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A RANGE-FINDING DEVELOPMENTAL INHALATION TOXICITY STUDY OF UNLEADED GASOLINE VAPOR CONDENSATE IN RATS AND MICE VIA WHOLE-BODY EXPOSURES

AN INHALATION DEVELOPMENTAL TOXICITY STUDY OF UNLEADED GASOLINE VAPOR CONDENSATE IN THE RAT VIA WHOLE-BODY EXPOSURE

Health and Environmental Sciences Department
Publication Numbers TR 412 and TR 414
April 1998



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A Range-Finding Developmental Inhalation Toxicity Study of Unleaded Gasoline Vapor Condensate in Rats and Mice via Whole-Body Exposures

Health and Environmental Sciences Department

API PUBLICATION NUMBER TR 412

PREPARED UNDER CONTRACT BY:

HUNTINGDON LIFE SCIENCES
METTLERS ROAD
PO Box 2360
EAST MILLSTONE, NJ 08875-2360

APRIL 1998

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ACKNOWLEDGMENTS

THE FOLLOWING PEOPLE ARE RECOGNIZED FOR THEIR CONTRIBUTIONS OF TIME AND EXPERTISE DURING THIS STUDY AND IN THE PREPARATION OF THIS REPORT:

API STAFF CONTACT

Richard Rhoden, Health and Environmental Sciences Department

MEMBERS OF THE DEVELOPMENTAL TOXICITY WORKGROUP

Linda Roberts, Chevron Research and Technology Company

Quang Bui, Unocal Corporation

Wayne Daughtrey, Exxon Biomedical Sciences Inc.

Francis Koschier, ARCO

Susan Rodney, Texaco, Inc.

David Steup, Shell Oil Company

PREFACE

This abridged publication consists of the full text and pertinent data from an initial range-finding study, *A Range-Finding Developmental Inhalation Toxicity Study of Regular Unleaded Gasoline in Rats and Mice via Whole Body Exposure*. The study was conducted by Huntingdon Laboratories under contract to API to determine the appropriate rodent species and exposure levels to be used in a second study. The complete appendices are too voluminous for distribution on a routine basis. The Table of Contents indicates omitted appendices in italics. The complete report is available through the API Information Specialist, 1220 L Street, Washington D.C., 20005.



ABSTRACT

This range-finding study was conducted for the American Petroleum Institute to determine concentration levels appropriate to assess the potential maternal toxicity and developmental toxicity of Unleaded Gasoline Vapor Condensate (API 94-02) administered by inhalation (whole-body exposure) to mated rats during the Day 6-19 gestation interval and to mated mice during the Day 6-17 gestation interval. Exposure levels were 0 (filtered air), 300, 1000, 3000 and 9000 ppm. These data will be used to identify the appropriate rodent species and exposure levels to be used in a definitive inhalation developmental toxicity study. In this study, no maternal or developmental toxicity in either rats or mice was seen up to an exposure level of 3000 ppm. At the highest exposure level evaluated (9000 ppm), maternal toxicity was seen only in the rat (reduced body weights, weight gain and food consumption during the treatment period). No evidence of developmental toxicity was seen in either species at the 9000 ppm exposure level.

Italicized sections in the Table of Contents have been omitted from this publication. These sections are on file at API and may be obtained by contacting the API Information Specialist, 1220 L St., Washington, D.C., 20005.

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STUDY NO. 95-6082

API STUDY NO. 08200-0601-SH9343

A RANGE-FINDING DEVELOPMENTAL INHALATION TOXICITY STUDY
OF UNLEADED GASOLINE VAPOR CONDENSATE (API 94-02) IN RATS AND MICE
VIA WHOLE-BODY EXPOSURE

LABORATORY

Huntingdon Life Sciences
Mettlers Road
P.O. Box 2360
East Millstone, N.J. 08875-2360

SPONSOR

American Petroleum Institute
Health and Environmental Sciences Department
1220 L Street, N.W.
Washington, D.C. 20005

FINAL REPORT

date final report is produced

LABORATORY SIGNATURE PAGE

This report constitutes a true and faithful account of the procedures adopted and the results obtained in the performance of this study.

Raymond E. Schroeder
 Raymond E. Schroeder, M.S., D.A.B.T.
 Study Director

29 September 97
 Date

Carol S. Auletta
 Carol S. Auletta, B.A., D.A.B.T.
 Senior Director, Toxicology

29 Sept 97
 Date

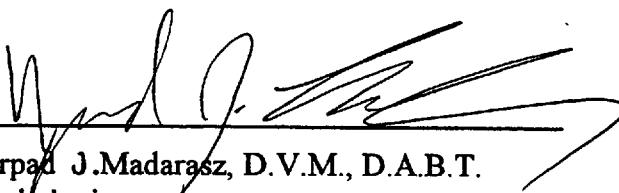
Ward R. Richter
 Ward R. Richter, D.V.M., M.S.,
 Diplomate, A.C.V.P.
 Vice President, Research and Pathology

29 Sep 97
 Date

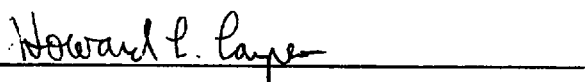
LABORATORY INDIVIDUAL SIGNATURE PAGE


 Gary M. Hoffman, B.A., D.A.B.T.
 Inhalation Toxicologist

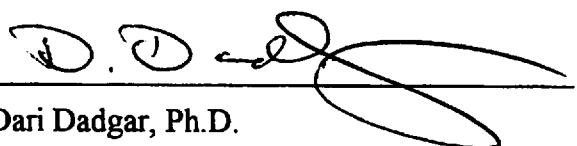
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 Date


 Arpad J. Madarasz, D.V.M., D.A.B.T.
 Pathologist

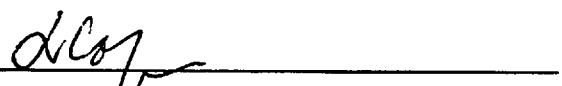
26 Sep 97
 Date


 Howard L. Carpenter, B.A.
 Scientist, Analytical Services

26 Sep 97
 Date


 Dari Dadgar, Ph.D.
 Vice President, Analytical Services

26/Sep/97
 Date


 Laura V. Cojocaru, B.S. Chem. Eng.
 Manager, Analytical Services

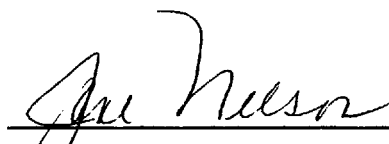
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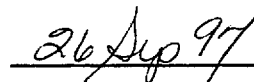
LABORATORY QUALITY ASSURANCE STATEMENT

Listed below are the dates that this study was inspected by the Quality Assurance Unit of Huntingdon Life Sciences, East Millstone, New Jersey, and the dates that findings were reported to the Study Director and Management.

Type of Inspection	Date(s) of Inspection(s)	Reported to Study Director	Reported to Management
GLP Protocol Review	8 Jun 95	09 Jun 95	19 Jun 95 and 30 Jun 95
Mating Observations	27 Jun 95	27 Jun 95	3 Jul 95 and 14 Jul 95
Exposure and Monitoring	26 Jun 95	27 Jun 95	30 Jul 95 and 23 Aug 95
Gestation Body Weights and Feeder Weights	7 Jul 95	7 Jul 95	14 Jul 95 and 23 Aug 95
Terminal Sacrifice	10 Jul 95	10 Jul 95	14 Jul 95 and 23 Aug 95
Final Analytical Report	16 Oct 95 to 19 Oct 95	19 Oct 95	20 Oct 95
Final In-Life and Pathology Report	16 Oct 95 to 20 Oct 95	20 Oct 95	20 Oct 95
Report Revisions	2 Apr 97	2 Apr 97	2 Apr 97
Report Revisions	26 Jun 97 to 1 Jul 97	7 Jul 97	7 Jul 97



Jane Nelson
Quality Assurance Senior Auditor



Date

LABORATORY COMPLIANCE STATEMENT

This study was conducted in compliance with the United States Environmental Protection Agency's Good Laboratory Practice Standards 40 CFR Part 792.

Raymond E. Schroeder
Raymond E. Schroeder, M.S., D.A.B.T.
Study Director

17 Mar 98
Date

Lorraine E. Twerdok
Lorraine E. Twerdok, Ph.D.
Sponsor Representative

3/30/98
Date

GOLDMAN ASSOCIATES INTERNATIONAL, INC.

Quality Assurance Statement

Study No. 08200-0601-SH9343

In accordance with the Good Laboratory Practice regulations published at 40 CFR 792, this study was monitored on behalf of the Sponsor, the American Petroleum Institute.

A Range Finding Developmental Inhalation Toxicity Study of Regular Unleaded Gasoline in Rats and Mice via Whole Body Exposure.

was monitored on

and reported to Sponsor on

June 6, 1995

June 6, 1995

June 30, 1995

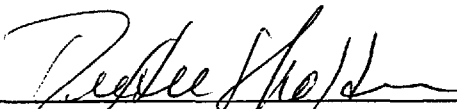
July 6, 1995

July 12, 1995

July 29, 1995

July 22, 1995

July 22, 1995


Dexter S. Goldman, Ph.D., President

P.O. Box 3374 • Gaithersburg, Maryland 20885-3374 • Voice: 301.963.0142 • Fax: 301.963.6713

200 Twelve Oaks Drive • Gaithersburg, Maryland 20878

Internet: dexterg@GLPMavenGAI.com

[http:// www.GLPMavenGAI.com](http://www.GLPMavenGAI.com)

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Section 1

SUMMARY

This inhalation study was performed to provide range-finding information on the maternal and developmental toxicity of Unleaded Gasoline Vapor Condensate (API 94-02) in rats and mice. Data from this study will be used to identify the appropriate rodent species and exposure levels to use in a definitive inhalation developmental toxicity study. Unleaded Gasoline Vapor Condensate (API 94-02) was administered as a vapor, via inhalation (whole-body) exposure, 6 hours/day to 40 mated rats (10/group) during Days 6-19 of gestation and to 40 mated mice (10/group) during Days 6-17 of gestation. Exposure levels were 300, 1000, 3000, and 9000 ppm. Ten mated rats and ten mated mice, which served as controls, were chamber-housed and received filtered room air only, 6 hours/day over the same treatment intervals.

Study animals were observed twice daily for mortality/morbidity and for obvious pharmacologic and/or toxicological effects. In addition, each animal was removed from its cage and given a detailed physical examination on Day 0 of gestation, daily both pre- and post-exposure during the treatment period and at terminal sacrifice. Body weights were recorded on Days 0, 3, 6, 9, 12, 15, 18, and 20 of gestation in the rat and Days 0, 3, 6, 9, 12, 15, and 18 of gestation in the mouse. Food consumption was recorded on Days 0-3, 3-6, 6-9, 9-12, 12-15, 15-18, and 18-20 of gestation for the rats and Days 0-3, 3-6, 6-8, 8-10, 10-12, 12-14, 14-16, and 16-18 of gestation for the mice.

Chamber exposure concentrations were evaluated daily using infrared spectrophotometry (hourly), gas chromatography (single grab sample, daily) and analysis of charcoal tubes (four samples/day). The latter were analyzed to monitor the ratio of the ten major components of the test material.

At terminal sacrifice (Day 18 of gestation for the mouse and Day 20 of gestation for the rat), animals were given a macroscopic postmortem examination. The gravid uterus with the ovaries attached was removed, weighed intact and evaluated for the number of fetuses and resorption sites. The number of *corpora lutea* on the ovaries was also recorded. Fetuses were removed from the uterus, weighed, sexed externally and evaluated for external irregularities. The fetuses were then sacrificed with an overdose of inhaled carbon dioxide and discarded.

The mean daily total hydrocarbon concentrations $\pm$ standard deviations from the infrared spectrophotometric evaluation over the entire exposure period for the 300, 1000, 3000 and 9000 ppm groups were 314 ± 31 , 1034 ± 63 , 3073 ± 146 and 9025 ± 417 ppm, respectively. Gas chromatograph analyses of syringe and charcoal tube samples of the exposure atmospheres, demonstrated a similarity among all exposure groups and a stability of the test material during each exposure day. In these data, nine major components of the test material were expressed as a ratio to isopentane, the largest component.

No mortality occurred in the control or treated animals for either species.

In the mouse, no maternal or developmental toxicity was seen at any exposure level of Unleaded Gasoline Vapor Condensate (API 94-02) evaluated.

In the rat, no maternal or developmental toxicity was seen at exposure levels up to and including 3000 ppm. At the 9000 ppm exposure level, the following statistically significant changes were seen in comparison to control data and were considered indicative of maternal toxicity: reduced body weights on Days 12, 15 and 20 of gestation; reduced weight gain over the Day 6-20 gestation interval using the actual Day 20 gestation weight; and reduced food consumption over the Day 6-9 gestation interval. No developmental toxicity was seen in the rat at the 9000 ppm exposure level.

Section 2

INTRODUCTION

The procedures and results obtained from a range-finding inhalation toxicity study of Unleaded Gasoline Vapor Condensate (API 94-02) in rats and mice via whole body exposure are presented in this report. This study was conducted at Huntingdon Life Sciences, Mettlers Road, P.O. Box 2360, East Millstone, New Jersey 08875-2360.

This inhalation study was performed to provide range-finding information on the maternal and developmental toxicity of Unleaded Gasoline Vapor Condensate (API 94-02) in rats and mice. Data from this study will be used to identify the appropriate rodent species and exposure levels to use in a definitive inhalation developmental toxicity study. Unleaded Gasoline Vapor Condensate (API 94-02) was administered as a vapor, via inhalation (whole-body) exposure, 6 hours/day to 40 mated rats (10/group) during Days 6-19 of gestation and to 40 mated mice (10/group) during Days 6-17 of gestation. Exposure levels were 300, 1000, 3000, and 9000 ppm. Ten mated rats and ten mated mice which served as controls, were chamber-housed and received filtered room air only, 6 hours/day over the same treatment intervals.

Procedures used during the study are presented in the Materials and Methods/References section of the report. Maternal and developmental toxicity data for the rat are presented in Appendices A through J and similar data for the mouse are presented in Appendices K through T. Data regarding the inhalation exposures are presented in Appendix U. Results, as well as methods used, for the chamber analyses performed by the Testing Facility's Metabolism and Analytical Department are presented in Appendix V. Historical control data for both rats and mice are presented in Appendix W. A copy of the study protocol and protocol amendments are presented in Appendix X.

Section 3

MATERIALS AND METHODS/REFERENCES

REGULATORY REFERENCES

Good Laboratory Practices

This study was conducted in compliance with Part 792 of 40 CFR (EPA Good Laboratory Practices - TSCA).

Animal Welfare Act Compliance

This study complied with all appropriate parts of the Animal Welfare Act Regulations: 9 CFR Parts 1 and 2 Final Rules, Federal Register, Volume 54, No. 168, August 31, 1989, pp. 36112-36163 effective October 30, 1989 and 9 CFR Part 3 Animal Welfare Standards; Final Rule, Federal Register, Volume 56, No. 32, February 15, 1991, pp. 6426-6505 effective March 18, 1991.

Facilities Management/Animal Husbandry

Currently acceptable practices of good animal husbandry were followed, e.g., Guide for the Care and Use of Laboratory Animals; DHHS Publication No. (NIH) 86-23, Revised 1985. Huntingdon Life Sciences, East Millstone, New Jersey is fully accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

STUDY MANAGEMENT

Sponsor

American Petroleum Institute
Health and Environmental Sciences Department
1220 L Street, N.W.
Washington, D.C. 20005

Sponsor Representative

Richard A. Rhoden, Ph.D.

Testing Facility

Huntingdon Life Sciences
 Mettlers Road
 P.O. Box 2360
 East Millstone, New Jersey 08875-2360

Study Director

Raymond E. Schroeder, M.S., D.A.B.T.

EXPERIMENTAL DESIGN

Group	Test Material	Exposure Level (ppm)	Treatment Schedule ^a		Number of Animals				Proportion of Fetuses/Litter Evaluated for External Malformations and/or Variations
			Rats (GD)	Mice (GD)	Mated		Sacrificed ^b		
					Rats	Mice	Rats (GD 20)	Mice (GD 18)	
I	Control	0	6-19	6-17	10	10	10	10	All
II	Unleaded Gasoline Vapor Condensate (API 94-02)	300	6-19	6-17	10	10	10	10	All
III	Unleaded Gasoline Vapor Condensate (API 94-02)	1000	6-19	6-17	10	10	10	10	All
IV	Unleaded Gasoline Vapor Condensate (API 94-02)	3000	6-19	6-17	10	10	10	10	All
V	Unleaded Gasoline Vapor Condensate (API 94-02)	9000 ^c	6-19	6-17	10	10	10	10	All

^aExposures were 6 hours/day.

^bComplete gross postmortem evaluations were performed on all animals.

GD = gestation day

^cThe Lower Explosive Limit (LEL) for Unleaded Gasoline Vapor Condensate (API 94-02) as reported in the American Petroleum Institute DTI Report 2509 was 1.25% (12,500 ppm). The high-exposure level of 9000 ppm represents approximately 75% of the LEL.

STUDY DATES

Study Initiation (Date Study Director signed the Protocol)

14 June 1995

Initiation of Mating (Experimental Start Date)

Rats: 19 June 1995

Mice: 21 June 1995

Initiation of Exposure

Rats: 26 June 1995

Mice: 28 June 1995

Termination of Exposure

Rats: 19 July 1995

Mice: 13 July 1995

Terminal Sacrifice

Rats: 10-20 July 1995

Mice: 10-14 July 1995

Experimental Termination (Date of Last Data Collection)

Rats: 20 July 1995

Mice: 14 July 1995

Study Termination

29 September 1997

TEST MATERIAL INFORMATION

TEST MATERIAL	LOT NO.	PURITY	DESCRIPTION	DATE RECEIVED	EXPIRATION DATE
Unleaded Gasoline Vapor Condensate (API 94-02)	API 94-02	Considered 100%	Clear liquid	6 March 1995	December 1999

Supplier

Chevron Research and Technology Company
 100 Chevron Way
 Richmond, California 94802-0627

Analysis

Documentation of the identity, strength, purity, composition; and synthesis, fabrication, and/or derivation of the test material was the responsibility of the Sponsor.

Stability

Documentation of the stability of the test material was the responsibility of the Sponsor.

Storage

Upon receipt, the test material was stored at ambient temperature in an outdoor solvent shed. When transferred indoors for use, the test material was stored at room temperature except during atmosphere generation when it was kept frozen at a temperature of -20°C.

Archival Sample

A sample of approximately 10 mL of test material is stored in the Archives of the Testing Facility.

Disposition

The unused portion of the test material, and any empty test material containers, are being retained at the Testing Facility until completion of the definitive study.

TEST ANIMAL INFORMATION

Test Animals

Rats: Albino (Outbred) VAF/Plus®

Strain: Sprague-Dawley derived (CD®) [CrI: CD® BR]

Mice: Albino

Strain: CD-1™ [CrI: CD-1®(ICR) BR]

Justification for Animal Selection

The rat and mouse are rodent animal models commonly used in developmental toxicity studies as recommended in the EPA-TSCA guidelines. In addition, historical control data are available for these species in this laboratory for comparative evaluation.

Number of Animals Purchased

Rats: 75 females

Mice: 90 females

All purchased animals were nulliparous and non-pregnant.

Number of Animals Placed On Test

Rats: 50 females

Mice: 50 females

Supplier - Males and Females

Charles River Laboratories
Portage, Michigan 49081

Date Received - Females

Rats and Mice: 22 May 1995

Date Received - Males

Rats and Mice: 1 May 1995

For rats, proven breeders were used solely for mating purposes (in-house breeding colony).

For mice, young males were received and used strictly for mating this study.

Age at Receipt - Females

Rats and Mice: 57 days old

Age at Receipt - Males

Rats: 50 days old

Mice: 43 days old

Age at Initiation of Mating - Females

Rats: 85 days old

Mice: 87 days old

Age at Initiation of Mating - Males

Rats: 99 days old

Mice: 92 days old

Weight of Mated Females Used on Test (Gestation Day 0)

	<u>Mean</u>	<u>Range</u>
	(grams)	(grams)
Rats:	260	219-293
Mice:	29	25-34

Acclimation Period - Females

Rats: 28 days

Mice: 30 days

SELECTION

More females than required for the study were purchased and acclimated. Animals considered unsuitable for the study on the basis of pretest physical examinations were eliminated prior to initiation of mating.

MATING

Rats

Females selected for mating were placed with male rats nightly in a 1:1 ratio. Vaginal smears were taken early in the morning following intervals of nightly cohabitation and females were considered to have mated if sperm was noted microscopically in the vaginal rinse and/or a plug was observed in the vaginal opening. The day on which evidence of mating was observed was defined as Day 0 of gestation. The evenings for cohabitation of males with females were scheduled to provide females at Day 20 gestation sacrifice during the Monday-to-Friday work week.

Mating was conducted on nine nights, 19-23, 26-29 June 1995.

Mice

Females selected for mating were placed with male mice nightly in a 1:1 ratio. In the morning, following the nightly interval of cohousing, females were evaluated for the presence of a vaginal copulatory plug. The day on which evidence of mating was observed was defined as Day 0 of gestation. The evenings for cohabitation of males with females were scheduled to provide females at Day 18 gestation sacrifice during the Monday-to-Friday work week.

Mating was conducted on five nights, 21-25 June 1995.

GROUP ASSIGNMENT

Females which mated were assigned to the groups daily in such a way as to provide an equal distribution of mated females among groups and equalize, as best possible, the Day 0 gestation mean body weights between groups.

ANIMAL IDENTIFICATION

Each female was assigned a temporary identification number upon receipt. Each mated female animal sorted into test groups was identified with a metal ear tag (rats) or a tail tattoo (mice) bearing its assigned animal number. This individual animal number plus the study number comprised a unique identification for each animal. Mated animals were either eartagged or tail tattooed on the day they were sorted into test groups, which was Day 0 of gestation. Each non-exposure cage was provided with a card that was color coded for exposure level identification, and contained the study number and animal number.

ANIMAL HUSBANDRY - NON-EXPOSURE

Housing

Rats were housed individually, except during mating, in elevated, stainless steel suspended cages with wire mesh floors and fronts.

Two mice of the same sex were housed together in stainless steel, suspended cages with wire mesh floors and fronts for the first week of acclimation. Thereafter, except during mating, animals were housed individually.

Food

Certified Rodent Diet, No. 5002; (Meal) (PMI Feeds, Inc., St Louis, MO) was available without restriction. Each animal's cage was fitted to retain a glass feeder jar with a stainless steel lid. During the acclimation period, fresh feed was provided weekly to the rats while the mice received fresh feed every three to four days. During gestation, fresh food was presented to the rats on Days 0, 6 and 12. The mice were provided with fresh food on Days 0, 3, 6, 10 and 14 of gestation. (Note: For the mice, one animal each from the control, 300, 1000 and 3000 ppm groups and two animals from the 9000 ppm groups were not provided with fresh feeders on Day 3 of gestation due to an oversight. This oversight was not considered to have compromised the validity or integrity of the study as the feeders contained sufficient feed for the animals to Day 6.)

Analyses of Feed

Analyses of each feed lot used during this study were performed by the PMI Feeds, Inc. These data are maintained on file at the Testing Facility.

Water

Tap water was available without restriction (supplier - Elizabethtown Water Company, Westfield, New Jersey, Raritan-East Millstone Plant) and provided to individual cages by an automated water delivery system.

Monthly Water Analyses

Monthly analyses of water supplied to this facility were provided by the Supplier. These data are maintained on file at the Testing Facility.

Biannual Water Analyses

Biannual chemical and microbiological analyses of water samples collected from representative rooms in the Testing Facility were conducted to assure that the water being provided met standards specified under the EPA National Primary Drinking Water Regulations (40 CFR Part 141). These data are maintained on file at the Testing Facility.

Contaminants

There were no known contaminants in the feed or water which were considered capable of interfering with the results of this study.

Environmental Conditions

Twelve hour light/dark cycle via automatic timer. During acclimation, the light cycle in the animal room was approximately 0700 to 1900 hours. On Day 6 of gestation, animals were transferred to a different room for the remainder of the study. In this room the light cycle was approximately 0600 to 1800 hours.

Temperature was monitored and recorded twice daily; relative humidity was monitored and recorded once daily.

	<u>Desired</u>	<u>Actual</u>
Temperature:	18 to 26°C	20 to 24°C
Relative Humidity:	40 to 70%	42 to 74%

ANIMAL HUSBANDRY DURING EXPOSURE

Housing

Animals were individually housed in wire mesh, stainless steel cages within a 1000 liter glass and stainless steel exposure chamber (see Appendix U for details).

Food and Water

None

Environmental Conditions

Chamber temperature and humidity were monitored and recorded every half hour during exposure and maintained, to the maximum extent possible, within the ranges presented below.

See Appendix U for monitoring equipment details.

	<u>Desired</u>	<u>Actual</u>
Temperature:	20 to 24°C	21 to 26°C
Relative Humidity:	40 to 60%	42 to 68%

TEST MATERIAL ADMINISTRATION

Route of Administration

Inhalation, as a vapor, via whole-body exposure.

Justification for Route of Administration

The inhalation route is one of the potential routes of human exposure to the test material.

Frequency and Duration Of Exposure

Rats were exposed for 6 hours daily over the Day 6-19 gestation period. Mice were exposed for 6 hours daily over the Day 6-17 gestation period.

Dates of Exposure

Day 6 of gestation. Rats: 26 June - 6 July 1995

Mice: 28 June - 2 July 1995

Day 19 of gestation. Rats: 9-19 July 1995

Day 17 of gestation. Mice: 9-13 July 1995

Prestudy Trials

Trials were performed to evaluate the optimal set of equipment and operating conditions to generate a stable atmosphere at the targeted exposure levels. See Appendix U, pages 23 and 24 for details of prestudy trials.

Chamber Operation

Chamber operation procedures as well as the chamber's airflow rate, time for air change and 99% equilibrium time (T_{99}) for each group are presented in Appendix U.

TEST MATERIAL PREPARATION

The test material was used as received.

EXPOSURE PROCEDURES

Complete exposure procedures for all groups, both species, are presented in Appendix U.

EXPOSURE CHAMBER SAMPLING

Total hydrocarbon levels were measured six times/exposure for the 300, 1000, 3000 and 9000 ppm groups and once daily for the controls using infrared spectrophotometry.

One sample/exposure for the 300, 1000, 3000 and 9000 ppm groups was analyzed using a syringe grab sample and a gas chromatographic (GC) procedure to characterize airborne vapor components. The ten major components listed below were identified for analysis:

isopentane, n-Butane; N-Pentane; *trans*-2 Pentene; 2-Methyl-2-butene; 2,3 Dimethylbutane; 2-Methylpentane; 3-Methylpentane; n-Hexane; Toluene.

Four samples/exposure for the 300, 1000, 3000 and 9000 ppm groups were collected in charcoal tubes and analyzed to monitor the ratio of the major components. The ten major components listed above were identified for analysis. Details of the actual sampling procedures are presented in Appendix U.

Nominal Concentration

A nominal exposure concentration was calculated daily for the 300, 1000, 3000, and 9000 ppm groups. The flow of air through the chamber was monitored using appropriate calibrated equipment. The test material consumed during the exposure was divided by the total volume of air passing through the chamber (volumetric flow rate multiplied by total exposure time) to give the nominal concentration using the following calculation:

$$\text{Nominal concentration (ppm)} = \frac{\Delta \text{ wt (g)}}{\text{Vol (L)}} \times \frac{24.457 \text{ L/mole}}{\text{MW (g/mole)}} \times 10^6 \mu\text{L/L.}$$

Particle Size Distribution Analysis

Particle size distribution measurements were performed once during each exposure to characterize the aerodynamic particle size distribution of any aerosol present. This measurement determined whether any aerosol present was due to background aerosol vs. test material aerosol. Complete procedural information is presented in Appendix U.

EXPERIMENTAL EVALUATIONS

Observations

Viability Checks (In-Cage). Observations for mortality, general appearance and signs of severe toxic or pharmacologic effects were made twice daily (morning and afternoon) on both species.

Physical Examinations. Each rat was removed from its cage and given a detailed physical examination on Days 0, 6-19 and 20 of gestation. In the mouse, these detailed examinations were performed on Days 0, 6-17 and 18 of gestation. During the exposure period, animals were evaluated both pre-exposure and post-exposure. The latter examination was performed approximately a half hour after exposures ceased when animals were removed from the chamber. Control animals were also removed from the chamber and examined at the same time as the test animals.

Body Weights

Each rat was weighed on Days 0, 3, 6, 9, 12, 15, 18 and 20 of gestation using a Mettler Balance, Model PE4000 (Mettler Instrument Corporation, Hightstown, New Jersey). Day 20 gestation body weights are presented as actual and corrected (the actual Day 20 gestation body weight minus the weight of the gravid uterus) values.

Each mouse was weighed on Days 0, 3, 6, 9, 12, 15 and 18 of gestation using a Sartorius Electric Top Loading Balance, Model Number U3600 (Sartorius Corporation, Edgewood, New York). Day 18 gestation body weights are presented as actual and corrected (the actual Day 18 gestation body weight minus the weight of the gravid uterus) values.

Food Consumption

Rats were presented with weighed feeders on Days 0, 3, 6, 9, 12, 15 and 18 of gestation. Feeders were removed on Days 3, 6, 9, 12, 15, 18 and 20 of gestation and weighed. All feeder weights were measured using a Mettler Balance, Model PE4000 (Mettler Instrument Corporation, Hightstown, New Jersey).

Mice were presented with weighed feeders on Days 0, 3, 6, 8, 10, 12, 14 and 16 of gestation. Feeders were removed on Days 3, 6, 8, 10, 12, 14, 16 and 18 of gestation and weighed. All feeder weights were measured using a Sartorius Electric Top Loading Balance, Model U3600 (Sartorius Corporation, Edgewood, New York).

Calculations. The resulting weight of the feeder at the end of the measurement interval was subtracted from the initial feeder weight. The resulting value represented the grams of feed consumed/interval. The following formula was used to calculate grams of feed per kilogram of body weight per day (g/kg/day).

$$\text{g/kg/day} = \frac{\text{grams of feed consumed}}{\text{previous body weight (kilograms)}} \div \text{Number of days}$$

Measurement intervals (i.e., the number of days over which food consumption was measured) and the body weight used to calculate food consumption are as follows:

Rats

- Day 0-3 = 3-day interval using Day 0 body weight.
- Day 3-6 = 3-day interval using Day 3 body weight.
- Day 6-9 = 3-day interval using Day 6 body weight.
- Day 9-12 = 3-day interval using Day 9 body weight.
- Day 12-15 = 3-day interval using Day 12 body weight.
- Day 15-18 = 3-day interval using Day 15 body weight.
- Day 18-20 = 2-day interval using Day 18 body weight.

Mice

- Day 0-3 = 3-day interval using Day 0 body weight.
- Day 3-6 = 3-day interval using Day 3 body weight.
- Day 6-8 = 2-day interval using Day 6 body weight.
- Day 8-10 = 2-day interval using Day 6 body weight.
- Day 10-12 = 2-day interval using Day 9 body weight.
- Day 12-14 = 2-day interval using Day 12 body weight.
- Day 14-16 = 2-day interval using Day 12 body weight.
- Day 16-18 = 2-day interval using Day 15 body weight.

Body weights and feeder weights were recorded to the nearest tenth of a gram and are presented in this report as a rounded whole number; the reported g/kg/day of food consumption was calculated using the unrounded body weights and feeder weights.

MATERNAL POSTMORTEM EXAMINATIONS

Macroscopic Postmortem Examinations

Complete macroscopic postmortem examinations were performed on all test animals. This included examination of all surfaces, all orifices, the cranial cavity, carcass, the external surface of the spinal cord and sectioned surfaces of the brain, nasal cavity and paranasal sinuses, the thoracic, abdominal and pelvic cavities and their viscera and the cervical tissues and organs. The carcass of each female was discarded at completion of the macroscopic postmortem examination. Only gross lesions were saved in 10% neutral buffered formalin.

Animals Sacrificed

All animals were exsanguinated following anesthesia with inhaled carbon dioxide. Rats were sacrificed on Day 20 of gestation during the period of 10-20 July 1995 and mice were sacrificed on Day 18 of gestation during the period of 10-14 July 1995.

Reproductive System

The intact uterus (ovaries attached) was removed from the abdominal cavity and weighed. The ovaries were dissected free to be examined for the presence and number of *corpora lutea*. The uteri were dissected longitudinally along the antimesometrial border and the number and location of the following were recorded for each horn: live fetuses (movement in response to touch); dead fetuses (absence of movement in response to touch with no visible degeneration); late resorptions (recognizable dead fetus undergoing degeneration regardless of size); early resorptions (evidence of implantation but no recognizable fetus); and implantation sites (total of live, dead and resorbed fetuses).

When no uterine implants were grossly apparent, the uterus was stained with ammonium sulfide (Salewski, 1964). When no uterine foci were visualized poststaining, the female was considered not pregnant.

FETAL EVALUATIONS

External Evaluations

All rat fetuses were weighed, using a Mettler Balance, Model No. PE4000 (Mettler Instrument Corporation, Hightstown, New Jersey) and all mouse fetuses were weighed using an AND Electronic Balance Model FX3200 (A&D Engineering, Inc., Milpitas, California). All fetuses were sexed externally (ano-genital distance) and given a macroscopic external examination for malformations and variations that included observations for palatal defects.

Following these examinations, all fetuses were euthanized via an overdose of carbon dioxide and discarded. Only fetuses with external malformations were saved in 10% neutral buffered formalin at the discretion of the Study Director.

Resorptions

Late resorptions were weighed, examined macroscopically for external malformations and discarded.

STATISTICAL ANALYSES

Continuous Data

The following parameters were analyzed statistically:

Rats

Mean body weights during gestation: Days 0, 3, 6, 9, 12, 15, 18 and 20.

Mean body weight change during gestation: Days 0-3, 3-6, 6-9, 9-12, 12-15, 15-18, 18-20 and cumulative to include Days 0-6 and 6-20. A cumulative weight gain for the Gestation Day 6-20 interval was also calculated for each animal using the corrected Day 20 gestation weights.

Mean food consumption values during gestation: Days 0-3, 3-6, 6-9, 9-12, 12-15, 15-18 and 18-20.

Mice

Mean body weights during gestation: Days 0, 3, 6, 9, 12, 15 and 18.

Mean body weight change during gestation: Days 0-3, 3-6, 6-9, 9-12, 12-15, 15-18 and cumulative to include Days 0-6 and 6-18. A cumulative weight gain for the Gestation Day 6-18 interval was also calculated for each animal using the corrected Day 18 gestation weights.

Mean food consumption values during gestation: Days 0-3, 3-6, 6-8, 8-10, 10-12, 12-14, 14-16, and 16-18.