



Health and Environmental
Sciences Department

An Inhalation Oncogenicity Study of Commercial Hexane in Rats and Mice

Part II—Mice

Final Abridged Report

MARCH 1995

**TOXICOLOGY REPORT NUMBER 405
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FOREWORD

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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:

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PREFACE

This publication highlights summary text and pertinent data from commercial hexane oncogenicity studies done on mice by Bio/dynamics, Inc. under contract to API. This abridged document has been prepared to provide study results and to call attention to the existence and availability of this research. The Study was conducted pursuant to section 4 (a) of the Toxic Substances Control Act (TSCA) [40 Code of Federal Regulations Part 799.2155].

The complete set of multi-volume reports from which this summary document has been compiled is titled, *An Inhalation Oncogenicity Study of Commercial Hexane in Rats and Mice*, and is available for viewing by the public at the API Library, 1220 L St., Washington D.C. 20005. The five parts that make up the complete study are too voluminous for distribution on a routine basis. For your convenience, the table of contents to the complete report is included as an appendix to this abridged document.

American Petroleum Institute
Health and Environmental Sciences Department

QUALITY ASSURANCE/GLP COMPLIANCE STATEMENT

Study Title: An Inhalation Oncogenicity Study of Commercial Hexane in Mice

Testing Facility: Bio/dynamics, Incorporated

Testing Facility Number: 88-8137

API Product Safety Number: PS-71

This study was reviewed by API Quality Assurance personnel under the direction of API Management on the dates indicated below for compliance with EPA TSCA Good Laboratory Practice (GLP) regulations. These studies were conducted in accordance with EPA TSCA GLP regulations with the following exemption. As indicated in a letter from M. Shapiro (EPA) to Dr. Drew (API) dated July 13, 1989, a conditional exemption to the GLP regulations for the disposal of the test substance storage containers was granted. Therefore, the test substance storage containers for this project were disposed of prior to the issuance of the final report. Appropriate records of this disposal were maintained, per M. Shapiro's letter.

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.

<u>Date(s) of Inspection/Review</u> (Reported to Management)	<u>Type of Inspection</u>
12/2/88 (12/2/88)	Protocol Evaluation
7/19/89 (7/19/89)	Protocol Evaluation
1/11/90 (1/16/90)	Pre-initiation inspection and data audit
1/25/90 (2/2/90)	In-life inspection and data audit
4/24/90 (4/24/90)	Progress report review
6/18-19/90 (6/19/90)	In-life inspection and data audit
9/5-7/90 (9/11/90)	In-life inspection and data audit
2/4-6/90 (2/11/90)	In-life inspection and data audit
7/23-25/91 (7/26/91)	In-life inspection and data audit

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9/13/91 (9/13/91)	Progress report
	review
12/18-19/91 (12/23/91)	In-life inspection
	and data audit
1/14-15/92 (1/16/92)	Sacrifice, data
	review
2/9-11/93 (2/26/93)	Draft report audit
5/18-19/93 (5/19/93)	Second draft report
	audit
5/26/93 (5/26/93)	Third draft report
	audit
5/28/93 (5/28/93)	Fourth draft report
	audit
6/2/93 (6/2/93)	Final Report
	Acceptance

Christine Sexsmith
 Christine Sexsmith, B.S.
 Quality Assurance Coordinator

6/2/93
 Date

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PROJECT NO. 88-8137M
 AN INHALATION ONCOGENICITY STUDY
 OF COMMERCIAL HEXANE IN RATS AND MICE
 PART II - MICE

ABSTRACT

This study, conducted for the American Petroleum Institute, was designed to assess the oncogenic effect of commercial hexane. The test substance was administered by whole-body inhalation as a vapor to Fischer 344 rats and B6C3F1 mice (50/sex/group/species). This report presents the results from the mouse portion of the study. The rat results are presented in a separate report. The test substance was administered for six hours per day, five days per week, for approximately two years at target concentrations of 900, 3000 and 9000 parts per million (ppm) of air. The 9000 ppm high exposure level was based upon the results of a 90-day subchronic study. In addition, 9000 ppm is approximately 80% of the lower explosive limit. Exposures were initiated on 23 January 1990 and completed on 20 January 1992. Exposure levels were analyzed hourly using an infrared spectrophotometer (IR). Gas chromatographic (GC) confirmation of the commercial hexane chamber exposure levels as well as analysis for the six major components were also conducted. Particle size distribution measurements of any background aerosol were made monthly. Detailed physical examinations were conducted weekly on all animals. Ophthalmoscopic examinations were performed on all animals pretest and prior to sacrifice. Body weight measurements were recorded pretest on Test Days -12, -7 and 0, weekly through Week 13, monthly through Week 101 and just prior to sacrifice. Differential white blood cell counts were analyzed pretest for all animals and at Month 12, Month 18 and prior to termination on Group I and IV survivors. Following the two years of exposure, all survivors were sacrificed. Complete macroscopic examinations were conducted for all animals. Microscopic examinations were performed in the lungs in all animals, in selected tissues in all Group I and IV animals, and in all animals which died or were sacrificed moribund prior to their scheduled sacrifice. In addition, microscopic examinations were conducted in the livers of Group II and III males and females and in the pituitaries of the Group II and III females.

The cumulative mean exposure concentrations as determined by IR were 900, 3000 and 9018 ppm. GC analyses confirmed these exposure levels and indicated commercial hexane was stable over the duration of the study with a mean composition (%) of: n-hexane (51.5), methylcyclopentane (16.0), 3-methylpentane (16.1), 2-methylpentane (12.9), cyclohexane (3.3) and 2,4-dimethylpentane (0.17). Particle size distribution determinations indicated that no significant test substance aerosol was present in the exposure chambers.

At termination of the study, in the control group survivorship was 85% in the males and 80% in the females. There was no significant difference in survivorship among any of control or exposure groups.

Physical observations, hematological and ophthalmoscopic examinations found no signs of any commercial hexane related effects.

Mean body weights and body weight gains in the exposed animals were not statistically different from control values in the male mice. However, food consumption in the 9018 ppm group was lower than controls on 29 of the 35 measurements obtained over the duration of the study.

In the females however, at 9018 ppm, body weight gain was reduced. The mean body weight in the 9018 ppm female group was significantly lower than control weights after week 29. By week 53 the 9018 ppm female mice weighed 14% less than the control mice. Similar to the males, mean food consumption values were not statistically different from control values in the mice exposed to the mid and low concentration of commercial hexane. However, in the 9018 ppm group, food consumption was lower than control values on 19 of the first 20 food consumption measurements at the beginning of the study.

Macroscopic examinations found an apparent treatment related increase in liver masses and nodules among the females in the 9018 ppm group but not among the males. There was a slight dose related decrease in the incidence of cysts in the uterus.

Microscopic examinations found a treatment related increase in hepatocellular neoplasms (adenoma and carcinoma) among females in the 9018 ppm group. Liver tumors among males were not treatment related. The incidence of liver tumors in the Group IV females is similar to the incidence in control males. Male mice of this strain have a higher spontaneous incidence of hepatocellular tumors than females.

There was also a treatment related decrease in the severity and a slight decrease in the incidence of cystic endometrial hyperplasia of the uterus among the females in the 9018 ppm group.

There was an increase in the incidence of pituitary proliferative changes (hyperplasia, adenoma and adenocarcinoma) among all treated groups of females but not among males. The treatment relationship of this observation is unclear because of a lack of dose response. The incidence of these changes is also within the historical range of data for control females.

In conclusion, under the exposure conditions of this study, commercial hexane is an oncogen in female mice. The NOEL for the neoplastic changes (hepatocellular tumors in females) was 3000 ppm.

I. INTRODUCTION:

This study, conducted for the American Petroleum Institute, was designed to assess the oncogenic effect of commercial hexane when administered by whole-body inhalation as a vapor to Fischer 344 rats and B6C3F1 mice (50/sex/group/species). This report presents the results from the mouse portion of the study. The rat results are presented in a separate report. The test substance was administered for six hours per day, five days per week, for approximately two years at target concentrations of 0, 900, 3000 and 9000 parts per million (ppm) of air. The 9000 ppm high exposure level was established based upon the results of a 90-day subchronic study. In addition, 9000 ppm is approximately 80 percent of the lower explosive limit.

Species and strain of test animal, method and route of test substance administration and target exposure levels were determined by the sponsor.

This study was mandated by an Environmental Protection Agency test rule (Federal Register, Vol 53, No 24, February 5, 1988; pp 3382-3395) issued under TSCA section 4(a) and was performed in compliance with 40 CFR Part 798.3300 as modified 799.2155 and 40 CFR part 792, the TSCA Good Laboratory Practice Regulations for Nonclinical Laboratory Studies. The facilities of Bio/dynamics, Inc., and this study were operated/conducted in accordance with the requirements and recommendations of the Animal Welfare Act (P.L. 89-544 as amended by P.L. 91-579 and P.L. 94-279), and other applicable federal, state and local laws, regulations and policies.

This study was conducted at Bio/dynamics, Inc., Mettlers Road, East Millstone, New Jersey 08875-2360. All raw data, specimens, the original study protocol and the original final report are stored in the Archives of Bio/dynamics, Inc.

II. MATERIALS AND METHODS:

A. Study Dates:

Study Initiation Date: 14 September 1989
(Date Study Director
signed the protocol)

Receipt of Test Animals: 27 December 1989

Initiation of Exposures: 23 January 1990
(Experimental Start
Date)

Termination of Exposures: 20 January 1992

Necropsy: 13-21 January 1992

Study Completion Date: Date final report is signed by Study
Director.

B. Test Substance:

Commercial Hexane
CAS: 110-54-3

Supplier: American Petroleum Institute (API),
manufacturing records are on file with
API.

Dates Received: A single batch of the test substance was
prepared, stored in 55-gallon drums and
then delivered on an as need basis. The
drums were received on:

7 February 1989 (Drum Nos. 3 - 10)
12 February 1990 (Drum Nos. 13 - 20)
13 March 1990 (Drum Nos. 21 - 25)
17 April 1990 (Drum Nos. 26 - 31)
25 May 1990 (Drum Nos. 32 - 37)
26 June 1990 (Drum Nos. 38 - 43)
24 July 1990 (Drum Nos. 44 - 49)
28 August 1990 (Drum Nos. 50 - 55)
27 September 1990 (Drum Nos. 56 - 61)
5 November 1990 (Drum Nos. 62 - 67)
4 December 1990 (Drum Nos. 68 - 73)
10 January 1991 (Drum Nos. 74 - 79)
14 February 1991 (Drum Nos. 80 - 85)
20 March 1991 (Drum Nos. 86 - 91)
22 April 1991 (Drum Nos. 92 - 97)
28 May 1991 (Drum Nos. 98 - 103)
27 June 1991 (Drum Nos. 104 - 109)
31 July 1991 (Drum Nos. 110 - 115)
9 September 1991 (Drum Nos. 116 - 121)



Documents

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II. MATERIALS AND METHODS (cont.):

B. Test Substance (cont.):

Dates Received (cont.): 10 October 1991 (Drum Nos. 122 - 127)
8 November 1991 (Drum Nos. 128 - 133)
13 December 1991 (Drum Nos. 134 - 137)

Lot No.: 1-26-89

Concentration: 100% Active Ingredient

Description: Clear, water-white liquid; mild, bland petroleum odor.

Analysis: The identity, strength, purity and composition; and synthesis, fabrication, and/or derivation of the test substance have been documented by the sponsor.

Physical Properties: The nature of the test substance and its solubility, melting/boiling point, vapor pressure and flammability have been documented by the sponsor.

Stability and Purity: The stability and purity of the test substance over the duration of the study were documented by the Department of Metabolism and Analytical Chemistry at Bio/dynamics, Inc. (See Appendix A, page A-9.)

Storage: The test substance was stored in an ambient storage shed in 55 gallon drums away from ignition sources. A nitrogen headspace was maintained in the drum as the test substance was being dispensed.

Disposition: All containers of the test substance were returned to the sponsor.

C. Test Animal:

Mouse

Strain:

B6C3F1

Justification for
Animal Selection:

Standard laboratory animal for inhalation toxicity studies. The B6C3F1 mouse was used due to its availability and due to the existing historical data base for comparative evaluation.



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II. MATERIALS AND METHODS (cont.):

C. Test Animal (cont.):

Number of Animals:

Received: 541 total (271 males, 270 females)

Placed on Test: 400 total (200 males, 200 females)

Supplier: Charles River Breeding Laboratories, Inc.
Portage, Michigan 49081

Date of Birth: 29 November 1989

Date Received: 27 December 1989

Age at Receipt (approx.): 4 weeks

Age at Initiation of
Exposure (approx.): 8 weeksWeight at Initiation of
Exposure (grams):

	Mean	Range
Males:	24	21 - 26
Females:	20	17 - 22

Acclimation Period: Animals were acclimated for 27 days
(27 December 1989 - 23 January 1990).
All animals were examined by the staff
veterinarian during the acclimation
period.Sentinel Control: A sentinel control group of 15 animals
per sex was exposed along with the test
animals to verify the health status of
the test animals. Three animals per sex
were sacrificed prestudy. Pretest
differential white blood counts, gross
necropsies were performed on all sentinel
animals. The surviving animals were
euthanized and their carcasses were
discarded at study termination.D. Selection: More animals than required for the study
were purchased and equilibrated. Animals
considered unsuitable for the study on
the basis of pretest physical
examinations, outlying body weight and
ophthalmoscopic examination were
eliminated prior to random selection for
group assignment.

II. MATERIALS AND METHODS (cont.):

E. Group Assignment:

Animals considered suitable for study were distributed into four groups of fifty animals per sex by a computerized random sort program so that body weight means for each group were comparable. Animals were assigned to control and exposure groups randomly.

F. Animal Identification:

Each animal was identified with a tail tattoo bearing its unique Bio/dynamics, Inc. animal number. In addition, each cage was provided with a color coded tape for dose level identification that contained the animal number.

G. Experimental Outline:

Group	Test Exposure ^a Level (ppm)	Number of Animals						
		On-Test (M/F)	Blood Smears ^b				Terminal Necropsy (M/F)	Histopathology ^c (M/F)
			Pretest (M/F)	Month 12 (M/F)	Month 18 (M/F)	Termination (M/F)		
I (Control)	0	50/51 ^d	50/50	47/50	46/45	41/39	41/39	50/50
II (Low)	900	50/50	50/50	46/48	46/43	40/37	40/37	50/50
III (Mid)	3000	50/53 ^e	50/50	48/48	45/46	43/35	43/35	50/50
IV (High)	9000	50/50	50/50	49/49	49/47	42/38	42/38	50/50
Sentinels	-	12/12	-	-	-	-	-	-

^aExposures were conducted for 6 hours/day, 5 days/week for approximately 2 years. If an exposure fell on a company holiday, the exposure was not conducted or made up. At no time was there ever more than four consecutive days without an exposure.

^bOnly slides from Groups I and IV were evaluated at Month 12, Month 18 and termination.

^cFull histopathologic examinations were conducted on all Group I and IV animals and all animals that died spontaneously or killed in extremis during the study. The lungs were evaluated in all animals sacrificed at study termination. Any target organs in Group II and III animals, as well as gross lesions and masses were evaluated.

^dOne Group I female died on day 11 and was replaced.

^eThree Group III females died on days 10, 22 and 26 and were replaced.

II. MATERIALS AND METHODS (cont.):

H. Husbandry:

During Non-Exposure
Periods:

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. Bio/dynamics is accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

Housing:

Animals were doubly housed in suspended stainless steel wire mesh cages during the first week of the acclimation period and were individually housed thereafter.

Food:

ad libitum; certified mash laboratory diet (Purina Certified Rodent Chow® Brand Animal Diet #5002). Fresh food was presented twice weekly. Analysis of each feed batch used during this study was performed by the Purina Mills, Inc. prior to receipt at Bio/dynamics, Inc. Results of the analyses are located in the study raw data.

Water:

ad libitum; by automated watering system. Municipal water supply (Elizabethtown Water Company). Monthly water analysis was provided by Elizabethtown Water Company, Westfield, New Jersey. Results are maintained on file at Bio/dynamics, Inc.

Environmental
Conditions:

Approximately 12 hour light/dark cycle (6 AM to 6 PM/6 PM to 6 AM) via automatic timer. Temperature and relative humidity were monitored and recorded twice daily and maintained, to the maximum extent possible, within the range presented below.

Temperature:

Desired: 20-24°C
Actual: 22±1°C ($\bar{X}$ ±SD)

Humidity:

Desired: 40-60%
Actual: 53±14% ($\bar{X}$ ±SD)

II. MATERIALS AND METHODS (cont.):

H. Husbandry (cont.):

During Exposure Periods:

Housing:	Animals were individually housed in stainless steel, wire mesh cages within 10,000 liters glass and stainless steel exposure chambers (see Figure 1).
Food:	None
Water:	None
Environmental Conditions:	Chamber temperature and relative humidity were recorded once upon initiation of exposure (T_0) and once every half-hour during each exposure.
Temperature:	Desired: 20-24°C Actual: 22±1°C ($\bar{X}$ ±SD)
Humidity:	Desired: 30-70% Actual: 50±10% ($\bar{X}$ ±SD)

I. Test Substance Administration and Chamber Operation:

Route:	Inhalation, administered into the breathing zone of the animals as a vapor.
Justification of Route of Administration:	Inhalation was chosen as the route of administration to simulate the potential exposure route during manufacture and/or use.
Target Exposure Levels:	0 (control), 900, 3000 and 9000 ppm.
Justification of Exposure Levels:	The high exposure level was based upon the results of a 90-day subchronic inhalation study and set at 9000 ppm. This is approximately 80% of commercial hexane's lower explosive limit.
Frequency of Exposure:	Daily (6 hours per day, 5 days per week for approximately 2 years).
Number of Exposures:	504
Dates of Exposure:	23 January 1990 - 20 January 1992

II. MATERIALS AND METHODS (cont.):

I. Test Substance Administration and Chamber Operation (cont.):

Animal Rotation:

The placement of each animal within the chamber was rotated weekly to assure uniform exposure of the animals.

Safety Precautions:

In order to safely handle and generate atmospheres of this flammable test substance, several precautionary procedures were adopted. The 55-gallon drums of commercial hexane were stored within a solvent shed detached from the main building. The commercial hexane was dispensed as needed for use in this study into 5 gallon containers. The drum and container were grounded to each other during this transfer. A nitrogen head space was maintained in all drums and containers. To generate the exposure atmosphere the commercial hexane was evaporated and diluted to below its LEL (lower explosive limit) using heated nitrogen. The temperature of the evaporation unit was continuously monitored with a monitor equipped with an audible alarm for any temperature excursions. The high exposure level for this study was set at 9000 ppm or approximately 80% of commercial hexane's LEL.

Pre-Study Trials:

Trials were performed to evaluate the optimal equipment and operating conditions in an attempt to generate a stable atmosphere at the targeted exposure levels. Results are located in the study raw data. In addition, measurements of the exposure levels within each chamber were done to confirm exposure level uniformity.

II. MATERIALS AND METHODS (cont.):

I. Test Substance Administration
and Chamber Operation
(cont.):

Chamber Operation:

The glass and stainless steel exposure chambers (Figure 1) in which the animals were exposed had a total volume of 10,000 liters. The exposure chambers using conditioned outside air were operated under slight negative pressure. The initial chamber airflow rate, N₂ generator flow rate, total flow rate, oxygen content, time for air change and time for equilibration (T₉₉) for each group are summarized below:

Group	Flow Rate (lpm)			O ₂ (%)	Air Change (min.)	T ₉₉ (min.)
	N ₂	Air	Total			
I	20	2000	2000	21	5.0	23.0
II	20	2010	2010	21	5.0	22.9
III	160	2110	2110	19	4.7	21.8
IV	150	2130	2130	19	4.7	21.6

This chamber size and airflow rate were adequate to maintain the oxygen concentration above 19% with an animal loading of less than 5% of the chamber volume. The chamber was exhausted through a coarse filter a HEPA filter, a charcoal filter and into a MOCO incinerator. A Carbtrol® charcoal drum was attached, via 2" flex tubing, to the exhaust pipe of each chamber as a back-up in case of incinerator shut down.

Recordings of chamber temperature, relative humidity, airflow rate and static pressure were made approximately every half-hour during exposure. See Figure 1 and Appendix A for equipment details.

Test Substance
Preparation:

The test substance was used as received.

II. MATERIALS AND METHODS (cont.):

I. Test Substance Administration and Chamber Operation (cont.):

Exposure Procedure:

Group I (Control):

Heated nitrogen was delivered through a Whitey® metering valve, via 1/4" Teflon® tubing, to a Dwyer® flowmeter regulated by a Nupro® metering valve and then into the turret of the exposure chamber. The animals remained in the exposure chamber for 30 minutes following the exposure to simulate chamber clearing, using conditioned air at the same airflow rate used during exposure. See Figure 1 and Appendix A for equipment details.

Group II (900 ppm):

An appropriate amount of the test substance was placed into a Protectoseal¹ laboratory can and connected to two FMI fluid metering pumps (two RPG-20 pumps with 3/8" pistons) connected in parallel with initial pump settings of 50%.¹ A nitrogen bag was attached to the can to maintain a nitrogen headspace. The test substance was fed from the pumps, via 1/8" Teflon® tubing, into the liquid inlet at the top of the counter current volatilization generator. The volatilization generator had a coiled glass rod insert heated internally by a nichrome heating element (approximately 100°F). The temperature of the heating element was controlled by a Glas-col® Minitrol variable autotransformer. Nitrogen delivered through a Whitey® metering valve heated by a Lindberg® Compact Tube furnace branched into a generation flow and a purge flow. The generator nitrogen flow was delivered, via 1/4" Teflon® tubing, to a Dwyer® flowmeter regulated by a Nupro® metering valve and into the bottom of the counter current unit where it flowed counter to the test material within the generator for volatilization of the test material. The purge nitrogen flow was

¹A 100% pump setting for an RPG-20 is rated at 12.8 ml/min for a 3/8" piston.

II. MATERIALS AND METHODS (cont.):

I. Test Substance Administration and Chamber Operation (cont.):

Exposure Procedure (cont.):

Group II (cont.):

delivered, via 1/4" Teflon[®] tubing, to a Dwyer[®] flowmeter regulated by a Nupro[®] metering valve and into the center of the heated coiled glass insert (at a flowrate of 2 lpm) to purge the heating element of oxygen. As a result, the heating and volatilization of the test substance was done under a nitrogen blanket and the test substance diluted to below its lower explosive limit prior to mixing with air in the exposure chamber. A Sentry[™] digital alarm module was used to monitor the temperature of the test substance laden nitrogen stream leaving the volatilization generator. The test atmosphere was directed into the turret of the exposure chamber which housed the animals, via 1/2" Teflon[®] tubing. The animals remained in the chamber for 30 minutes following the exposure to allow the chamber to clear, using conditioned air at the same air flowrate used during the exposure. See Figures 1 and 2 and Appendix A for equipment details.

Group III (3000 ppm):

An appropriate amount of the test substance was placed into a Protectoseal[®] laboratory can and connected to a FMI fluid metering pump (RPG-400 with a 1/4" piston) with an initial pump setting of 22%.² A nitrogen bag was attached to the can to maintain a nitrogen headspace. The test substance was fed from the pump, via 1/8" Teflon[®] tubing, into the liquid inlets at the top of two counter current volatilization generators connected in parallel. The volatilization generators had coiled glass rod inserts heated internally by nichrome heating elements (approximately 100°F). The temperature of the heating elements was controlled by a Staco variable autotransformer and a

²A 100% pump setting for an RPG-400 is rated at 108 ml/min for a 1/4" piston.

II. MATERIALS AND METHODS (cont.):

I. Test Substance Administration and Chamber Operation (cont.):

Exposure Procedure (cont.):

Group III (cont.):

Glas-col® Minitrol variable autotransformer. Nitrogen delivered through a Whitey® metering valve heated by a Lindberg® Compact Tube furnace branched into a generation flow and a purge flow. The generator nitrogen flow was delivered, via 1/4" Teflon® tubing, to two Dwyer® flowmeters regulated by Nupro® metering valves and into the bottom of each counter current unit where it flowed counter to the test material within the generator for volatilization of the test material. The purge nitrogen flow was delivered, via 1/4" Teflon® tubing, to two Dwyer® flowmeters regulated by Nupro® metering valves and into the center of each heated coiled glass insert (at a flowrate of 2 lpm) to purge the heating elements of oxygen. As a result, the heating and volatilization of the test substance was done under a nitrogen blanket and the test substance diluted to below its lower explosive limit prior to mixing with air in the exposure chamber. Two Sentry™ digital alarm modules were used to monitor the temperature of the test substance laden nitrogen streams leaving the volatilization generators. The test atmosphere was directed into the turret of the exposure chamber which housed the animals, via 1/2" Teflon® tubing. The animals remained in the chamber for 30 minutes following the exposure to allow the chamber to clear, using conditioned air at the same air flowrate used during the exposure. See Figures 1 and 2 and Appendix A for equipment details.

**Documents**

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II. MATERIALS AND METHODS (cont.):

I. Test Substance Administration and Chamber Operation (cont.):

Exposure Procedure (cont.):

Group IV (9000 ppm):

An appropriate amount of the test substance was placed into three Protectoseal[®] laboratory cans and connected to three FMI fluid metering pumps (three RPG-400 pumps with two 3/8" pistons and one 1/4" piston). A nitrogen bag was attached to each can to maintain a nitrogen headspace. The test substance was fed from the pumps, via 1/8" Teflon[®] tubing, into the liquid inlets at the top of three counter current volatilization generators connected in parallel. The volatilization generators had coiled glass rod inserts heated internally by nichrome heating elements (approximately 100°F). The temperature of the heating elements was controlled by a Staco variable autotransformer and two Glascol[®] Minitrol variable autotransformers. Nitrogen delivered through a Whitey[®] metering valve heated by a Lindberg[®] Compact Tube furnace branched into a generation flow and a purge flow. The generator nitrogen flow was delivered, via 1/4" Teflon[®] tubing, to three Dwyer[®] flowmeters regulated by Nupro[®] metering valves and into the bottom of each counter current unit where it flowed counter to the test material within the generator for volatilization of the test material. The purge nitrogen flow was delivered, via 1/4" Teflon[®] tubing, to three Dwyer[®] flowmeters regulated by Nupro[®] metering valves and into the center of each heated coiled glass insert (at a flowrate of 2 lpm) to purge the heating elements of oxygen. As a result, the heating and volatilization of the test substance was done under a nitrogen

II. MATERIALS AND METHODS (cont.):

I. Test Substance Administration
and Chamber Operation
(cont.):Exposure Procedure
(cont.):

Group IV (cont.):

blanket and the test substance diluted to below its lower explosive limit prior to mixing with air in the exposure chamber. Three Sentry™ digital alarm modules were used to monitor the temperature of the test substance laden nitrogen streams leaving the volatilization generators. The test atmosphere was directed into the turret of the exposure chamber which housed the animals, via 1/2" Teflon® tubing. The animals remained in the chamber for 30 minutes following the exposure to allow the chamber to clear, using conditioned air at the same air flowrate used during the exposure.

Pump settings were selected in order to achieve the target exposure level. Initial pump settings for each fluid metering pump are summarized below:

<u>Pump</u>	<u>Initial Pump Setting^a (%)</u>	<u>Piston Size (inch)</u>
A	20	3/8
B	10	3/8
C	18	1/4

^aA 100% pump setting for a RPG-400 is rated at 250 ml/min for a 3/8" piston and 108 ml/min for a 1/4" piston. See Figures 1 and 2 and Appendix A for equipment details.



II. MATERIALS AND METHODS (cont.):

J. Exposure Chamber Sampling:

Chamber Sampling:

Samples for determination of commercial hexane exposure levels were analyzed using a MIRAN® 1A Ambient Air Analyzer equipped with a Fisher Recordall® Series 5000 strip chart recorder. The test atmosphere was drawn either through 1/4" Teflon® tubing into a Wilkes Multipoint Sampler and then into the MIRAN® or into the MIRAN® directly. A Thomas pump was used to draw the sample through the MIRAN®. Samples were withdrawn hourly during each exposure from the normal sampling portal (designated H-1 in Figure 1). The resultant absorbance was read off of a Micronta® LCD Bench Top digital multimeter. The exposure levels were determined by comparison of the resultant absorbance to a calibrated response curve constructed using the same instrument settings.

Additionally, samples for the determination of the proportionality of the components in the commercial hexane vapor were collected in two charcoal tubes in tandem (T-1 and T-2) from the breathing zone in each exposure chamber monthly during the study. The tube tips were filed off and the inlet side was inserted into a stopper at the H-1 sampling portal of the exposure chamber (See Figure 1 for location). Samples were pulled through the outlet end of the tube, via 1/4" Teflon® tubing, by a Neptune Dyna-pump® monitored by a Dwyer® flowmeter regulated by a Nupro® metering valve. The samples were then assayed by gas chromatography by the Department of Metabolism and Analytical Chemistry at Bio/dynamics, Inc. See Appendix A, page A-9.

II. MATERIALS AND METHODS (cont.):

J. Exposure Chamber Sampling
(cont.):

Chamber Distributions:

Distribution samples were taken during exposures. Samples were taken using a MIRAN® 1A Ambient Air Analyzer from the H-4, H-6, H-7, H-9, H-12, H-14, H-15 and H-17 sampling portals within the chamber. See Figure 1 for location of sampling portals.

Particle Size
Distribution Analysis:

Particle size distribution measurements were performed monthly for chamber air using a TSI Aerodynamic Particle Sizer. An IBM PS/2 Model 30 computer was used to program the system to the appropriate settings prior to sampling. The particle size distributions were calculated by the computer and printed out on an EPSON LQ-500 dot matrix printer based on the amount of material collected by the particle sizer. See Appendix A for equipment details.

Oxygen Content
Determinations:

Oxygen content determination measurements were performed for each exposure chamber monthly during the study using a Gastechtor O₂ monitor. See Appendix A for equipment details.

Nominal Concentration:

The nominal concentration was determined by weighing the generation apparatus containing the test substance before and after the exposure and dividing the difference in these weights by the total volume of air delivered during exposure (volumetric flow rate times total exposure time).

II. MATERIALS AND METHODS (cont.):

K. Physical Observations:

For Mortality and Gross
Signs of Toxicologic or
Pharmacologic Effects:

Twice daily, once in the morning and once
in the afternoon.

Detailed Physical
Examinations:

Pretest and weekly thereafter. See
Appendix A for Methodology and
References.

L. Ophthalmoscopic Examination: Ophthalmoscopic evaluations were performed at the time intervals listed below. Examinations were performed by Lionel F. Rubin, V.M.D., Diplomate, American College of Veterinary Ophthalmologists. See Appendix A for Methodology and References.

Time Intervals

Pretest: 10 January 1990
Termination: 10 January 1992

M. Body Weight:

Three times pretest (Test Days -12, -7 and 0), weekly through Week 13, monthly through Week 101 and prior to termination. See Appendix A for Methodology and References.

N. Food Consumption:

Twice pretest, weekly through Week 13 and monthly through Week 101. See Appendix A for Methodology and References.

O. Laboratory Studies:

At the interim bleeding intervals blood was obtained via a tail nick in unanesthetized animals. At termination, blood was obtained via venipuncture of the orbital sinus (retrobulbar venous plexus) under light ether anesthesia. Animals were not sacrificed until after the samples were collected. See Appendix A for Methodology and References.

At termination, blood samples from all surviving animals were also taken and the plasma frozen at approximately -80°C for possible further analysis. The analyses and final disposition of these sera will be determined by the sponsor.

II. MATERIALS AND METHODS (cont.):

O. Laboratory Studies
(cont.):Time Intervals

Pretest: 10 January - 16 January 1990
Month 12: 28 January - 1 February 1991
Month 18: 23 July - 26 July 1991
Termination: 13 January - 21 January 1992

Parameter Evaluated:

differential leukocyte counts

P. Postmortem:

Gross Postmortem
Examination:

Performed on all animals found dead, accidentally killed, sacrificed in a moribund condition or killed at study termination. Examinations included the external surface, all orifices, the nasal cavity, the cranial cavity, external surfaces of the brain and spinal cord, the thoracic, abdominal and pelvic cavities with their associated organs and tissues, the neck with its associated organs and tissues and the carcass. Animals were fasted prior to the scheduled sacrifice.

Terminal Necropsy:

13 January - 21 January 1992

Sacrifice Method:

Exsanguination while under ether anesthesia.

Tissues Preserved:

The following tissues were preserved for all animals. Number in parentheses indicates number of organs preserved.

abdominal aorta
adrenals (2)
bone (femur and sternum)
bone marrow smears
brain (3 sections including medulla/pons, cerebral cortex and cerebellar cortex)
esophagus
exorbital lacrimal gland
eyes
gall bladder
gonads(ovaries or testes with epididymides)
heart

II. MATERIALS AND METHODS (cont.):

P. Postmortem (cont.):

Tissues Preserved (cont.): intestine
cecum
colon
duodenum
ileum
jejunum
rectum
kidneys (2)
larynx
liver (left lateral and median lobes)
lungs (with mainstem bronchi)³
lymph nodes (mesenteric and
peribronchial)
mammary gland (right inguinal with skin)
nasopharyngeal tissues
nerve (sciatic with muscle biceps
femoris)
pancreas
pituitary
prostate
salivary gland (sub-mandibular)
seminal vesicles
skin (with mammary gland)
spinal cord (cervical, mid-thoracic and
lumbar sections)
spleen
stomach
thymic region
thyroid/parathyroid
trachea
urinary bladder³
uterus (corpus and cervix uteri)
vagina/penis
gross lesions (including a section of
normal-appearing portion of same tissue)
tissue masses

³Inflated with fixative to ensure fixation.

II. MATERIALS AND METHODS (cont.):

P. Postmortem (cont.):

Tissues Examined
Histopathologically:

The lungs were examined in all animals. In addition, the following tissues were examined for animals found dead, accidentally killed, sacrificed in a moribund condition and all Group I and IV animals sacrificed at study termination. Number in parentheses indicates number of organs/sections examined.

abdominal aorta (1)
adrenals (1 each)
bone (femur and sternum)
bone marrow (femur and sternum)
brain (3 sections including frontal cortex and basal ganglia, parietal cortex and thalamus and cerebellum and pons)
esophagus (1)
exorbital lacrimal gland
eyes (1 each)
gall bladder
gonads (1 each ovaries or testes with epididymides)
heart
intestine (1 each)
cecum
colon
duodenum
ileum
jejunum
rectum
kidneys (1 each)
larynx (1)
liver (2 - from separate lobes)
lungs (2 - each lobe and mainstem bronchi)
lymph nodes (2 - mesenteric and peribronchial)
nasopharyngeal tissues (4)
nerve (1 - optic and 1 - sciatic with biceps femoris)

II. MATERIALS AND METHODS (cont.):

P. Postmortem (cont.):

Tissues Examined Histopathologically (cont.):

pancreas (1)
pituitary (1)
prostate (1)
salivary gland (1 - sub-mandibular)
seminal vesicles (1)
skin (with mammary gland)
spinal cord (3)
spleen
stomach (1)
thymic region (1)
thyroid/parathyroid (1)
trachea (1)
urinary bladder (1)
uterus (2 - horns and cervix)
gross lesions (including a section of
normal-appearing portion of same tissue)
tissue masses

In addition, the livers in males and females and pituitary in females were considered target organs and were also evaluated in Groups II and III.

Preservatives:

Blood Smears: Methanol
All other tissues: 10% neutral buffered formalin (eyes, testes and epididymides were placed in Bouin's solution for the initial 48 - 72 hours).

Stain:

Tissues: Hematoxylin and Eosin
Smears: Wright (Camco). See Appendix A for Methodology and References.

Q. Statistical Analysis:

Body weight, body weight change from week 0 and mean food consumption values were analyzed. Mean values of all exposure groups were compared to control at each time interval. Statistically significant differences from control are indicated on mean tables of appendices. See Appendix A for Methodology and References.

In addition, survival data was analyzed and is located in Appendix I.

II. MATERIALS AND METHODS (cont.):

R. Protocol Deviations:

The following deviations were not considered to have adversely affected the results of this study:

1. The results from 16 feed analyses were not available from the 131 batches of feed used during the study.
2. The non-exposure and exposure period temperatures and relative humidities deviated from the protocol desired ranges, on occasion.
3. Chamber distribution samples were performed during exposure and not pretest.
4. Histopathology was not performed on pretest sentinel animals.

III. RESULTS AND DISCUSSION:

A. Commercial Hexane:

Analyses of commercial hexane from the storage drums (Appendix B-56) and in the exposure atmospheres (Appendix B-54) indicated that the composition of the test substance in the exposure chambers was the same as the original material. In addition, the commercial hexane was stable over the duration of this study with a composition of:

<u>Component</u>	<u>Percentage</u>
n-hexane	51.5
methylcyclopentane	16.0
3-methylpentane	16.1
2-methylpentane	12.9
cyclohexane	3.3
2,4-dimethylpentane	0.17

Therefore, the composition of the commercial hexane met the criteria of 40-55% n-hexane and greater than 10% methylcyclopentane established in the Environmental Protection Agency's test rule (Fed. Register, Vol 53, No. 130; May 27, 1988; pp 19315-19316).

B. Chamber Monitoring:

The analytical and nominal concentrations (mean $\pm$ standard deviation) of c-hexane (commercial hexane) for each exposure group are presented in Appendix B, plotted in Figure 3 and are summarized below:

<u>Group</u>	<u>Test Substance</u>	<u>Target Concentration (ppm)</u>	<u>Analytical Concentration (ppm)</u>	<u>Nominal Concentration (ppm)</u>
I	Control	0	0.039 $\pm$ 0.890	-
II	C-Hexane	900	900 $\pm$ 38	904 $\pm$ 74
III	C-Hexane	3000	3000 $\pm$ 117	2839 $\pm$ 196
IV	C-Hexane	9000	9018 $\pm$ 446	9232 $\pm$ 685

The achieved mean exposure concentration for each group was very close