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Document Title

RANGE-FINDING INHALATION TOXICITY STUDY IN MICE WITH C9 AROMATIC HYDROCARBONS

Chemical Category

O-XYLENE (95-47-6)

EPA-OTS
[redacted]
0006174349

8L-890000283
CONTAINS NO CBI

REPORT NO 380

SUBMISSION OF LISTS AND COPIES OF HEALTH AND SAFETY STUDIES

NAMES OF REQUESTED SUBSTANCES(S)	CAS NUMBER(S)
XYLENE, O-	000095-47-6
CUMENE	000098-82-8
ETHYLTOLUENE, 4-	000622-96-8
ETHYLTOLUENE, 2-	000611-14-3
TRIMETHYLBENZENE, 1,3,5-	000108-67-8
TRIMETHYLBENZENE, 1,2,4-	000095-63-6
TRIMETHYLBENZENE, 1,2,3-	000526-73-8
ETHYLTOLUENE, 3-	000620-14-4

OTHER COMPONENT(S)

SUBMITTED BY

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HEE-88-0097

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American Petroleum Institute
Health and Environmental Sciences Department

QUALITY ASSURANCE STATEMENT

Study Title: Range-Finding Inhalation Toxicity Study In Mice with C9 Aromatic Hydrocarbons

Testing Facility Number: IRDC 418-031

API Product Safety Number: PS-64

This study was reviewed by API Quality Assurance personnel on the dates indicated below for compliance with applicable Good Laboratory Practice regulations.

Copies of reports by API Quality Assurance personnel are available upon written request to the Director of the Health and Environmental Sciences Department of the American Petroleum Institute or his designee.

Date(s) of Inspection/Review	Type of Inspection
September 14-16, 1987	Preliminary raw data audit and data inventory
November 23-24, 30 and December 1, 1987	Draft final report and raw data audit
February 11, 1988	Final report review Amendment to report requested
April 4, 1988	Final report accepted

Rodney C. Anderson

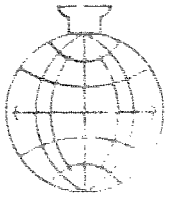
Rodney C. Anderson, M.S.
Quality Assurance Associate

5 April 1988
Date

86-890000229

CONTAINS NO CBI

HSE - 88 - 0129



International Research and Development Corporation

MATERIAL SPECIFICATION
ASTM D3734 TYPE I
C-9 AROMATIC HYDROCARBONS

CONSCI

American Petroleum Institute
1220 L Street N.W.
Washington, D.C. 20005

February 14, 1986

Attn: Dr. C. E. Holdsworth

Certificate # : 51219001
Sample Discription : C9 Aromatic Naphtha
Date Received : December 19, 1985

CAPILLARY GAS CHROMATOGRAPHY ANALYSIS *

Component Name	Wt.%	LV.%	
Total non-aromatics	< 0.10	< 0.12	
C6 + C7 aromatics	0.01	0.01	
m-Xylene	0.05	0.05	
p-Xylene	0.02	0.02	
o-Xylene	3.17	3.14	
Isopropylbenzene	2.76	2.79	
n-Propylbenzene	3.95	3.99	
1-Methyl-3-ethylbenzene	15.85	15.98	
1-Methyl-4-ethylbenzene	6.13	6.19	
1,3,5-Trimethylbenzene	8.09	8.14	
1-Methyl-2-ethylbenzene	5.78	5.72	
1,2,4-Trimethylbenzene	39.18	39.13	
tert-Butylberzene	<0.20	<0.20	#
Isobutylbenzene	0.12	0.12	
sec-Butylbenzene	0.11	0.11	
1-Methyl-2-isopropylbenzene	0.01	0.01	
1,2,3-Trimethylbenzene	5.49	5.35	
1-Methyl-4-isopropylbenzene	0.07	0.07	
Indane (2,3-Dihydroindene)	0.96	0.87	
1,3-Diethylbenzene	1.16	1.18	
1-Methyl-3-n-propylbenzene	0.60	0.60	
1-Methyl-4-n-propylbenzene/n-Butylbenzene	0.82	0.83	
1,2-Diethylbenzene	0.89	0.89	
1,4-Diethylbenzene/1,3-Dimethyl-5-ethylbenzene	0.12	0.12	
1-Methyl-2-n-propylbenzene	0.17	0.17	
1,4-Dimethyl-2-ethylbenzene	0.48	0.48	
1,3-Dimethyl-4-ethylbenzene	0.59	0.59	
1,2-Dimethyl-4-ethylbenzene	2.28	2.28	
1,3-Dimethyl-2-ethylbenzene	0.04	0.04	
1,2-Dimethyl-3-ethylbenzene	0.23	0.22	

Component Name	Wt.%	LV.%
1,2,4,5-Tetramethylbenzene	0.18	0.18
Unidentified C11 compounds	0.14	0.17
1,2,3,5-Tetramethylbenzene	0.19	0.19
5-Methylindan	0.04	0.03
Naphthalene	0.02	0.02
	100.00	100.00

* All peak ID's confirmed by GC/MS analysis.
# Quantified by GC/MS due to coelution with 1,2,4 TMB peak.

ASTM D-86 Distillation

Volume % Distilled	deg F @760mm *
IBP	320
5	322
10	323
20	324
30	325
40	326
50	327
60	328
70	329
80	331
90	335
95	339
Dry Point	346
End Point	350
Recovered	99.0%
Residue	1.0%
Loss	0.0%

* Average of duplicate runs.

International Research
and Development Corporation
MATTAWAN, MICHIGAN U.S.A. 49071 TELEPHONE (616) 668-3336

Amendment

to the

Final Report

SPONSOR:	American Petroleum Institute
TEST ARTICLE:	C_9 Aromatic Hydrocarbons
SUBJECT:	Range-Finding Inhalation Toxicity Study in Mice with C_9 Aromatic Hydrocarbons
DATE OF SUBMISSION:	March 30, 1988

418-031

Reason for Amendment

This amendment is for clarification of data in the final report.

The following revisions have been made and revised pages are attached.

1. Pages 27 and 28: A note has been added to each page to explain how g/kg/day food consumption was calculated for the overall gestation interval (days 0-18).
2. Page 29: A footnote has been added to indicate that pregnancy could not be determined for the two animals which were sacrificed on gestation day 6.

James L. Schardein, M.S.
Scientific Director, Reproduction and Teratology Division
Study Director

3/28/88
Date

Margery J. Wirth, B.S.
Director of Quality Assurance

3/25/88
Date

418-031

TABLE

Summary of Maternal Food Consumption Values

	C_9 Aromatic Hydrocarbons (Desired Concentrations) (ppm)														
	0 (Control)					100					250				
Days of Gestation	g/ animal/ day	S.D.	g/ kg/ day	S.D.	N	g/ animal/ day	S.D.	g/ kg/ day	S.D.	N	g/ animal/ day	S.D.	g/ kg/ day	S.D.	N
0- 6	4.5	0.77	154.7	20.56	4	4.2	0.55	160.2	15.12	5	4.5	0.49	160.8	12.61	4
6- 9	4.7	0.27	153.5	15.25	4	5.4	0.17	176.7	10.27	4	5.0	0.27	171.6	17.06	4
9-12	4.6	1.26	146.1	27.56	4	6.1	1.07	179.8	35.69	4	5.8	0.19	171.1	16.08	4
12-16	6.6	0.43	165.0	24.12	4	6.5	0.50	149.6	12.58	5	6.2	0.24	148.0	10.36	4
16-18	6.6	1.75	146.5	8.64	4	6.6	0.82	134.3	15.81	5	6.1	0.75	148.5	27.47	4
6-18	5.6	0.61	128.3	19.75	4	6.1	0.39	125.2	10.06	5	5.8	0.20	142.4	34.89	4
0-18	5.2	0.22	121.0[a]	27.77	4	5.5	0.43	111.4[a]	11.32	5	5.4	0.27	131.8[a]	33.01	4

S.D. - Standard deviation
N - Number of animals
[a]0-18 day g/kg/day food consumption = $\frac{\text{total food consumption (days 0-18)}}{\text{day 18 body weight}}$ / 18 (days)

418-031

James L. Schardein, M.S.
Study Director

Date

Revised Page 27

TABLE

Summary of Maternal Food Consumption Values

C9 Aromatic Hydrocarbons (Desired Concentrations) (ppm)

Days of Gestation	500 g/animal/day	S.D.	500 g/kg/day	S.D.	N	1,000 g/animal/day	S.D.	1,000 g/kg/day	S.D.	N	1,500 g/animal/day	S.D.	1,500 g/kg/day	S.D.	N
0- 6	4.4	0.82	190.3	22.31	4	4.5	0.49	163.2	16.68	5	4.[illegible]	0.21	160.7	4.70	4
6- 9	4.4	1.45	142.2	46.69	4	4.7	0.86	155.8	17.55	5	3.7	0.00	120.3	2.79	2
9-12	5.3	0.47	152.2	7.90	4	5.4	0.55	159.7	9.11	5	5.3	0.47	159.1	10.71	2
12-16	6.1	0.72	142.7	8.34	4	5.9	0.52	145.4	5.32	5	5.4	0.18	132.7	2.05	2
16-18	9.6	2.78	114.[illegible]	46.52	4	5.8	0.76	130.3	15.88	5	6.0	1.41	131.6	29.04	2
6-18	5.4	1.11	116.4	1.92	4	5.5	0.49	122.2	6.59	5	5.0	0.41	110.7	7.34	2
0-18	5.1	0.96	109.4[a]	4.47	4	5.1	0.45	119.5[a]	8.28	5	4.6	0.39	107.4[a]	5.24	2

S.D. - Standard deviation
N - Number of animals
[a]0-18 day g/kg/day food consumption = total food consumption (days 0-18) / day 18 body weight / 18 (days)

418-031

James L. Schardein, M.S.
Study Director
3/29/82
Date

Revised Page 28

TABLE

Summary of Maternal and Fetal Observations at Cesarean Section

	C_9 Aromatic Hydrocarbons (Desired Concentrations) (ppm)											
	0 (Control)		100		250		500		1,000		1,500	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Animals on study:	5	-	5	-	5	-	5	-	5	-	5	-
Animals that were gravid:	4	-	5	-	4	-	4	-	5	-	2	-
Animals that were sacrificed:	0	-	0	-	0	-	0	-	0	-	2[d]	-
Nongravid:	-	-	-	-	-	-	-	-	-	-	-	-
Gravid:	-	-	-	-	-	-	-	-	-	-	-	-
Animals that delivered:	0	-	0	-	1	-	0	-	0	-	0	-
Animals examined at Cesarean section:	5	-	5	-	4	-	5	-	5	-	3	-
Nongravid:	1	-	0	-	1	-	1	-	0	-	1	-
Gravid:	4	-	5	-	3	-	4	-	5	-	2	-
Dams with resorptions only:	1	-	0	-	0	-	0	-	0	-	0	-
Dams with viable fetuses:	3	-	5	-	3	-	4	-	5	-	2	-
Viable fetuses/dam:	7.8	-	11.2	-	9.3	-	10.8	-	9.6	-	11.0	-
Postimplantation loss/dam:	2.3	-	0.6	-	2.7	-	1.0	-	1.0	-	1.0	-
Total implantations/dam:	10.0	-	11.8	-	12.0	-	11.8	-	10.6	-	12.0	-
Corpora lutea/dam:	12.3	-	14.8	-	12.3	-	13.8	-	10.8	-	14.5	-
Group mean preimplantation loss (%)[a]:	-	10.8[c]	-	20.3	-	2.7	-	14.5	-	1.9	-	17.2
Group mean postimplantation loss (%)[b]:	-	6.1	-	5.1	-	22.2	-	8.5	-	9.4	-	8.3
Mean fetal body weight (grams):	1.36	-	1.27	-	1.28	-	1.17	-	1.09	-	0.94	-

418-031

[a] Total No. Corpora Lutea − Total No. Implantations / Total No. Corpora Lutea × 100

[b] Total No. Implantations − Total No. Viable Fetuses / Total No. Implantations × 100

[c] Does not include data from dams with regressing corpora lutea

[d] Unable to determine if pregnant

- - Not applicable

No. - Number

James L. Schardein, M.S.
Study Director

3/29/88
Date

Revised Page 29

International Research
and Development Corporation
MATTAWAN, MICHIGAN, U.S.A. 49071 TELEPHONE (616) 668-3336

SPONSOR: American Petroleum Institute

TEST ARTICLE: C_9 Aromatic Hydrocarbons

SUBJECT: Range-Finding Inhalation Toxicity Study in Mice with C_9 Aromatic Hydrocarbons

DATE OF SUBMISSION: February 1, 1988

418-031

"credence through research"

TABLE OF CONTENTS

		Page
I.	Quality Assurance Statement	1
II.	Synopsis	2
III.	Introduction	3
	A. Objective	3
	B. Test Article Identification	3
IV	Methods and Procedures	4
	A. Experimental Design	4
	1. Animal Receipt and Maintenance	4
	2. Rationale for Selection of Species and Strain	5
	3. Mating	5
	4. Organization of Test Groups	6
	B. Test Article Administration	6
	1. Animal Exposure	6
	2. Generation of Exposure Atmospheres	7
	3. Analysis of Exposure Atmospheres	8
	a. Nominal	8
	b. Actual	8
	c. Gas Chromatographic Analysis of Chamber Atmospheres	12
	C. Test Material Analysis	15
	D. Maternal Observations	16
	1. Survival, Appearance and Behavior	16
	2. Body Weights	16
	3. Food Consumption	16
	4. Uterine Examination Observations	16
	5. Statistical Analysis	17
	E. Data Retention	17
V.	Results	18
	A. Maternal Observations	18
	1. Survival, Appearance and Behavior	18
	2. Body Weights	18
	3. Food Consumption	18
	4. Uterine Examination Observations	19

TABLE OF CONTENTS
(Continued)

	Page
B. Exposure Concentrations	20
1. Exposure Chamber Environment	20
2. Exposure Concentrations	20
VI. Signatures	22
VII. Discussion and Conclusion	23
FIGURE NO.	
1. Schematic Diagram of Generation and Exposure System	24
TABLE NO.	
1. Summary of Maternal Antemortem and Necropsy Observations	25
2. Summary of Group Mean Maternal Body Weights and Body Weight Changes	26
3. Summary of Maternal Food Consumption Values	27-28
4. Summary of Maternal and Fetal Observations at Cesarean Section	29
5. Summary of the Incidence of External Fetal Malformations	30
APPENDIX A	
Individual Experimental Data	31-43
APPENDIX B	
Chamber Environment	44-47
APPENDIX C	
Gas Bag Checks of Analytical System	48-49
APPENDIX D	
Gas Chromatographic Analysis of Chamber Atmospheres	50-51
APPENDIX E	
Daily Nominal and Actual Exposure Concentrations	52-54
APPENDIX F	
Historical Control Data	55-57

I. QUALITY ASSURANCE STATEMENT

Study Title: Range-Finding Inhalation Toxicity Study in Mice with C_9 Aromatic Hydrocarbons

Test Article: C_9 Aromatic Hydrocarbons

This report has been reviewed by the International Research and Development Corporation Quality Assurance Department in accordance with the United States Environmental Protection Agency Good Laboratory Practice Standards of May 2, 1984.

Approved By: Margery J. Wirth 2/1/88
Margery J. Wirth, B.S. Date
Director of Quality Assurance

418-031

II. SYNOPSIS

Thirty Charles River CD®-1 mice, consecutively assigned to one control and five treatment groups of five animals each, were used in this range-finding study to determine concentration levels of C_9 Aromatic Hydrocarbons for an inhalation developmental toxicity study. Concentration levels of 100, 250, 500, 1,000 and 1,500 ppm were administered by whole-body inhalation exposure daily for a six-hour period on days 6 through 15 of gestation. The control group received filtered air only. Uterine examinations were performed on all surviving females on gestation day 18. Fetuses were individually examined for external malformations and variations.

Test article induced maternal toxicity was present at the 1,500 ppm concentration level as exemplified by clinical observations of labored breathing and lacrimation, and by reductions in mean absolute and relative food consumption. Two animals at this level appeared moribund and were sacrificed *in extremis* on gestation day 6 following the initial exposure.

A less severe, toxic effect was seen at the 1,000 ppm concentration level as demonstrated by clinical observations of lacrimation.

Dose responsive reductions in mean fetal body weights, indicating possible fetotoxic effects, were noted at the 500, 1,000 and 1,500 ppm concentration levels.

In conclusion, when exposed to C_9 Aromatic Hydrocarbons by whole-body inhalation, Charles River CD®-1 mice expressed maternal toxicity at 1,500 and 1,000 ppm. Probable fetotoxicity was evident at concentrations of 500 ppm and greater. Based on the results of this study, concentration levels of 100, 500 and 1,500 ppm were selected for the developmental toxicity study with C_9 Aromatic Hydrocarbons in mice.

III. INTRODUCTION

A. OBJECTIVE

The objective of this study was to establish concentration levels of the test article for a developmental toxicity study in mice.

B. TEST ARTICLE IDENTIFICATION

The test article was received in two shipments from Experimental Pathology Laboratories, Inc., Herndon, Virginia as indicated below:

Receipt Date	IRDC Test Article Identification Number	Label	Description
July 1, 1986	8953 Drum number 6	C-9 AROMATIC NAPHTHA ASTM D-3734 TYPE 1 UN-1255 FLASH 117°F EPL - 368 Additional label on container FLAMMABLE LIQUID Drum number 6 labeled as above.	clear liquid [a](6 55-gallon drums received)
February 17, 1987	8953B Drum number 1	C-9 AROMATIC NAPHTHA ASTM D-3734 TYPE 1 Additional information on barrel UN-1255 FLASH 117°F	clear liquid [a](6 55-gallon drums received)

[a]Only portions of drum number 6 from the first shipment and drum number 1 from the second shipment were used on this study.

IV. METHODS AND PROCEDURES

This study was conducted in accordance with the protocol as approved by the Sponsor and in compliance with the Standard Operating Procedures of International Research and Development Corporation (IRDC). Procedures pertinent to this study are described herein.

A. EXPERIMENTAL DESIGN

1. Animal Receipt and Maintenance

Forty eight untreated, sexually mature, 70-day old, virgin female Charles River CD®-1 mice were received from The Charles River Breeding Laboratories, Inc., Portage, Michigan on April 13, 1987. Upon receipt, the animals were assigned temporary animal numbers and housed individually in suspended stainless steel wire-mesh cages. During the 14-day acclimation period, the animals were carefully observed for changes in appearance and behavior. From acquisition until sacrifice, each animal was provided with basal laboratory diet of Purina® Certified Rodent Chow® #5002 and tap water available ad libitum, except during the exposure period when diet was not available.

The basal laboratory diet was analyzed by the manufacturer for the presence of pesticides, heavy metals and aflatoxins; each diet-lot used was recorded. The drinking water at IRDC is analyzed quarterly for the presence of pesticides, heavy metals and coliforms. The results of these analyses are stored in the IRDC Archives in Mattawan, Michigan and are available upon request. The Study Director was not aware of any potential contaminants of either diet or drinking water which might have interfered with the results of this study.

When not housed in the exposure chambers, all animals were housed in an environmentally controlled room. Temperature ranged between 73°F and 78°F, with a mean temperature ± standard deviation of 76 ± 1.0°F; humidity ranged between 41% and 50%, with a mean humidity ± standard

deviation of 45 ± 5.5%. Fluorescent lighting provided illumination 12 hours per day. Mice were housed individually in suspended stainless steel wire-mesh cages from receipt until sacrifice except during mating. Nesting material was not provided since the females were sacrificed prior to delivery. Each female was identified by cage, group and individually by toe clip. The individual animal toe clip number plus the IRDC study number comprised a unique identification number for each animal. The females were 84 days old at the time of mating and weighed between 23 and 29 g on gestation day 0.

Mating was initiated on April 27, 1987 and the last uterine examination was performed on May 19, 1987.

2. Rationale for Selection of Species and Strain

The mouse is an acceptable model for inhalation developmental toxicity studies according to the OTS Health Effects Test Guidelines for Inhalation. This laboratory has historical control data on the incidence of fetal malformations and variations in this strain from this source. This strain is susceptible to known teratogenic agents.

3. Mating

At the end of the acclimation period, all animals were weighed and subjected to a detailed physical examination. At this time, animals considered suitable for study were cohabitated with stock males used exclusively for this purpose.

One female and one male mouse of the same strain and source were placed together for mating. The occurrence of copulation was determined by daily inspection for a copulatory plug. The day evidence of mating was detected was designated day 0 of gestation and the female was returned to an individual cage, assigned a permanent animal number and properly identified by toe clip.

4. Organization of Test Groups

Mated females were consecutively assigned in a block design to one control and five treatment groups consisting of five mice each by the following procedure. The order in which the mated females were assigned corresponded to the day evidence of copulation was observed and the order in which the animal appeared on the breeding record. The first mated female on the breeding record was assigned to the first group, the second mated female assigned to the second group; all remaining animals were assigned in this manner until the required number of mated females had been placed into each group.

B. TEST MATERIAL ADMINISTRATION

1. Animal Exposure

The selected route of administration was whole body inhalation exposure because this was considered the most closely equivalent to the typical route of human exposure.

Animals were exposed to the test article daily for a 6-hour period. Dosing began on day 6 of gestation and continued through day 15 of gestation.

The animals were exposed in six $16m^3$ stainless steel and glass exposure chambers. Each chamber was dedicated for use with one group for the duration of exposure. The animals were housed in the exposure chambers approximately 24 hours per day. During the exposure phase of the study the mice were individually housed in stainless steel wire-mesh cages. Before each daily exposure the animals were removed from the chamber, excreta pans were removed from the cage racks, the food jars were capped and excreta was removed from the chamber. The animals were then placed in the exposure chamber for the duration of the exposure. After the completion of the daily exposure, the animals were again removed from the chamber, the chambers were cleaned, excreta pans were

returned, and caps on the food jars were removed. The animals were then returned to the chamber. Chamber ventilation air was provided from an HVAC system separate from the general laboratory air-handling system. Chamber airflow rate, temperature and relative humidity were recorded at approximately half-hour intervals during each day's exposure.

2. Generation of Exposure Atmospheres

The exposure atmospheres were generated with the system shown schematically in Figure 1. Nitrogen, heated to 200°C by passage through a 1L stainless steel cylinder fitted with a 1500 watt band heater was introduced at the bottom of a glass column 7.6 cm in diameter and 30 cm long which was packed with glass beads. The liquid test material was delivered by a fluid metering pump (FMI) from a stainless steel safety can, through Teflon® tubing, to the bottom quarter of the column. The test material was vaporized as it flowed up the column co-current with the nitrogen flow. The vapors were passed to the chamber inlet where they were diluted with chamber ventilation air to the desired exposure concentration. The operating parameters for the generation system are shown in the following table:

Chamber Number	C-4	C-9	C-11	C-10	C-12	C-3
Desired Conc. (ppm)	0	100	250	500	1,000	1,500
FMI Pump Type:	-	RPG-20-1/4	RPG-20-1/4	RPG-50-3/8	RPG-50-3/8	SYX-72-3/8
FMI Pump Setting:	-	3.0	8.5	2.5	5.0	5.5
Approximate Liquid Flow Rate (ml/min):	-	2.1	5.1	9.0	17.8	27.4
Ammeter Setting for heater (amps):	-	10	10	10	10	10
Nitrogen Flow Rate (L/min):	150	150	150	150	150	150
Chamber Airflow Rate (L/min):	3200-4000	3200-4000	3200-4000	3200-4000	3200-4000	3200-4000
Ammeter Setting for heat tape (amps):	N/A	N/A	N/A	N/A	N/A	2.

3. Analysis of Exposure Atmospheres

a. Nominal

A nominal exposure concentration was determined for each exposure by weighing the test material reservoir before and after each day's exposure and dividing the difference in weight by the total volume of air. The total air volume was calculated by multiplying the arithmetic mean of the half-hourly measurements of chamber flowrate by the exposure duration. The nominal concentration was converted from g/L to ppm by use of the following equation:

$$ppm = \frac{g}{L} \times \frac{R}{M} \times \frac{T}{P} \times 10^6$$

$$= g/L \times 2.06 \times 10^5$$

Where: g = weight of test material used during the exposure, in grams

L = volume of air that passed through the chamber during the exposure, in liters

R = universal gas constant, $\frac{62.36L - mm\ Hg}{mole - {}^\circ K}$

M = gram molecular weight of the test material, 120 g/mole

T = nominal laboratory temperature, 294 °K

P = nominal laboratory barometric pressure, 740 mm Hg

10^6 = ppm conversion factor

b. Actual

The actual exposure concentrations were monitored with a gas-phase infrared spectrophotometer (IR). Each chamber, including the control, was sampled by drawing chamber atmosphere through a Teflon® sample line, through an automatic sampling system, into the IR. The automatic sampling system allowed, by means of solenoid valves, each

chamber to be sampled sequentially for approximately seven (7) minutes of every hour. Both the automatic sampling system and the recording of actual concentrations were controlled by a Hewlett-Packard Model 3388A laboratory computer. The operating conditions for the IR were as follows:

Instrument:	Miran 1A
Wavelength:	3.44 mcm
Range:	1 Absorbance Unit Full Scale
Slit Width:	1 mm
Closed-Loop Volume:	5.64L
Pathlength Setting:	1.54
Gain Setting:	X10
Cell Pressure:	-1 psig
Meter Response Time:	10 seconds
Zero Gas:	Chamber Supply Air

The IR was calibrated by use of the closed loop technique as recommended by the manufacturer. The closed-loop calibration consisted of injections of various volumes of the test material into the closed loop. The concentration of test material in the closed loop was calculated as follows:

$$\text{conc. (ppm)} = \frac{V}{L} \times \frac{R \times T}{M} \times \frac{D}{P} \times 10^{-3} \times 10^{6}$$

$$= V \times 34.176$$

V = liquid volume of test material, in mcl

L = volume of closed loop, 5.64L

R = universal gas constant, $\frac{62.36L - mmHg}{mole - °K}$

M = gram molecular weight of the test material, 120 g/mole

T = nominal laboratory temperature, 294 °K

D = density of test material, 0.868 g/ml

P = IR cell pressure, 1 psig below laboratory barometric pressure or 688 mmHg

10^{-3} = mL to mcl conversion factor

10^{6} = ppm conversion factor

The volumes of test material injected into the closed loop and the corresponding vapor concentrations are shown in the following table:

Volume of Test Material Injected (mcl)	Cumulative Injection Volume (mcl)	Vapor Concentration in closed loop (ppm)
1.5	1.5	51
1.5	3	103
5	8	273
5	13	444
10	23	786
10	33	1130
10	43	1470
10	53	1810

Each calibration point was replicated three (3) times, and a standard curve of mean instrument response versus closed-loop vapor concentration was generated. Before each day's exposure the standard curve was verified by the injection of liquid test material into the closed loop at the concentrations shown in the following table:

Volume of Test Material Injected (mcl)	Cumulative Injection Volume (mcl)	Vapor Concentration in Closed Loop (ppm)
3	3	103
12	15	513
30	45	1540

If the measured concentrations for all three (3) points were within ±10% of the actual closed-loop concentrations, the IR was considered to be in calibration.

In addition the accuracy of the analytical system was confirmed by the use of vapor standards prepared by vaporizing liquid test material into Tedlar® gas bags and then analyzing the bag atmosphere. The gas bag was sampled from the end of the sample line within the appropriate chamber. The concentration of the test material vapors in the gas bags were calculated as follows:

$$ppm = \frac{V}{L} \times \frac{R \times T}{M} \times \frac{D}{P} 10^{-3} \times 10^{-6}$$

$$= \frac{V}{L} \times 179.2$$

Where: V = liquid volume of test material, in mcl
L = volume of air added to gas bag, in liters
R = universal gas constant, $\frac{62.36\ L\text{-}mmHg}{mole - {}^{\circ}K}$
M = gram molecular weight, 120 g/mole
T = nominal laboratory temperature, 294 °K
P = nominal laboratory barometric pressure, 740 mmHg
10^{-3} = conversion factor, ml to mcl
10^{-6} = ppm conversion factor

The gas bags were prepared as shown below:

Desired Exposure Concentration (ppm)	Bag Volume (L)	Volume of Liquid Test Material (mcl)	Calculated Bag Conc. (ppm)	Acceptable Analyzed Range (ppm)
100	100	56	100	90-110
250	100	140	251	226-276
500	100	280	502	450-550
1000	100	560	1000	900-1100
1500	100	840	1510	1350-1660

If the analyzed concentration of the gas bag was not within ±10% of the calculated concentration, a second bag was prepared and analyzed. If the second bag was also not within ± 10% of the calculated concentration, the Study Director was notified. The results of the gas bag checks are presented in Appendix C.

c. Gas Chromatographic Analysis of Chamber Atmospheres

Once each day for the first five (5) exposure days and then once during the second week of exposure, the composition (on a weight percentage basis) of the test material within each exposure chamber was determined by use of gas chromatography (GC).

The compositional analysis was performed to demonstrate complete vaporization of the test material, not to define exposure concentrations.

The chamber samples for GC analysis were collected on two-stage (400 mg stage 1, 200 mg stage 2) charcoal tubes (SKC, Inc.) under the following sampling regimens.

Desired Exposure Conc. (ppm)	Sample Flow Rate (cc/min)	Sample Duration (min)	Total Volume of Sample (L)
0	200	300	60
100	200	300	60
250	200	150	30
500	200	75	15
1000	200	37	7.4
1500	200	25	5

After the samples were collected, the charcoal from each stage was placed in separate 5 cc vials. Internal Standard Solution, prepared by adding 300 mcl of styrene to a 200 ml volumetric flask and diluting to the mark with carbon disulfide, was added to each vial (4 ml for stage 1, 1 ml for stage 2). Each vial was then capped and the charcoal was allowed to desorb on a shaker for at least 150 min. Approximately 1 mcl aliquots were drawn from the vials and injected into a calibrated GC (see below for details of calibration). The ratio of the peak area for the component to peak area of internal standard was determined for the following compounds/components: o-xylene, cumene, n-propylbenzene (PB), 4-ethyltoluene (4-ET), 3-ethyltoluene (3-ET), 2-ethyltoluene (2-ET),

1,3,5-trimethylbenzene (1,3,5-TMB), 1,2,4-trimethylbenzene (1,2,4-TMB) and 1,2,3-trimethylbenzene (1,2,3-TMB). In addition, unidentified components with retention times greater than 1,2,3-TMB were defined as $\geq$C10's. The weight percent for each compound was calculated by dividing the amount of each compound by the sum of the amounts of the individually quantitated compound and multiplying by 100 minus the weight percent of the $\geq$C10's. The calculations are shown below:

$$RFy = \frac{[CM]}{[ISS]} \times \frac{AIS}{Ay} \times \frac{wt.\%yCM}{1}$$

$$Amt.\ y = \frac{Ay \times RFy \times [ISS] \times EV}{AIS}$$

$$wt.\ \% \geq C10 = \frac{\Sigma AC10}{\Sigma Ay + \Sigma AC10} \times 100$$

$$wt.\ \%\ y = \frac{Amt.\ y}{\Sigma\ Amt.\ y} \times (100 - wt.\ \% \geq C10)$$

Where:
- RFy = response factor for compound y
- [CM] = concentration of calibration mixture in working standard in mg/ml
- [ISS] = concentration of internal standard, 1.36 mg/ml
- AIS = area of internal standard from chromatogram
- Ay = area from chromatogram for an individual compound (o-xylene, cumene, PB, 1,3,5-TMB, 1,2,3-TMB, 1,2,4-TMB, 2-ET, 3-ET, 4-ET)
- wt.%yCM = weight percent of individual compound y in calibration mixture
- Amt. y = amount of individual compound y, in mg
- EV = elution volume, 4 ml for stage 1 samples and 1 ml for stage 2 samples
- wt. % $\geq$ C10 = weight percent of compounds with retention times > 1,2,3-TMB
- AC10 = area from chromatogram for compounds with retention times > 1,2,3-TMB
- wt. % y = weight percent of individual compound y

Both stages of the charcoal tubes were evaluated. At no time was there any evidence of breakthrough into the second stage.

The GC was calibrated with a mixture of known composition prepared to be similar to the test material.

The GC operating parameters for the charcoal tube analysis and for analysis of the neat liquid (as described above under Test Material) are shown in the following table:

Instrument:	Varian 2400 Gas Chromatograph
Column:	6' x 2mm ID glass
Packing:	5% SP-1200/1.75% Bentone 34 on 100/120 mesh supelcoport
Detector:	Flame ionization
Temperatures	
Injector:	160°C
Column:	75°C
Detector:	200°C
Gas Flow Rates	
N_2:	30 cc/min at 60 psig
H_2:	40 cc/min at 40 psig
Air:	300 cc/min at 60 psig
Electrometer Attenuation	
Setting:	$16x10^{-11}A$
Injection Volume:	1mcl
Integrator:	HP3388A
Chart Speed:	0.5 cm/min
Chart Display	
0-15 min	26
15-40 min	22
Threshold Setting	
0-15 min	6
15-40 min	1

The results of the GC analysis of the vapor composition of the exposure atmospheres are presented in Appendix D.

C. TEST MATERIAL ANALYSIS

The composition of the test material in each drum used during the animal exposures was analyzed using methods described below. As each drum was opened, (not more than two (2) weeks before use) three 1 ml aliquots were accurately weighed into separate 50 ml volumetric flasks, diluted to the mark with internal standard solution, and then diluted again 5:10. Each solution was injected into the GC and the weight percent of each compound, including the C10's was calculated as described above. The weight percents for the three aliquots were averaged and the results were reported to the Study Director before the drum was used on study. The results for all drums used on study were in agreement with the test material composition defined by the protocol, which is shown in the following table.

Compound	Weight Percent
o-xylene	3.20
cumene	2.74
n-propylbenzene	3.97
4-ethyltoluene	7.05
3-ethyltoluene	15.1
2-ethyltoluene	5.44
1,3,5 - trimethylbenzene	8.37
1,2,4 - trimethylbenzene	40.5
1,2,3 - trimethylbenzene	6.18
≥C10's	6.19
	98.74

D. MATERNAL OBSERVATIONS

1. Survival, Appearance and Behavior

Throughout the study the females were observed twice daily for mortality and overt changes in appearance and behavior. The presence and duration of clinical signs of toxicity were recorded once daily on days 6 to 15 of gestation and prior to uterine examination.

Any mouse showing signs of severe debility or toxicity, particularly if death appeared imminent, was sacrificed to prevent loss of tissues through autolysis. All mice sacrificed in extremis were subjected to a routine necropsy procedure. Normally developing implantations from these dams were discarded. Any female showing signs of abortion or premature delivery was sacrificed and necropsied on the day such evidence was observed and the aborted tissue was examined and discarded.

2. Body Weights

Individual maternal body weights were recorded daily on gestation days 0, 6, 9, 12, 16 and 18.

3. Food Consumption

Individual maternal food consumption was recorded for all dams on gestation days 0, 6, 9, 12, 16 and 18. Mean food consumption (gram/animal/day) and relative food consumption (g/kg of body weight/day) were calculated for the gestation intervals 0-6, 6-9, 9-12, 12-16, 16-18, 6-18 and 0-18.

4. Uterine Examination Observations

On gestation day 18, all surviving females were sacrificed by i.p. injection of sodium pentobarbital and exsanguination via the abdominal aorta. Immediately following sacrifice, the uterus and ovaries were exposed by an abdominal incision and the number and location of viable and nonviable fetuses, early and late resorptions and the total number of implantations and corpora lutea were recorded. The uterus was then

excised, weighed and the fetuses removed. The abdominal and thoracic cavities and organs of the females were examined for grossly evident morphological changes and the carcasses discarded. Uteri from females that appeared nongravid were opened and placed in 10% ammonium sulfide solution for detection of implantations[a]. All fetuses were individually weighed, examined for external malformations and variations and discarded.

5. Statistical Analysis

Statistical analysis was not required due to the limited number of animals used.

E. DATA RETENTION

All raw data, a sample of the test article and copies of the final report are retained in the Archives of International Research and Development Corporation, 500 N. Main, Mattawan, Michigan, 49071.

[a]Kopf, R., Lorenz, D., and Salewski, E. (1964). The effects of thalidomide on the fertility of rats studied in two generations. Naunyn Schmiedebergs Arch. Pharmacol., 247:121-135.

V. RESULTS

A. MATERNAL OBSERVATIONS

1. Survival, Appearance and Behavior (Table 1)

Two dams from the 1,500 ppm concentration level were sacrificed *in extremis* on gestation day 6. This mortality may have resulted from the shock of initial test article exposure. Survival was 100% in the 100, 250, 500 and 1,000 ppm concentration levels as well as in the control group.

Observations noted in both mice that were sacrificed *in extremis* included labored breathing, hindlimbs rotated laterally and moribund appearance. Additional observations noted in one or the other mouse included tremors, ataxia, hunched posture, prostration and closed eyes. No gross lesions were evident at the necropsy of these animals.

An increase in lacrimation was noted in mice at the 1,000 and 1,500 ppm concentration levels. Although a dose-related pattern was lacking, the observations were considered a treatment effect. Incidental clinical observations noted in one or more study groups included hair loss, scabbing, staining and material around the nose.

The only necropsy finding, cystic ovarian bursa, was noted in one animal each in the control and 250 ppm concentration groups.

2. Body Weights (Table 2)

Evaluation of maternal body weight data showed no meaningful differences in mean body weights or body weight gains in any treated group when compared to the control group.

3. Food Consumption (Table 3)

Slight to moderate reductions in maternal food consumption (g/kg/day and g/animal/day) were noted at the high-concentration level during the first and third intervals of treatment (gestation days 6 to 9 and 12 to 16). Food consumption during the resulting overall treatment and gestation intervals was also reduced at this level. No meaningful effects on food consumption were seen at the 100, 250, 500 and 1,000 ppm concentrations.

4. Uterine Examination Observations

The only indication of a possible treatment effect was a slight reduction in mean fetal body weights at the 500, 1,000 and 1,500 ppm concentration levels. The values were notably lower than the concurrent control value but were within the range of the historical control.

The mean numbers of corpora lutea and/or total implantations were increased in several treated groups when compared to the control group. However, these parameters occurred prior to test article administration and were thus due to random occurrence.

One dam at the 250 ppm concentration level delivered on the day of scheduled uterine examination.

No meaningful differences in mean postimplantation loss were evident at any treated level when compared to the control group.

No indications of abnormal fetal development attributable to the test article were evident. One fetus at the 100 ppm level had hindlimb flexure and one fetus at the 250 ppm level had encephalocele.

0 0 3 4

B. EXPOSURE CONCENTRATIONS

1. Exposure Chamber Environment

The chamber environment data (airflow, temperature and relative humidity) are summarized as the mean and standard deviation of the daily means, in the following table:

Chamber Number	Air Flow (L/min)		Temperature (°C)		Relative Humidity (%RH)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
C-4	3390	110	24	1.0	56	8.6
C-9	3690	130	23	0.9	43	5.7
C-11	3640	90	24	1.0	42	5.4
C-10	3220	60	23	1.0	46	3.6
C-12	3300	70	23	1.2	46	5.9
C-3	3630	140	23	1.6	51	7.6

The daily means (± S.D.) for the chamber environment data are presented in Appendix B.

2. Exposure Concentrations

Nominal and actual exposure concentrations for each exposure day can be found in Appendix E, and are presented in summary (mean ± S.D. of the daily nominal and the daily mean actual concentration) below:

Chamber Number	Desired Conc.	Nominal		Actual	
		Mean	S.D.	Mean	S.D.
C-4	0	Control Chamber			
C-9	100	104	2.8	104	1.8
C-11	250	246	7.7	252	4.3
C-10	500	454	30.6	492	14.2
C-12	1,000	950	43.7	1000	27.9
C-3	1,500	1360	56.6	1520	36.1

S.D. - Standard deviation

Due to maintenance on the air handling systems, only 5 hours of exposure were achieved on the first exposure day.

The fact that the nominal exposure concentrations were lower than the actual measured levels, particularly at the highest level, was quite unusual. After the completion of the study, it was determined that the calibration of the analytical instrument was slightly in error. Based on the gas bag data collected a few days prior to study initiation, this error was on the order of 10%, or possibly slightly less. Due to the inherent limitations of the methods used, the absolute accuracy of measurement for both nominal and actual concentrations were no better than ±5%. Therefore, differences between nominal and actual concentrations on the order of 10%, cannot be meaningfully differentiated, and thus, the actual concentrations were considered the best estimate of animal exposure levels.

VI. SIGNATURES

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Date

418-031

VII. DISCUSSION AND CONCLUSION

Test article induced maternal toxicity was present at the 1,500 ppm concentration level as exemplified by clinical observations of labored breathing and lacrimation, and by reductions in mean absolute and relative food consumption. Two animals at this level appeared moribund and were sacrificed *in extremis* on gestation day 6 following the initial exposure.

A similar, though less severe, toxic effect was seen at the 1,000 ppm concentration level as demonstrated by clinical observations of lacrimation.

A slight reduction in mean fetal body weights, indicating probable fetotoxicity, was noted at the 500, 1,000 and 1,500 ppm concentration levels.

In conclusion, when exposed to C_9 Aromatic Hydrocarbons by whole-body inhalation, Charles River CD®-1 mice expressed maternal toxicity at 1,500 and 1,000 ppm. Probable fetotoxicity was also evident at the 500 ppm concentration level and greater. Based on the results of this study, concentration levels of 100, 500 and 1,500 ppm were selected for the developmental toxicity study with C_9 Aromatic Hydrocarbons in mice.

To the best of my knowledge, there were no significant deviations from the Good Laboratory Practice Regulations which affected the quality and integrity of the study. This study was conducted in conformance with the Good Laboratory Practice Regulations. This report accurately reflects the raw data obtained during the performance of the study.

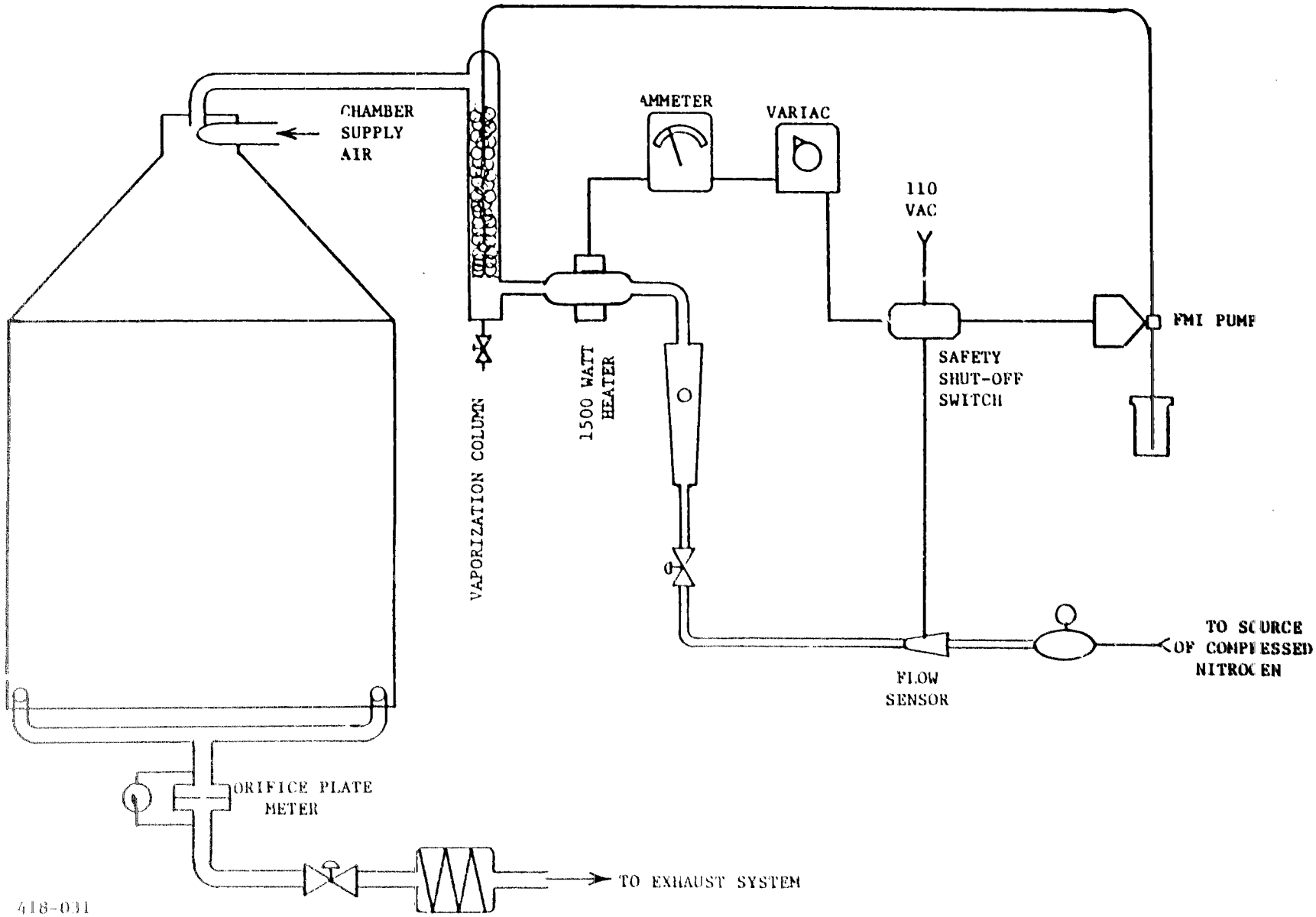
James L. Schardein, M.S.
Scientific Director, Reproduction and
Teratology Division
Study Director

2/1/88
Date

418-031

0 0 3 8

FIGURE NO. 1 SCHEMATIC DIAGRAM OF GENERATION AND EXPOSURE SYSTEM



418-031

TABLE 1. Summary of Maternal Antemortem and Necropsy Observations

Observation	C_9 Aromatic Hydrocarbons (Desired Concentrations) (ppm)											
	0 (Control)		100		250		500		1,000		1,500	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Number of animals observed:	5	-	5	-	5	-	5	-	5	-	5	-
No visible abnormalities[a]	5	100	5	100	4	80	3	60	2	40	1	20
Sacrificed in extremis											2	40
Delivered					1	10						
Antemortem Observations:												
Hair loss							2	40	2	40		
Scabbing									1	20		
Staining									1	20		
Material, nose									1	20		
Lacrimation									2	40	2	40
Both eyes closed									1	20	1	20
Labored breathing											2	40
Moved in circles											1	20
Hunched posture											1	20
Hindlimbs rotate laterally											2	40
Ataxia											1	20
Moribund											2	40
Necropsy Observations												
No gross lesions	4	80	5	100	4	80	5	100	5	100	5	100
Cystic ovarian bursa	1	20			1	20						

[a]Includes animals with no visible abnormalities during the entire observation period

No. - Number

% - Percent based on number of animals at beginning of observation period

- - Not applicable

418-031

TABLE 2.

Summary of Group Mean Maternal Body Weights and Body Weight Changes

Day of Gestation	Group Mean Maternal Body Weights (grams)					
	C_9 Aromatic Hydrocarbons (Desired Concentrations) (ppm)					
	0 (Control)	100	250	500	1,000	1,500
	Mean	Mean	Mean	Mean	Mean	Mean
0	26	25	26	26	25	27
6	29	26	28	29	28	28
9	31	30	29	31	30	31
12	31	34	34	35	34	34
16	41	44	42	43	40	41
18	45	49	47	46	45	46
18 (Adjusted)[b]	31.8	31.5	31.5	29.8	30.6	30.9
Days of Gestation	Group Mean Maternal Body Weight Change (grams)[a]					
0 to 6	3	1	3	3	3	1
6 to 9	2	3	1	2	2	3
9 to 12	0.5	4	5	4	4	3
12 to 16	10	10	8	8	6	7
16 to 18	4	6	5	4	4	5
0 to 18	19	24	21	20	19	18
0 to 13 (Adjusted)[b]	5.6	6.5	5.5	3.8	5.4	2.9

[a] Values represent the mean of the individual changes in maternal body weight for these intervals
[b] Gestation day 18 body weight minus weight of gravid uterus and contents

TABLE 3. Summary of Maternal Food Consumption Values

	C_9 Aromatic Hydrocarbons (Desired Concentrations) (ppm)														
	0 (Control)					100					250				
Days of Gestation	g/ animal/ day	S.D.	g/ kg/ day	S.D.	N	g/ animal/ day	S.D.	g/ kg/ day	S.D.	N	g/ animal/ day	S.D.	g/ kg/ day	S.D.	N
0- 6	4.5	0.77	154.7	20.56	4	4.2	0.55	160.2	15.12	5	4.5	0.49	160.8	12.61	4
6- 9	4.7	0.27	153.5	15.25	4	5.4	0.17	176.7	10.27	4	5.0	0.27	171.6	17.06	4
9-12	4.6	1.26	146.1	27.56	4	6.1	1.07	179.8	35.69	4	5.8	0.19	171.1	16.08	4
12-16	6.6	0.43	165.0	24.12	4	6.5	0.50	149.6	12.58	5	6.2	0.24	148.0	10.36	4
16-18	6.6	1.75	146.5	8.64	4	6.6	0.82	134.3	15.81	5	6.1	0.75	148.5	27.47	4
6-18	5.6	0.61	128.3	19.75	4	6.1	0.39	125.2	10.06	5	5.8	0.20	142.4	34.89	4
0-18	5.2	0.22	121.0	27.77	4	5.5	0.43	111.4	11.32	5	5.4	0.27	131.8	33.01	4

S.D. - Standard deviation
N - Number of animals

418-031

TABLE 3. Cont.

Summary of Maternal Food Consumption Values

	C_9 Aromatic Hydrocarbons (Desired Concentrations) (ppm)														
	500					1,000					1,500				
Days of Gestation	g/ animal/ day	S.D.	g/ kg/ day	S.D.	N	g/ animal/ day	S.D.	g/ kg/ day	S.D.	N	g/ animal/ day	S.D.	g/ kg/ day	S.D.	N
0- 6	4.4	0.82	150.3	22.31	4	4.5	0.49	163.2	16.68	5	4.5	0.21	160.7	4.70	4
6- 9	4.4	1.45	142.2	46.69	4	4.7	0.86	155.8	17.55	5	3.7	0.00	120.3	2.79	2
9-12	5.3	0.47	152.2	7.90	4	5.4	0.55	159.7	9.11	5	5.3	0.47	159.1	10.71	2
12-16	6.1	0.72	142.7	8.34	4	5.9	0.52	145.4	5.32	5	5.4	0.18	132.7	2.05	2
16-18	5.6	2.78	114.9	46.52	4	5.8	0.76	130.3	15.88	5	6.0	1.41	131.6	29.04	2
6-18	5.4	1.11	116.4	1.92	4	5.5	0.49	122.2	6.59	5	5.0	0.41	110.7	7.34	2
0-18	5.1	0.96	109.4	4.47	4	5.1	0.45	115.5	8.28	5	4.6	0.39	107.4	5.24	2

S.D. - Standard deviation
N - Number of animals

418-031

TABLE 4. Summary of Maternal and Fetal Observations at Cesarean Section

	C_9 Aromatic Hydrocarbons (Desired Concentrations) (ppm)											
	0 (Control)		100		250		500		1,000		1,500	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Animals on study:	5	-	5	-	5	-	5	-	5	-	5	-
Animals that were gravid:	4	-	5	-	4	-	4	-	5	-	4	-
Animals that were sacrificed:	0	-	0	-	0	-	0	-	0	-	2	-
Nongravid:	-	-	-	-	-	-	-	-	-	-	0	-
Gravid:	-	-	-	-	-	-	-	-	-	-	0	-
Animals that delivered:	0	-	0	-	1	-	0	-	0	-	0	-
Animals examined at Cesarean section:	5	-	5	-	4	-	5	-	5	-	3	-
Nongravid:	1	-	0	-	1	-	1	-	0	-	1	-
Gravid:	4	-	5	-	3	-	4	-	5	-	2	-
Dams with resorptions only:	1	-	0	-	0	-	0	-	0	-	0	-
Dams with viable fetuses:	3	-	5	-	3	-	4	-	5	-	2	-
Viable fetuses/dam:	7.8	-	11.2	-	9.3	-	10.8	-	9.6	-	11.0	-
Postimplantation loss/dam:	2.3	-	0.6	-	2.7	-	1.0	-	1.0	-	1.0	-
Total implantations/dam:	10.0	-	11.8	-	12.0	-	11.8	-	10.6	-	12.0	-
Corpora lutea/dam:	12.3	-	14.8	-	12.3	-	13.8	-	10.8	-	14.5	-
Group mean preimplantation loss (%)[a]:	-	10.8[c]	-	20.3	-	2.7	-	14.5	-	1.9	-	17.2
Group mean postimplantation loss (%)[b]:	-	6.1	-	5.1	-	22.2	-	8.5	-	9.4	-	8.3
Mean fetal body weight (grams):	1.36	-	1.27	-	1.28	-	1.17	-	1.09	-	0.94	-

418-031

[a] $\frac{\text{Total No. Corpora Lutea} - \text{Total No. Implantations}}{\text{Total No. Corpora Lutea}} \times 100$

[b] $\frac{\text{Total No. Implantations} - \text{Total No. Viable Fetuses}}{\text{Total No. Implantations}} \times 100$

[c] Does not include data from dams with regressing corpora lutea

- - Not applicable

No. - Number

TABLE 5. Summary of the Incidence of External Fetal Malformations

	C_9 Aromatic Hydrocarbons (Desired Concentrations) (ppm)					
	0 (Control)	100	250	500	1,000	1,500
No. of litters examined:	3	5	4	4	5	2
No. of fetuses examined externally:	31	56	39	43	49	22
External malformations observed						
Encephalocele			1 (1)			
Hindlimb flexture		1 (1)				
Total fetuses (litters) with malformations:	0 (0)	1 (1)	1 (1)	0 (0)	0 (0)	0 (0)

APPENDIX A

Individual Experimental Data

Individual Maternal Antemortem and Necropsy Observations

Test Article, Desired Concentrations Dam Number	Gestation Day	Antemortem Observations	Day of Sacrifice	Necropsy Observations
0 ppm (Control):				
23686	6-18	No visible abnormalities	18	No gross lesions Uterus: nongravid[a]
23687	6-18	No visible abnormalities	18	No gross lesions
23688	6-18	No visible abnormalities	18	No gross lesions
23689	6-18	No visible abnormalities	18	Cystic ovarian bursa: bilateral, slight
23690	6-18	No visible abnormalities	18	No gross lesions
C_9 Aromatic Hydrocarbons 100 ppm:				
23691	6-18	No visible abnormalities	18	No gross lesions
23692	6-18	No visible abnormalities	18	No gross lesions
23693	6-18	No visible abnormalities	18	No gross lesions
23694	6-18	No visible abnormalities	18	No gross lesions
23695	6-18	No visible abnormalities	18	No gross lesions
C_9 Aromatic Hydrocarbons 250 ppm:				
23696	6-18	No visible abnormalities	18	No gross lesions
23697	6-18	No visible abnormalities	18	No gross lesions
23698	6-18	No visible abnormalities	18	No gross lesions Uterus: nongravid[a]
23699	6-18	No visible abnormalities	18	No gross lesions
23700	6-18 18	No visible abnormalities Delivered	18	Cystic ovarian bursa: bilateral, slight
C_9 Aromatic Hydrocarbons 500 ppm:				
23701	6-18	No visible abnormalities	18	No gross lesions Uterus: nongravid[a]
23702	6-18	No visible abnormalities	18	No gross lesions
23703	6-12 13-18	No visible abnormalities Hair loss, focal	18	No gross lesions
23704	6-12 13-18	No visible abnormalities Hair loss, focal	18	No gross lesions
23705	6-18	No visible abnormalities	18	No gross lesions

[a]Confirmed by ammonium sulfide test

Individual Maternal Antemortem and Necropsy Observations

Test Article, Desired Concentration Dam Number	Gestation Day	Antemortem Observations	Day of Sacrifice	Necropsy Observations
C_9 Aromatic Hydrocarbons 1,000 ppm:				
23706	6 7-18 7-9 10-16	No visible abnormalities Hair loss, right forelimb Scabbing, right forelimb Material, nose, dark red	18	No gross lesions
23707	6-7 8-18 9-15 12-15	No visible abnormalities Hair loss, top of head Staining, head, yellow Lacrimation, right eye	18	No gross lesions
23708	6-18	No visible abnormalities	18	No gross lesions
23709	6-7 8,10-18 9	Lacrimation, right eye No visible abnormalities Both eyes partially closed	18	No gross lesions
23710	6-18	No visible abnormalities	18	No gross lesions
C_9 Aromatic Hydrocarbons 1,500 ppm:				
23711	6,16-18 7-15	No visible abnormalities Lacrimation, right eye	18	No gross lesions
23712	6-18	No visible abnormalities	18	No gross lesions Uterus: nongravid[a]
23713	6-10,18 11-17	No visible abnormalities Lacrimation, both eyes	18	No gross lesions
23714	6	Prostration Both eyes closed Labored breathing Both hindlimbs rotated laterally Moribund	6	No gross lesions
23715	6	Tremors Hunched posture Moved in circles to right Ataxia Hindlimbs rotated laterally Labored breathing Moribund	6	No gross lesions

[a]Confirmed by ammonium sulfide test

Individual Maternal Body Weights (grams)

Test Article, Desired Concentration, Dam Number	Day of Gestation 0	6	9	12	16	18	Uterus Weight (g)	Adjusted[d] Body Weight (g)
0 ppm (Control):								
23686[a]	24	19	28	25	26	28	-	-
23687	24	27	31	33	42	44	12.67	31.33
23688	29	31	32	28	48	54	20.25	33.75
23689	25	27	29	34	44	51	19.25	31.75
23690	27	30	30	29	30	31	0.56	30.44
C_9 Aromatic Hydrocarbons 100 ppm:								
23691	24	25	30	34	46	51	19.48	31.52
23692	24	27	28	33	42	48	16.00	32.00
23693	26	30	32	36	46	51	19.30	31.70
23694	23	25	26	31	38	44	15.01	28.99
23695	28	25	33	36	46	52	18.97	33.03
C_9 Aromatic Hydrocarbons 250 ppm:								
23696	24	25	28	31	37	41	12.87	28.13
23697	28	31	31	36	47	52	17.43	34.57
23698[a]	24	25	27	27	26	26	-	-
23699	26	28	28	35	42	48	16.34	31.66
23700	24	28	30	35	42	29[b]	c	-
C_9 Aromatic Hydrocarbons 500 ppm:								
23701[a]	24	26	28	27	29	28	-	-
23702	25	28	30	33	36	33	12.25	20.75
23703	24	28	30	35	46	52	20.36	31.64
23704	27	31	32	35	42	48	13.52	34.48
23705	28	29	32	37	46	52	19.78	32.22

[a]Nongravid, not included in calculation of mean
[b]Delivered weight, not included in calculation of mean
[c]Delivered, uterus weight not recorded
- - Not applicable
[d]Dam weight on gestation day 18 minus gravid uterus weight

Individual Maternal Body Weights (grams)

Test Article, Desired Concentration, Dam Number	Day of Gestation 0	6	9	12	16	18	Uterus Weight (g)	Adjusted[d] Body Weight (g)
C_9 Aromatic Hydrocarbons 1,000 ppm:								
23706	24	27	30	31	36	39	8.94	30.06
23707	24	26	27	32	41	46	16.37	29.63
23708	25	29	32	36	42	47	14.53	32.47
23709	26	27	29	33	40	44	14.59	29.41
23710	27	30	33	37	42	47	15.35	31.65
C_9 Aromatic Hydrocarbons 1,500 ppm:								
23711	28	28	30	33	40	45	14.13	30.87
23712[a]	26	28	27	26	27	25	-	-
23713	27	28	31	34	41	46	e	-
23714	25	26	Sacrificed					
23715	26	29	Sacrificed					

[a]Nongravid, not included in calculation of mean
[d]Dam weight on gestation day 18 minus gravid uterus weight
[e]Inadvertently not weighed
- - Not applicable

Individual Maternal Food Consumption (grams)

Test Article, Desired Concentration, Dam Number	Gestation Days 0-6	6-9	9-12	12-16	16-18
0 ppm (Control):					
23686[a]	16	19	16	20	8
23687	22	14	17	27	12
23688	27	13	11	28	17
23689	25	15	17	27	15
23690	33	14	10	24	9
C_9 Aromatic Hydrocarbons 100 ppm:					
23691	22	16	-	25	11
23692	29	16	23	27	14
23693	28	16	17	25	15
23694	26	-	16	24	12
23695	22	17	17	29	14
C_9 Aromatic Hydrocarbons 250 ppm:					
23696	23	15	18	24	11
23697	28	14	17	26	14
23698[a]	12	18	13	16	8
23699	30	16	18	25	13
23700	27	15	17	24	11
C_9 Aromatic Hydrocarbons 500 ppm:					
23701[a]	24	13	15	18	9
23702	20	7	15	20	3
23703	26	17	16	26	14
23704	32	14	15	26	13
23705	27	15	18	25	15

[a]Nongravid, not included in calculation of mean
- - Not available due to technical error

418-031

Individual Maternal Food Consumption (grams)

Test Article, Desired Concentration, Dam Number	Gestation Days 0-6	6-9	9-12	12-16	16-18
C_9 Aromatic Hydrocarbons 1,000 ppm:					
23706	26	13	14	20	10
23707	23	11	15	23	13
23708	28	15	18	25	10
23709	31	14	17	24	13
23710	28	18	17	25	12
C_9 Aromatic Hydrocarbons 1,500 ppm:					
23711	27	11	15	21	10
23712[a]	23	7	10	18	9
23713	28	11	17	22	14
23714	25	Sacrificed			
23715	27	Sacrificed			

[a]Nongravid, not included in calculation of mean

Individual Maternal and Fetal Observations at Cesarean Section

Test Article, Desired Concentration, Dam Number	Fetuses		Resorptions		Post-Implantation Loss	Total Implantations	Corpora Lutea	Mean Fetal Body Weight
	Viable	Nonviable	Late	Early				
	No.	No.	No.	No.	No.	No.	No.	Grams
0 ppm (Control):								
23686	Nongravid							
23687	7	0	0	2	2	9	11	1.42
23688	12	0	0	0	0	12	12	1.35
23689	12	0	0	0	0	12	14	1.31
23690	0	0	0	7	7	7	regressing	
Total	31	0	0	9	9	40	37	-
C_9 Aromatic Hydrocarbons 100 ppm:								
23691	13	0	0	1	1	14	19	1.19
23692	10	0	0	1	1	11	12	1.27
23693	12	0	0	0	0	12	13	1.32
23694	9	0	0	0	0	9	13	1.31
23695	12	0	0	1	1	13	17	1.27
Total	56	0	0	3	3	59	74	-
C_9 Aromatic Hydrocarbons 250 ppm:								
23696	8	0	0	4	4	12	12	1.25
23697	11	0	0	3	3	14	15	1.25
23698	Nongravid							
23699	9	0	0	1	1	10	10	1.33
23700	Delivered							
Total	28	0	0	8	8	36	37	-

418-051

- - Not applicable
No. - Number

Individual Maternal and Fetal Observations at Cesarean Section

Test Article, Desired Concentration, Dam Number	Fetuses		Resorptions		Post-implantation Loss	Total Implantations	Corpora Lutea	Mean Fetal Body Weight
	Viable	Nonviable	Late	Early				
	No.	No.	No.	No.	No.	No.	No.	Grams
C_9 Aromatic Hydrocarbons 500 ppm:								
23701	Nongravid							
23702	10	0	0	0	0	10	11	0.92
23703	13	0	0	0	0	13	17	1.18
23704	8	0	0	4	4	12	14	1.28
23705	12	0	0	0	0	12	13	1.30
Total	43	0	0	4	4	47	55	-
C_9 Aromatic Hydrocarbons 1,000 ppm:								
23706	5	1	0	2	3	8	8	1.21[a]
23707	12	0	0	0	0	12	12	1.03
23708	9	0	1	1	2	11	11	1.14
23709	10	0	0	0	0	10	11	1.18
23710	12	0	0	0	0	12	12	0.90
Total	48	1	1	3	5	53	54	-
C_9 Aromatic Hydrocarbons 1,500 ppm:								
23711	12	0	0	0	0	12	12	0.81
23712	Nongravid							
23713	10	0	0	2	2	12	17	1.07
23714	Sacrificed gestation day 6, unable to determine if pregnant							
23715	Sacrificed gestation day 6, unable to determine if pregnant							
Total	22	0	0	2	2	24	29	-

[a]Mean does not include weight of nonviable pup

- - Not applicable
No. - Number

Individual External Fetal Malformations and Variations

Test Article, Desired Concentration, Dam Number	Number of Fetuses Examined	Number of Fetuses With Malformations	Fetus Number	Description of Malformation	Number of Fetuses With Variations	Fetus Number	Description of Variation
0 ppm (Control):							
23687	7	0	-	No malformations observed	0	-	No variations observed
23688	12	0	-	No malformations observed	0	-	No variations observed
23689	12	0	-	No malformations observed	0	-	No variations observed
Total	31	0	-	-	0	-	-
C_9 Aromatic Hydrocarbons 100 ppm:							
23691	13	0	-	No malformations observed	0	-	No variations observed
23692	10	1	10	Hindlimb flexture	0	-	No variations observed
23693	12	0	-	No malformations observed	0	-	No variations observed
23694	9	0	-	No malformations observed	0	-	No variations observed
23695	12	0	-	No malformations observed	0	-	No variations observed
Total	56	1	-	-	0	-	-
C_9 Aromatic Hydrocarbons 250 ppm:							
23696	8	0	-	No malformations observed	0	-	No variations observed
23697	11	0	-	No malformations observed	0	-	No variations observed
23699	9	0	-	No malformations observed	0	-	No variations observed
23700	11	1	6[a]	Encephalocele	0	-	No variations observed
Total	39	1	-	-	0	-	-
C_9 Aromatic Hydrocarbons 500 ppm:							
23702	10	0	-	No malformations observed	0	-	No variations observed
23703	15	0	-	No malformations observed	0	-	No variations observed
23704	8	0	-	No malformations observed	0	-	No variations observed
23705	12	0	-	No malformations observed	0	-	No variations observed
Total	45	0	-	-	0	-	-

- = Not applicable
[a]Delivered

Individual External Fetal Malformations and Variations

Test Article, Desired Concentration, Dam Number	Number of Fetuses Examined	Number of Fetuses With Malformations	Fetus Number	Description of Malformation	Number of Fetuses With Variations	Fetus Number	Description of Variation
C_9 Aromatic Hydrocarbons 1,000 ppm:							
23706	6[b]	0	-	No malformations observed	0	-	No variations observed
23707	12	0	-	No malformations observed	0	-	No variations observed
23708	9	0	-	No malformations observed	0	-	No variations observed
23709	10	0	-	No malformations observed	0	-	No variations observed
23710	12	0	-	No malformations observed	0	-	No variations observed
Total	49	0	-	-	0	-	-
C_9 Aromatic Hydrocarbons 1,500 ppm:							
23711	12	0	-	No malformations observed	0	-	No variations observed
23713	10	0	-	No malformations observed	0	-	No variations observed
Total	22	0	-	-	0	-	-

- - Not applicable
[b] Includes one nonviable fetus

Individual Fetal Body Weights (grams)

Test Article, Desired Concentration, Dam Number	Fetus Number 1	2	3	4	5	6	7	8	9	10	11	12	13	14
0 ppm (Control):														
23686	Nongravid													
23687	1.48	1.42	1.46	1.47	1.31	1.38	ER	1.44	ER					
23688	1.38	1.26	1.42	1.38	1.37	1.34	1.35	1.39	1.36	1.35	1.33	1.31		
23689	1.34	1.30	1.18	1.25	1.24	1.39	1.27	1.28	1.33	1.44	1.35	1.31		
23690	ER	ER	ER	ER	ER	ER	ER							
C_9 Aromatic Hydrocarbons 100 ppm:														
23691	1.11	1.02	1.22	1.24	1.20	1.25	1.21	ER	1.18	1.14	1.20	1.17	1.26	1.33
23692	1.33	1.27	ER	1.34	1.21	1.03	1.38	1.27	1.39	1.19	1.24			
23693	1.32	1.33	1.29	1.35	1.45	1.45	1.23	1.26	1.30	1.17	1.34	1.32		
23694	1.40	1.34	1.35	1.28	1.27	1.37	1.26	1.24	1.32					
23695	1.28	ER	1.41	1.13	1.16	1.20	1.29	1.16	1.15	1.34	1.42	1.31	1.39	
C_9 Aromatic Hydrocarbons 250 ppm:														
23696	1.22	1.25	ER	1.21	ER	1.31	1.20	ER	1.29	1.25	1.27	ER		
23697	1.27	1.27	1.23	ER	ER	1.24	1.29	1.26	1.22	ER	1.23	1.27	1.21	1.23
23698	Nongravid													
23699	ER	1.33	1.24	1.30	1.33	1.38	1.32	1.42	1.29	1.38				
23700	Delivered													
C_9 Aromatic Hydrocarbons 500 ppm:														
23701	Nongravid													
23702	0.87	0.85	0.94	0.95	0.98	0.74	0.97	0.94	0.98	0.99				
23703	1.29	1.24	1.13	1.10	1.16	1.10	1.09	1.09	1.16	1.26	1.23	1.24	1.26	
23704	1.29	1.22	ER	1.27	ER	ER	1.28	ER	1.26	1.38	1.22	1.32		
23705	1.37	1.23	1.32	1.36	1.26	1.05	1.27	1.31	1.34	1.33	1.41	1.40		

ER - Early resorption

Individual Fetal Body Weights (grams)

Test Article, Desired Concentration, Dam Number	Fetus Number 1	2	3	4	5	6	7	8	9	10	11	12
C_9 Aromatic Hydrocarbons 1,000 ppm:												
23706	ER	1.05	1.20	1.28	ER	0.79[a]	1.29	1.24				
23707	1.06	1.14	1.03	0.92	0.95	0.96	1.04	1.03	1.10	1.03	0.97	1.07
23708	1.18	ER	1.12	1.17	1.14	1.24	1.08	1.09	LR	1.12	1.14	
23709	1.12	1.17	1.19	1.20	1.19	1.13	1.14	1.27	1.13	1.28		
23710	0.92	0.93	0.90	0.84	0.93	0.86	0.95	0.96	0.88	0.97	0.93	0.77
C_9 Aromatic Hydrocarbons 1,500 ppm:												
23711	0.80	0.75	0.79	0.82	0.87	0.84	0.85	0.84	0.78	0.68	0.81	0.86
23712	Nongravid											
23713	1.03	ER	1.08	0.91	0.97	1.02	1.09	ER	1.10	1.13	1.12	1.27
23714	Sacrificed											
23715	Sacrificed											

[a]Nonviable fetus
ER - Early resorption
LR - Late resorption

APPENDIX B
Chamber Environment

Daily Mean Chamber Environment Data

Chamber Number	Date of Exposure	Temp. (°C) Mean	Temp. (°C) S.D.	Relative Humidity (%) Mean	Relative Humidity (%) S.D.	Chamber Air Flow (LPM) Mean	Chamber Air Flow (LPM) S.D.
C-4	MON MAY 4 1987	23	0.8	37	1.2	3150	665
	TUE MAY 5 1987	23	0.4	63	0.8	3380	19
	WED MAY 6 1987	24	0.5	62	1.4	3330	17
	THU MAY 7 1987	25	0.5	59	3.6	3560	123
	FRI MAY 8 1987	24	0.5	53	4.1	3520	30
	SAT MAY 9 1987	25	2.3	59	8.4	3390	40
	SUN MAY 10 1987	23	1.2	67	4.2	3250	13
C-9	MON MAY 4 1987	23	0.9	43	5.6	3370	764
	TUE MAY 5 1987	23	0.4	40	3.8	3720	76
	WED MAY 6 1987	23	0.5	39	2.7	3740	24
	THU MAY 7 1987	24	0.7	41	5.5	3690	53
	FRI MAY 8 1987	23	0.4	38	2.0	3640	21
	SAT MAY 9 1987	24	2.0	39	3.1	3670	35
	SUN MAY 10 1987	26	2.0	43	0.8	3630	23
C-10	MON MAY 4 1987	23	0.7	38	0.6	3030	672
	TUE MAY 5 1987	23	1.0	47	0.3	3250	14
	WED MAY 6 1987	24	0.7	47	0.8	3200	18
	THU MAY 7 1987	24	0.5	47	0.5	3200	54
	FRI MAY 8 1987	23	0.3	46	1.7	3200	17
	SAT MAY 9 1987	24	2.2	48	3.7	3180	21
	SUN MAY 10 1987	26	1.7	49	2.1	3250	15
C-3	MON MAY 4 1987	22	0.7	56	1.6	3590	668
	TUE MAY 5 1987	23	0.5	51	6.6	3810	51
	WED MAY 6 1987	23	0.4	53	6.0	3430	32
	THU MAY 7 1987	24	0.5	53	5.9	3410	41
	FRI MAY 8 1987	23	0.6	41	8.6	3590	127
	SAT MAY 9 1987	24	1.9	42	5.8	3870	165
	SUN MAY 10 1987	26	1.9	49	2.1	3630	131
C-11	MON MAY 4 1987	23	1.5	29	0.9	3550	818
	TUE MAY 5 1987	23	1.0	45	0.3	3760	14
	WED MAY 6 1987	24	0.8	45	1.1	3720	22
	THU MAY 7 1987	24	1.1	44	0.7	3700	63
	FRI MAY 8 1987	24	0.5	40	2.5	3640	18
	SAT MAY 9 1987	25	2.4	44	5.8	3570	25
	SUN MAY 10 1987	26	2.1	43	2.9	3520	13

S.D. - Standard deviation

Daily Mean Chamber Environment Data

Chamber Number	Date of Exposure	Temp. (°C) Mean	Temp. (°C) S.D.	Relative Humidity (%) Mean	Relative Humidity (%) S.D.	Chamber Air Flow (LPM) Mean	Chamber Air Flow (LPM) S.D.
C-12	MON MAY 4 1987	23	1.2	26	1.5	3120	709
	TUE MAY 5 1987	23	0.7	44	0.6	3380	30
	WED MAY 6 1987	23	0.8	42	1.3	3330	19
	THU MAY 7 1987	23	0.8	44	1.4	3290	49
	FRI MAY 8 1987	22	0.5	39	2.5	3250	18
	SAT MAY 9 1987	24	1.9	42	5.1	3290	67
	SUN MAY 10 1987	26	1.7	41	3.5	3240	23
C-4	MON MAY 11 1987	21	0.0	50	2.8	3410	15
	TUE MAY 12 1987	24	0.3	55	2.7	3520	56
	WED MAY 13 1987	24	0.3	59	0.6	3440	17
	THU MAY 14 1987	24	0.3	67	2.1	3390	19
	FRI MAY 15 1987	24	0.3	45	9.4	3410	12
	SAT MAY 16 1987	24	0.5	58	9.7	3300	17
C-9	MON MAY 11 1987	24	0.5	38	0.4	3630	14
	TUE MAY 12 1987	23	0.6	47	2.2	3680	75
	WED MAY 13 1987	23	0.7	50	0.9	3660	16
	THU MAY 14 1987	23	0.4	56	1.6	3760	122
	FRI MAY 15 1987	23	0.7	39	6.9	3860	81
	SAT MAY 16 1987	23	0.8	50	7.5	3920	17
C-10	MON MAY 11 1987	24	0.3	41	0.8	3250	29
	TUE MAY 12 1987	23	0.7	45	1.1	3260	77
	WED MAY 13 1987	23	0.7	48	0.7	3230	13
	THU MAY 14 1987	23	0.6	51	1.4	3240	48
	FRI MAY 15 1987	22	0.9	42	2.9	3280	26
	SAT MAY 16 1987	22	0.3	47	3.8	3270	38
C-3	MON MAY 11 1987	24	0.3	42	2.1	3650	53
	TUE MAY 12 1987	23	0.7	51	2.3	3560	73
	WED MAY 13 1987	23	0.7	55	1.2	3560	49
	THU MAY 14 1987	23	0.3	61	1.7	3580	30
	FRI MAY 15 1987	22	0.6	42	0.8	3670	123
	SAT MAY 16 1987	19	0.0	65	10.2	3790	32

S.D. - Standard deviation

418-031

Daily Mean Chamber Environment Data

Chamber Number	Date of Exposure	Temp. (°C) Mean	Temp. (°C) S.D.	Relative Humidity (%) Mean	Relative Humidity (%) S.D.	Chamber Air Flow (LPM) Mean	Chamber Air Flow (LPM) S.D.
C-11	MON MAY 11 1987	25	0.5	36	1.2	3550	36
	TUE MAY 12 1987	23	0.9	41	1.7	3660	62
	WED MAY 13 1987	23	1.0	44	0.6	3610	32
	THU MAY 14 1987	24	0.9	49	1.3	3610	96
	FRI MAY 15 1987	23	0.9	36	5.7	3750	55
	SAT MAY 16 1987	23	0.7	44	5.9	3730	19
C-12	MON MAY 11 1987	24	0.5	33	1.5	3300	53
	TUE MAY 12 1987	22	1.0	40	2.3	3330	87
	WED MAY 13 1987	22	0.9	43	0.5	3330	10
	THU MAY 14 1987	22	0.6	48	1.2	3300	11
	FRI MAY 15 1987	22	0.7	33	6.6	3360	33
	SAT MAY 16 1987	22	0.7	43	6.3	3380	56

S.D. - Standard deviation

Appendix C

Gas Bag Checks of Analytical System

Gas Bag Checks of Analytical System

Date	Calculated Concentration (ppm)	Instrument Response (MV)	Measured Concentration (ppm)	% of Calculated Concentration
4-29-87	100	77.7	98	98
4-29-87	251	151.7	244	97
4-29-87	502	381.9	529	105
4-29-87	1000	592.8	1080	108
4-29-87	1510	988.3	1630	108

APPENDIX D

Gas Chromatographic Analysis of Chamber Atmospheres

Gas Chromatographic Analysis of Chamber Atmospheres (wt. %)

Chamber	Compound	Date Sample Collected					
		5-4-87	5-5-87	5-6-87	5-7-87	5-8-87	5-11-87
C-9	o-xylene	3.17	3.16	3.13	3.17	3.17	3.15
(100 ppm desired)	cumene	2.83	2.81	2.80	2.82	2.83	2.81
	NPB	4.08	4.09	4.07	4.08	4.08	4.07
	4ET	7.31	7.27	7.30	7.27	7.31	7.29
	3ET	15.6	15.5	15.5	15.5	15.6	15.5
	2ET	5.40	5.39	5.40	5.40	5.40	5.39
	1,3,5 TMB	8.31	8.30	8.31	8.30	8.33	8.28
	1,2,4 TMB	40.5	40.7	40.7	40.7	40.6	40.6
	1,2,3 TMB	5.93	5.93	5.91	5.94	5.91	5.97
	>C10's	6.86	6.89	6.89	6.84	6.78	6.93
C-11	o-xylene	3.20	3.16	3.16	3.16	3.15	3.15
(250 ppm desired)	cumene	2.83	2.83	2.82	2.84	2.81	2.82
	NPB	4.05	4.07	4.07	4.07	4.09	4.06
	4ET	7.28	7.30	7.31	7.31	7.30	7.27
	3ET	15.4	15.5	15.5	15.5	15.5	15.5
	2ET	5.43	5.41	5.42	5.41	5.40	5.41
	1,3,5 TMB	8.33	8.31	8.32	8.31	8.32	8.29
	1,2,4 TMB	40.2	40.6	40.5	40.4	40.7	40.5
	1,2,3 TMB	6.06	5.97	5.96	5.95	5.94	5.99
	>C10's	7.17	6.84	6.87	7.09	6.80	7.06
C-10	o-xylene	2.15	3.18	3.18	3.17	3.17	3.18
(500 ppm desired)	cumene	1.72	2.82	2.83	2.83	2.82	2.83
	NPB	3.25	4.08	4.07	4.08	4.08	4.08
	4ET	6.27	7.31	7.29	7.31	7.29	7.34
	3ET	12.8	15.5	15.5	15.5	15.5	15.5
	2ET	5.31	5.40	5.40	5.41	5.41	5.40
	1,3,5 TMB	7.88	8.31	8.29	8.31	8.30	8.27
	1,2,4 TMB	44.3	40.5	40.5	40.6	40.6	40.5
	1,2,3 TMB	7.28	5.96	5.99	5.95	5.96	5.97
	>C10's	9.04	6.91	7.04	6.78	6.83	7.01
C-12							
(1,000 ppm desired)	o-xylene	3.14	3.15	3.15	3.15	3.14	3.17
	cumene	2.82	2.83	2.80	2.82	2.81	2.85
	NPB	4.07	4.09	4.08	4.09	4.08	4.10
	4ET	7.32	7.34	7.33	7.32	7.30	7.31
	3ET	15.6	15.6	15.6	15.5	15.5	15.6
	2ET	5.39	5.40	5.41	5.39	5.41	5.41
	1,3,5 TMB	8.30	8.28	8.33	8.28	8.32	8.32
	1,2,4 TMB	40.9	40.8	40.8	40.6	40.5	40.5
	1,2,3 TMB	5.92	5.94	5.95	5.94	5.96	5.92
	>C10's	6.55	6.53	6.56	6.84	6.94	6.80
C-3							
(1,500 ppm desired)	o-xylene	3.17	3.18	3.19	3.19	3.18	3.17
	cumene	2.82	2.83	2.83	2.83	2.83	2.85
	NPB	4.08	4.09	4.09	4.09	4.08	4.08
	4ET	7.30	7.32	7.32	7.32	7.31	7.30
	3ET	15.5	15.6	15.6	15.5	15.5	15.6
	2ET	5.40	5.41	5.41	5.42	5.41	5.42
	1,3,5 TMB	8.32	8.32	8.33	8.33	8.32	8.28
	1,2,4 TMB	40.6	40.6	40.7	40.6	40.6	40.5
	1,2,3 TMB	5.95	5.92	5.93	5.97	5.95	5.95
	>C10's	6.83	6.73	6.65	6.79	6.81	6.90

418-031

APPENDIX E

Daily Nominal and Actual Exposure Concentrations

Daily Actual Exposure Concentrations in ppm (Mean ± Standard Deviation

Date	C-9		C-11		C-10		C-12		C-3	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
5-04-87	104	0.5	248	0.5	492	7.2	1010	0.0	1500	44.2
5-05-87	103	5.1	247	0.4	497	2.8	997	11.6	1520	13.4
5-06-87	103	1.9	250	1.1	499	3.1	1010	4.5	1550	7.7
5-07-87	102	1.2	250	1.8	477	6.4	917	245.6	1560	14.8
5-08-87	105	1.8	253	1.2	469	2.4	1030	6.3	1580	97.3
5-09-87	102	2.6	256	1.2	491	8.5	1020	21.6	1570	130.5
5-10-87	104	1.9	257	1.3	462	2.1	1020	4.5	1470	64.3
5-11-87	107	2.0	259	1.6	509	7.5	1010	17.9	1490	10.0
5-12-87	107	1.1	254	1.5	501	2.1	1020	14.8	1480	6.3
5-13-87	105	3.0	258	2.2	507	1.4	1000	0.4	1520	14.8
5-14-87	102	5.6	254	6.0	499	12.7	1010	7.7	1500	14.8
5-15-87	102	4.6	247	2.8	498	4.5	997	11.3	1520	54.8
5-16-87	104	1.1	248	0.6	496	4.3	996	19.0	-	-

S.D. - Standard deviation
- - Not applicable

Daily Nominal Exposure Concentrations in ppm

Date	C-9	C-11	C-10	C-12	C-3
5-04-87	103	236	456	953	1280
5-05-87	102	241	469	949	1290
5-06-87	103	243	470	957	1440
5-07-87	102	244	453	819	1430
5-08-87	104	248	446	982	1360
5-09-87	103	252	359	972	1270
5-10-87	106	264	437	1000	1380
5-11-87	106	251	470	970	1350
5-12-87	107	247	465	958	1400
5-13-87	106	252	477	959	1390
5-14-87	100	249	466	966	1400
5-15-87	101	242	466	950	1370
5-16-87	110	235	466	921	-

- - Not applicable

APPENDIX F

Historical Control Data

Individual studies have been subjected to Quality Assurance audit; the following historical compilation of data has not.

File 51
IRDC Historical Control Data - Charles River CD®-1 Mice

Summary of Maternal and Fetal Observations at Cesarean Section

No. of animals in the historical control:	194
No. of animals that died:	4
No. of animals that aborted/delivered:	0
No. of animals examined at Cesarean section:	190
No. nongravid:	18
No. gravid:	172
No. of dams with resorptions only:	6
No. of dams with live fetuses:	166
No. of live fetuses/dam:	10.7[7.6-11.5]
No. of late resorptions/dam:	0.3[0.1-0.4]
No. of early resorptions/dam:	0.9[0.2-3.0]
No. of postimplantation losses/dam:	1.2[0.6-3.2]
No. of implantations/dam:	11.9[10.7-12.6]
No. of corpora lutea/dam:	12.9[11.4-14.0]
Fetal sex ratio: Male:Female	925:917
Range - %Male	39.7-52.7
%Female	47.3-60.3
Mean fetal body weight (g):	0.87[0.73-0.94]

[ ] - Range of mean
The historical control data compiled April 7, 1984 includes data from studies terminating between September, 1978 to October, 1983.

418-031

Summary of the Incidence of Malformations and of Developmental Variations

No. of litters examined:	166
Total no. of fetuses examined externally:	1842
Total no. of fetuses examined skeletally:	1128
Total no. of fetuses examined viscerally:	714

Malformations Observed	No. Fetuses (Litters)	[Range of Fetuses % (Litters %)]
Exencephaly:	2 (2)	[0.0(0.0)-1.5(12.5)]
Malpositioned pinnae:	1 (1)	[0.0(0.0)-1.5(12.5)]
Nares absent:	1 (1)	[0.0(0.0)-0.4(4.3)]
Anotia:	1 (1)	[0.0(0.0)-1.5(12.5)]
Aglossia:	1 (1)	[0.0(0.0)-1.5(12.5)]
No palate:	1 (1)	[0.0(0.0)-1.5(12.5)]
Cleft palate:	7 (6)	[0.0(0.0)-1.0(8.3)]
Gastroschisis:	1 (1)	[0.0(0.0)-0.5(5.6)]
Omphalocele:	1 (1)	[0.0(0.0)-0.5(5.3)]
Genital tubercle enlarged:	1 (1)	[0.0(0.0)-0.5(5.3)]
Hydrocephaly:	2 (1)	[0.0(0.0)-2.8(5.6)]
Fused esophagus and trachea:	1 (1)	[0.0(0.0)-4.5(12.5)]
Malformed kidney:	1 (1)	[0.0(0.0)-1.3(5.3)]
Cryptorchidism:	1 (1)	[0.0(0.0)-1.3(5.3)]
Vertebral anomalies with or without associated rib anomalies:	13 (10)	[0.0(0.0)-3.6(16.7)]
Extra cervical arch:	1 (1)	[0.0(0.0)-1.0(7.1)]
Forked cervical arch(es):	10 (6)	[0.0(0.0)-7.1(31.6)]
Extra site of ossification in vertebrae	2 (2)	[0.0(0.0)-1.0(7.1)]
Fused sternebrae:	1 (1)	[0.0(0.0)-0.7(5.3)]
Bent ilium:	1 (1)	[0.0(0.0)-0.5(5.3)]

Developmental Variations Observed		
27 presacral vertebrae:	1 (1)	[0.0(0.0)-2.2(12.5)]
25 presacral vertebrae:	23 (17)	[0.0(0.0)-5.7(26.1)]
Greater than normal (13 pairs) ribs:	724 (98)	[13.1(42.9)-41.3(87.5)]
Fewer than normal (13 pairs) ribs:	1 (1)	[0.0(0.0)-0.7(5.3)]
7th cervical rib(s):	181 (71)	[2.7(14.3)-31.2(75.7)]
6th cervical rib(s):	2 (2)	[0.0(0.0)-2.2(12.5)]
Accessory skull bone:	6 (2)	[0.0(0.0)-2.6(5.0)]
Skull reduced in ossification:	195 (76)	[5.1(28.6)-33.3(69.9)]
Hyoid unossified:	24 (17)	[0.7(4.2)-13.0(25.0)]
Sternebrae #5 and/or #6 unossified:	231 (89)	[9.9(29.2)-60.9(100.0)]
Other sternebrae unossified:	34 (23)	[0.0(0.0)-21.7(87.5)]
Entire sternum unossified:	9 (5)	[0.0(0.0)-13.0(25.0)]
Sternebrae misaligned:	12 (9)	[0.0(0.0)-3.6(16.7)]
Extra sternebra:	1 (1)	[0.0(0.0)-0.7(5.6)]
Vertebrae reduced in ossification:	21 (19)	[0.0(0.0)-11.8(47.4)]
Metacarpals and/or metatarsals unossified:	6 (4)	[0.0(0.0)-8.7(25.0)]
Entire skeleton reduced in ossification:	1 (1)	[0.0(0.0)-0.6(4.3)]
Pelvic bone reduced in ossification:	1 (1)	[0.0(0.0)-0.7(4.2)]
Renal papillae not developed and/or distended ureter(s):	5 (5)	[0.0(0.0)-2.8(11.1)]
Gallbladder variation:	1 (1)	[0.0(0.0)-0.9(4.8)]

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