data - Table 20)

(a) Malformations Possibly Related to the Test
Substance

Two 250 mg/kg/day dosage group fetuses (9262-7; 9271-11) and three 500 mg/kg/day dosage group fetuses (9278-13; 9289-4; 9293-1) had gross external malformations. Although there was no clear dosage-dependency, these observations were considered possibly related to the test substance because: 1) they occurred only in the two highest dosage groups; 2) the incidence in the 250 mg/kg/day dosage group exceeded the historical control ranges^a; and 3) the reduced incidences in the 500 mg/kg/day dosage group, as compared to the 250 mg/kg/day dosage group, may be secondary to the increased embryo-fetal mortality. These fetuses are described below.

One 250 mg/kg/day dosage group fetus (9262-7) had depressed eye bulges, cleft palate, agnathia, anasarca, short digits in the right hindpaw, a kinked tail and an outward rotated right hindlimb. Skeletal examination of this fetus revealed small eye sockets, short nasal bones, incompletely ossified premaxillae bones, absent mandibles, fusion of the exoccipital bone and the atlas, incomplete ossification of the palate, short 1st through 5th right hindpaw digits and short right hindpaw phalanges. The other 250 mg/kg/day dosage group fetus (9271-11) had depressed eye bulges, a short snout, absent nares, cleft palate, microstomia and micrognathia. Skeletal examination of this fetus revealed small eye sockets, fused and short nasal bones, incomplete ossification of the left premaxilla bone and the palate,

a. See APPENDIX B (HISTORICAL CONTROL DATA).

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short mandibles, irregularly shaped right mandible and fusion of the right portion of the exoccipital bone to the atlas.

One 500 mg/kg/day dosage group fetus (9278-13) had edema in the neck region, an umbilical hernia, absent digits in the right forepaw, short hindlimbs and absent digits in both hindpaws. Soft tissue examination of the fetus revealed slight dilation of the lateral and third ventricles of the brain. Fetus 9289-4 in the 500 mg/kg/day dosage group had a cleft palate, anasarca, an umbilical hernia and short digits in the right hindpaw. Skeletal examination of this fetus revealed incomplete ossification of the palate, short mandibles, not ossified pubes, short 1st through 5th right hindpaw digits and short right hindpaw phalanges. The other 500 mg/kg/day dosage group fetus (9293-1) had depressed eye bulges, a divided snout (three parts) and agnathia. Skeletal examination of this fetus revealed small eye sockets, fused nasals (where the palate should be), not ossified premaxillae bones, fused maxillae bones (where the nasals and premaxillae should be), short and fused mandibles, fused tympanic rings and a variation in sternal ossification (not ossified 1st sternebra).

(b) Malformations Unrelated to the Test Substance

The 50 mg/kg/day dosage group fetus (9230-1) had an umbilical hernia as its only alteration. This observation was considered unrelated to the test substance because the incidence was within the range observed historically^a.

a. See APPENDIX H (HISTORICAL CONTROL DATA).

3. Fetal Soft Tissue Alterations (Summary - Table 10;
Individual Data - Table 20)

a. Malformations Possibly Related to the Test
Substance

Two 250 mg/kg/day dosage group littermates (9264-3,-15) and four** fetuses (9280-9; 9281-4; 9287-12; 9297-8) in four** 500 mg/kg/day dosage group litters had marked dilation of the left or right renal pelvis (hydronephrosis). One 500 mg/kg/day dosage group fetus (9297-8) also had a variation in renal development (moderate dilation of the right renal pelvis). The increase and significant increases ($P < 0.01$) in the litter and/or fetal incidences of this observation were considered possible effects of the test substance because: 1) it occurred in the two highest dosage groups; or 2) the values were statistically significant.

b. Malformation Unrelated to the Test Substance

One control group fetus (9210-11) had a ventricular septal defect and situs inversus of the stomach. Another control group fetus (9222-18) had a diaphragmatic hernia as its only alteration.

c. Variations

One externally malformed 500 mg/kg/day dosage group fetus (9278-13) had slight dilation of the lateral and third ventricles of the brain, as described previously.

Moderate dilation of the pelvis of one kidney, a reversible developmental delay⁽¹⁸⁾, occurred in one control group fetus (9208-14),

** Significantly different from the vehicle control group value ($P < 0.01$).

five 50 mg/kg/day dosage group fetuses (9228-10; 9238-14; 9240-8; 9242-4; 9248-4), three 250 mg/kg/day dosage group fetuses (9254-10; 9260-14; 9271-8) and one 500 mg/kg/day dosage group fetus (9297-8). The 500 mg/kg/day dosage group fetus (9297-8) also had marked dilation of the left renal pelvis, as described previously. These observations were considered unrelated to the test substance because: 1) the incidences were not dosage-dependent; and 2) the incidences were not significant, as compared with the control group incidence.

4. Fetal Skeletal Alterations (Summaries - Tables 11 and 12; Individual Data - Table 20)

a. Malformations Possibly Related to the Test Substance

Two externally malformed 250 mg/kg/day dosage group fetuses (9262-7; 9271-11) and two externally malformed 500 mg/kg/day dosage group fetuses (9289-4; 9293-1) had skull malformations (small eye socket and/or incompletely ossified palate) and were fully described previously.

b. Malformations Unrelated to the Test Substance

(1) Rib/Vertebrae

Rib/vertebral malformations occurred in one control group fetus (9218-7). This fetus had unilateral (left) ossification of the 3rd and 5th thoracic vertebral centra, small arches (right) of the 3rd and 5th thoracic vertebrae, fused 5th and 6th left ribs, absent 3rd and 5th right ribs and variations in sternal ossification (incompletely or not ossified 1st and 2nd sternbrae).

(2) Hindlimbs

One externally malformed 250 mg/kg/day dosage group fetus (9262-7) and one externally malformed 500 mg/kg/day dosage group fetus (9289-4) had short digits and phalanges, as described previously. These observations were considered unrelated to the test substance because they occurred in one fetus in each of the 250 and 500 mg/kg/day dosage groups.

c. Variations

As described in the following information, all skeletal variations were reversible delays in fetal ossification⁽¹⁹⁾, with the exception of cervical rib, a common variation in this strain of rat.

(1) Variations Related to the Test Substance(a) Vertebrae

A bifid thoracic vertebral centrum occurred in 1, 3, 7 and 14** fetuses in 1, 3, 6** and 8** litters in the 0(Vehicle), 50, 250 and 500 mg/kg/day dosage groups. The increases or significant increases ($P \leq 0.01$) in the litter and fetal incidences in the 250 and 500 mg/kg/day dosage groups were considered effects of the test substance because: 1) the values were significant; or 2) the incidences exceeded the range observed historically*.

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

a. See APPENDIX H (HISTORICAL CONTROL DATA).

(b) Sternal Centers

Delayed sternal ossification (incompletely ossified or unossified sternabrae) occurred in 3, 0, 1 and 6** fetuses from 2, 0, 1 and 5** litters in the 0(Vehicle), 50, 250 and 500 mg/kg/day dosage groups, respectively. The significant increases ($P \leq 0.01$) in litter and fetal incidences were considered related to the test substance because:

- 1) the values were significant, as compared to the control group values;
- 2) the event occurred in the highest dosage group; and 3) the incidences exceeded the ranges observed historically^a.

One 0(Vehicle) group fetus (9218-7), one 250 mg/kg/day dosage group fetus (9260-1) and two 500 mg/kg/day dosage group littermates (9293-1,-8) had other observations that were described previously. One 50 mg/kg/day dosage group fetus (9231-10) had a duplicated manubrium and 1st through 3rd sternabrae. This fetus had a variation in vertebral ossification, as described previously.

(c) Fetal Ossification Sites

Significant reductions ($P \leq 0.01$) in the average numbers of ossified caudal vertebrae occurred in the 250 and 500 mg/kg/day dosage groups, and significant reductions ($P \leq 0.05$ to $P \leq 0.01$) in the average numbers of ossified sternal centers, metacarpals, metatarsals and hindpaw phalanges occurred in the 500 mg/kg/day dosage group. These events were considered related to the test substance and associated with delays in ossification commonly observed with reduced fetal body weights.

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

a. See APPENDIX H (HISTORICAL CONTROL DATA).

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(2) Variations Unrelated to the Test Substance(a) Vertebrae

Variations in ossification of the thoracic and/or lumbar vertebrae (unilateral ossification or bifid vertebral centra) occurred in two 0(Vehicle) group fetuses (9218-7; 9224-15), two fetuses (9260-1; 9275-8) in the 250 mg/kg/day dosage group and three fetuses (9276-11; 9293-8; 9298-12) in the 500 mg/kg/day dosage group. One control group fetus (9224-15), one 250 mg/kg/day dosage group fetus (9260-1) and one 500 mg/kg/day dosage group fetus (9293-8) also had variations in sternal or pelvic ossification (incompletely ossified, not ossified or duplicated sternbrae, manubrium or pubes). The control group fetus (9218-7) was described previously.

(b) Ribs

Incompletely ossified (hypoplastic) and wavy ribs occurred in one control group fetus (9219-13).

Unilateral or bilateral cervical ribs at the 7th cervical vertebra, a common observation in this strain of rat, occurred in two 0(Vehicle) group fetuses (9208-13; 9216-1) and four 50 mg/kg/day dosage group fetuses (9231-6; 9236-1,-11; 9242-15).

(c) Pelvis

Incompletely ossified or unossified ischia and/or pubes occurred in 9, 0**, 0** and 1** fetuses in 5, 0**, 0** and 1** litters in the 0(Vehicle), 50, 250 and 500 mg/kg/day dosage groups, respectively.

** Significantly different from the vehicle control group value (P<0.01).

These significant reductions ($P \leq 0.01$) were considered unrelated to the test substance because: 1) increases in fetal variations, rather than reductions, are expected effects of a developmental toxicant; and 2) the values were not dosage-dependent. One 0 (Vehicle) group fetus (9224-15) and one 500 mg/kg/day dosage group fetus (9289-4) had other observations, as described previously.

(d) Fetal Ossification Sites

There were no statistically significant or biologically important differences among the four dosage groups in the average numbers of ossification sites per fetus for the hyoid, vertebrae (cervical, thoracic, lumbar and sacral), sternum (manubrium and xiphoid), forepaws (carpals and phalanges) or the hindpaws (tarsals).

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