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Petroleum
Institute

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
CAIS NO. 41-33232

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.

Date(s) of Inspection/Review (Reported to Management)	Type of Inspection
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 Mice

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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Bio/dynamics, Inc.

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.

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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: (Date Study Director signed the protocol)	14 September 1989
Receipt of Test Animals:	27 December 1989
Initiation of Exposures: (Experimental Start Date)	23 January 1990
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)

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.

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 weeks

Weight 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]: Pretest (M/F)	Blood Smears[b]: Month 12 (M/F)	Blood Smears[b]: Month 18 (M/F)	Blood Smears[b]: Termination (M/F)	Terminal Necropsy (M/F)	Histopathology[c] (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	-	-	-	-	-	-

[a] Exposures 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.
[b] Only slides from Groups I and IV were evaluated at Month 12, Month 18 and termination.
[c] Full 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.
[d] One Group I female died on day 11 and was replaced.
[e] Three 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 dispenced 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, N_2 generator flow rate, total flow rate, oxygen content, time for air change and time for equilibration (T_{99}) for each group are summarized below:

Group	Flow Rate (lpm) N_2	Air	Total	O_2 (%)	Air Change (min.)	T_{99} (min.)
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 Protectosea'® 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

[1]A 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

[2]A 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 Glas-col® 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:

Pump	Initial Pump Setting[a] (%)	Piston Size (inch)
A	20	3/8
B	10	3/8
C	18	1/4

[a]A 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 O_2 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 (retrobular venous plexsus) 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 bladder[3]
- uterus (corpus and cervix uteri)
- vagina/penis
- gross lesions (including a section of normal-appearing portion of same tissue)
- tissue masses

[3]Inflated 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 affecte e results of this study:

1. The results from 16 feed analyse: 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:

Component	Percentage
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 ± standard deviation) of c-hexane (commercial hexane) for each exposure group are presented in Appendix B, plotted in Figure 3 and are summarized below:

Group	Test Substance	Target Concentration (ppm)	Analytical Concentration (ppm)	Nominal Concentration (ppm)
I	Control	0	0.039 ± 0.890	-
II	C-Hexane	900	900 ± 38	904 ± 74
III	C-Hexane	3000	3000 ± 117	2839 ± 196
IV	C-Hexane	9000	9018 ± 446	9232 ± 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 two days 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-57) showed there was no significant gradient of the commercial hexane exposure level within any chamber. Oxygen levels (Appendix B-58) were 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	2.2	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	4.9	2.3	0.011
		Mean:[b]	3.1	2.6	0.0057

[a]MMAD = Mass Median Aerodynamic Diameter;
GSD = Geometric Standard Deviation.
[b]Mean 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 85% survival in the control males and 80% 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 exposure groups.

D. Physical Observations:

Weekly detailed observations (Appendix D) found no commercial hexane related effects.

E. Ophthalmology:

Ophthalmoscopic examination found no commercial hexane related ocular effects.

F. Body Weights and Food Consumption:

Mean body weights and body weight gains in the exposed animals (Appendix F, Volume I) were not statistically different from control values in the male mice. However, food consumption in the 9000 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 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.

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:

Macroscopic:

There was an increase in liver masses and nodules among Group IV (9018 ppm) females but not among treated males. There was a slight dose- related decrease in the incidence of cysts of the uterus among females, as shown in the table below. The significance of these observations will be discussed under the microscopic section.

Macroscopic Incidence of Uterine Cysts

Group	I	II	III	IV
No. Examined	50	50	49	50
Uterine Cysts	21	18	14	11

There were no other treatment-related macroscopic changes. The other macroscopic observations were not unusual for mice of this strain and age.

Microscopic:

Treatment-related changes were present in the liver (neoplastic) and uterus (non-neoplastic) of Group IV (9018 ppm) females. There was no similar treatment-related neoplastic change in the liver of males. There was an increase in proliferative lesions of the pituitary among females (hyperplasia, adenoma and adenocarcinoma) at all three treatment levels when compared to the controls. This change may not be treatment-related because of an unusually low incidence among controls.

III. RESULTS AND DISCUSSION (cont.):

H. Macroscopic and Microscopic Pathology (cont.):

Microscopic (cont.):

The incidence of liver tumors for males and females is tabulated below:

Liver Neoplasia Females

Group	I	II	III	IV
No. Examined	50	50	49	50
Hepatocellular Adenoma	4	6	4	10
Hepatocellular Carcinoma	3	2	5	6
Total Neoplasms	7	8	9	16

Liver Neoplasia Males

Group	I	II	III	IV
No. Examined	49	50	50	50
Hepatocellular Adenoma	10	5	7	10
Hepatocellular Carcinoma	7	11	10	3
Total Neoplasms	17	16	17	13

The increase in hepatocellular adenomas and carcinomas for females, individually or combined as total hepatocellular neoplasms, is considered to be treatment-related. For females, the incidence of benign liver tumors was statistically significant for trend at the 0.04 level. There were no significant pairwise differences. The incidence of malignant liver tumors was not significant for trend or pairwise comparisons. When benign and malignant tumors were combined there was a statistically significant trend at the 0.01 level and a statistically significant pairwise comparison of the high dose group and control group at the 0.06 level by Cox's test and 0.04 level by the GB/KW test. It should be noted that the incidence among Group IV females is similiar to the incidence among control males.

Liver tumors among males were not treatment-related. There was no treatment-related increase in any other lesions of the liver, including foci of cellular alteration, among males or females.

III. RESULTS AND DISCUSSION (cont.):

H. Macroscopic and Microscopic Pathology (cont.):

Microscopic (cont.):

There was a treatement-related decrease in the severity and a slight decrease in the incidence of cystic endometrial hyperplasia of the uterus among Group IV females when compared to controls. The decrease in severity was statistically significant but the slight decrease in incidence was not statistically significant. This correlated with the dose related decrease in uterine cysts observed at necropsy among Group IV females when compared to controls. Cystic endometrial hyperplasia of the uterus is a common age change among mice of this strain and is thought to be related to a change in the hormonal balance with aging. The incidence and severity of this change is tabulated below:

Incidence & Severity Cystic Endometrial Hyperplasia

Group	I	II	III	IV
No. Examined	50	32	32	50
Cystic Endometrial Hyperplasia	48	29	27	44
trace	3	4	3	10
mild	14	13	8	19
moderate	23	10	15	15
severe	8	2	1	0

There was an increase in the incidence of proliferative lesions of the pituitary (hyperplasia, adenoma and carcinoma) among all treated groups of females when compared to controls. A similar increase was not present among males. There was no dose related response among the females. The incidence was similar among all three treated groups. The treatment relationship of this observation is unclear.

III. RESULTS AND DISCUSSION (cont.):

H. Macroscopic and Microscopic Pathology (cont.):

Microscopic (cont.):

It may be related to an unusually low incidence among the control females. The incidence of pituitary lesions for females is tabulated below:

Incidence of Pituitary Proliferative Lesions for Females

Group	I	II	III	IV
No. Examined	45	48	48	49
Hyperplasia	2	4	4	6
Adenoma	0	6	7	5
Adenocarcinoma	0	0	1	0
Total Neoplasms	0	6	8	5
Total Proliferative Lesions	2	10	12	11

There were no other treatment related neoplastic or non neoplastic changes present among males or females on this study. All of the other changes and lesions were spontaneous, agonal or technique related. The lesions observed were not unusual for mice of this strain and age. There was an increase in the incidence of focal lymphocytic infiltration in the Group IV lacrimal glands, but this was considered to be spurious. This lesion is a common spontaneous finding in mice and it was trace in severity.

The decrease in the severity of cystic endometrial hyperplasia of the uterus in the Group IV females may indicate a possible treatment-related alteration in the hormonal balance such as a decrease in estrogenic stimulation of the uterus. 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. The evidence for a treatment-related hormonal effect on the uterus in the females may suggest that the females are expressing the normal male incidence for hepatocellular tumors.

III. RESULTS AND DISCUSSION (cont.):

H. Macroscopic and Microscopic Pathology (cont.):

Microscopic (cont.):

The significance of the pituitary proliferative changes is unclear. The lack of dose response (the incidence is similar for all treated groups), suggests that the change may not be treatment related. The apparent increase could be a result of a low incidence among control females. Control data for B6C3F1 mice published by Charles River (P.L. Lang, 1989) indicates a mean of 7.9% with a range of 0 to 29% for adenomas of the pars distalis. This compares to 10% for Group IV females and 14% for Group III females on this study.

I. Time to Tumor Analyses:

Time to Tumor analyses was conducted in the males for: malignant liver carcinoma, benign liver adenomas and combined liver carcinoma and adenomas. The results (Appendix I) found the test substance was not related to benign or malignant tumor production or time to tumor.

In the females four groups of tumors were tested: benign pituitary adenomas, benign liver adenomas, malignant liver carcinomas and combined liver carcinomas and adenomas. Statistically there was a marginal effect in the high exposure group for each benign and malignant tumor and a definite effect only when they were combined.

J. Sentinel Animal Data:

Pretest differential leukocyte counts were performed on all sentinel animals (Appendix J). These evaluations showed that all animals were healthy. Six animals died prior to study termination. All other sentinel animals were euthanized at study termination and their carcasses were discarded.

IV. CONCLUSION:

B6C3F1 mice were exposed for six hours per day, five days per week for 24 months to 0, 900, 3000 and 9018 ppm of commercial hexane. These exposures produced an effect in the females at 9018 ppm, body weight gain was reduced and the mean body weight was significantly lower than control after week 29. By week 53 the female mice weighed 14% less than control. Mean food consumption values were not significantly effected by the commercial hexane exposures. However, there was a dose related trend on many days. For the females with the lower body weight in the 9018 ppm group food consumption was lower than control 19 out of 20 times at the beginning of the study. The commercial hexane exposures produced no test substance related changes in ophthalmology, hematology parameters or morphologic findings. Survivorship at 24 months in the control group was 85% in the males and 80% in the females. Survivorship was not affected by the commercial hexane exposures. Macroscopic examinations found an increase in liver masses and nodules among the females in the 9018 ppm group but not among the males.

Microscopic examinations found a treatment related increase in hepatocellular neoplasms (adenoma and carcinoma) among females in the 9018 ppm group. A similar change was not present in the males. The incidence of these neoplasms in the females in the 9018 ppm group was similar to the incidence in the control males. The NOEL for the neoplastic changes was 3000 ppm.

IV. CONCLUSION (cont.):

There was 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 mice.

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APPENDIX 1

Mortality Summary[a]
Male Mice

Group (ppm)	Number of Animals on Test	Month 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Total	Percent Survival
I Control	50	0	0 (1)	0	0 (1)	0	0	0	0	0	0	1	0	0	0	0	0	0	1	2	1	1	0	1	0	7	85
II 900	50	0	0 (1)	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	3	0	0	2	0	2	9	82
III 3000	50	0	0 (1)	0	0	0	0	0	0	0	0	0	0 (1)	0	1	0	0	0	2	0	0	0	1	1	0	5	90
IV 9000	50	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2	0	1	2	2	8	84

[a]Includes spontaneous deaths and moribund sacrifices that were not accidentally related; accidental or "other" deaths are presented in parentheses and were not included in the calculation for percent survival.

Mortality Summary[a]
Female Mice

Group (ppm)	Number of Animals on Test	Month 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Total	Percent Survival
I Control	51[b]	1[b]	0	0	0	0	0 (1)	0	0	0	0	0	0	0	0	2	0	0	2	0	0	1	1	2	2	10	80
II 900	50	0	0 (1)	0	0 (1)	0 (1)	0	0	0	0	0	0	0	1	0	1	0	2	0	1	1	1	0	0	3	10	79
III 3000	53[c]	3[c]	0	0 (1)	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	3	2	3	1	14	71
IV 9000	50	0	0	0	0	0	0 (1)	0	0	0	0	0	0	0	0	0	0	0	2	1	2	2	1	2	1	11	78

[a]Includes spontaneous deaths and moribund sacrifices that were not accidentally related; accidental or "other" deaths are presented in parentheses and were not included in the calculation for percent survival.
[b]One Group I female died on Day 11 and was replaced. This animal was not included in the the calculation for percent survival.
[c]Three Group III females died on Days 10, 22 and 26 and were replaced. These animals were not included in the calculation for percent survival.

Appendix C (cont.)
An Inhalation Oncogenicity Study
of Commercial Hexane in Rats and Mice

Animal Termination History - Male Mice

Group (ppm)	An. No.	Date of Death	Type of Death[d]	Days on Test[e]	An. No.	Date of Death	Type of Death[d]	Days on Test[e]
I	1050	8/7/91	M	562	1075	1/15/92	T	723
Control	1051	1/13/92	T	721	1076	1/15/92	T	723
	1052	3/2/90	A	39	1077	1/15/92	T	723
	1053	1/13/92	T	721	1078	1/16/92	T	724
	1054	1/13/92	T	721	1079	5/10/90	E	108
	1055	9/23/91	S	609	1080	1/16/92	T	724
	1056	1/13/92	T	721	1081	1/16/92	T	724
	1057	1/13/92	T	721	1082	1/16/92	T	724
	1058	1/13/92	T	721	1083	1/16/92	T	724
	1059	7/16/91	M	540	1084	1/16/92	T	724
	1060	1/13/92	T	721	1085	1/16/92	T	724
	1061	12/9/91	S	686	1086	1/17/92	T	725
	1062	1/14/92	T	722	1087	1/17/92	T	725
	1063	1/14/92	T	722	1088	1/17/92	T	725
	1064	1/14/92	T	722	1089	1/17/92	T	725
	1065	1/14/92	T	722	1090	1/17/92	T	725
	1066	1/14/92	T	722	1091	1/17/92	T	725
	1067	1/14/92	T	722	1092	1/17/92	T	725
	1068	1/14/92	T	722	1093	1/20/92	T	728
	1069	1/15/92	T	723	1094	10/13/91	S	629
	1070	1/15/92	T	723	1095	1/20/92	T	728
	1071	12/17/90	M	329	1096	1/20/92	T	728
	1072	1/15/92	T	723	1097	1/20/92	T	728
	1073	8/16/91	S	571	1098	1/20/92	T	728
	1074	1/15/92	T	723	1099	1/21/92	T	729

[d]A = Accidental death, M = Moribund sacrifice, E = Animal escaped, S = Spontaneous death and T = Terminal sacrifice.
[e]Days on Test includes days on which animals were not exposed to the test substance.

Appendix C (cont.)
An Inhalation Oncogenicity Study
of Commercial Hexane in Rats and Mice

Animal Termination History - Male Mice (cont.)

Group (ppm)	An. No.	Date of Death	Type of Death[d]	Days on Test[e]	An. No.	Date of Death	Type of Death[d]	Days on Test[e]
II	2050	1/13/92	T	721	2075	1/16/92	T	724
900	2051	1/13/92	T	721	2076	1/15/92	S	723
	2052	1/13/92	T	721	2077	1/16/92	T	724
	2053	11/19/91	S	666	2078	1/17/92	T	725
	2054	1/13/92	T	721	2079	1/17/92	T	725
	2055	1/13/92	T	721	2080	9/29/90	S	250
	2056	1/13/92	T	721	2081	11/6/91	S	653
	2057	1/2/92	S	710	2082	7/24/91	S	548
	2058	1/14/92	T	722	2083	1/17/92	T	725
	2059	1/14/92	T	722	2084	3/1/90	A	38
	2060	1/14/92	T	722	2085	1/17/92	T	725
	2061	1/14/92	T	722	2086	1/17/92	T	725
	2062	1/14/92	T	722	2087	1/17/92	T	725
	2063	1/14/92	T	722	2088	1/20/92	T	728
	2064	1/15/92	T	723	2089	8/4/91	S	559
	2065	1/15/92	T	723	2090	1/20/92	T	728
	2066	1/15/92	T	723	2091	1/20/92	T	728
	2067	1/15/92	T	723	2092	1/20/92	T	728
	2068	1/15/92	T	723	2093	1/20/92	T	728
	2069	7/26/90	S	185	2094	1/20/92	T	728
	2070	1/15/92	T	723	2095	1/21/92	T	729
	2071	1/16/92	T	724	2096	1/21/92	T	729
	2072	1/16/92	T	724	2097	1/21/92	T	729
	2073	8/11/91	S	566	2098	1/21/92	T	729
	2074	1/16/92	T	724	2099	1/21/92	T	729

[d]A = Accidental death, S = Spontaneous death and T = Terminal sacrifice.
[e]Days on Test includes days on which animals were not exposed to the test substance.

Appendix C (cont.)
An Inhalation Oncogenicity Study
of Commercial Hexane in Rats and Mice

Animal Termination History - Male Mice (cont.)

Group (ppm)	An. No.	Date of Death	Type of Death[d]	Days on Test[e]	An. No.	Date of Death	Type of Death[d]	Days on Test[e]
III	3050	1/13/92	T	721	3075	1/16/92	T	724
3000	3051	1/13/92	T	721	3076	1/16/92	T	724
	3052	1/13/92	T	721	3077	1/16/92	T	724
	3053	1/13/92	T	721	3078	1/17/92	T	725
	3054	1/13/92	T	721	3079	1/17/92	T	725
	3055	1/13/92	T	721	3080	1/17/92	T	725
	3056	11/26/91	S	673	3081	1/17/92	T	725
	3057	1/14/92	T	722	3082	1/17/92	T	725
	3058	1/14/92	T	722	3083	1/17/92	T	725
	3059	1/14/92	T	722	3084	1/20/92	T	728
	3060	1/14/92	T	722	3085	1/20/92	T	728
	3061	1/14/92	T	722	3086	1/20/92	T	728
	3062	1/14/92	T	722	3087	1/20/92	T	728
	3063	1/15/92	T	723	3088	1/20/92	T	728
	3064	1/15/92	T	723	3089	12/26/90	A	338
	3065	1/15/92	T	723	3090	1/20/92	T	728
	3066	1/15/92	T	723	3091	1/21/92	T	729
	3067	3/3/91	S	405	3092	11/13/91	S	660
	3068	1/15/92	T	723	3093	1/21/92	T	729
	3069	1/15/92	T	723	3094	1/21/92	T	729
	3070	1/16/92	T	724	3095	1/21/92	T	729
	3071	1/16/92	T	724	3096	1/21/92	T	729
	3072	7/6/91	S	530	3097	3/8/90	A	45
	3073	1/16/92	T	724	3098	1/21/92	T	729
	3074	7/21/91	S	545	3099	1/21/92	T	729

[d]A = Accidental death, M = Moribund sacrifice, S = Spontaneous death and T = Terminal sacrifice.
[e]Days on Test includes days on which animals were not exposed to the test substance.

Appendix C (cont.)
An Inhalation Oncogenicity Study
of Commercial Hexane in Rats and Mice

Animal Termination History - Male Mice (cont.)

Group (ppm)	An. No.	Date of Death	Type of Death[d]	Days on Test[e]	An. No.	Date of Death	Type of Death[d]	Days on Test[e]
IV	4050	1/13/92	T	721	4075	1/16/92	T	724
9000	4051	1/13/92	T	721	4076	1/17/92	T	725
	4052	1/13/92	T	721	4077	1/17/92	T	725
	4053	1/13/92	T	721	4078	1/17/92	T	725
	4054	1/13/92	T	721	4079	1/17/92	T	725
	4055	1/13/92	T	721	4080	11/29/91	M	676
	4056	1/14/92	T	722	4081	1/17/92	T	725
	4057	1/14/92	T	722	4082	1/17/92	T	725
	4058	1/14/92	T	722	4083	9/12/91	M	598
	4059	1/14/92	T	722	4084	9/24/90	S	245
	4060	12/15/91	S	692	4085	1/20/92	T	728
	4061	1/14/92	T	722	4086	1/20/92	T	728
	4062	1/14/92	T	722	4087	1/20/92	T	728
	4063	1/15/92	T	723	4088	1/17/92	M	725
	4064	1/15/92	T	723	4089	1/20/92	T	728
	4065	1/15/92	T	723	4090	1/20/92	T	728
	4066	1/15/92	T	723	4091	1/6/92	S	714
	4067	11/21/91	S	668	4092	1/21/92	T	729
	4068	1/15/92	T	723	4093	1/21/92	T	729
	4069	1/15/92	T	723	4094	1/21/92	T	729
	4070	1/16/92	T	724	4095	9/23/91	S	609
	4071	1/16/92	T	724	4096	1/21/92	T	729
	4072	1/16/92	T	724	4097	1/21/92	T	729
	4073	1/16/92	T	724	4098	1/21/92	T	729
	4074	1/16/92	T	724	4099	1/21/92	T	729

[d]M = Moribund sacrifice, S = Spontaneous death and T = Terminal sacrifice.
[e]Days on Test includes days on which animals were not exposed to the test substance.

Appendix C (cont.)
An Inhalation Oncogenicity Study
of Commercial Hexane in Rats and Mice

Animal Termination History - Female Mice

Group (ppm)	An. No.	Date of Death	Type of Death[d]	Days on Test[e]	An. No.	Date of Death	Type of Death[d]	Days on Test[e]
I	1550	1/13/92	T	721	1575	7/12/90	A	171
Control	1551	1/13/92	T	721	1576	1/16/92	T	724
	1552	1/13/92	T	721	1577[f]	2/2/90	A	11
	1553	1/13/92	T	721	1578	1/16/92	T	724
	1554	4/12/91	M	445	1579	1/16/92	T	724
	1555	1/13/92	T	721	1580	12/16/91	M	693
	1556	1/13/92	T	721	1581	1/16/92	T	724
	1557	1/13/92	T	721	1582	9/30/91	M	616
	1558	1/14/92	T	722	1583	4/15/91	M	448
	1559	1/14/92	T	722	1584	12/27/91	M	704
	1560	1/14/92	T	722	1585	1/17/92	T	725
	1561	1/14/92	T	722	1586	1/17/92	T	725
	1562	1/14/92	T	722	1587	1/17/92	T	725
	1563	1/14/92	T	722	1588	1/8/92	M	716
	1564	1/14/92	T	722	1589	1/17/92	T	725
	1565	1/15/92	T	723	1590	1/17/92	T	725
	1566	1/15/92	T	723	1591	12/7/91	S	684
	1567	1/15/92	T	723	1592	1/17/92	T	725
	1568	11/14/91	S	661	1593	7/13/91	S	537
	1569	1/15/92	T	723	1594	1/20/92	T	728
	1570	1/15/92	T	723	1595	6/30/91	S	524
	1571	1/15/92	T	723	1596	1/20/92	T	728
	1572	1/15/92	T	723	1597	1/20/92	T	728
	1573	1/16/92	T	724	1598	1/20/92	T	728
	1574	1/16/92	T	724	1599	1/21/92	T	729
					1600[g]	1/16/92	T	713

[d]A = Accidental death, M = Moribund sacrifice, S = Spontaneous death and T = Terminal sacrifice.
[e]Days on Test includes days on which animals were not exposed to the test substance.
[f]Animal replaced.
[g]Replaced Animal Number 1577.

Appendix C (cont.)
An Inhalation Oncogenicity Study
of Commercial Hexane in Rats and Mice

Animal Termination History - Female Mice (cont.)

Group (ppm)	An. No.	Date of Death	Type of Death[d]	Days on Test[e]	An. No.	Date of Death	Type of Death[d]	Days on Test[e]
II	2550	1/13/92	T	721	2575	1/16/92	T	724
900	2551	1/13/92	T	721	2576	1/16/92	T	724
	2552	1/13/92	T	721	2577	1/16/92	T	724
	2553	1/13/92	T	721	2578	1/16/92	T	724
	2554	1/13/92	T	721	2579	1/16/92	T	724
	2555	6/6/90	A	135	2580	1/16/92	T	724
	2556	1/13/92	T	721	2581	10/10/91	S	626
	2557	1/14/92	T	722	2582	1/17/92	T	725
	2558	6/19/91	S	513	2583	1/17/92	T	725
	2559	8/12/91	S	567	2584	1/17/92	T	725
	2560	1/14/92	T	722	2585	3/26/91	S	428
	2561	1/14/92	T	722	2586	1/17/92	T	725
	2562	5/23/90	A	121	2587	12/27/91	S	704
	2563	1/14/92	T	722	2588	1/17/92	T	725
	2564	1/28/91	M	371	2589	1/17/92	T	725
	2565	1/14/92	T	722	2590	1/20/92	T	728
	2566	1/14/92	T	722	2591	1/20/92	T	728
	2567	1/15/92	S	723	2592	1/20/92	T	728
	2568	1/15/92	T	723	2593	1/20/92	T	728
	2569	1/15/92	T	723	2594	1/20/92	T	728
	2570	9/9/91	S	595	2595	2/26/90	A	35
	2571	1/15/92	T	723	2596	1/20/92	T	728
	2572	1/15/92	T	723	2597	1/21/92	T	729
	2573	6/19/91	S	513	2598	1/21/92	T	729
	2574	1/15/92	T	723	2599	1/20/92	M	728

[d]A = Accidental death, M = Moribund sacrifice, S = Spontaneous death and T = Terminal sacrifice.
[e]Days on Test includes days on which animals were not exposed to the test substance.

Appendix C (cont.)
An Inhalation Oncogenicity Study
of Commercial Hexane in Rats and Mice

Animal Termination History - Female Mice (cont.)

Group (ppm)	An. No.	Date of Death	Type of Death[d]	Days on Test[e]	An. No.	Date of Death	Type of Death[d]	Days on Test[e]
III	3550	5/21/90	S	119	3575	1/16/92	T	724
3000	3551	1/13/92	T	721	3576	1/16/92	T	724
	3552	1/13/92	T	721	3577	1/16/92	T	724
	3553	1/13/92	T	721	3578[f]	2/17/90	O	26
	3554	1/13/92	T	721	3579	1/16/92	T	724
	3555	11/11/91	S	658	3580[f]	2/1/90	A	10
	3556	1/13/92	T	721	3581	1/17/92	T	725
	3557	1/13/92	T	721	3582	1/17/92	T	725
	3558	1/14/92	T	722	3583	9/26/91	S	612
	3559	12/3/91	M	680	3584	4/19/90	E	87
	3560	1/14/92	T	722	3585	9/5/91	M	591
	3561	1/14/92	T	722	3586	1/17/92	T	725
	3562	1/14/92	T	722	3587	1/17/92	T	725
	3563	12/11/91	M	688	3588	1/17/92	T	725
	3564	1/14/92	T	722	3589	1/17/92	T	725
	3565	3/25/91	M	427	3590	1/20/92	T	728
	3566	1/14/92	T	722	3591	11/18/91	M	665
	3567	1/12/92	S	720	3592	7/26/91	M	550
	3568	10/17/91	S	633	3593[f]	2/13/90	A	22
	3569	9/29/91	S	615	3594	1/20/92	T	728
	3570	1/15/92	T	723	3595	6/13/91	M	507
	3571	1/15/92	T	723	3596	1/20/92	T	728
	3572	1/15/92	T	723	3597	1/20/92	T	728
	3573	1/15/92	T	723	3598	12/9/91	M	686
	3574	1/15/92	T	723	3599	1/20/92	T	728
					3600[h]	1/16/92	T	714
					3601[i]	1/20/92	T	706
					3602[j]	1/16/92	T	698

[d]A = Accidental death, E = Animal escaped, M = Moribund sacrifice, O = Animal sacrificed after giving birth, S = Spontaneous death and T = Terminal sacrifice.
[e]Days on Test includes days on which animals were not exposed to the test substance.
[f]Animal replaced.
[h]Replaced Animal Number 3580.
[i]Replaced Animal Number 3593.
[j]Replaced Animal Number 3578.

Appendix C (cont.)
An Inhalation Oncogenicity Study
of Commercial Hexane in Rats and Mice

Animal Termination History - Female Mice (cont.)

Group (ppm)	An. No.	Date of Death	Type of Death[d]	Days on Test[e]	An. No.	Date of Death	Type of Death[d]	Days on Test[e]
IV	4550	1/13/92	T	721	4575	1/16/92	T	724
9000	4551	12/18/91	S	695	4576	8/20/91	S	575
	4552	1/13/92	T	721	4577	1/16/92	T	724
	4553	1/13/92	T	721	4578	1/16/92	T	724
	4554	1/13/92	T	721	4579	1/16/92	T	724
	4555	7/8/91	S	532	4580	1/16/92	T	724
	4556	1/13/92	T	721	4581	1/17/92	T	725
	4557	1/13/92	T	721	4582	1/17/92	T	725
	4558	1/14/92	T	722	4583	1/17/92	T	725
	4559	1/14/92	T	722	4584	10/20/91	S	636
	4560	9/13/91	S	599	4585	1/17/92	T	725
	4561	1/14/92	T	722	4586	1/17/92	T	725
	4562	1/14/92	T	722	4587	1/17/92	T	725
	4563	1/14/92	T	722	4588	10/9/91	S	625
	4564	1/14/92	T	722	4589	7/23/91	S	547
	4565	1/15/92	T	723	4590	1/20/92	T	728
	4566	11/9/91	S	656	4591	1/20/92	T	728
	4567	1/15/92	T	723	4592	12/9/91	S	686
	4568	1/15/92	T	723	4593	1/20/92	T	728
	4569	7/5/90	A	164	4594	9/22/91	S	608
	4570	1/15/92	T	723	4595	1/20/92	T	728
	4571	1/15/92	T	723	4596	1/20/92	T	728
	4572	1/15/92	T	723	4597	1/20/92	T	728
	4573	1/16/92	T	724	4598	1/21/92	T	729
	4574	1/2/92	M	710	4599	1/21/92	T	729

[d]A = Accidental death, M = Moribund sacrifice, S = Spontaneous death and T = Terminal sacrifice.
[e]Days on Test includes days on which animals were not exposed to the test substance.

APPENDIX 2

Appendix D
An Inhalation Oncogenicity Study
of Commercial Hexane in Rats and Mice

Physical Observations
Preface

Detailed physical observations were performed prior to exposure on Test Days -1 and 0 (see week 0). Physical observations were performed post-exposure weekly thereafter.

Number of animals examined represents the total number of animals observed and animals which were found dead, died accidentally or were killed in a moribund condition for a given interval. Physical observations are presented weekly for Weeks 0 through 4 and monthly thereafter. When observations are presented monthly and an animal dies between intervals, the death of the animal is listed under the next current interval. For example, if observations are presented for Weeks 8, 12, etc. and an animal dies in Week 11, the death would appear in Week 12.

One Group I female died on day 11 and was replaced and three Group III females died on days 10, 22 and 26 and were replaced. Pretest observations presented include the replacement animals in Week 0. Observations for replacement animals begin to be presented the week they were placed on-test. Therefore, the number of animals is less than 50.

If more than one degree was noted in data for different locations, the most extreme degree was presented (i.e., slight lacrimation, right eye and moderate lacrimation, left eye were recorded, moderate lacrimation was presented in the report).

For summarization purposes, descriptive comments [i.e., location of alopecia, scab(s) and sore(s), etc.] are not presented in this appendix. These data are contained in the study raw data if needed.

Corresponding exposure levels for each group were as follows:

Group I - 0 ppm
Group II - 900 ppm
Group III - 3000 ppm
Group IV - 9000 ppm

APPENDIX D (cont.)
AN INHALATION ONCOGENICITY STUDY
OF COMMERCIAL HEXANE IN RATS AND MICE

SUMMARY OF IN-LIFE PHYSICAL OBSERVATIONS - MALE MICE

	WEEK:	0	1	2	3	4	8	12	16	20	24	28	32	36	40	44
# OF ANIMALS EXAMINED	I	50	50	50	50	50	50	49	49	48	48	48	48	48	48	48
	II	50	50	50	50	50	50	49	49	49	49	49	48	48	47	47
	III	50	50	50	50	50	50	49	49	49	49	49	49	49	49	49
	IV	50	50	50	50	50	50	50	50	50	50	50	50	50	49	49
NORMAL																
NO SIGNIFICANT CLINICAL OBSERVATIONS	I	50	50	50	50	50	47	47	46	40	39	34	26	29	25	28
	II	50	50	50	50	50	46	45	45	40	42	39	37	32	30	37
	III	50	50	50	50	50	45	40	42	39	36	34	33	31	30	35
	IV	50	50	50	50	50	50	47	48	44	45	44	41	35	34	37
DEAD																
..PED	I	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SPONTANEOUS DEATH	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
ACCIDENTAL	I	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EYES																
OPACITY	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0
EXCESS LACRIMATION	I	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0

APPENDIX D (cont.)
AN INHALATION ONCOGENICITY STUDY
OF COMMERCIAL HEXANE IN RATS AND MICE

SUMMARY OF IN-LIFE PHYSICAL OBSERVATIONS - MALE MICE

	WEEK:	0	1	2	3	4	8	12	16	20	24	28	32	36	40	44
# OF ANIMALS EXAMINED	I	50	50	50	50	50	50	49	49	48	48	48	48	48	48	48
	II	50	50	50	50	50	50	49	49	49	49	49	48	48	47	47
	III	50	50	50	50	50	50	49	49	49	49	49	49	49	49	49
	IV	50	50	50	50	50	50	50	50	50	50	50	50	50	49	49
SWOLLEN AREA AROUND EYE(S)	I	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MISCELLANEOUS																
TAIL PROBLEM(S)	I	0	0	0	0	0	1	1	1	1	1	2	4	2	2	1
	II	0	0	0	0	0	0	0	0	0	0	2	2	1	1	1
	III	0	0	0	0	0	1	0	1	2	2	3	3	3	3	2
	IV	0	0	0	0	0	0	0	0	0	0	1	2	2	2	1
SWOLLEN ANO-GENITAL AREA	I	0	0	0	0	0	0	0	0	4	3	1	4	6	6	2
	II	0	0	0	0	0	0	0	0	5	2	2	2	6	8	1
	III	0	0	0	0	0	0	0	0	2	1	1	3	3	3	0
	IV	0	0	0	0	0	0	0	0	4	2	1	4	8	7	2
UNDESCENDED TESTES	I	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	3	1	0	0	0	0	0	0	0
STRANGULATED PENIS	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DISTENDED ABDOMEN	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TISSUE MASS(ES)	I	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	2	1	1
HUNCHED APPEARANCE	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX D (cont.)
AN INHALATION ONCOGENICITY STUDY
OF COMMERCIAL HEXANE IN RATS AND MICE

SUMMARY OF IN-LIFE PHYSICAL OBSERVATIONS - MALE MICE

	WEEK:	0	1	2	3	4	8	12	16	20	24	28	32	36	40	44
# OF ANIMALS EXAMINED	I	50	50	50	50	50	50	49	49	48	48	48	48	48	48	48
	II	50	50	50	50	50	50	49	49	49	49	49	48	48	47	47
	III	50	50	50	50	50	50	49	49	49	49	49	49	49	49	49
	IV	50	50	50	50	50	50	50	50	50	50	50	50	50	49	49
SWOLLEN THORAX	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SWOLLEN FACIAL AREA	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NOSE																
MUCOID NASAL DISCHARGE	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ORAL-BUCCAL																
TEETH PROBLEM(S)	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	II	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SKIN/FUR																
ALOPECIA, GENERAL	I	0	0	0	0	0	0	1	1	4	6	11	8	7	14	14
	II	0	0	0	0	0	2	1	2	4	5	5	4	6	6	7
	III	0	0	0	0	0	3	3	4	6	10	11	9	9	15	14
	IV	0	0	0	0	0	0	0	0	1	1	4	3	3	6	9
ALOPECIA, EXTREMITIES/SNOUT	I	0	0	0	0	0	0	0	0	0	0	0	14	9	8	5
	II	0	0	0	0	0	0	0	0	0	0	0	7	5	6	4
	III	0	0	0	0	0	0	1	0	1	0	0	10	11	10	11
	IV	0	0	0	0	0	0	0	0	1	1	1	4	2	4	4
SCAB(S)	I	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
	III	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

APPENDIX D (cont.)
AN INHALATION ONCOGENICITY STUDY
OF COMMERCIAL HEXANE IN RATS AND MICE

SUMMARY OF IN-LIFE PHYSICAL OBSERVATIONS - MALE MICE

	WEEK:	48	52	56	60	64	68	72	76	80	84	88	92	96	100	103
# OF ANIMALS EXAMINED	I	48	47	47	47	47	47	47	47	47	46	44	43	42	42	41
	II	47	47	47	47	47	47	47	47	47	45	44	44	44	42	42
	III	49	49	48	48	47	47	47	47	46	45	45	45	45	44	43
	IV	49	49	49	49	49	49	49	49	49	49	49	47	47	46	44
NORMAL																
NO SIGNIFICANT CLINICAL OBSERVATIONS	I	27	28	23	26	21	24	22	21	21	18	18	19	18	18	15
	II	32	36	30	30	28	25	23	23	18	18	15	15	15	14	12
	III	32	28	27	27	26	27	22	21	17	16	20	18	17	17	15
	IV	36	35	34	34	30	33	30	30	24	25	22	23	16	17	17
DEAD																
SPONTANEOUS DEATH	I	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0
	II	0	0	0	0	0	0	0	0	2	1	0	0	2	0	2
	III	0	0	0	1	0	0	0	1	1	0	0	0	1	1	0
	IV	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1
MORIBUND - EUTHANIZED	I	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1
ACCIDENTAL	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DEPRESSION																
LETHARGIC	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
EXCITATION																
HEAD TILT	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
	III	0	0	0	0	0	0	0	0	0	0	0	0	3	1	2
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX D (cont.)
AN INHALATION ONCOGENICITY STUDY
OF COMMERCIAL HEXANE IN RATS AND MICE

SUMMARY OF IN-LIFE PHYSICAL OBSERVATIONS - MALE MICE

	WEEK:	48	52	56	60	64	68	72	76	80	84	88	92	96	100	103
# OF ANIMALS EXAMINED	I	48	47	47	47	47	47	47	47	47	46	44	43	42	42	41
	II	47	47	47	47	47	47	47	47	47	45	44	44	44	42	42
	III	49	49	48	48	47	47	47	47	46	45	45	45	45	44	43
	IV	49	49	49	49	49	49	49	49	49	49	49	47	47	46	44
EYES																
OPACITY	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0
	IV	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1
MISSING EYE(S)	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	II	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0
EXCESS LACRIMATION	I	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3
	II	0	0	0	0	0	0	0	0	1	6	1	2	0	0	2
	III	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0
	IV	0	2	0	1	2	1	0	0	2	1	0	1	0	0	4
ULCERATION ON EYE(S)	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MISCELLANEOUS																
TAIL PROBLEM(S)	I	1	1	2	2	2	2	2	2	2	2	3	3	3	3	3
	II	0	0	2	2	2	2	1	2	2	2	2	2	1	1	1
	III	2	3	3	3	3	3	3	3	3	3	3	3	3	2	3
	IV	0	1	1	2	2	2	1	1	1	1	1	2	3	3	3
SWOLLEN ANO-GENITAL AREA	I	2	1	0	0	0	0	1	1	1	0	0	0	0	0	0
	II	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	2	3	1	0	0	0	1	2	1	1	1	0	0	0	0
	IV	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
STRANGULATED PENIS	I	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	III	0	0	1	1	1	0	0	0	0	0	1	1	1	1	1
	IV	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0

88-8137M

SUMMARY OF IN-LIFE PHYSICAL OBSERVATIONS - MALE MICE

	WEEK:	48	52	56	60	64	68	72	76	80	84	88	92	96	100	103
# OF ANIMALS EXAMINED	I	48	47	47	47	47	47	47	47	47	46	44	43	42	42	41
	II	47	47	47	47	47	47	47	47	47	45	44	44	44	42	42
	III	49	49	48	48	47	47	47	47	46	45	45	45	45	44	43
	IV	49	49	49	49	49	49	49	49	49	49	49	47	47	46	44
DISTENDED ABDOMEN	I	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
	II	0	0	0	0	0	0	0	0	1	0	0	2	1	1	2
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	1	1	2	3
TISSUE MASS(ES)	I	1	1	1	1	2	2	1	1	1	2	3	3	3	5	6
	II	2	1	2	2	1	3	3	2	4	6	4	5	6	8	7
	III	0	1	2	2	3	3	5	5	5	5	5	4	4	7	8
	IV	3	1	2	2	3	2	5	5	3	4	2	4	5	7	4
HUNCHED APPEARANCE	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SWOLLEN FACIAL AREA	I	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	III	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
	IV	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0
POOR CONDITION	I	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
EMACIATED	I	0	0	0	0	0	0	1	1	0	0	0	1	1	1	1
	II	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
SWOLLEN CERVICAL AREA	I	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EAR PROBLEM(S)	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	1	1	1	1	2
	IV	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1

APPENDIX D (cont.)
AN INHALATION ONCOGENICITY STUDY
OF COMMERCIAL HEXANE IN RATS AND MICE

SUMMARY OF IN-LIFE PHYSICAL OBSERVATIONS - MALE MICE

	WEEK:	48	52	56	60	64	68	72	76	80	84	88	92	96	100	103
# OF ANIMALS EXAMINED	I	48	47	47	47	47	47	47	47	47	46	44	43	42	42	41
	II	47	47	47	47	47	47	47	47	47	45	44	44	44	42	42
	III	49	49	48	48	47	47	47	47	46	45	45	45	45	44	43
	IV	49	49	49	49	49	49	49	49	49	49	49	47	47	46	44
SWOLLEN SNOUT	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ENLARGED TESTES	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SWOLLEN PENIS	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	2	1	1	1
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ORAL-BUCCAL																
TEETH PROBLEM(S)	I	0	3	3	3	5	5	4	5	4	4	3	3	3	3	3
	II	0	1	1	1	5	5	8	8	8	7	5	7	7	7	7
	III	0	1	1	1	1	1	2	2	3	2	2	2	2	2	2
	IV	1	1	1	1	1	1	2	2	2	2	2	2	1	1	1
RESPIRATION																
DYSPNEA	I	0	0	0	0	0	1	1	1	0	0	0	1	0	1	1
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
SKIN/FUR																
ALOPECIA, GENERAL	I	17	14	16	14	13	14	18	18	16	15	15	15	18	15	16
	II	10	8	12	11	12	13	13	12	12	12	13	13	12	13	12
	III	16	16	17	17	17	17	17	18	16	18	18	18	16	18	19
	IV	9	9	12	11	12	10	12	12	15	14	10	13	12	15	13
ALOPECIA, EXTREMITIES/SNOUT	I	5	11	12	8	17	12	3	15	14	12	8	10	6	12	13
	II	4	6	8	9	10	11	1	10	10	12	8	10	8	12	11
	III	12	11	15	13	14	17	6	17	12	15	13	15	15	15	16
	IV	6	5	10	9	11	9	2	11	9	14	9	9	8	13	12

APPENDIX D (cont.)
AN INHALATION ONCOGENICITY STUDY
OF COMMERCIAL HEXANE IN RATS AND MICE

SUMMARY OF IN-LIFE PHYSICAL OBSERVATIONS - MALE MICE

	WEEK:	48	52	56	60	64	68	72	76	80	84	88	92	96	100	103
# OF ANIMALS EXAMINED	I	48	47	47	47	47	47	47	47	47	46	44	43	42	42	41
	II	47	47	47	47	47	47	47	47	47	45	44	44	44	42	42
	III	49	49	48	48	47	47	47	47	46	45	45	45	45	44	43
	IV	49	49	49	49	49	49	49	49	49	49	49	47	47	46	44
OPEN SORE(S)	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	III	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
SCAB(S)	I	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
YELLOW ANO-GENITAL STAINS	I	0	0	0	0	0	0	1	1	5	7	7	4	6	3	6
	II	0	0	0	0	0	1	0	3	4	6	9	5	6	3	5
	III	0	0	0	0	0	0	0	2	8	9	7	6	6	2	0
	IV	0	0	0	0	0	1	0	1	6	6	17	5	14	2	6
MATTED COAT	I	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1
	III	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
BROWN ANO-GENITAL STAINS	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX D (cont.)
AN INHALATION ONCOGENICITY STUDY
OF COMMERCIAL HEXANE IN RATS AND MICE

SUMMARY OF IN-LIFE PHYSICAL OBSERVATIONS - FEMALE MICE

	WEEK:	0	1	2	3	4	8	12	16	20	24	28	32	36	40	44
# OF ANIMALS EXAMINED	I	50	49	49	50	50	50	50	50	50	50	50	49	49	49	49
	II	50	50	50	50	50	50	49	49	49	47	47	47	47	47	47
	III	50	47	48	48	49	50	50	50	49	48	48	48	48	48	48
	IV	50	50	50	50	50	50	50	50	50	50	49	49	49	49	49
NORMAL																
NO SIGNIFICANT CLINICAL OBSERVATIONS	I	50	49	49	50	50	50	49	47	44	40	35	36	33	31	33
	II	50	50	50	50	50	46	42	41	39	39	34	36	31	31	33
	III	50	47	48	48	49	49	47	43	36	37	36	38	33	32	37
	IV	50	50	50	50	48	42	42	41	36	36	35	35	33	33	31
DEAD																
ESCAPED	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SPONTANEOUS DEATH	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ACCIDENTAL	I	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	II	0	0	0	0	0	1	0	0	2	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
EYES																
MISSING EYE(S)	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MISCELLANEOUS																
TAIL PROBLEM(S)	I	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
	II	0	0	0	0	0	1	1	1	1	1	0	1	1	2	1
	III	0	0	0	0	0	0	0	0	1	1	1	2	2	1	1
	IV	0	0	0	0	0	0	0	1	1	1	1	3	3	2	3

APPENDIX D (cont.)
AN INHALATION ONCOGENICITY STUDY
OF COMMERCIAL HEXANE IN RATS AND MICE

SUMMARY OF IN-LIFE PHYSICAL OBSERVATIONS - FEMALE MICE

	WEEK:	0	1	2	3	4	8	12	16	20	24	28	32	36	40	44
# OF ANIMALS EXAMINED	I	50	49	49	50	50	50	50	50	50	50	50	49	49	49	49
	II	50	50	50	50	50	50	49	49	49	47	47	47	47	47	47
	III	50	47	48	48	49	50	50	50	49	48	48	48	48	48	48
	IV	50	50	50	50	50	50	50	50	50	50	49	49	49	49	49
SKIN/FUR																
ALOPECIA, GENERAL	I	0	0	0	0	0	0	1	2	5	7	10	12	13	11	9
	II	0	0	0	0	0	2	6	7	7	6	11	10	11	11	10
	III	0	0	0	0	0	1	2	5	9	10	10	9	11	11	9
	IV	0	0	0	0	2	8	8	5	13	12	13	13	14	13	12
ALOPECIA, EXTREMITIES/SNOUT	I	0	0	0	0	0	0	0	3	2	7	8	8	14	14	12
	II	0	0	0	0	0	0	2	5	4	5	7	10	15	11	6
	III	0	0	0	0	0	0	3	3	10	7	6	9	14	11	7
	IV	0	0	0	0	0	0	5	8	11	10	11	13	16	14	14

APPENDIX D (cont.)
AN INHALATION ONCOGENICITY STUDY
OF COMMERCIAL HEXANE IN RATS AND MICE

SUMMARY OF IN-LIFE PHYSICAL OBSERVATIONS - FEMALE MICE

	WEEK:	48	52	56	60	64	68	72	76	80	84	88	92	96	100	103
# OF ANIMALS EXAMINED	I	49	49	49	49	49	47	47	47	46	45	45	44	44	43	41
	II	47	47	47	46	46	45	45	45	43	43	42	41	40	40	40
	III	48	48	48	48	48	47	47	47	46	45	45	42	41	39	36
	IV	49	49	49	49	49	49	49	49	48	47	46	44	42	41	39
NORMAL																
NO SIGNIFICANT CLINICAL OBSERVATIONS	I	34	35	30	26	25	23	26	25	24	26	6	1	23	22	22
	II	34	33	28	26	24	24	23	23	23	24	4	0	19	17	20
	III	37	35	28	28	26	30	23	32	33	31	14	0	25	24	22
	IV	36	36	31	33	32	30	25	27	27	27	14	0	25	20	21
DEAD																
SPONTANEOUS DEATH	I	0	0	0	0	0	0	0	1	1	0	0	0	1	1	0
	II	0	0	0	0	1	0	0	2	0	1	1	1	0	0	2
	III	0	0	0	0	0	0	0	0	0	0	2	1	1	0	1
	IV	0	0	0	0	0	0	0	1	1	1	2	2	1	2	0
MORIBUND - EUTHANIZED	I	0	0	0	0	2	0	0	0	0	0	1	0	0	1	2
	II	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
	III	0	0	0	0	1	0	0	1	1	0	1	0	1	3	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
DEPRESSION																
LETHARGIC	I	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EXCITATION																
TREMORS	I	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
HEAD TILT	I	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1
	IV	0	0	0	0	0	0	0	0	0	0	0	0	1	3	3

APPENDIX D (cont.)
AN INHALATION ONCOGENICITY STUDY
OF COMMERCIAL HEXANE IN RATS AND MICE

SUMMARY OF IN-LIFE PHYSICAL OBSERVATIONS - FEMALE MICE

	WEEK:	48	52	56	60	64	68	72	76	80	84	88	92	96	100	103
# OF ANIMALS EXAMINED	I	49	49	49	49	49	47	47	47	46	45	45	44	44	43	41
	II	47	47	47	46	46	45	45	45	43	43	42	41	40	40	40
	III	48	48	48	48	48	47	47	47	46	45	45	42	41	39	36
	IV	49	49	49	49	49	49	49	49	48	47	46	44	42	41	39
HYPERACTIVITY	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
EYES																
MISSING EYE(S)	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EXCESS LACRIMATION	I	0	0	0	0	1	0	0	0	0	0	1	2	1	0	1
	II	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
	III	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
EXOPHTHALMOS	I	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MISCELLANEOUS																
TAIL PROBLEM(S)	I	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2
	II	1	1	1	2	2	1	0	0	0	0	1	1	1	3	3
	III	1	1	2	2	2	2	2	2	2	2	2	2	2	3	3
	IV	1	1	2	2	3	4	4	4	4	4	4	3	3	5	4
DISTENDED ABDOMEN	I	0	0	0	0	1	0	0	0	0	1	1	1	1	1	1
	II	0	0	0	0	0	1	1	1	1	1	0	0	1	1	1
	III	0	0	0	0	0	0	0	0	0	0	0	0	1	2	3
	IV	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1
TISSUE MASS(ES)	I	0	0	0	1	1	1	1	0	0	0	1	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0
	IV	0	0	0	0	0	0	0	0	0	1	0	0	0	2	1

APPENDIX D (cont.)
AN INHALATION ONCOGENICITY STUDY
OF COMMERCIAL HEXANE IN RATS AND MICE

SUMMARY OF IN-LIFE PHYSICAL OBSERVATIONS - FEMALE MICE

	WEEK:	48	52	56	60	64	68	72	76	80	84	88	92	96	100	103
# OF ANIMALS EXAMINED	I	49	49	49	49	49	47	47	47	46	45	45	44	44	43	41
	II	47	47	47	46	46	45	45	45	43	43	42	41	40	40	40
	III	48	48	48	48	48	47	47	47	46	45	45	42	41	39	36
	IV	49	49	49	49	49	49	49	49	48	47	46	44	42	41	39
HUNCHED APPEARANCE	I	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
	II	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
POOR CONDITION	I	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
	II	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EMACIATED	I	0	0	0	0	1	0	1	0	0	0	2	1	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	1	1	1	1	1	1	1	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
RED EXUDATE FROM ANO-GENITAL AREA	I	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IMPAIRED LIMB(S)	I	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NOSE																
RED NASAL DISCHARGE	I	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DRIED RED NASAL DISCHARGE	I	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0

APPENDIX D (cont.)
AN INHALATION ONCOGENICITY STUDY
OF COMMERCIAL HEXANE IN RATS AND MICE

SUMMARY OF IN-LIFE PHYSICAL OBSERVATIONS - FEMALE MICE

	WEEK:	48	52	56	60	64	68	72	76	80	84	88	92	96	100	103
# OF ANIMALS EXAMINED	I	49	49	49	49	49	47	47	47	46	45	45	44	44	43	41
	II	47	47	47	46	46	45	45	45	43	43	42	41	40	40	40
	III	48	48	48	48	48	47	47	47	46	45	45	42	41	39	36
	IV	49	49	49	49	49	49	49	49	48	47	46	44	42	41	39
ORAL-BUCCAL																
TEETH PROBLEM(S)	I	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RESPIRATION																
DYSPNEA	I	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	II	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0
	IV	0	0	0	0	0	0	0	0	1	0	0	1	0	1	1
MOIST RALES	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	II	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SKIN/FUR																
ALOPECIA, GENERAL	I	14	13	18	21	21	21	15	13	14	16	28	41	11	14	12
	II	12	13	17	17	17	20	17	15	15	15	35	40	12	15	15
	III	10	10	17	17	17	15	11	10	9	10	22	39	8	6	6
	IV	12	11	14	13	13	14	11	13	12	13	26	39	11	11	12
ALOPECIA, EXTREMITIES/SNOUT	I	4	13	12	20	22	22	18	19	18	15	32	42	10	12	12
	II	4	12	15	20	20	21	21	20	20	18	28	40	15	14	15
	III	3	11	17	16	19	16	23	12	10	10	20	38	8	4	7
	IV	8	12	15	14	15	17	23	18	17	17	23	41	13	11	12
YELLOW ANO-GENITAL STAINS	I	0	0	0	0	0	0	0	0	0	0	10	15	1	2	0
	II	0	0	1	0	0	0	0	0	0	0	10	14	1	3	1
	III	0	0	0	0	0	0	1	0	0	0	5	19	0	1	0
	IV	0	0	0	0	0	0	0	0	1	0	3	12	0	0	0
PALE EXTREMITIES	I	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX D (cont.)
AN INHALATION ONCOGENICITY STUDY
OF COMMERCIAL HEXANE IN RATS AND MICE

SUMMARY OF IN-LIFE PHYSICAL OBSERVATIONS - FEMALE MICE

	WEEK:	48	52	56	60	64	68	72	76	80	84	88	92	96	100	103
# OF ANIMALS EXAMINED	I	49	49	49	49	49	47	47	47	46	45	45	44	44	43	41
	II	47	47	47	46	46	45	45	45	43	43	42	41	40	40	40
	III	48	48	48	48	48	47	47	47	46	45	45	42	41	39	36
	IV	49	49	49	49	49	49	49	49	48	47	46	44	42	41	39
MATTED COAT	I	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	1	7	1	0	0
	III	0	0	0	0	0	0	0	0	0	0	1	7	1	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0
BROWN ANO-GENITAL STAINS	I	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	2	3	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
WET FUR	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DRIED BROWN MATERIAL ON TAIL	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

APPENDIX 3

Appendix H

An Inhalation Oncogenicity Study of Commercial Hexane in Rats and Mice

Pathology Report

Appendix H
An Inhalation Oncogenicity Study of Commercial Hexane in Rats and Mice Pathology Report

Table of Contents

An Inhalation Oncogenicity Study of Commercial Hexane in Rats and Mice Pathology Report

INTRODUCTION:

This study was designed to evaluate the oncogenic effects of Commercial Hexane administered by inhalation to mice 6 hours per day, 5 days per week for 24 months.

Four hundred B6C3F1 mice[1] (200 males, 200 females) were randomly distributed into 4 groups (50/sex/group). Mice in Groups II, III, and IV were exposed, whole-body, to Commercial Hexane at dose levels of 900, 3000 and 9018 ppm respectively, evaporated with heated nitrogen and administered in their breathing air, for 6 hours per day, 5 days per week for approximately 24 months. Animals in Group I, the controls, were similarly exposed to heated nitrogen only in their breathing air. The animals were approximately 8 weeks of age at the initiation of exposure.

A number of animals that died or were sacrificed early in the study were replaced. Information regarding the identification numbers of the animals involved is listed below. The data for the original animals will be kept on file but will not be presented in this report.

Original Animal Number	Study Day of Death	Replacement Animal Number
3580	10	3600
1577	11	1600
3593	22	3601
3578	26	3602

For this study, a number of animals from all of the groups were designated as sentinel animals (15 per sex). Prior to the initiation of the study and at various intervals throughout the study, these animals were designated to be killed. A list of the animals that died unscheduled deaths and were examined postmortem is presented in the preface to Table I of Appendix J.

[1]Supplied by Charles River Breeding Laboratories, Portage, MI.

An Inhalation Oncogenicity Study
of Commercial Hexane in Rats and Mice
Pathology Report

PATHOLOGY METHODS:

Primary test animals found in a moribund condition during the course of the study and all of the survivors at the end of the study were killed by exsanguination under ethyl ether anesthesia. One female from Group III (3000 ppm) was similarly killed following parturition. Immediately after death, the external surfaces, all orifices, the thoracic, abdominal and pelvic cavities and their associated tissues and organs, the cranial cavity and the external surfaces of the brain and spinal cord, the nasal cavity, the neck and its tissues and organs and the remaining carcass were examined for the presence of macroscopic morphologic abnormalities. The liver, spleen, kidneys and the heart were incised in order to ascertain the presence of such abnormalities, including neoplasms, which would not have been visible when only the surfaces of these organs were examined. All of the animals which died during the course of the study, were examined similarly as soon as possible.[2]

Sentinel animals which were killed prior to the initiation of exposure and in extremis (exsanguination under ethyl ether anesthesia) as well as those which were found dead during the study were examined as described above. Sentinel animals which survived to the end of the exposure period were killed and discarded without postmortem examination.

The following tissues and organs were sampled from all of the primary test animals as well as from sentinel animals which were killed prior to exposure and in extremis or were found dead during the course of the study.

---

[2] Animal number 3584 was missing from its cage; when the carcass was found, it was discarded. Its date of death is recorded in laboratory data as 4/19/90. Animal #1079 was missing from its cage and never found. Laboratory data notes this effective 5/10/90.

An Inhalation Oncogenicity Study
of Commercial Hexane in Rats and Mice
Pathology Report

PATHOLOGY METHODS (cont.):

List of Preserved Tissues:

abdominal aorta
adrenal glands
*blood smears
bone and bone marrow (sternum and femur)
*bone marrow smear
brain (three sections, including medulla/pons, cerebral cortex, and cerebellar cortex)
esophagus
exorbital lacrimal gland
eyes (with optic nerve)
gallbladder
gonads (ovaries, or testes with epididymides)
heart
intestine (duodenum, jejunum, ileum, cecum, colon and rectum)
kidneys
larynx
liver (left lateral and median lobes)
lungs (with mainstem bronchi and each lobe)
lymph nodes (mesenteric and peribronchial)
mammary gland (right inguinal, with skin)
nasopharyngeal tissue
nerve (sciatic) with Biceps femoris
pancreas
pituitary gland
prostate gland
salivary glands (submandibular)
seminal vesicles
skeletal muscle (Biceps femoris)
skin (with mammary gland)
spinal cord (cervical, midthoracic and lumbar sections)
spleen
stomach
thymic region
thyroid gland with parathyroid glands
trachea
urinary bladder
uterus (horns and cervix)
vagina/penis
macroscopic lesions and tissue masses (with border of normal tissue)

An Inhalation Oncogenicity Study
of Commercial Hexane in Rats and Mice
Pathology Report

PATHOLOGY METHODS (cont.):

The smear preparations of the peripheral blood and sternal bone marrow were made from all of the primary test (non-sentinel) animals which were killed in extremis or were killed at the end of the exposure period. These smear preparations were air dried, fixed in absolute methanol, and stained using Wright's stain. The smear preparations of the peripheral blood of animals from Groups I and IV were examined by light microscopy; the results and their analysis are presented in the report of the antemortem findings. The remaining smear preparations of peripheral blood as well as the smear preparations of the bone marrow were stored for future reference, if necessary.

The eyes, testes and epididymides were preserved in Bouin's solution for 48-72 hours, washed in three consecutive changes of 70% Synosol® (each of 24 hours duration) and transferred to 10% neutral buffered formalin prior to processing. The lungs and the urinary bladder were infused with 10% neutral buffered formalin prior to their immersion into a larger volume of the same fixative. All of the other tissues and organs were preserved in 10% neutral buffered formalin. Bones were decalcified in RDO®, a commercially available decalcifying solution.

After preservation, the aforementioned tissues (except those indicated with an asterisk) from all of the primary test animals in Groups I (0 ppm) and IV (9018 ppm) and from all primary test animals from Groups II (900 ppm) and III (3000 ppm) that died unscheduled deaths, were routinely processed and embedded in paraffin, cut at a microtome setting of three to seven microns, mounted on glass slides, stained with hematoxylin and eosin and examined by light microscopy. Tissues and organs which had macroscopic morphologic abnormalities and the lungs from all primary test animals from Groups II (900 ppm) and III (3000 ppm) were processed and examined similarly. Additionally, the livers from primary test animals from Groups II (900 ppm) and III (3000 ppm) and the pituitary glands from the females in those groups, were processed and examined by light microscopy.

An Inhalation Oncogenicity Study
of Commercial Hexane in Rats and Mice
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PATHOLOGY METHODS (cont.):

The portion of the skull containing the nasopharyngeal tissues, preserved intact in 10% neutral buffered formalin and decalcified in RDO®, was cut transversely into four sections according to the following schedule. Cross sections of the nasopharyngeal tissues were examined for the presence of macroscopic morphologic abnormalities.

Section 1: included the area between the upper incisor tooth and the incisive papilla;
Section 2: included the area between the incisive papilla and the first palatal ridge;
Section 3: included the areas between the second palatal ridge and the first upper molar tooth;
Section 4: included the area between the first upper molar tooth and nasopharynx.

Tissues and organs sampled from the sentinel animals were stored for future reference, if necessary.

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RESULTS:

Mortality:

Mortality Summary

	MALES				FEMALES			
GROUP:	I	II	III	IV	I	II	III	IV
FOUND DEAD	4	9	5	5	4	8	6	10
SACRIFICED MORIBUND	3	-	-	3	6	2	8	1
ACCIDENTAL	1	1	2	-	2	3	2	1
OTHER	-	-	-	-	-	-	1	-
TOTAL UNSCHEDULED DEATHS	8	10	7	8	12	13	17	12
TOTAL SCHEDULED DEATHS	41	40	43	42	39	37	35	38
TOTAL ANIMALS	49	50	50	50	51	50	52	50

Sentinel Animals:

There were no macroscopic morphologic abnormalities in the sentinel animals killed prior to exposure to the test material.

During the course of the study, six sentinel animals died or were killed in extremis. The macroscopic morphologic findings in these animals were typical of those seen in mice of this strain and age, and did not indicate a compromise of their health. The sentinel animals were identified as "Group V" for organizational clarity. An incidence summary of macroscopic findings in the sentinel animals which were killed in extremis or found dead during the exposure period is presented in Table I of Appendix J; findings in the individual animals are presented in Table II. Tissues and organs from these animals were not examined by light microscopy, and the cause(s) of death and moribundity were not determined.

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RESULTS (cont.):

Primary Test Animals:

Macroscopic:

There was an increase in liver masses and nodules among Group IV (9018 ppm) females but not among treated males. There was a slight dose-related decrease in the incidence of cysts of the uterus among females, as shown in the figure below. The significance of these observations will be discussed under the microscopic section.

Macroscopic Incidence of Uterine Cysts

Group	I	II	III	IV
No. Examined	50	50	49	50
Uterine Cysts	21	18	14	11

There were no other treatment-related macroscopic changes. The other macroscopic observations were not unusual for mice of this strain and age.

Microscopic:

Treatment-related changes were present in the liver (neoplastic) and uterus (non-neoplastic) of Group IV (9018 ppm) females. There was no similar treatment-related neoplastic change in the liver of males.

There was an increase in proliferative lesions of the pituitary among females (hyperplasia, adenoma, adenocarcinoma) at all three treatment levels when compared to the controls. This change may not be treatment-related because of an unusually low incidence among controls.

The incidence of liver tumors for males and females is tabulated as follows.

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RESULTS (cont.):

Microscopic (cont.):

Liver Neoplasia, Females

Group	I	II	III	IV
No. Examined	50	50	49	50
Hepatocellular Adenoma	4	6	4	10
Hepatocellular Carcinoma	3	2	5	6
Total Neoplasms	7	8	9	16

Liver Neoplasia, Males

Group	I	II	III	IV
No. Examined	49	50	50	50
Hepatocellular Adenoma	10	5	7	10
Hepatocellular Carcinoma	7	11	10	3
Total Neoplasms	17	16	17	13

The increase in hepatocellular adenomas and carcinomas for females, individually or combined as total hepatocellular neoplasms, is considered to be treatment-related. For females, the incidence of benign liver tumors was statistically significant for trend at the 0.04 level. There were no significant pairwise differences. The incidence of malignant liver tumors was not significant for trend or pairwise comparisons. When benign and malignant tumors were combined there was a statistically significant trend at the 0.01 level and a statistically significant pairwise comparison of the high dose group and control group at the 0.06 level by Cox's test and 0.04 level by the GB/KW test (see Appendix I for details). It should be noted that the incidence among Group IV females is similar to the incidence among control males.

Liver tumors among males were not treatment-related.

There was no treatment-related increase in any other lesions of the liver, including foci of cellular alteration, among males or females.

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RESULTS (cont.):

Microscopic (cont.):

There was a treatment-related decrease in the severity and a slight decrease in the incidence of cystic endometrial hyperplasia of the uterus among Group IV females when compared to controls. The decrease in severity was statistically significant but the slight decrease in incidence was not statistically significant (see the statistical analysis following the Conclusions). This correlated with the dose-related decrease in uterine cysts observed at necropsy among Group IV females when compared to controls. The microscopic examination of the uterus was not complete for the low and mid dose groups (gross lesions only). Cystic endometrial hyperplasia of the uterus is a common age change among mice of this strain and is thought to be related to a change in the hormonal balance with aging. The incidence and severity of this change is tabulated below.

Incidence and Severity of Cystic Endometrial Hyperplasia

Group	I	II	III	IV
No. Examined	50	32	32	50
Cystic Endometrial Hyperplasia	48	29	27	44
trace	3	4	3	10
mild	14	13	8	19
moderate	23	10	15	15
severe	8	2	1	0

There was an increase in the incidence of proliferative lesions of the pituitary (hyperplasia, adenoma, carcinoma) among all treated groups of females when compared to the controls. A similar increase was not present among males. There was no dose-related response among the females. The incidence was similar among all three treated groups. The treatment relationship of this observation is unclear. It may be related to an unusually low incidence among the control females. The incidence of pituitary lesions for females is tabulated below:

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RESULTS (cont.):

Microscopic (cont.):

Incidence of Pituitary Proliferative Lesions for Females

Group	I	II	III	IV
No. Examined	45	48	48	49
Hyperplasia	2	4	4	6
Adenoma	0	6	7	5
Adenocarcinoma	0	0	1	0
Total Neoplasms	0	6	8	5
Total Proliferative Lesions	2	10	12	11

There were no other treatment-related neoplastic or non-neoplastic changes present among males or females on this study. All of the other changes and lesions were spontaneous, agonal or technique-related. The lesions observed were not unusual for mice of this strain and age. There was an increase in the incidence of focal lymphocytic infiltration in the Group IV lacrimal glands, but this was considered to be spurious. This lesion is a common spontaneous findings in mice, and it was trace in severity.

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DISCUSSION:

The decrease in the severity of cystic endometrial hyperplasia of the uterus in Group IV females indicates a possible treatment-related alteration in the hormonal balance such as a decrease in estrogen stimulation of the uterus. 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. The evidence for a treatment-related hormonal effect on the uterus in the females suggests that the females may be expressing the normal male incidence for hepatocellular tumors.

The significance of the pituitary proliferative changes is unclear. The lack of dose response (the incidence is similar for all treated groups), suggests that the change may not be treatment-related. The apparent increase could be a result of a low incidence among control females. Control Data for B6C3F1 mice published by Charles River (P.L. Lang, 1989) indicates a range of 0 to 29% and a mean of 7.9% for adenomas of the pars distalis. This compares to 10% for Group IV females and 14% for Group III females on this study.

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CONCLUSIONS:

1. There was an increase in liver masses and nodules observed among Group IV (9018 ppm) females, but not males, at necropsy.

2. There was a treatment-related increase in hepatocellular neoplasms (adenoma and carcinoma) among Group IV (9018 ppm) females. A similar change was not present among any male treatment groups.

3. There was a statistically-significant treatment-related decrease in the severity and a slight decrease in the incidence of cystic endometrial hyperplasia of the uterus among Group IV females (9018 ppm).

4. The NOEL for the neoplastic changes (hepatocellular tumors) was 3000 ppm.

5. There was an increase in the incidence of pituitary proliferative changes (hyperplasia, adenoma, 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.

6. All other changes observed microscopically or macroscopically were considered to be spontaneous, agonal or technique-related. They were unrelated to treatment with the compound.

Ward R Richter 6-1-93

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

An Inhalation Oncogenicity Study of Commercial Hexane in Rats and Mice

Pathology Report
Statistical Analysis of Cystic Endometrial Hyperplasia Severity Ratings

Statistical Analysis of Cystic Endometrial Hyperplasia Severity Ratings
Pharmaco LSR Study 88-8137

Statistical analyses of cystic endometrial hyperplasia severity scores from female rats were conducted on data from Pharmaco LSR Study 88-8137. Measures were available from the control group and high dose group. The measures for analysis were the presence of cystic endometrial hyperplasia, and an ordinal severity score of the hyperplasia. The severity data are presented in Table I.

Table I
Severity Scores for
Cystic Endometrial Hyperplasia

	Group	
	Control	High Dose
Number Examined	51	50
Not Present	3	6
Trace	3	10
Mild	14	19
Moderate	23	15
Severe	8	0

STATISTICAL ANALYSES

The analyses considered (1) the number of animals with any sign of endometrial hyperplasia and (2) the number of animals with a given severity score. The analysis of simple incidence was by Fisher's Exact Test. The statistical model for the severity score analysis was a logistic regression or proportional odds model with the levels of severity coded as 0 to 4 as the scores ranged from not present to severe.

The Fisher Exact Test for incidence was based on 48 of 51 (94%) affected animals in the control group and 44 of 50 (88%) affected animals in the high dose group. There were no statistically significant differences between the two groups for the number of affected animals.

The analysis of severity scores indicated a highly statistically significant difference in severity scores between the two groups at the 0.0002 level, with the control group having higher severity scores than the high dose group. The test for model assumptions indicated the proportional odds model was appropriate for these data. The plot of the cumulative scores shows that the control group had a higher proportion of larger severity scores relative to the dosed group for all scores; for example 61% of the control group animals had a score of moderate or worse while the high dose group had only 30% of the animals at moderate or worse.

CONCLUSION

The compound tested significantly reduces the severity of cystic endometrial hyperplasia scores in female rats. The incidence of the hyperplasia was not statistically different in the two groups.

Mark J. Nicolich, PhD
Statistician
March 26, 1993

REFERENCES:

Bradley, J.V., **Distribution Free Statistical Tests**, Prentice Hall, Englewod Cliffs, NJ. 1968. (Fisher Exact Test).

McCullagh and Nelder, **Generalized Linear Models, 2nd edition**, Chapman & Hall, 1989. (Logistic regression).

An Inhalation Oncogenicity Study of Commercial Hexane in Rats and Mice Pathology Report

Analysis of Cystic Endometrial Hyperplasia
Fisher Exact Test for Presence/Absence of Endometrial Hyperplasia

TABLE OF GROUP BY HYP

GROUP	HYP		
Frequency Percent Row Pct Col Pct	n	y	Total
I	3 2.97 5.88 33.33	48 47.52 94.12 52.17	51 50.50
IV	6 5.94 12.00 66.67	44 43.56 88.00 47.83	50 49.50
Total	9 8.91	92 91.09	101 100.00

STATISTICS FOR TABLE OF GROUP BY HYP

Statistic	DF	Value	Prob
Chi-Square	1	1.164	0.281
Likelihood Ratio Chi-Square	1	1.183	0.277
Continuity Adj. Chi-Square	1	0.532	0.466
Mantel-Haenszel Chi-Square	1	1.153	0.283
Fisher's Exact Test (Left)			0.234
(Right)			0.925
(2-Tail)			0.318
Phi Coefficient		-0.107	
Contingency Coefficient		0.107	
Cramer's V		-0.107	

Sample Size = 101
WARNING: 50% of the cells have expected counts less than 5. Chi-Square may not be a valid test.

An Inhalation Oncogenicity Study of Commercial Hexane in Rats and Mice Pathology Report

Analysis of Cystic Endometrial Hyperplasia Logistic Regression on Severity Scores

The LOGISTIC Procedure

Data Set: WORK.INN
Response Variable: SCORE
Response Levels: 5
Number of Observations: 101
Link Function: Logit

Response Profile

Ordered Value	SCORE	Count
1	0	9
2	1	13
3	2	33
4	3	38
5	4	8

Simple Statistics for Explanatory Variables

Variable	Mean	Standard Deviation	Minimum	Maximum
GROUP2	0.495050	0.502469	0	1.00000

Score Test for the Proportional Odds Assumption

Chi-Square = 3.3672 with 3 DF (p=0.3384)

Criteria for Assessing Model Fit

Criterion	Intercept Only	Intercept and Covariates	Chi-Square for Covariates
AIC	293.519	282.093	.
SC	303.979	295.169	.
-2 LOG L	285.519	272.093	13.425 with 1 DF (p=0.0002)
Score	.	.	12.898 with 1 DF (p=0.0003)

Analysis of Maximum Likelihood Estimates

Variable	Parameter Estimate	Standard Error	Wald Chi-Square	Pr > Chi-Square	Standardized Estimate
INTERCP1	-3.2091	0.4442	52.1897	0.0001	.
INTERCP2	-2.1160	0.3516	36.2199	0.0001	.
INTERCP3	-0.5150	0.2804	3.3737	0.0662	.
INTERCP4	1.9385	0.3871	25.0809	0.0001	.
GROUP2	1.3795	0.3877	12.6589	0.0004	0.382159

An Inhalation Oncogenicity Study of Commercial Hexane in Rats and Mice Pathology Report

Analysis of Cystic Endometrial Hyperplasia Logistic Regression on Severity Scores

The LOGISTIC Procedure

Association of Predicted Probabilities and Observed Responses

Concordant	= 39.4%	Somers' D	= 0.273
Discordant	= 12.1%	Gamma	= 0.530
Tied	= 48.6%	Tau-a	= 0.199
(3677 pairs)		c	= 0.636

An Inhalation Oncogenicity Study
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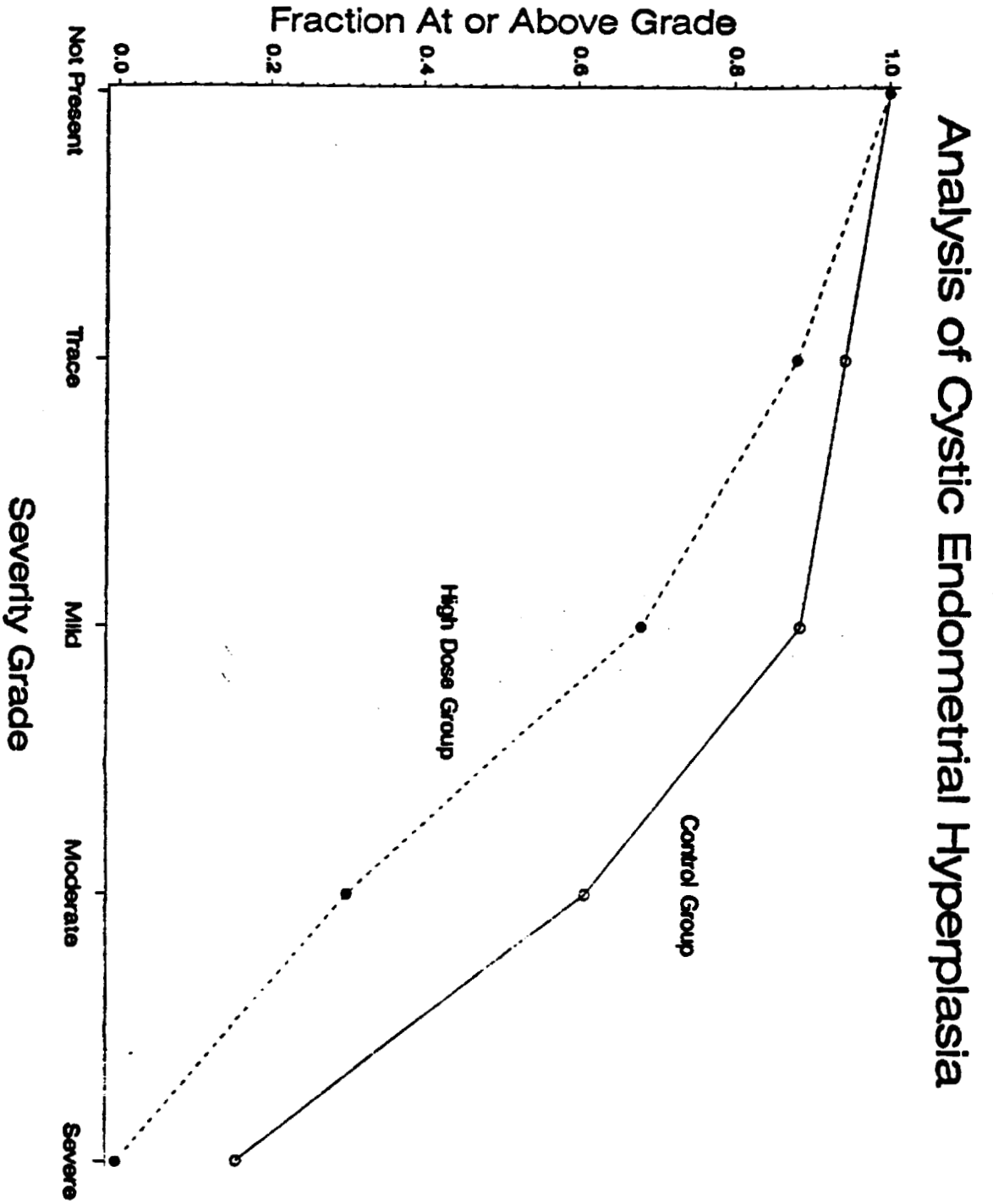

Analysis of Cystic Endometrial Hyperplasia

APPENDIX 4

Appendix I
An Inhalation Oncogenicity Study
of Commercial Hexane in Rats and Mice

Survivorship and Time to Tumor Analyses
Preface

Key to Group Designations on Tables:

Group 0 = Group I (Dose = 0 ppm)

Group 1 = Group II (Dose = 900 ppm)

Group 2 = Group III (Dose = 3000 ppm)

Group 3 = Group IV (Dose = 9000 ppm)

The terminal sacrifice started on Test day 721, January 13, 1992 and continued through 21 January 1992. Animals 1079 and 3584 escaped, 3584 was sacrificed prior to the scheduled sacrifice and 1079 was never found; both were entered as accidentals. Animal 2567 died spontaneously after day 721 but prior to the scheduled sacrifice. Animals 1600, 3600, 3601 and 3602 were replacement animals and had test days (actual days on test) less than 721. Therefore, to accommodate the Survivorship Analysis program their deaths and or test days were entered as a terminal sacrifice on day 721. All accidental deaths were not included in the survivorship analyses.

Time to Tumor analysis were conducted on the following organs and tumors:

Organ	Tumor
Liver	Hepatocellular Adenoma
	Hepatocellular Carcinoma
Pituitary	Adenoma

Statistical Analysis of Survivorship Data
Bio/dynamics Study 88-8137M

A series of statistical analyses of survivorship data were conducted separately for male and female B6C3F1 mice from Bio/dynamics Study 88-8137M. The study design consisted of four groups each with 50 animals per sex. The exposure groups were control, 900, 3000 and 9000 ppm. The analyses were performed using the Thomas, Breslow and Gart analyses which tests for both incidence of death (chi-square and Fisher tests) and survivorship (Kaplan-Meier curves, Cox's Tests and the Gehan-Breslow/Kruskal-Wallis Analyses).

For the male mice there were no statistically significant differences among the exposure groups with respect to death incidence nor survivorship.

For the female mice there were no statistically significant differences among the exposure groups with respect to death incidence nor survivorship.

The conclusion is that the compound tested did not affect survivorship in male or female mice.

Mark J. Nicolich, PhD
Statistician
October 2, 1992

Ref: Thomas, D.G., Breslow, N. and Gart, J.J., "Trend and homogeneity analysis of proportions and life table data", Computers and Biomedical Research, 10, 1977, pg. 373-381.

APPENDIX 5

Appendix L
An Inhalation Oncogenicity Study of Commercial Hexane in Rats and Mice

Quality Assurance Statement

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

Dates of Inspection	Reported to Study Director	Reported to Management
9/12/89	9/12/89	9/14/89 and 9/26/89
1/21/90	1/22/90	1/22/90
1/30/90 to 2/2/90	2/7/90	2/9/90 and 2/23/90
2/2/90	2/7/90	2/9/90 and 2/23/90
3/6/90	3/12/90	3/13/90 and 3/29/90
4/11/90	4/12/90	4/12/90
6/11/90	7/10/90	7/10/90 and 7/12/90
5/3/90 to 5/7/90	5/15/90	5/15/90 and 6/11/90
6/14/90	6/25/90	6/25/90 and 7/23/90
7/29/90	7/30/90	7/31/90 and 8/15/90
7/26/90 to 7/27/90	8/7/90	8/1/90 and 8/15/90
8/17/90	8/20/90	8/20/90 and 8/27/90
9/13/90	9/20/90	9/20/90 and 10/17/90
10/23/90 and 10/24/90	10/25/90	1/10/90 and 11/13/90
11/6/90	11/7/90	11/8/90 and 12/5/90
12/4/90 to 12/6/90	12/10/90	12/10/90 and 12/31/90
1/25/91	1/25/91	1/30/91 and 2/19/91
3/5/91	3/5/91	3/6/91 and 3/7/91
6/14/91	6/17/91	6/18/91 and 6/21/91
7/24/91	7/24/91	7/26/91 and 8/5/91
8/13/91 to 8/15/91	8/15/91	8/15/91 and 9/4/91
11/15/91	11/19/91	12/12/91 and 1/7/91
12/17/91	12/17/91	12/17/91
1/17/92	1/17/92	2/6/92 and 2/13/92
1/22/92	1/24/92	2/10/92 and 2/13/92
1/28/92	1/28/92	2/10/92 and 2/13/92
10/8/92 to 10/20/92	10/21/92	11/6/92 and 11/23/92
10/19/92 to 10/22/92	10/22/92	11/6/92, 11/18/92 and 11/20/92
10/22/92 to 11/9/92	11/9/92	11/18/92 and 11/25/92
11/6/92 to 11/10/92	11/11/92	11/17/92, 11/18/92 and 11/20/92
11/10/92 to 11/18/92	11/19/92	12/11/92 and 1/18/93
11/17/92 and 11/18/92	11/18/92	11/20/92 and 11/23/92
1/12/93 and 1/13/93	1/13/93	1/14/93
1/13/93 to 1/15/93	1/15/93	1/18/93

Appendix L (cont.)
An Inhalation Oncogenicity Study
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Quality Assurance Statement (cont.)[a]

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

Dates of Inspection	Reported to Study Director	Reported to Management
5/12/93	5/12/93	5/25/93 and 5/27/93

Jane Pasquito 5/27/93

Jane Pasquito, B.S. Date
Quality Assurance Group Leader

[a]Quality Assurance Statement was originally signed on 18 January 1993, statement was re-signed because of additional statistical analysis of cystic endometrial hyperplasia severity ratings.

APPENDIX 6

Appendix M
An Inhalation Oncogenicity Study of Commercial Hexane in Rats and Mice

Statement of Compliance

Results of inspections made by the Quality Assurance Unit at Bio/dynamics, Inc. indicate that this study was conducted in compliance with the United States Environmental Protection Agency's Good Laboratory Practice Standards and applicable Standard Operating Procedures.

Paul E Newton 6/1/93

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

APPENDIX 7

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