EXXON CHEMICAL COMPANY

FDCN: 88970000094

Safety and Environmental Affairs Department
David J. Johnson
MANAGER, SAFETY PROGRAMS

8EHQ-0198-13844

January 8, 1998

Document Control Office (7407)
Attn: TSCA Section 8(e) Coordinator
Office of Pollution Prevention and Toxics
U. S. Environmental Protection Agency
401 M Street, S. W.
Washington, D. C. 20460-0001

Contains No CBI

Re: TSCA Section 8(e) Document Control Number 8EHQ-1296-13844

Dear Sir or Madam:

On December 19, 1996, Exxon Chemical Company submitted a notification of substantial risk under the provisions of TSCA Section 8(e). The initial submission (TSCA Section 8(e) Document Control Number 8EHQ-1296-13844) described the results of a dose range-finding developmental toxicity study in rats on a substance identified as 1,2-benzenedicarboxylic acid, di-C_{6-8}-branched alkyl esters, C_7 rich (CAS Registry Number 71888-89-6). This was followed by a submission dated May 12, 1997, which described preliminary results on a full developmental toxicity study in rats on the same substance. At the time the preliminary results were submitted, the study had been completed but the data and report had not passed through a quality assurance review. The purpose of this submission is to complete the record on these studies by providing final reports from both the dose range-finding developmental toxicity study and the full developmental toxicity study.

The results from both of these studies were reported in summary form in the submissions referenced above. Consequently, results summaries have not been provided with this submission, the purpose of which is only to provide copies of the final reports. In both reports, the test material is identified with the code name MRD-96-676. This code name corresponds to the substance identified by chemical name and CASRN above.

If you have any questions or need additional information, please feel free to contact me on (281) 870-6874.

Sincerely yours,

Steven G. Hentges

8EHQ-96-13844

SGH/jad
Enclosures

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P.O. Box 3272, Houston, Texas 77253-3272
Tel: (281) 870-6212 Fax: (281) 870-6661
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EXXON BIOMEDICAL SCIENCES, INC.

FINAL REPORT

PROJECT NUMBER: 167633

TEST MATERIAL: MRD-96-676

DEVELOPMENTAL TOXICITY RANGEFINDING STUDY IN RATS WITH MRD-96-676

PERFORMED FOR:

EXXON CHEMICAL AMERICAS
13501 Katy Freeway
Houston, Texas 77079-1398

PERFORMED AT:

EXXON BIOMEDICAL SCIENCES, INC.
Toxicology Laboratory
Mettlers Road
CN 2350
East Millstone, New Jersey 08875-2350

COMPLETION DATE: December 5, 1997

REVISION 1: December 31, 1997

APPROVAL SIGNATURES

J. J. Freeman, Ph.D., D.A.B.T.
Mammalian Toxicology Laboratory Director

24 Dec 97
Date

D. J. Letinski, M.S.
Analytical Chemistry Supervisor

31 Dec 97
Date

I hereby accept responsibility for the validity of these data and declare that to the best of my knowledge, the study contained herein was performed under my supervision in compliance with the EU Council Decision on Compliance with Principles of Good Laboratory Practice and the EPA Good Laboratory Practice standards set forth in 40 CFR Part 792.

G. W. Trimmer, B.A.
Study Director

31 Dec 97
Date

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QUALITY ASSURANCE STATEMENT

STUDY NUMBER: 167633

TEST SUBSTANCE/ARTICLE: MRD-96-676

STUDY SPONSOR: Exxon Chemical Americas

Listed below are the dates that this study was inspected by the Quality Assurance Unit of Exxon Biomedical Sciences, Inc., and the dates findings were reported to the Study Director and Management.

Date(s) of Inspection	Reported to Study Director	Reported to Management
17 Oct 96	17 Oct 96	24, 26 Oct 96
01 Nov 96	01 Nov 96	04, 05 Nov 96
06 Jan 97	06 Jan 97	24, 25 Jan 97
18 - 30 May 97	30 May 97	18, 19 Jun 97
16 Jun 97	17 Jun 97	18, 19 Jun 97

Joanne R. Jackson, B.S.
Quality Assurance Supervisor

22 Dec 97
Date

PERSONNEL

Study Director:	G. W. Trimmer, B.A.
Sponsor:	Exxon Chemical Americas 13501 Katy Freeway Houston, Texas 77079-1398
Sponsor Representative:	L. H. Keller, Ph.D.
Mammalian Toxicology Laboratory Director:	J. J. Freeman, Ph.D., D.A.B.T.
Toxicology and Animal Care Supervisor:	R. C. Forgash, B.S.
Report Preparation Supervisor:	E. R. Frank, B.A.
Compound Preparation Supervisor:	M. A. Elliott, B.S.
Analytical Chemistry Supervisor:	D. J. Letinski, M.S.
Quality Assurance/Archives Supervisor:	J. R. Jackson, B.S.
Maintenance Supervisor:	J. L. McGrath, A.S.
Veterinarian:	R. L. Harris, D.V.M.

SUMMARY

This study was conducted to evaluate the developmental toxicity of MRD-96-676 administered via oral gavage to pregnant rats during the period of major organogenesis and fetal growth, and to select appropriate dose levels for a subsequent definitive developmental toxicity study.

The test material was administered by oral gavage to 4 groups of Crl:CDBR female rats at doses of 250, 500, 750 and 1000 mg/kg. Group 1 served as a control and received the carrier (corn oil) only. Mated females were dosed once daily from Gestation Day (GD) 6 through 20. Dosing volumes were 5 ml/kg for all groups and were based on the animals' most recent body weight.

Males and females were paired and housed overnight until confirmation of mating (sperm/plug = GD 0). Each mated female was returned to its own cage and new females were placed in the males' cages until the required number of mated females was obtained. Mated females were assigned to dose groups using a computer generated body weight sorting program using their GD 0 body weights.

Clinical observations were made daily during gestation. Body weight and food consumption measurements were made on GD 0, 6, 9, 12, 15, 18, 20 (body weight only) and 21. On GD 21, animals were euthanized and caesarean sections were performed. Gross necropsies were performed, uterine weights with ovaries attached were recorded, uterine contents were examined, and the required uterine implantation data were recorded. All live fetuses were weighed, examined externally to determine sex and for gross malformations, and subsequently discarded without further examination.

All maternal animals survived to scheduled study termination on GD 21 and there were no treatment-related clinical signs observed during gestation.

There was a decrease in the mean GD 21 body weight in the 750 and 1000 mg/kg dams compared with controls. These decreases in GD 21 body weights were attributed to decreases in the mean uterine weights in both groups, since all mean GD 21 body weights after correction for the uterine weights were equivalent to control group weight. In addition, suppressed body weight gain was observed in the 750 and 1000 mg/kg dams during the majority of the gestation intervals, during the overall treatment period, as well as the overall gestation period (17% and 32%, respectively), compared with controls. The suppression in body weight gain after GD 15 was probably related to the smaller uterine weights. The decreased uterine weights were directly related to the increase in resorptions in the 750 and 1000 mg/kg groups. However, the suppression in body weight gain during the GD 6-9 and 9-12 intervals for the 1000 mg/kg dams was probably due to slight maternal toxicity. Mean body weights and body weight gain of the 250 and 500 mg/kg dose groups appeared comparable with controls throughout the entire gestation period.

SUMMARY (CONT'D)

There were no biologically significant differences in mean gestation food consumption between treated and control dams at any interval during the gestation period. There were no postmortem findings which were attributed directly to treatment.

There was an increase in the mean number of resorptions per litter, resorptions per implantation sites, and percent post-implantation loss in the 750 and 1000 mg/kg dose groups compared with the controls. Consequently, a decrease in the mean number of live fetuses per litter and mean number of fetuses per implantation sites was observed in the 750 and 1000 mg/kg dose groups compared with controls. Additionally, the incidence of dams with malformed and affected (resorptions + dead + malformed) fetuses was significantly increased in the 750 and the 1000 mg/kg dose groups compared with controls. There was also an apparent difference in the fetal sex ratio, as determined by external exam, in the 750 and 1000 mg/kg litters. The number of fetuses identified as males was reduced and the number of fetuses identified as female was increased compared with controls. However, results from the follow-up main study with the test material (EBSI, 1997), which included an internal exam, indicated that this effect was probably due to difficulty with the external sexing procedure in the smaller fetuses and subsequent potential misclassification. There were no apparent differences in implantation parameters between the 250 and 500 mg/kg group and the control group.

The mean fetal body weight of the 750 and 1000 mg/kg fetuses was substantially lower (14-18%) than controls and lower than the laboratory historical range. External malformations were observed in the 750 (4 fetuses, 3 litters) and 1000 (3 fetuses, 2 litters) mg/kg groups and included single or few occurrences of encephalocele, cleft palate, filamentous tail, atresia ani, acaudia, exencephaly, and anasarca. Other findings included cranial hematomas or protruding tongue. Additionally, eight fetuses (four litters) in the 750 mg/kg dose group and nine fetuses (four litters) in the 1000 mg/kg group were stunted (body weight $\leq$ 4.0 grams). There were no external abnormalities seen in any fetuses in the control, 250 mg/kg, or 500 mg/kg groups and mean fetal body weights of the 250 and 500 mg/kg dose groups were essentially equivalent to controls.
In conclusion, signs of developmental toxicity were apparent at the 750 and 1000 mg/kg dose levels, as indicated by a decrease in mean total number of live fetuses and mean number of fetuses per implantation sites; and an increase in the mean number of resorptions per litter, mean number of resorptions per implantation sites, mean percent post-implantation loss, and incidence of dams with malformed and affected (resorptions + dead + malformed) fetuses compared with controls. Additionally, mean fetal body weights were substantially decreased in the 750 and 1000 mg/kg fetuses compared with controls and lower than the laboratory historical control range. Malformations also were observed in the 750 and 1000 mg/kg dose group fetuses. In the dams, there was a suppression in body weight gain in the 1000 mg/kg dams during the GD 6-9 and GD 9-12 intervals which was indicative of slight maternal toxicity.

SUMMARY (CONT'D)

Due to excessive fetal lethality at the 1000 mg/kg dose, 750 mg/kg was selected as the high dose for the subsequent definitive developmental toxicity study in rats. The 750 mg/kg dose was expected to provide a sufficient number of fetuses for an adequate evaluation of the developmental toxicity of the test material. The low and mid doses selected were 100 and 300 mg/kg, respectively, with the low dose expected to produce a No Observable Adverse Effect Level (NOAEL).

INTRODUCTION

This study was conducted to evaluate the developmental toxicity of MRD-96-676 administered via oral gavage to pregnant rats during the period of major organogenesis and fetal growth, and to select appropriate dose levels for a subsequent definitive developmental toxicity study.

This study was conducted for Exxon Chemical Americas, 13501 Katy Freeway, Houston, Texas 77079-1398 (subsequently referred to as the Sponsor).

The study was conducted by Exxon Biomedical Sciences, Inc. (EBSI) Toxicology Laboratory, Mettlers Road, CN 2350, East Millstone, New Jersey 08875-2350. The EBSI Toxicology Laboratory is an American Association for Accreditation of Laboratory Animal Care (AAALAC) accredited facility and a Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF) certified facility.

Study Initiation (Protocol Signature Date)

October 21, 1996

Inlife Test Period

October 22, 1996 to November 15, 1996

Justification for Selection of Test System

The rat is among the species of choice for teratogenicity testing according to the EC Methods for Determination of Toxicity, "Teratogenicity Test -Rodent and Non-Rodent" (EC, 1987), OECD Guideline for Testing of Chemicals, "Teratogenicity" (OECD, 1981) and the U.S. EPA TSCA Test Guidelines for Developmental Toxicity Studies (EPA, 1985).

Justification of Dosing Route

Oral intubation is an accepted or preferred route of administration according to EC (1987), OECD (1981) and U.S. EPA TSCA (1985) guidelines, and ingestion represents a possible route of human exposure.

INTRODUCTION (CONT'D)

Compliance

This study was conducted in general agreement with the following guidelines and standards:

Animal Welfare Act of 1966 (P.L. 89-544), as amended in 1970, 1976, and 1985. Code of Federal Regulations, Title 9 [Animals and Animal Products], Subchapter A - Animal Welfare Parts 1, 2, and 3.

Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, National Academy Press, Washington, D.C., 1996.

MATERIALS AND METHODS

TEST MATERIAL

Material Identification

EBSI Identification:	MRD-96-676
Supplier:	Exxon Chemical
Date Received:	September 25, 1996
Lot #:	011249
Expiration Date:	January 4, 1997
Description:	Colorless liquid
Storage Condition:	Room temperature

Adjustments in the dose calculations were not made to correct for the purity of the test material. The test material, as received, was considered the "pure" material for the purpose of dosing.

Characterization of the Test Material

The stability, identity, strength, purity, and composition or other characteristics which appropriately identify the test material was performed by the testing laboratory (EBSI). Documentation is maintained at Exxon Biomedical Sciences, Inc., East Millstone, New Jersey.

The methods of synthesis, fabrication, and/or derivation of the test material are the responsibility of the Sponsor. Documentation is maintained at Exxon Baton Rouge Chemical Plant, 4999 Scenic Highway, Baton Rouge, LA 70821.

TEST MATERIAL (CONT'D)

Analysis of Mixtures

Analyses for the stability, uniformity, and concentration of the test and control material mixtures were performed by the testing laboratory. Analyses for stability and uniformity were initiated concurrent with the experimental start. Concentration analyses were performed on the second and third dosing mixture preparations.

Solubility

The test material was soluble in the carrier at the concentrations required for this study.

Sample Retention

One archival sample of the undiluted test material, as well as the carrier, was collected by the Compound Preparation Department and stored at room temperature.

Carrier

Corn Oil

Supplier:	Best Foods CPC International
Batch:	Mar2897B; 801B6
Date Received:	October 8, 1996; November 1, 1996
Description:	Yellow liquid
Storage Condition:	Room temperature
Expiration Date:	March 28, 1997; November, 2001

TEST SYSTEM

Test Animal

Species:	Rat
Strain/stock:	Crl:CD BR VAF/Plus
Supplier:	Charles River Laboratories, Inc. Portage, Michigan

Animal Receipt Information (Females)

Receipt Date:	October 10, 1996
Purchase Order Number:	96GWT1059

Quarantine and Acclimation Period

12 days; animals were checked for viability at least once daily.

Number and Sex

75 sexually mature virgin females

35 females were allocated to study groups after confirmation of mating; additional animals received for mating purposes only.

50 sexually mature males (received for mating purposes only)

Age at Initiation of Gestation (Designated GD 0)

Females: Approximately 13 weeks

Weight at Initiation of Gestation (Designated GD 0)

Females: 231 to 295 grams

TEST SYSTEM (CONT'D)

Animal Identification

Individual ear tags and corresponding cage identification.

Selection

More animals than required for the conduct of the study were purchased and acclimated. Animals determined to be unsuitable for inclusion on this study because of poor health, outlying body weights, or other abnormalities were excluded from selection by the attending Veterinarian, Study Director, and/or technical staff.

Husbandry

Feed

Certified Rodent Diet #5002 (meal), ad libitum

Manufacturer:	PMI Feeds Inc. Richmond, Indiana
Analysis:	Performed by PMI Feeds Inc. Copies of the feed analyses are maintained in the EBSI Toxicology Laboratory.
Contaminants:	There were no known contaminants in the feed believed to have been present at levels that may have interfered with this study.

The availability of feed was checked daily for all animals.

TEST SYSTEM (CONT'D)

Water

Automatic watering system, ad libitum

Supplier:	Elizabethtown Water Company Bound Brook, New Jersey.
Analysis:	Periodic analysis is the responsibility of EBSI. A copy of the results is maintained at EBSI.
Contaminants:	There were no known contaminants in the water believed to have been present at levels that may have interfered with this study.

The availability of water was checked daily for all animals.

Housing

Room Number:	512
Housing:	Single housed during the study period, except during mating.
Caging:	Suspended stainless steel and wire mesh with absorbent paper below cages.

Environmental Conditions

Temperature:	68 to 76 degrees Fahrenheit
Humidity:	40 to 70 percent relative humidity
Lighting:	Approximately 12 hours light (0700 to 1900 hours) and 12 hours dark (1900 to 0700 hours) by automatic timer.

Monitored at least once daily.

EXPERIMENTAL DESIGN

Mating

On the initial scheduled mating day, females were placed in males' cages in a 1:1 (male: female) ratio. Males and females were paired based on sequential Physical Identification Number. A sufficient number of animals were co-housed in an attempt to produce an acceptable number of mated animals to accommodate lab scheduling. Mating was confirmed on the following morning by observation of a copulatory plug (vaginal) and/or by the presence of sperm in a vaginal rinse. The day on which mating was confirmed was considered Day 0 of gestation (GD 0). After confirmation of mating, each mated female was returned to its own cage. New females then were placed in the males' cages until the required number of mated females was obtained by continuous cohabitation in consideration of lab scheduling. Mated females subsequently were assigned to dose groups by a computer generated body weight sorting program using the GD 0 body weights to ensure mean body weight was similar between all groups on GD 0.

Preparation of Test Material Mixtures

The undiluted test material was thoroughly mixed in carrier prior to dispensing.

The test material/carrier mixtures were prepared weekly. Dosing solutions were prepared at the following doses.

Experimental Groups

Group Number	Number of Females per Group	Dose (mg/kg/day)
1 (Carrier Control)	7	0
2 Low	7	250
3 Intermediate 1	7	500
4 Intermediate 2	7	750
5 High	7	1000

EXPERIMENTAL DESIGN (CONT'D)

Administration of Test Material and Treatment Schedule

Mated females were dosed once daily by oral gavage from GD 6 through GD 20. Dosing volumes were 5 ml/kg for all groups and were based on the most recent individual body weights. Animals were dosed at approximately the same time each day (11:00 ± 2 hours).

Experimental Evaluation

Animals were examined for viability at least twice daily during the treatment period and at least once daily at other times during the study.

Body weights were taken prior to selection, and on GD 0, 6, 9, 12, 15, 18, 20, and 21.

Food consumption was measured for mated females on GD 6, 9, 12, 15, 18, and 21.

A clinical examination was given to each female prior to selection, and daily during gestation.

Euthanasia and Caesarean Section

Mated females were euthanized on GD 21. Euthanasia was by CO_2 asphyxiation and exsanguination. A gross necropsy was performed on all mated females.

Body weight was recorded on the day of necropsy. Uterine weight with ovaries attached was recorded at the time of necropsy. Uterine contents were examined and the numbers and locations of implantation sites, early and late resorptions, live fetuses, and dead fetuses were counted and recorded. Corpora lutea also were counted. The uteri of all apparently non-pregnant females were stained to confirm pregnancy status.

EXPERIMENTAL DESIGN (CONT'D)

Examination of Fetuses

Each live fetus was weighed and examined externally for gross malformations. Fetal sex was determined by external examination.

Tissue Preservation

Tissues from mated adult females were not preserved since there were no significant abnormal findings observed. Selected abnormal fetal heads were preserved in Bouin's fixative or ethanol for possible future examination

Records

A copy of the protocol, final report, raw data, computer generated listings of raw data, supporting documentation, sample(s) of test material, and tissue specimens are maintained in the EBSI Toxicology Laboratory Archives.

Statistical Analysis

Statistical analyses included means and standard deviations of appropriate parameters (Snedecor and Cochran, 1989).

RESULTS

1. CLINICAL INLIFE OBSERVATIONS AND SURVIVAL

Incidence of Gestation Observations: Table 1
Individual Gestation Observations: Appendix A

All maternal animals survived to scheduled study termination on GD 21. All dams in all dose groups were free of observable abnormalities during the entire gestation period with the exception of one 1000 mg/kg dam observed with red vaginal material on GD 21.

2. GESTATION BODY WEIGHT

Mean Gestation Body Weight and Body Weight Change: Table 2
Individual Gestation Body Weight: Appendix B

In the 750 and 1000 mg/kg dams, there was a decrease in mean body weight on GD 21 (8% and 14%, respectively) and in uterine weight (31% and 52%, respectively) compared with controls. Additionally, suppressed body weight was observed in the 750 and 1000 mg/kg dams during the majority of the gestation intervals, during the overall treatment period, and the overall gestation period (17% and 32%, respectively) compared with controls. The suppression in body weight gain after GD 15 was probably related to the smaller uterine weights. However, the suppression in body weight gain during the GD 6-9 and 9-12 intervals for the 1000 mg/kg dams was probably due to slight maternal toxicity.

Mean body weights of the 250 and 500 mg/kg dose groups appeared comparable with controls throughout the entire gestation period.

3. GESTATION FOOD CONSUMPTION

Mean Gestation Food Consumption: Table 3
Individual Gestation Food Consumption: Appendix C

There were no biologically significant differences in mean gestation food consumption between treated and control dams at any interval during the gestation period.

RESULTS (CONT'D)

4. GROSS POSTMORTEM OBSERVATIONS

Incidence of Gross Postmortem Observations: Table 4
Individual Gross Postmortem Observations: Appendix D

Postmortem findings were limited to one 750 mg/kg dam with a dilated renal pelvis and one 1000 mg/kg dam with situs inversus of the stomach, gastrointestinal tract, pancreas, and spleen. These single occurrences were considered incidental. All other dams were free of abnormalities.

5. UTERINE IMPLANTATION DATA

Mean Uterine Implantation Data: Table 5
Individual Uterine Implantation Data: Appendix E

There was an increase in the mean number of resorptions per litter and resorptions per implantation sites in the 750 (5.0 and 0.32, respectively) and 1000 (9.86 and 0.57, respectively) mg/kg dose groups compared with the controls (1.71 and 0.10, respectively). Consequently, decreases in the mean number of live fetuses per litter were observed in the 750 (10.83) and 1000 (7.43) mg/kg dose groups compared with controls (14.86). Coinciding decreases in the mean number of live males, mean number of live females, and mean number of fetuses per implantation sites also were observed in the 750 and 1000 mg/kg dose groups. These differences were directly related to the increase in percent post-implantation loss in the 750 (31.6) and 1000 (57.0) mg/kg dose groups compared with the controls (10.3). There was also an apparent difference in the fetal sex ratio, as determined by external exam, in the 750 and 1000 mg/kg litters. The number of fetuses identified as males was reduced and the number of fetuses identified as female was increased compared with controls. However, results from the follow-up main study with the test material (EBSI, 1997), which included an internal exam, indicated that this effect was probably due to difficulty with the external sexing procedure in the smaller fetuses and subsequent potential misclassification.

The incidence of dams with malformed, variant, and affected (resorptions + dead + malformed) fetuses was significantly increased in the 750 (0.67, 0.2, and 5.7, respectively) and the 1000 (0.43, 0.1, and 10.3, respectively) mg/kg dose groups compared with controls (0.0, 0.0, and 1.7, respectively).

There also were differences in the percent pre-implantation loss in some treated groups compared with controls. However, these differences were not attributed to treatment with the test material since dosing occurred from GD 6-20, which is after implantation is completed (GD 5-6; Manson and Kang, 1989).

RESULTS (CONT'D)

5. *UTERINE IMPLANTATION DATA (cont'd)*

There were no apparent differences in the implantation parameters between the 250 and 500 mg/kg groups and the control group. Additionally, there were no dead fetuses in any group. One 500 mg/kg female and one 750 mg/kg female were not pregnant.

6. *FETAL BODY WEIGHT*

Mean Fetal Body Weight: Table 6
Individual Fetal Body Weight: Appendix F

The mean fetal body weights of the 750 (males: 4.54 g; females 4.58 g) and 1000 (males: 4.73 g; females: 4.58 g) mg/kg fetuses were substantially lower (14-18%) than controls (males: 5.52 g; females: 5.43 g) and lower than the laboratory historical range (males: 5.26-5.85 g; females: 4.96-5.62 g).

Mean fetal body weights of the 250 and 500 mg/kg dose groups were essentially equivalent to controls and within the laboratory historical range.

7. *FETAL EXTERNAL OBSERVATIONS*

Incidence of Fetal External Observations: Table 7
Individual Fetal External Observations: Appendix G

External malformations were observed in the 750 (4 fetuses, 3 litters) and 1000 (3 fetuses, 2 litters) mg/kg dose groups and included single or few occurrences of encephalocele, cleft palate, filamentous tail, atresia ani, acaudia, exencephaly, and anasarca. Other findings included cranial hematomas or protruding tongue. Additionally, eight fetuses (four litters) in the 750 mg/kg dose group and nine fetuses (four litters) in the 1000 mg/kg group were stunted (body weight $\leq$ 4.0 grams).

There were no external abnormalities seen in any fetuses in the 250 mg/kg, 500 mg/kg, or control groups. One 250 mg/kg fetus was stunted. This finding was considered spurious.

RESULTS (CONT'D)

8. ANALYTICAL CHEMISTRY

Analytical Chemistry Report: Appendix H

Satisfactory uniformity was observed for the test material at 2% and 20% w/v. The relative standard deviation ranged from 1.3 to 1.8%. The test material was stable in corn oil for at least 9 days. Concentration verification analyses indicated that the test material concentrations were within 7% of the nominal value.

CONCLUSION

Signs of developmental toxicity were apparent at the 750 and 1000 mg/kg dose levels, as indicated by a decrease in mean total number of live fetuses and mean number of fetuses per implantation sites; and an increase in the mean number of resorptions per litter, mean number of resorptions per implantation sites, mean percent post-implantation loss, and incidence of dams with malformed and affected (resorptions + dead + malformed) fetuses compared with controls. Additionally, mean fetal body weights were substantially decreased in the 750 and 1000 mg/kg fetuses compared with controls and lower than the laboratory historical control range. Malformations also were observed in the 750 and 1000 mg/kg dose group fetuses. In the dams, there was a suppression in body weight gain in the 1000 mg/kg dams during the GD 6-9 and GD 9-12 intervals which was indicative of slight maternal toxicity.

Due to excessive fetal lethality at the 1000 mg/kg dose, 750 mg/kg was selected as the high dose for the subsequent definitive developmental toxicity study in rats. The 750 mg/kg dose was expected to provide a sufficient number of fetuses for an adequate evaluation of the developmental toxicity of the test material. The low and mid doses selected were 100 and 300 mg/kg, respectively, with the low dose expected to produce a No Observable Adverse Effect Level (NOAEL).

PROTOCOL EXCEPTION

CONCENTRATION VERIFICATION: Per protocol, concentration analyses for all dose levels were to be performed on the first dosing mixture preparations. Concentration analyses were actually performed on the second and third dosing mixture preparations. This deviation did not adversely affect the study results or integrity.

No other circumstances occurred that would have affected the quality or integrity of the data.

REFERENCES

Animal Welfare Act of 1966 (P.L. 89-544), as amended in 1970, 1976, and 1985. Code of Federal Regulations, Title 9 [Animals and Animal Products], Subchapter A -Animal Welfare Parts 1, 2, and 3.

EBSI, Exxon Biomedical Sciences, Inc, Developmental Toxicity Study in Rats with MRD-96-676: 167634, unpublished report, 1997.

EC, Official Journal of the European Communities, No. L 133, Part B. Methods for Determination of Toxicity, "Teratogenicity", Annex V, adopted November, 1987.

EC, Official Journal of the European Communities, Council Decision on Principles of Good Laboratory Practice, (89/569/EEC), 1989.

EPA, U.S. Environmental Protection Agency, 40 CFR Part 792, Toxic Substances Control Act (TSCA), Good Laboratory Practice Standards (GLPs), Final Rule, 1989.

EPA, U.S. Environmental Protection Agency, 40 CFR Part 798, Toxic Substances Control Act (TSCA), Test Guidelines for Developmental Toxicity Studies, 1985.

EPA, U.S. Environmental Protection Agency, Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), Test guideline 83-3, Teratogenicity study and the draft guideline OPPTS 870.3700, 1996.

Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, National Academy Press, Washington, D.C., 1996.

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OECD, Organization for Economic Cooperation and Development, Principles of Good Laboratory Practice, 1981.

OECD, Organization for Economic Cooperation and Development, Guidelines for the Testing of Chemicals, Test Guideline 414, 1981.

Snedecor, G.W., and Cochran, W.G., Statistical Methods, 8th ed., Iowa State University Press, Ames, Iowa, 1989.

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EXXON BIOMEDICAL SCIENCES, INC.

FINAL REPORT

PROJECT NUMBER: 167634

TEST MATERIAL: MRD-96-676

DEVELOPMENTAL TOXICITY STUDY IN RATS
WITH MRD-96-676

PERFORMED FOR:

EXXON CHEMICAL AMERICAS
13501 Katy Freeway
Houston, Texas 77079-1398

PERFORMED AT:

EXXON BIOMEDICAL SCIENCES, INC.
Toxicology Laboratory
Mettlers Road
CN 2350
East Millstone, New Jersey 08875-2350

COMPLETION DATE: December 5, 1997

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APPROVAL SIGNATURES

J. J. Freeman, Ph.D., D.A.B.T.
Mammalian Toxicology Laboratory Director

24 Dec 97
Date

D. J. Letinski, M.S.
Analytical Chemistry Supervisor

31 Dec 97
Date

I hereby accept responsibility for the validity of these data and declare that to the best of my knowledge, the study contained herein was performed under my supervision in compliance with the EU Council Decision on Compliance with Principles of Good Laboratory Practice and the EPA Good Laboratory Practice standards set forth in 40 CFR Part 792.

G. W. Trimmer, B.A.
Study Director

31 DEC 97
Date

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QUALITY ASSURANCE STATEMENT

STUDY NUMBER: 167634

TEST SUBSTANCE/ARTICLE: MRD-96-676

STUDY SPONSOR: Exxon Chemical Americas

Listed below are the dates that this study was inspected by the Quality Assurance Unit of Exxon Biomedical Sciences, Inc., and the dates findings were reported to the Study Director and Management.

Date(s) of Inspection	Reported to Study Director	Reported to Management
06 DEC 96	06 DEC 96	07 APR 97
16 DEC 96 - 03 JAN 97	06 JAN 97	24,25 JAN 97
31 DEC 96, 03 JAN 97	06 JAN 97	24,25 JAN 97
11,28 FEB 97	28 FEB 97	21 APR 97
20 AUG 97 - 04 SEP 97	08 SEP 97	24 NOV 97 02 DEC 97
17-21 NOV 97	24 NOV 97	04,05 DEC 97

Joanne R. Jackson, B.S.
Quality Assurance Supervisor

22 December 97
Date

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PERSONNEL

Study Director:	G. W. Trimmer, B.A.
Sponsor:	Exxon Chemical Americas 13501 Katy Freeway Houston, Texas 77079-1398
Sponsor Representative:	L. H. Keller, Ph.D.
Mammalian Toxicology Laboratory Director:	J. J. Freeman, Ph.D., D.A.B.T.
Toxicology and Animal Care Supervisor:	R. C. Forgash, B.S.
Report Preparation Supervisor:	E. R. Frank, B.A.
Compound Preparation Supervisor:	M. A. Elliott, B.S.
Analytical Chemistry Supervisor:	D. J. Letinski, M.S.
Quality Assurance/Archives Supervisor:	J. R. Jackson, B.S.
Maintenance Supervisor:	J. L. McGrath, A.S.
Veterinarian:	R. L. Harris, D.V.M.
Reproductive Toxicology Consultant:	S. B. Harris, Ph.D.

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SUMMARY

This study was conducted to evaluate the potential developmental toxicity of MRD-96-676 diluted in corn oil and administered via oral gavage to pregnant rats during the period of major organogenesis and fetal growth. The test material was administered by oral gavage to 25 confirmed-mated Crl:CDBR female rats at doses of 0 (corn oil only), 100, 300, and 750 mg/kg at a dose volume of 5 ml/kg once daily from Gestation Day (GD) 6 through GD 20. These doses were selected based on a previously conducted rangefinding study with the test material performed at EBSI (EBSI, 1997). In the rangefinding study, the test material was administered orally by gavage to groups of 7 confirmed-mated Crl:CDBR female rats at doses of 250, 500, 750 and 1000 mg/kg (1000 mg/kg is the limit dose for developmental toxicity studies as per OECD guidelines). Since excessive embryolethality and fetotoxicity was observed at a dose of 1000 mg/kg in the rangefinding study, a dose of 1000 mg/kg in the main study would most likely have produced an insufficient number of fetuses for a valid assessment of visceral and skeletal alterations. Thus, 750 mg/kg was selected as the high dose for the subsequent definitive developmental toxicity study in rats with the test material. The 750 mg/kg dose was expected to provide a sufficient number of fetuses for an adequate evaluation of the developmental toxicity of the test material. The low dose (100 mg/kg) was expected to produce a No Observable Adverse Effect Level (NOAEL) and 300 mg/kg was selected for the mid dose.

Clinical observations were made daily during gestation. Body weight and food consumption measurements were made on GD 0, 6, 9, 12, 15, 18, and 21. On GD 21, animals were sacrificed and cesarean sections were performed. Gross necropsies were performed, liver weights and uterine weights with ovaries attached were recorded, uterine contents were examined, and the required uterine implantation data were recorded. All live fetuses were weighed, sexed externally, and examined externally for gross malformations.

The viscera of approximately one-half of the fetuses of each litter were examined by fresh dissection. After these fetuses were examined, they were decapitated. The heads were preserved in Bouin's solution for at least two weeks, rinsed, and subsequently stored in 70% ethanol. The fetal heads were sectioned and examined with a dissecting microscope for the presence of abnormalities. The remaining fetuses were eviscerated, processed for skeletal staining, stained for bone and cartilage, and examined for the presence of skeletal malformations and variations.

The majority of dams survived to scheduled terminal sacrifice on GD 21 and were free of clinical or postmortem effects attributable to treatment. One control dam and one 300 mg/kg dam were euthanized after early deliveries on Days 20 and 21, respectively.

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SUMMARY (CONT'D)

There was a statistically significant decrease in mean GD 21 body weight (7%) and uterine weight (30%) of the 750 mg/kg dams compared with controls. Additionally, suppressed body weight gain was observed in the 750 mg/kg dams during the GD 15/18 and GD 18/21 intervals, the overall treatment period from GD 6/21, and the overall gestation period (GD 0/21) compared with controls. The lower body weights and suppression in body weight gain during the later part of gestation were due to the decreased uterine weights, since the mean GD 21 corrected body weight and corrected body weight gain of the 750 mg/kg dams was essentially equivalent to controls. The decreased uterine weights were directly related to the increased embryonic death and smaller fetuses in the 750 mg/kg group. Mean body weights and body weight gain of the 100 and 300 mg/kg dose groups were comparable with controls throughout gestation. There were no statistically significant differences in mean food consumption between any treated group and the control group during the gestation period.

There were dose-related increases in mean absolute and relative maternal liver weights that were statistically significant in the 300 and 750 mg/kg dams compared with controls. These increases were consistent with the known ability of certain other alkyl esters of 1,2 benzenedicarboxylic acid to cause peroxisome proliferation (Boorman et al., 1990) and are considered to be a physiological adaptation. Thus, under the condition of this study, no clear maternal toxicity was identified.

There was a statistically significant increase in the mean number of resorptions per litter and resorptions per implantation site in the 750 mg/kg group (4.70 and 0.31, respectively) compared with the controls (0.74 and 0.05, respectively). Consequently, the mean number of live fetuses and mean number of fetuses/implantation site were significantly decreased compared with controls. These differences were directly related to the significant increase in post-implantation loss in the 750 mg/kg dose group (31.1%) compared with the controls (4.6%). The incidence of dams with malformed fetuses, fetuses with developmental variations, and affected (resorptions + dead + malformed) fetuses per litter was statistically significantly increased in the 750 mg/kg dose group (5.13, 5.1, and 9.8, respectively) compared with controls (0.26, 1.5, and 1.0, respectively). There were no statistically significant differences in implantation parameters between the 100 and 300 mg/kg group and the control group.

The mean male and female fetal body weights of the 750 mg/kg group were statistically significantly decreased compared with controls and also substantially lower than the laboratory historical control range. These differences were considered treatment-related. The 100 mg/kg and 300 mg/kg group mean fetal body weights were essentially equivalent to controls and within the laboratory historical control range.

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SUMMARY (CONT'D)

There was a statistically significant increase in the incidence of fetuses with external, visceral, and skeletal malformations and/or variations in the 750 mg/kg dose group compared with controls on a per litter and/or per fetus basis. The most common malformations were anophthalmia, microphthalmia, ectopic testes/ovaries, abnormal origin or agenesis of the blood vessels, and agenesis, misshapen, fused, or malformed bones of the skull, sternebrae, ribs, or vertebrae. Additionally, stunted fetuses (less than 4 grams) were observed in approximately one half the 750 mg/kg group litters. These findings were considered treatment-related. In the controls, 100 mg/kg, and 300 mg/kg groups, there were low incidences of fetal external, visceral and/or skeletal observations, but none of these were considered treatment-related or biologically important.

In conclusion, evidence of growth retardation and increased embryo/fetal death was observed in the high dose (750 mg/kg) group compared with controls. Additionally, there was an increased incidence of external, visceral and skeletal malformations/variations in the 750 mg/kg compared with control. Therefore, the test material was considered embryolethal, fetotoxic, and a selective developmental toxicant at a dose of 750 mg/kg under the conditions of this study. At doses of 300 mg/kg or less, there were no treatment-related or biologically important fetal malformations, embryolethality, or fetal weight reduction. Accordingly, the developmental NOAEL (No Observable Adverse Effect Level) was established at 300 mg/kg.

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INTRODUCTION

This study was conducted to evaluate the developmental toxicity of MRD-96-676 administered via oral gavage to pregnant rats during the period of major organogenesis and fetal growth.

This study was conducted for Exxon Chemical Americas, 13501 Katy Freeway, Houston, Texas 77079-1398 (subsequently referred to as the Sponsor).

The study was conducted by Exxon Biomedical Sciences, Inc. (EBSI) Toxicology Laboratory, Mettlers Road, CN 2350, East Millstone, New Jersey 08875-2350. The EBSI Toxicology Laboratory is an American Association for Accreditation of Laboratory Animal Care (AAALAC) accredited facility and a Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF) certified facility.

Study Initiation (Protocol Signature Date)

December 6, 1996

Inlife Test Period

December 9, 1996 to January 15, 1997

Justification for Selection of Test System

The rat is among the species of choice for teratogenicity testing according to the EC Methods for the Determination of Toxicity, "Teratogenicity Test - Rodent and Non-Rodent" (EC, 1987), OECD Guideline for the Testing of Chemicals 414, "Teratogenicity" (OECD, 1981) and the U.S. EPA TSCA Test Guidelines for Developmental Toxicity Studies (EPA, 1985).

Justification of Dosing Route

Oral intubation is an accepted or preferred route of administration according to EC (1987), OECD (1981) and U.S. EPA TSCA (1985) guidelines, and ingestion represents a possible route of human exposure.

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INTRODUCTION (CONT'D)

Justification of Dose Selection

Doses were selected based on a previously conducted rangefinding study with MRD-96-676 performed at EBSI. In the rangefinding study, the test material was administered by oral gavage to groups of 7 Crl:CDBR mated female rats at doses of 250, 500, 750 and 1000 mg/kg. The high dose was selected because it was the limit dose for developmental toxicity studies as per OECD guidelines (OECD, 1981).

In the rangefinding study with a limited number of animals per group, the following signs of toxicity were apparent at the 750 and 1000 mg/kg dose levels: adverse effects on maternal uterine implantation parameters, decreased mean fetal body weight when compared with controls, and external fetal malformations. Additionally, suppression of maternal body weight gain was observed in the dams at 1000 mg/kg during the GD 6-9 and 9-12 intervals which may have been indicative of slight, transient maternal toxicity.

Due to excessive fetal lethality at a dose level of 1000 mg/kg, 750 mg/kg was selected as the high dose for the definitive developmental toxicity study in rats. The 750 mg/kg dose was expected to provide a sufficient number of fetuses for an adequate evaluation of the developmental toxicity of the test material. The low and mid doses selected were 100 and 300 mg/kg, respectively. The 100 mg/kg dose level was expected to produce a No Observable Adverse Effect Level.

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INTRODUCTION (CONT'D)

Compliance

This study was conducted in general agreement with the following guidelines and standards:

Animal Welfare Act of 1966 (P.L. 89-544), as amended in 1970, 1976, and 1985. Code of Federal Regulations, Title 9 [Animals and Animal Products], Subchapter A - Animal Welfare Parts 1, 2, and 3.

EC, Official Journal of the European Communities, No. L 133, Part B. Methods for Determination of Toxicity, "Teratogenicity". Annex V, adopted November, 1987.

EPA, U.S. Environmental Protection Agency, 40 CFR Part 798, Toxic Substance Control Act (TSCA), Test Guidelines for Developmental Toxicity Studies, 1985.

EPA, U.S. Environmental Protection Agency, Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), Test guideline 83-3, Teratogenicity study and the draft guideline OPPTS 870.3700, 1996.

Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, National Academy Press, Washington, D.C., 1996.

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MATERIALS AND METHODS

TEST MATERIAL

Material Identification

EBSI Identification:	MRD-96-676
Supplier:	Exxon Chemical
Date Received:	September 25, 1996
Lot #:	011249
Expiration Date:	January 2001
Description:	Colorless liquid
Storage Condition:	Room temperature

Adjustments in the dose calculations were not made to correct for the purity of the test material. The test material, as received, was considered the "pure" material for the purpose of dosing.

Characterization of the Test Material

The stability, identity, strength, purity, and composition or other characteristics which appropriately identified the test material was performed by the testing laboratory (EBSI). Documentation is maintained at Exxon Biomedical Sciences, Inc., East Millstone, New Jersey.

The methods of synthesis, fabrication, and/or derivation of the test material are the responsibility of the Sponsor. Documentation is maintained at Exxon Baton Rouge Chemical Plant, 4999 Scenic Highway, Baton Rouge, LA 70821.

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TEST MATERIAL (CONT'D)

Analysis of Mixtures

Analyses for the stability, uniformity, and concentration of the test and control material mixtures were performed by the testing laboratory. Analyses for stability and uniformity were initiated concurrent with the start of this study. Concentration analyses were performed on the first and third dosing mixture preparations.

Solubility

The test material was soluble in the carrier at the concentrations required for this study.

Sample Retention

One archival sample of the undiluted test material, as well as the carrier, was collected by the Compound Preparation Department and stored at room temperature.

Carrier

Corn Oil

Supplier:	Best Foods CPC International
Batch:	801B6
Date Received:	November 1, 1996
Description:	Yellow liquid
Storage Condition:	Room temperature
Expiration Date:	November 2001

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TEST SYSTEM

Test Animal

Species:	Rat
Strain/stock:	Crl:CD BR VAF/Plus
Supplier:	Charles River Laboratories, Inc. Portage, Michigan

Animal Receipt Information (Females)

Receipt Date: November 26, 1996
Purchase Order Number: 6CR11496R

Quarantine and Acclimation Period

14 days; animals were checked for viability at least once daily.

Number and Sex

150 sexually mature virgin females

100 females were allocated to study groups after confirmation of mating; additional animals received for mating purposes only.

50 sexually mature males (received for mating purposes only)

Age at Initiation of Gestation (Designated GD 0)

Females: Approximately 13-16 weeks

Weight at Initiation of Gestation (Designated GD 0)

Females: 240 to 315 grams

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TEST SYSTEM (CONT'D)

Animal Identification

Individual ear tags and corresponding cage identification.

Selection

More animals than required for the conduct of the study were purchased and acclimated. Animals determined to be unsuitable for inclusion on this study because of poor health, outlying body weights, or other abnormalities were excluded from selection by the attending Veterinarian, Study Director, and/or technical staff.

Husbandry

Feed

Certified Rodent Diet Meal 5002, ad libitum

Manufacturer:	PMI Feeds Inc. Richmond, Indiana
Analysis:	Performed by PMI Feeds Inc. Copies of the feed analyses are maintained in the EBSI Toxicology Laboratory.
Contaminants:	There were no known contaminants in the feed believed to have been present at levels that may have interfered with this study.

The availability of feed was checked daily for all animals.

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TEST SYSTEM (CONT'D)

Water

Automatic watering system, ad libitum

Supplier:	Elizabethtown Water Company Bound Brook, New Jersey.
Analysis:	Periodic analysis is the responsibility of EBSI. A copy of the results is maintained at EBSI.
Contaminants:	There were no known contaminants in the water believed to have been present at levels that may have interfered with this study.

The availability of water was checked daily for all animals.

Housing

Room Number:	511
Housing:	Single housed during the study period, except during mating.
Caging:	Suspended stainless steel and wire mesh with absorbent paper below cages.

Environmental Conditions

Temperature:	64 to 72 degrees Fahrenheit
Humidity:	30 to 70 percent relative humidity
Lighting:	Approximately 12 hours light (0700 to 1900 hours) and 12 hours dark (1900 to 0700 hours) by automatic timer.

Monitored continuously.

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EXPERIMENTAL DESIGN

Mating

On the initial scheduled mating day, females were placed in males' cages in a 1:1 (male:female) ratio. Males and females were paired based on sequential Physical Identification Numbers. A sufficient number of animals were co-housed in an attempt to produce an acceptable number of mated animals to accommodate lab scheduling. Mating was confirmed on the following morning by observation of a copulatory plug (vaginal) and/or by the presence of sperm in a vaginal rinse. The day on which mating was confirmed was considered Day 0 of gestation (GD 0). After confirmation of mating, each mated female was returned to its own cage. New females then were placed in the males' cages until the required number of mated females was obtained by continuous cohabitation in consideration of lab scheduling. Mated females subsequently were assigned to dose groups by a computer generated body weight sorting program using the GD 0 body weights to ensure mean body weight was similar between all groups on GD 0.

Preparation of Test Material Mixtures

The undiluted test material was thoroughly mixed in carrier prior to dispensing.

The test material/carrier mixtures were prepared once a week. Dosing solutions were prepared at the following doses.

Experimental Groups

Group Number	Number of Females per Group	Dose (mg/kg/day)
1 (Carrier Control)	25	0
2 Low	25	100
3 Intermediate	25	300
4 High	25	750

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EXPERIMENTAL DESIGN (CONT'D)

Administration of Test Material and Treatment Schedule

Mated females were dosed once daily by oral gavage from GD 6 through GD 20. Dosing volumes were 5 ml/kg for all groups and were based on the most recent individual body weights, with the exception of the GD 20 body weight. Animals were dosed at approximately the same time each day (11:00 ± 2.5 hours).

Experimental Evaluation

Animals were examined for viability at least twice daily during the treatment period and at least once daily at other times during the study.

Body weights were taken prior to selection, and on GD 0, 6, 9, 12, 15, 18, 20, and 21.

Food consumption was measured for mated females on GD 6, 9, 12, 15, 18, and 21.

A clinical examination was given to each female prior to selection, and daily during gestation.

Euthanasia and Cesarean Section

Euthanasia was by CO_2 asphyxiation followed by exsanguination. A gross necropsy was performed on all mated females.

Mated females were euthanized on GD 21. Body weights were recorded on the day of necropsy. Uterine weights with ovaries attached and liver weights were recorded at the time of necropsy. Uterine contents were examined and the numbers and locations of implantation sites, early and late resorptions, live and dead (alive or dead *in utero*) fetuses were counted. Corpora lutea also were counted. The uteri of all apparently non-pregnant females were stained to confirm pregnancy status.

Evaluations of dams during cesarean section were conducted without knowledge of treatment group in order to minimize bias.

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EXPERIMENTAL DESIGN (CONT'D)

Euthanasia and Cesarean Section (cont'd)

Females that delivered prior to scheduled cesarean section were sacrificed. The animals were weighed and examined for gross lesions. The number of implantation sites or conceptuses in each uterine horn was counted and each pup was sexed externally and counted. No further examination of the pups was performed. The pups were sacrificed by asphyxiation with CO_2.

Examination of Fetuses

Each live fetus (alive *in utero*) was weighed and examined externally for gross malformations. Fetal sex was determined by external examination and confirmed internally only on those fetuses receiving visceral examinations.

The viscera of approximately one-half of the live fetuses (alive *in utero*) of each litter were examined by fresh dissection (Staples, 1974; Stuckhardt and Poppe, 1984). After these fetuses were examined, they were decapitated. The heads were preserved in Bouin's solution for at least two weeks, then rinsed and subsequently stored in 70% ethanol. Free-hand razor blade sections of the Bouin's-fixed fetal heads were examined for the presence of abnormalities. The remaining live fetuses (alive *in utero*) were eviscerated, processed by double staining with Alizarin red and Alcian blue (Tyl, 1996), and examined for the presence of bone and cartilage malformations and ossification variations. Representative malformations were photographed at the discretion of the Study Director or his designee.

Fetal evaluations were conducted without knowledge of treatment group in order to minimize bias.

Tissue Preservation

The livers of all confirmed mated females were preserved in 10% neutral buffered formalin for possible future histologic examination. Abnormal tissues from mated adult females were saved at the discretion of the Study Director or his designee and preserved in 10% neutral buffered formalin for possible future histologic examination. Fetal heads were saved in ethanol.

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EXPERIMENTAL DESIGN (CONT'D)

Records

A copy of the protocol, final report, raw data, computer generated listings of raw data, supporting documentation, sample(s) of test material, and tissue specimens are maintained in the EBSI Toxicology Laboratory Archives.

Statistical Analysis

Statistical treatment of the results was conducted where appropriate. Statistical evaluation of equality of means was done by an appropriate one way analysis of variance and a test for ordered response in the dose groups. First, Bartlett's Test was performed to determine if the dose groups had equal variance (Snedecor and Cochran, 1989). If the variances were equal, the testing was done using parametric methods, otherwise nonparametric techniques were used.

Where applicable, percentages were calculated and transformed by Cochran's transformation, followed by the arc sine transformation (Snedecor and Cochran, 1989). The raw percentages and the transformed percentages both were tested for statistical significance. Transformations were not reported since they were not statistically significant.

For the parametric procedures, a standard one way ANOVA using the F distribution to assess significance was used (Snedecor and Cochran, 1989). If significant differences among the means were indicated, Dunnett's Test was used to determine which treatment groups differed significantly from control (Dunnett, 1964). In addition to the ANOVA, a standard regression analysis for linear response in the dose groups was performed. The regression also tested for linear lack of fit in the model.

For the nonparametric procedures, the test of equality of means was performed using the Kruskal-Wallis Test (Hollander and Wolfe, 1973). If significant differences among the means were indicated, Dunn's Summed Rank Test was used to determine which treatment groups differed significantly from the control (Hollander and Wolfe, 1973). In addition to the Kruskal-Wallis Test, Jonckheere's Test for monotonic trend in the dose response was performed.

Bartlett's Test for equal variance was conducted at the 1% level of significance. All other tests were conducted at the 5% and 1% level of significance.

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EXPERIMENTAL DESIGN (CONT'D)

Statistical Analysis (Cont'd)

Fetal weight was analyzed by a standard nested analysis of covariance (Snedecor and Cochran, 1989) with fetuses nested within dams and with dams nested within doses, and litter size (both sexes combined) as the covariate. If differences in groups were identified, the Least Significant Difference (LSD) technique was used to determine which groups differed from the control group (Snedecor and Cochran, 1989). Male and female fetuses were tested separately (the covariate was combined sexes in each analysis). All tests were reported at the 5% or 1% level of significance.

Fetal malformation and variation incidence data were analyzed for statistical significance as follows: First, a standard chi-square analysis was performed to determine if the proportions of incidences differ between the groups tested (Snedecor and Cochran, 1989). In keeping with standard statistical practice, if any one cell had an expected value less than 5, this step was not reported. Next, each treatment group was compared to the control group using a 2 x 2 Fisher Exact Test (Bradley, 1968). Thirdly, Armitage's test for linear trend in the dosage groups was performed (Snedecor and Cochran, 1989). All tests were reported at the 5% or 1% level of significance.

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RESULTS

1. CLINICAL INLIFE OBSERVATIONS AND SURVIVAL

Incidence of Gestation Observations: Table 1
Individual Gestation Observations: Appendix A

All maternal animals survived to scheduled study termination on GD 21 with the exception of one control dam and one 300 mg/kg dam which were euthanized after early deliveries on Days 20 and 21, respectively.

The majority of dams in all dose groups were free of observable abnormalities during the entire gestation period. Adverse clinical signs were limited to one 750 mg/kg dam with anogenital staining, dry rales, red material around the snout, soft stool, and little sign of food consumption and/or stool beginning on Day 9. These signs may have been related to dosing trauma, although there was no evidence of a dosing accident at necropsy.

There was a single or low incidence of dental abnormalities, a mechanically induced truncated digit, swollen snout, and/or ocular discharge observed sporadically. These findings were considered incidental and unrelated to treatment.

2. GESTATION BODY WEIGHT

Mean Gestation Body Weight and Body Weight Change: Table 2
Individual Gestation Body Weight: Appendix B

There was a statistically significant decrease in mean GD 21 body weight (7%) and uterine weight (30%) of the 750 mg/kg dams compared with controls. Additionally, suppressed body weight gain was observed in the 750 mg/kg dams during the GD 15/18 (27%) and GD 18/21 (23%) intervals, during the overall treatment period from GD 6/21 (21%), and the overall gestation period, GD 0/21 (16%), compared with controls. The lower body weights and suppression in body weight gain during the later part of the study was due to the decreased uterine weights, since the mean GD 21 corrected body weight and corrected body weight gain of the 750 mg/kg dams was essentially equivalent to the controls. The decreased uterine weights were directly related to the increased embryonic death and smaller fetuses in the 750 mg/kg group.

Mean body weights and body weight gain of the 100 and 300 mg/kg dose groups appeared comparable with controls throughout the entire gestation period.

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RESULTS (CONT'D)

3. GESTATION FOOD CONSUMPTION

Mean Gestation Food Consumption: Table 3
Individual Gestation Food Consumption: Appendix C

There were no statistically significant differences in mean gestation food consumption between treated and control dams at any interval during the gestation period.

4. GROSS POSTMORTEM OBSERVATIONS

Incidence of Gross Postmortem Observations: Table 4
Individual Gross Postmortem Observations: Appendix D

The majority of dams were free of abnormalities at postmortem examination. One 300 mg/kg animal which was not pregnant had enlarged adrenal glands, a hole in the diaphragm, enlarged discolored kidneys, and an enlarged spleen. There were also single occurrences of dilated renal pelvis (300 mg/kg), anogenital staining (750 mg/kg), truncated digit (750 mg/kg), an esophageal tear (100 mg/kg) which was the result of a dosing error, and several animals with dental abnormalities. All findings were considered either incidental or accidental and not related to treatment with the test material.

5. LIVER AND RELATIVE LIVER WEIGHT DATA

Mean Organ Weight: Table 5
Mean Relative Organ Weight: Table 6
Individual Organ and Relative Weights: Appendices E and F

There were dose-related increases in mean absolute and relative liver weights that were statistically significant in the 300 (11% and 8%, respectively) and 750 (20% and 16%, respectively) mg/kg dams compared with controls. These increases were small, consistent with the known ability of certain other alkyl esters of 1,2 benzenedicarboxylic acid to cause peroxisome proliferation (Boorman et al., 1990), and are considered to be a physiological adaptation. Thus, under the condition of this study, no clear maternal toxicity was identified.

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RESULTS (CONT'D)

6. UTERINE IMPLANTATION DATA

Mean Uterine Implantation Data: Table 7
Individual Uterine Implantation Data: Appendix G

There was a substantial, statistically significant increase in the mean number of resorptions per litter and resorptions per implantation site in the 750 mg/kg group (4.70 and 0.31, respectively) compared with the controls (0.74 and 0.05, respectively). Consequently, statistically significant decreases in the mean number of live fetuses per litter were observed in the 750 mg/kg dose group (10.65) compared with controls (15.09). Coinciding statistically significant decreases in the mean number of live males, mean number of live females, and mean number of live fetuses per implantation site also were observed in the 750 mg/kg dose groups compared with controls. These differences were directly related to the substantial statistically significant increase in post-implantation loss in the 750 mg/kg dose group (31.1%) compared with the controls (4.6%).

The incidence of dams with malformed fetuses, fetuses with developmental variations, and affected (resorptions + dead + malformed) fetuses was statistically significantly increased in the 750 mg/kg dose group (5.13, 5.1, and 9.8, respectively) compared with controls (0.26, 1.5, and 1.0, respectively).

There were no statistically significant differences in the implantation parameters between the 100 and 300 mg/kg group and the control group. Additionally, there were no dead fetuses in any group.

One control female, four 100 mg/kg females, two 300 mg/kg females, and two 750 mg/kg females were not pregnant.

7. FETAL BODY WEIGHT

Mean Fetal Body Weight: Table 8
Individual Fetal Body Weight: Appendix H

There was a statistically significant decrease in the mean fetal body weights of the 750 mg/kg (males: 4.73 g; females 4.53 g) fetuses compared with controls (males: 5.30 g; females: 5.02 g). These weights were also substantially lower than the laboratory historical control range (males: 5.25-5.85 g; females: 4.96-5.62 g).

Mean fetal body weights of the 100 and 300 mg/kg dose groups were essentially equivalent to controls and within the laboratory historical control range.

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RESULTS (CONT'D)

8. FETAL OBSERVATIONS

Incidence of Fetal Observations: Table 9
Individual Fetal Observations: Appendix I

External Findings: External malformations were observed primarily in the 750 mg/kg dose group (22 fetuses, 13 litters) and included anasarca, red amniotic fluid, exencephaly, cranioschisis, apparent anophthalmia, apparent microphthalmia, cleft palate, atresia ani, and malformed tail (corkscrew, filamentous, kinked or fleshy tab tail). The overall incidence of 750 mg/kg fetuses with external malformations was statistically significantly increased compared with controls on both a per fetus and per litter basis. Specifically, the incidences of apparent anophthalmia and apparent microphthalmia were statistically significantly increased compared with controls on a per fetuses basis and on a per litter basis for apparent anophthalmia only. Other external findings in the high dose group included protruding tongue (external variation) and short umbilical cord. Additionally, there was a statistically significant increase in the incidence (25 fetuses, 12 litters) of stunted fetuses (body weight < 4.0 grams) compared with controls on both a per fetus and per litter basis. These findings were considered treatment-related.

Other external malformations were limited to one 100 mg/kg fetus with apparent anophthalmia and another 100 mg/kg fetus with a fleshy tab tail and atresia ani. Additionally, two control fetuses (two litters), one fetus each from the 100 and 300 mg/kg dose groups were stunted, and one control fetus had a cleft palate. In the absence of a clear dose response, these limited findings were considered incidental and unrelated to treatment.

Visceral Findings: There was a statistically significant increase in the incidence of fetuses with visceral malformations in the 750 mg/kg dose group compared with controls on both a per litter and per fetus basis. The incidence of abnormal origin of the subclavian artery, agenesis of the innominate artery, and ectopic testes or ovaries were statistically significantly increased compared with controls on both a per litter and per fetuses basis; and of elongated ovaries and hypoplastic uterus on a per fetus basis only. Other visceral malformations observed in the 750 mg/kg dose groups included single incidences of misshapen diencephalon, agenesis of the oviducts or aortic arch, elongated innominate artery, abnormal origin of the carotid artery or descending aorta, and small kidneys, and low incidences of hydroureter and hydronephrosis.

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RESULTS (CONT'D)

Fetal Observations (cont'd):

Similarly, there was a statistically significant increase in the incidence of fetuses with visceral variations in the 750 mg/kg dose group compared with controls on a per fetus basis only. Visceral variations in the high dose group included dilated cerebral ventricles, umbilical artery on left side of urinary bladder and small atrium. Other visceral findings included a statistically significant increase in the incidence of fetuses with discolored livers in the 750 mg/kg dose group compared with controls on both a per litter and per fetus basis. One high dose fetus was observed with discolored lungs.

There were single or low incidences of visceral malformations in the control, low, or mid dose groups and included agenesis of the innominate artery (100 mg/kg), abnormal origin of carotid artery (100 mg/kg), hydroureter (control), retinal coloboma (100 mg/kg), and abnormal origin of the subclavian artery (control, 100 mg/kg). Additionally, there was a low incidence of folded retina observed in all groups, including controls. Other visceral findings included single incidences of misshapen heart (control), small atrium (100 mg/kg), and discolored liver (300 mg/kg). Additionally, there was a low incidence of umbilical artery on left side of urinary bladder (fetal variation) observed in all groups, including controls. These findings were considered incidental.

Skeletal Findings: There was a statistically significant increase in the incidence of fetuses with skeletal malformations in the 750 mg/kg dose group compared with controls on both a per litter and per fetus basis. The incidence of fused or malformed sternebrae, fused ribs, thoracic centra/arch agenesis, and fused or short (not connected to sternebrae) rib anlage were statistically significantly increased compared with controls on both a per litter and per fetuses basis; and of malformed skull or vertebral bones, agenesis of the thoracic ribs, malformed thoracic rib anlage, and sternebrae/rib anlage fused on a per fetus basis only.

There was also a low incidence of other malformations in the 750 mg/kg dose group which were not statistically significantly increased compared with controls. These consisted primarily of agenesis of the skull bones, vertebral cervical or lumbar arches, sternebrae fused to ribs, ribs fused with rib anlage, malformed ribs, short/rudimentary ribs with no anlage, or short ribs (not connected to sternum), multiple vertebral bones absent, vertebral centra malformed, and centra/arches fused.

Skeletal malformations in other groups were limited to one 100 mg/kg dose fetus with multiple vertebral bones absent. This single occurrence was considered incidental.

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RESULTS (CONT'D)

Fetal Observations (cont'd):

There was a statistically significant increase in the incidence of fetuses with skeletal variations in the 750 mg/kg dose group compared with controls on a per fetus basis only. The incidence of asymmetric, bifid, or hypoplastic sternebrae; rudimentary or well-formed lumbar ribs; thoracic centra bifid or hypoplastic; lumbar centra dumbbell/8-shaped; and bifid or dumbbell/8-shaped vertebral anlage; were statistically significantly increased compared with controls on both a per litter and per fetuses basis; and of unossified hindpaw, dumbbell/8-shaped or misshapen sternebrae, or dumbbell/8-shaped thoracic centra on a per fetus basis only.

There was a statistically significant increase in rudimentary lumbar ribs observed in the 300 mg/kg group compared with controls on both a per fetus and/or per litter basis. The incidence of rudimentary lumbar ribs in the 300 mg/kg dose group (18% fetal and 50% litter) was well within the historical control range of this laboratory (4-29% fetal and 13-81% litter). Additionally, there was a statistically significant increase in the incidence of unossified hindpaw in the 300 mg/kg dose group compared with controls on a per fetus basis only. This incidence (5.9%) was also within the historical control range of this laboratory (0.7-6.1%). Thus, these skeletal variations in the 300 mg/kg dose group were considered biologically unimportant.

Skeletal variations or observations in the control, low, or mid dose groups consisted primarily of hypoplastic, misshapen, unossified, supernumerary elements, or advanced bones of the skull, sternebrae, ribs, vertebrae, or paws. These findings were considered incidental. The frequency of these findings was generally low or essentially equivalent between treated and control groups and thus, considered incidental.

9. ANALYTICAL CHEMISTRY

Analytical Chemistry Report: Appendix J

Satisfactory uniformity was observed for the test material at 2% and 20% w/v. The relative standard deviation ranged from 1.3 to 1.8%. The test material was stable in corn oil for at least 9 days. Concentration verification analyses indicated that the test material concentrations were within 9% of the nominal value.

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DISCUSSION

There was no clear evidence of maternal toxicity in this study at any dose level tested. Maternal liver weights were increased in a dose-dependent way, but the size of the increase was small and this was judged to be an adaptive response. Reductions in mean body weight and body weight gain were apparent in the high dose (750 mg/kg) dams during the latter part of gestation; however, these decreases were attributed to the increased embryonic death and smaller fetuses in the 750 mg/kg group. After correcting for the uterine weight, the mean GD 21 body weight and body weight gain of the 750 mg/kg dams was essentially equivalent to the controls. It is important to note that limited toxicity testing has been performed on this material. Thus, it is unknown if maternal toxicity manifested itself in ways not investigated in this study. However, under conditions of this study, no clear sign of maternal toxicity was observed.

Even though there were no apparent maternal effects, overt signs of fetal toxicity were apparent at the 750 mg/kg dose level, as indicated by adverse effects on uterine implantation parameters. These included a decrease in mean number of live fetuses and mean number of fetuses per implantation site together with an increase in the mean number of resorptions per litter, mean number of resorptions per implantation site, mean percent post-implantation loss, and incidence of dams with malformed fetuses, fetuses with developmental variations, and affected (resorptions + dead + malformed) fetuses compared with controls. There was also an increased incidence of specific fetal external, visceral, and/or skeletal malformations/variations compared with controls. Additionally, mean fetal body weights were substantially decreased in the 750 mg/kg fetuses compared with controls and lower than the laboratory historical control range.

There was no evidence of fetal toxicity at dose levels of 100 mg/kg or 300 mg/kg. There was a statistically significant increase in rudimentary lumbar ribs observed in the 300 mg/kg group compared with controls on both a per fetus and per litter basis. However, the incidence of rudimentary lumbar ribs in the 300 mg/kg dose group (18% fetal and 50% litter) was well within the historical control range of this laboratory (4-29% fetal and 13-81% litter) and thus, not considered toxicologically significant. Variations in skeletal structure, particularly rudimentary ribs, are so common that they are regarded as alternative normal patterns and not regarded as harmful developmental toxic effects (Kimmel and Wilson, 1973; Khera, 1981; Harris and DeSesso, 1994). Rudimentary ribs are the most common skeletal variation observed in normal rat fetuses (MARTA/MTA, 1995), and studies have shown that in rats these variations do not persist during postnatal development (Wickramaratne, 1988; Chernoff et al., 1991). Furthermore, rudimentary ribs are not considered biologically significant in the absence of other conventional signs of embryotoxicity (Holson et al., 1976; Aliverti et al., 1979; Khera, 1981).

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DISCUSSION (CONT'D)

There was also a statistically significant increase in the incidence of unossified hindpaw in the 300 mg/kg dose group compared with controls on a per fetus basis only. This incidence (5.9%) was within the historical control range of this laboratory (0.7-6.1%) and thus, not considered toxicologically significant. The absence of a statistically significant or dose-dependent increase in the litter-based incidence of unossified hindpaw provides additional evidence that the observed increase in this parameter was not biologically meaningful, since the litter is the preferred unit of measure in developmental toxicology.

In conclusion, evidence of growth retardation and increased embryo/fetal death was observed in the high dose (750 mg/kg) group compared with controls. Additionally, there was an increased incidence of external, visceral and skeletal malformations/variations in the 750 mg/kg compared with control. Therefore, the test material was considered embryolethal, fetotoxic, and a selective developmental toxicant at a dose of 750 mg/kg under the conditions of this study. At doses of 300 mg/kg or less, there were no treatment-related or biologically important fetal malformations, embryolethality, or fetal weight reduction. Accordingly, the developmental NOAEL (No Observable Adverse Effect Level) was established at 300 mg/kg.

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PROTOCOL EXCEPTIONS/NOTES

POST-LIFE DATA: Although not required by protocol, all post-life data (dam's terminal sacrifice data, fetal external, head, visceral, and skeletal examination data) was collected with the technicians blinded to the treatment group of the animals. Due to concerns with dosing errors, all inlife data was collected with the technicians knowing the treatment group of the animals.

TEMPERATURE: On December 30, 1996 the temperature in the animal room exceeded the acceptable limit specified in the protocol (72°F) for approximately 32 minutes. The highest temperature reached was 80.2°F. This deviation had no adverse effect on the study since there was no high temperature of significant magnitude or duration to affect the health or well being of the animals.

No other circumstances occurred that would have affected the quality or integrity of the data.

NOTES:

RED AMNIOTIC FLUID: Red amniotic fluid was observed in the litter of dam IGA116. The dam was observed with no observable abnormalities at gross postmortem examination. This finding could not accurately be attributed to a fetus(es), but had to have affected at least one fetus. Therefore, this observation was tallied for one fetus for this litter and the litter was tallied for having this observation for statistical analysis of this observation.

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