



Health and Environmental
Sciences Department

An Inhalation Oncogenicity Study of Commercial Hexane in Rats and Mice

Part I—Rats

Final Abridged Report

MARCH 1995

**TOXICOLOGY REPORT NUMBER 404
CAIS NO. 41-33231**

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 rats 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 seven 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
RE-ISSUED 12/14/93

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

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.

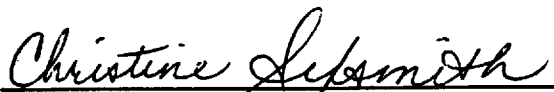
Copies of reports by API Quality Assurance personnel are available upon written request to the Director of the Health and Environmental Sciences Department of the American Petroleum Institute or his designee.

<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

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

Page 2

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
1/11-14/93 (1/20/93)	Draft report audit
4/1-2/93 (4/2/93)	Second draft report audit
4/16/93 (4/16/93)	Final Report Acceptance
ADDED:	
10/14/93 (10/15/93)	Final Report Amendment #1 Review
12/2/93 (12/2/93)	Protocol Amendment #7 Review
12/14/93 (12/14/93)	Final Report Amendment #1 Acceptance


Christine Sexsmith, B.S.
Quality Assurance Coordinator

12/14/93
Date

TABLE OF CONTENTS (abridged report)

<u>Section</u>	<u>Page</u>
Abstract	i
Introduction	1
Materials and Methods	2
Results and Discussion	23
Conclusion	29
Amendments to Final Report	Appendix 1
Mortality Summaries	Appendix 2
Physical Observations	Appendix 3
Body Weight Changes	Appendix 4
Pathology Report	Appendix 5
Laboratory Quality Assurance	Appendix 6
Statement of Compliance	Appendix 7
Table of Contents to Unabridged Report	Appendix 7





PROJECT NO. 88-8137R

AN INHALATION ONCOGENICITY STUDY

OF COMMERCIAL HEXANE IN RATS AND MICE

PART I - RATS

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 rat portion of the study. The mouse 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 29 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 -11, -5 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 of 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 of the lungs in all animals and of selected tissues in all Group I and IV animals and for all animals which died or were sacrificed moribund prior to their scheduled sacrifice.

The cumulative mean exposure concentrations as determined by IR were 900, 3000 and 9016 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 the control group survivorship was 67% in the males and 76% in the females. There was no significant difference in survivorship among the control or exposure groups.

Physical observations, hematological and ophthalmoscopic examinations found no signs of any commercial hexane related effects except for excess lacrimation which was increased in the commercial hexane exposed males at 3000 and 9016 ppm.

Body weight gain was significantly reduced in the 3000 and 9016 ppm exposure groups. At study termination, the mean male and female body weights in the 9016 ppm group were 7 and 11 percent lower than control values respectively. In the 3000 ppm group, mean male and female body weights were 5 and 3 percent lower than control values, respectively, at study termination. Food consumption was reduced in the commercial hexane exposure groups but was not dose related.

There were no macroscopic morphologic abnormalities which were considered to be related to exposure to commercial hexane.

Microscopic morphologic abnormalities which were considered related to commercial hexane were found in the nasal turbinates and the larynx.

Hyperplasia of the respiratory epithelium was seen most frequently in males and females from Group IV (9016 ppm). Hypertrophy/hyperplasia of goblet cells was seen most frequently in males and females from Group IV, followed by Group III (3000 ppm); the severity, greater in the exposure groups than in the controls, was severe in Group IV. Both findings were considered to be an expected response to mucosal irritation and injury.

Intracytoplasmic eosinophilic material in the respiratory epithelial cells and sustentacular cells of the olfactory epithelium was seen in almost all of the males and females from the exposure groups for which the nasoturbinal tissues were examined, and in numerous control animals. When compared to controls, the severity was increased and comparable in Groups III (3000 ppm) and IV (9016 ppm), followed by Group II (900 ppm). While a common finding in aging and aged rats, the exact nature of this material (presumed to be secretory) and reasons for its increase following exposure to irritants are not known.

The following changes were also seen: Inflammatory cells/cell debris in the nasal lumen [males, Groups II (900 ppm), III (3000 ppm) and IV (9016 ppm); and females, Group III]; and subacute (chronic active)/chronic inflammation in the nasal mucosa, the incidence of which was greatest in males and females from Group IV.

Squamous/squamoid metaplasia/hyperplasia of the pseudostratified columnar epithelium was seen in a small number of animals from the exposure and control groups. In the males, the incidence was highest in Group IV (9016 ppm), followed by Group III (3000 ppm). In the females, the incidence in Groups III and IV was comparable and greater than that seen in Group I (0 ppm). This finding was considered to be a localized response indicative of irritation. There was no indication of any oncogenic effect.

In conclusion, under the exposure conditions of this study, commercial hexane is not an oncogen in the rat.

I. INTRODUCTION:

This study, conducted for the American Petroleum Institute, was designed to assess the oncogenic effect of commercial hexane vapor when administered by whole-body inhalation to Fischer 344 rats (50/sex/group) and B6C3F1 mice (50/sex/group). This report presents the results from the rat portion of the study. The mouse 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: 28 December 1989

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

Termination of Exposures: 29 January 1992

Necropsy: 22-30 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)

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:

Rat

Strain:

Fischer 344

Justification for
Animal Selection:

Standard laboratory animal for inhalation toxicity studies. The Fischer 344 rat was used due to its availability and due to the existing historical data base for comparative evaluation.

II. MATERIALS AND METHODS (cont.):

C. Test Animal (cont.):

Number of Animals:

Received:	557 total (279 males, 278 females)									
Placed on Test:	400 total (200 males, 200 females)									
Supplier:	Charles River Breeding Laboratories, Inc. Raleigh, North Carolina 27610									
Date of Birth:	30 November 1989									
Date Received:	28 December 1989									
Age at Receipt (approx.):	4 weeks									
Age at Initiation of Exposure (approx.):	8 weeks									
Weight at Initiation of Exposure (grams):	<table><thead><tr><th></th><th>Mean</th><th>Range</th></tr></thead><tbody><tr><td>Males:</td><td>167</td><td>120 - 181</td></tr><tr><td>Females:</td><td>119</td><td>111 - 126</td></tr></tbody></table>		Mean	Range	Males:	167	120 - 181	Females:	119	111 - 126
	Mean	Range								
Males:	167	120 - 181								
Females:	119	111 - 126								

Acclimation Period: Animals were acclimated for 26 days (28 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 (see page 20) were performed on all sentinel animals. The remaining 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	50/50	50/50	48/50	46/49	33/38	33/38	50/50
II (Low)	900	50/50	50/50	49/48	46/46	30/39	30/39	50/50
III (Mid)	3000	50/50	50/50	49/50	48/49	32/36	32/36	50/50
IV (High)	9000	51 ^d /50	50/50	50/50	48/48	33/39	33/39	50/50
Sentinels	-	12/13 ^e	-	-	-	-	-	-

^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 IV male died on day 8 and was replaced.

^eOne Sentinel female died on day 7 and was 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 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:	511
Dates of Exposure:	23 January 1990 - 29 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 electrically 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 Protectoseal® 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.

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
Month 5: 19 July 1990 (10 animals only)
Termination: 17 January 1992

M. Body Weight:

Three times pretest (Test Days -11, -5 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 scheduled intervals blood was obtained via a tail nick in unanesthetized animals. See Appendix A for Methodology and References.

Time Intervals

Pretest: 8 January - 11 January 1990
Month 12: 22 January - 1 February 1991
Month 18: 29 July - 1 August 1991
Termination: 22 January - 30 January 1992

II. MATERIALS AND METHODS (cont.):

O. Laboratory Studies (cont.):

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: 22 January - 30 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
gonads(ovaries or testes with epididymides)
heart
intestine
cecum
colon
duodenum
ileum
jejunum
rectum

II. MATERIALS AND METHODS (cont.):

P. Postmortem (cont.):

Tissues Preserved (cont.): kidneys (2)
larynx
liver (left lateral and mediag 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 all 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)
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

Preservatives:

Blood and bone 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 of 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. Pretest histopathologic evaluations of the sacrificed sentinel animals were not performed.



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III. RESULTS AND DISCUSSION:

A. Commercial Hexane:

Analyses of commercial hexane from the storage drums (Appendix B-60) and in the exposure atmospheres (Appendix B-58) 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	0.043 $\pm$ 0.973	-
II	C-Hexane	900	900 $\pm$ 38	904 $\pm$ 74
III	C-Hexane	3000	3000 $\pm$ 116	2842 $\pm$ 197
IV	C-Hexane	9000	9016 $\pm$ 445	9241 $\pm$ 685

The achieved mean exposure concentration for each group was very close

III. RESULTS AND DISCUSSION (cont.):

B. Chamber Monitoring (cont.):

to the respective target and nominal concentrations. Commercial hexane was measured in the control chamber on one day but this may have been produced by a technical problem with the instrumentation and may not have been indicative of an actual exposure.

Chamber distribution samples (Appendix B-61) showed there was no significant gradient of the commercial hexane exposure level within any chamber. Oxygen level (Appendix B-62) was at least 19 percent in all chambers. Temperatures and humidities were acceptable.

Monthly particle size distributions (Appendix B) are summarized below:

Group	Test Substance	Target Concentration (ppm)	Average Particle Sizing Data		
			MMAD (μm)	GSD	Total Mass Concentration (mg/m^3)
I	Control	0	2.1	2.5	0.0089
II	c-Hexane	900	2.0	3.0	0.0028
III	c-Hexane	3000	3.1	2.5	0.0032
IV	c-Hexane	9000	4.8	2.3	0.011
		Mean: ^b	3.3	2.6	0.0057

^aMMAD = Mass Median Aerodynamic Diameter;
GSD = Geometric Standard Deviation.

^bMean of test substance exposed groups.

The slightly increased mass concentration at 9000 ppm verses the other groups indicates there may have been some commercial hexane aerosol present in that group. However, because the concentration is so small, this amount of aerosol is not significant.

III. RESULTS AND DISCUSSION (cont.):

C. Mortality:

At termination of the study, there was approximately 67% survival in the control males and 76% in the control females (Appendix C-1 and 2 and Figure 4-1 and 2). Survivorship Analyses (Appendix I) found no statistically significant difference in survivorship among the control or exposure groups.

D. Physical Observations:

Excess lacrimation was observed beginning at week 2 in the 9016 ppm males and then at week 3 in the other groups including control (Appendix D). Subsequently, excess lacrimation was observed in all groups over the duration of the study with an increased incidence in the 3000 and 9016 ppm groups. In the females, excess lacrimation was observed at week 1 in all commercial hexane exposed groups and was subsequently observed in all groups, including control, over the duration of the study. However, in the females, the incidence appeared equal among all the groups and not commercial hexane related.

E. Ophthalmology:

Because of the excess lacrimation and a few corneal scars being observed, an interim ophthalmoscopic examination of the eyes from 10 animals was conducted at month 5. This examination found corneal dystrophy which is common in Fischer 344 rats (Appendix E-2). There was no indication at month 5 or at the study termination of any commercial hexane related ocular effects.

III. RESULTS AND DISCUSSION (cont.):

F. Body Weights and Food Consumption:

Mean body weights and body weight gains (Appendix F and Figure 5, Volume I) were decreased in both males and females exposed to commercial hexane. At 900 ppm the decreased body weight effect was transient. The mean body weights, or change in body weights, were significantly lower than control values for several intervals between weeks 1 to 13 in the females and weeks 6 to 25 in the males. At 3000 ppm mean body weights, or body weight gains, were significantly lower than control values for several intervals between 2 and 33 weeks of exposure in the females and at 6 and 105 weeks in the males. At 9016 ppm, mean body weights or gains were significantly lower than control values for the entire study beginning with week 3 in the females and week 10 in the males.

The mean body weights and percent difference between the commercial hexane exposed groups and the control group at 13 and 105 weeks are given below. There is a small but dose related decrease.

Group ^a	Body Weights (grams)			
	13 weeks		105 weeks	
	Males	Females	Males	Females
Control	290.2	179.4	345.8	250.6
900 ppm	283.4(-2.3%)*	174.4(-2.8%)**	342.8(-0.8%)*	250.7(0%)
3000 ppm	281.3(-3.1%)*	174.9(-2.5%)*	327.3(-5.3%)*	243.3(-2.9%)
9000 ppm	279.3(-3.8%)**	174.3(-2.8%)**	321.3(-7.1%)**	222.5(-11.2%)**

^aSignificantly different from control, $p \leq 0.05$ = * and $p \leq 0.01$ = **.

Food consumption (Appendix F and Figure 6, Volume I) was frequently reduced in all three commercial hexane groups as compared to the control group values. The differences were small, a few percent, and there was no clear dose related response, however, over the duration of the study they could explain the decrease in body weight gains.

III. RESULTS AND DISCUSSION (cont.):

G. Laboratory Studies:

There was no indication (Appendix G) of any commercial hexane related effects in differential leukocyte count or erythrocyte morphology.

H. Macroscopic and Microscopic Pathology:

There were no macroscopic morphologic abnormalities which were considered to be related to exposures to commercial hexane. Microscopic morphologic abnormalities which were considered to be related to commercial hexane were seen in the nasoturbinal tissues and larynx.

1. Nasoturbinal Tissues:

Hyperplasia of the respiratory epithelium was seen most frequently in males and females from Group IV (9016 ppm). Hypertrophy/hyperplasia of goblet cells was seen most frequently in males and females from Group IV, followed by Group III (3000 ppm); the severity, greater in the exposure groups than in the controls, was severe in Group IV. Both findings were considered to be an expected response to mucosal irritation and injury.

Intracytoplasmic eosinophilic material in the respiratory epithelial cells and sustentacular cells of the olfactory epithelium was seen in almost all of the males and females from the exposure groups for which the nasoturbinal tissues were examined, and in numerous control animals. When compared to controls, the severity was increased and comparable in Groups III (3000 ppm) and IV (9016 ppm), followed by Group II (900 ppm). While a common finding in aging and aged rats, the exact nature of this material (presumed to be secretory) and reasons for its increase following exposure to irritants are not known.

III. RESULTS AND DISCUSSION (cont.):

H. Macroscopic and Microscopic Pathology (cont.):

1. Nasoturbinal Tissues (cont.):

The following changes were also seen: Inflammatory cells/cell debris in the nasal lumen [males, Groups II (900 ppm), III (3000 ppm) and IV (9016 ppm); and females, Group III]; and subacute (chronic active)/chronic inflammation in the nasal mucosa, the incidence of which was greatest in males and females from Group IV.

2. Larynx:

Squamous/squamoid metaplasia/hyperplasia of the pseudostratified columnar epithelium was seen in a small number of animals from the exposure and control groups. In the males, the incidence was highest in Group IV (9016 ppm), followed by Group III (3000 ppm). In the females, the incidence in Groups III and IV was comparable and greater than that seen in Group I (0 ppm). This finding was considered to be a localized response indicative of irritation.

Other morphologic findings in tissues and organs which were examined by light microscopy occurred with comparable incidence and severities in animals from the control and exposure groups or they occurred sporadically. None of these were considered to be related to the whole-body exposure to atmospheres which contained commercial hexane.

Exposures to commercial hexane at levels up to 9016 ppm had no oncogenic effect in Fischer 344 Rats.

I. Sentinel Animal Data:

Pretest differential leukocyte counts were performed on all sentinel animals (Appendix J). These evaluations showed that all animals were healthy. One sentinel animal died on Test Day 7 and was replaced; three additional animals died spontaneously during the study. All other sentinel animals were euthanized at study termination and their carcasses were discarded.

IV. CONCLUSION:

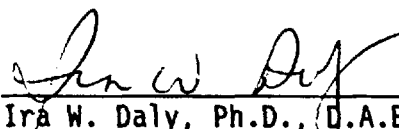
Fischer 344 rats were exposed for six hours per day, five days per week for 24 months to 0, 900, 3000 and 9016 ppm of commercial hexane. These exposures produced a decrease in body weight gain in the 3000 and 9016 ppm exposure groups and transiently in the 900 ppm group. Excess lacrimation was observed in the males at 3000 and 9016 ppm. Survivorship at 24 months in the control group was 67% in the males and 76% in the females. Survivorship was not affected by the commercial hexane exposures. The commercial hexane exposures produced no test substance related changes in ophthalmology, hematology parameters or macroscopic findings. Microscopically, localized responses indicative of irritation were seen in the 9016 ppm group in the nasoturbinal tissues and the larynx. These exposures did not produce an oncogenic effect. In conclusion, under the exposure conditions of this study, commercial hexane is not an oncogen in the rat.



Paul E. Newton, Ph.D., D.A.B.T.
Study Director
Director of Inhalation Toxicology

4/13/93

Date



Ira W. Daly, Ph.D., D.A.B.T.
Senior Vice President and
Director of Toxicology

4/14/93

Date

APPENDIX 1

Appendix 1 of this report presents additional microscopic evaluation of nasal tissue not included in the Final report. At API's request, mid-dose animals were re-evaluated microscopically. For diagnostic consistency, unscheduled deaths in previously examined control and high-dose group animals as well as mid-dose animals were re-read. The results are presented in this amendment.



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PROJECT NO. 88-8137R
(SPONSOR NO. API-PS-71-600-9)

AN INHALATION ONCOGENICITY STUDY
OF COMMERCIAL HEXANE IN RATS

Part I - Rats

Final Report

VOLUME I OF VII

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

Attn: Dr. Richard Rhoden

Date: April 14, 1993

An Inhalation Oncogenicity Study
of Commercial Hexane in Rats and Mice

Report Amendments

The following is a list of changes made to the final report.

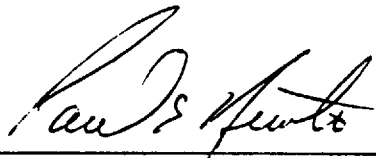
Page No.	Change no. 1	Reason for Change
Volumes I, V, VI, VII:		
Tables of Contents	Pagination, Appendix H.	*Additional histopathologic evaluations of rat nasopharyngeal tissue.
Volumes I, IV, V, VI, VII:		
Tables of Contents	Addition of "H-" to pages referencing the pathology appendix.	For consistency with other appendices; to clarify which pages are included in the pathology appendix.
Volume I:		
Table of Contents and Appendix N	Relocation of Report Amendments listing and deletion of reference to that listing as "Appendix N".	Request of Sponsor to have this list of changes relocated.
Abstract, all pages	Revision of paragraphs involving the discussion of microscopic findings in the nasoturbinal tissues and the larynx.	*Additional histopathologic evaluations of rat nasopharyngeal tissue.
p. 20	Revision of the beginning of the paragraph, amended to read, "The lungs and nasal turbinates were examined...."	Clarification of work done.

*At the Sponsor's request, the nasal turbinates from the mid-dose groups were to be evaluated histopathologically. For diagnostic consistency, the previously examined control and high dose group nasal turbinates and those of mid-dose unscheduled deaths were reread at the time that the additional work was done. This report presents the re-evaluation of the corresponding nasal turbinate tissue and the original diagnoses for the mid-dose nasal turbinates.

An Inhalation Oncogenicity Study
of Commercial Hexane in Rats and Mice

Report Amendments (cont.)

<u>Page No.</u>	<u>Change no. 1</u>	<u>Reason for Change</u>
Volume I (cont.):		
pp. 27-30	Revision of paragraphs involving the discussion of microscopic findings in the nasoturbinal tissues and the larynx.	*Additional histopathologic evaluations of rat nasopharyngeal tissue.
Appendix H pp. 4-15	Revision of paragraphs involving the discussion of microscopic findings in the nasoturbinal tissues and the larynx.	*Additional histopathologic evaluations of rat nasopharyngeal tissue.
Appendix L	Revised and re-signed.	Audit of additional histopathology and report amendments.
Volumes I, V, VI, VII:		
Tables IV, VIII, IX, X	Revised histopathology data	*Additional histopathologic evaluations of rat nasopharyngeal tissue.


Paul E. Newton, Ph.D., D.A.B.T.
Study Director
Director of Inhalation Toxicology

12/10/83
Date

*At the Sponsor's request, the nasal turbinates from the mid-dose groups were to be evaluated histopathologically. For diagnostic consistency, the previously examined control and high dose group nasal turbinates and those of mid-dose unscheduled deaths were reread at the time that the additional work was done. This report presents the re-evaluation of the corresponding nasal turbinate tissue and the original diagnoses for the mid-dose nasal turbinates.



PROJECT NO. 88-8137R

AN INHALATION ONCOGENICITY STUDY

OF COMMERCIAL HEXANE IN RATS AND MICE

PART I - RATS

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 rat portion of the study. The mouse 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 29 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 -11, -5 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 of 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 of the lungs and nasal turbinates in all animals and of selected tissues in all Group I and IV animals and for all animals which died or were sacrificed moribund prior to their scheduled sacrifice.

The cumulative mean exposure concentrations as determined by IR were 900, 3000 and 9016 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 the control group survivorship was 67% in the males and 76% in the females. There was no significant difference in survivorship among the control or exposure groups.

Physical observations, hematological and ophthalmoscopic examinations found no signs of any commercial hexane related effects except for excess lacrimation which was increased in the commercial hexane exposed males at 3000 and 9016 ppm.

Body weight gain was significantly reduced in the 3000 and 9016 ppm exposure groups. At study termination, the mean male and female body weights in the 9016 ppm group were 7 and 11 percent lower than control values respectively. In the 3000 ppm group, mean male and female body weights were 5 and 3 percent lower than control values, respectively, at study termination. Food consumption was reduced in the commercial hexane exposure groups but was not dose related.

There were no macroscopic morphologic abnormalities which were considered to be related to exposure to commercial hexane.

Microscopic morphologic abnormalities which were considered related to commercial hexane were found in the nasal turbinates and the larynx. The incidence and/or severities of these findings in the exposure groups were increased when compared to the respective controls and were considered to be the expected responses to mucosal irritation and injury. In the nasoturbinal tissues:

1. Hypertrophy/hyperplasia of goblet cells, seen in numerous males and females, tended to occur more frequently in the exposure groups than in the controls. The severity showed a dose related increase.
2. Hyperplasia of the respiratory epithelium was seen more frequently and with greater severity in the exposure groups, where the incidence showed a dose related increase, as compared to controls.
3. Intracytoplasmic eosinophilic material in the respiratory and submucosal glandular epithelium and in the sustentacular cells of the olfactory epithelium were seen more frequently and with greater severity in the exposure groups than in the controls. In the males, the severities in Groups III and IV (3000 and 9016 ppm, respectively) were comparable and greater than that seen in Group II (900 ppm). In the females the severities in Groups II, III and IV were essentially similar.
4. Microscopic findings associated with inflammatory changes seen in a number of males and females from the exposure and control groups occurred most frequently as follows: a) subacute (chronic active)/chronic inflammation of the nasal mucosa in the males, followed by the females, from Group IV (9016) ppm; b) inflammatory cells/cell debris in the nasal lumen in males from Group IV; c) edema of the nasal mucosa in males from Group IV.
5. A no observable effect level (NOEL) was not seen in the nasoturbinal tissues of the rats in this study.

In the larynx, squamous/squamoid metaplasia/hyperplasia of the pseudostratified columnar epithelium was seen in a small number of animals from the control and exposure groups. In the males, the highest incidence was seen in Group IV (9016 ppm), followed by Group III (3000 ppm). In the females, the incidence in Groups III and IV was comparable, and greater than that seen in Group I (0 ppm). This finding was considered to be a localized response indicative of irritation. In all of the affected animals, the metaplastic epithelium was well differentiated and organized, with no evidence of atypia or dysplasia.

There was no indication of any oncogenic effect.

In conclusion, under the exposure conditions of this study, commercial hexane is not an oncogen in the rat.

**Documents**

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TABLE OF CONTENTS

VOLUME I

REPORT AMENDMENTS

ABSTRACT

I. INTRODUCTION.....1

II. MATERIALS AND METHODS

A. Study Dates.....	2
B. Test Substance.....	2
C. Test Animal.....	3
D. Selection.....	4
E. Group Assignment.....	5
F. Animal Identification.....	5
G. Experimental Outline.....	5
H. Husbandry.....	6
I. Test Substance Administration and Chamber Operation.....	7
J. Exposure Chamber Sampling.....	15
K. Physical Observations.....	17
L. Ophthalmoscopic Examination.....	17
M. Body Weight.....	17
N. Food Consumption.....	17
O. Laboratory Studies.....	17
P. Postmortem.....	18
Q. Statistical Analysis.....	21
R. Protocol Deviations.....	22

III. RESULTS AND DISCUSSION

A. Commercial Hexane.....	23
B. Chamber Monitoring.....	23
C. Mortality.....	25
D. Physical Observations.....	25
E. Ophthalmology.....	25
F. Body Weights and Food Consumption.....	26
G. Laboratory Studies.....	27
H. Macroscopic and Microscopic Pathology.....	27
I. Sentinel Animal Data.....	28

IV. CONCLUSION.....29

FIGURES

1. Diagram of 10,000 Liter Chamber.....	F 1-1
2. Diagram of Generation System Group II - IV.....	F 2-1
