AFAMRL-TR-81-126
ADA 110 587

TOXIC HAZARDS RESEARCH UNIT

Annual Technical Report: 1981

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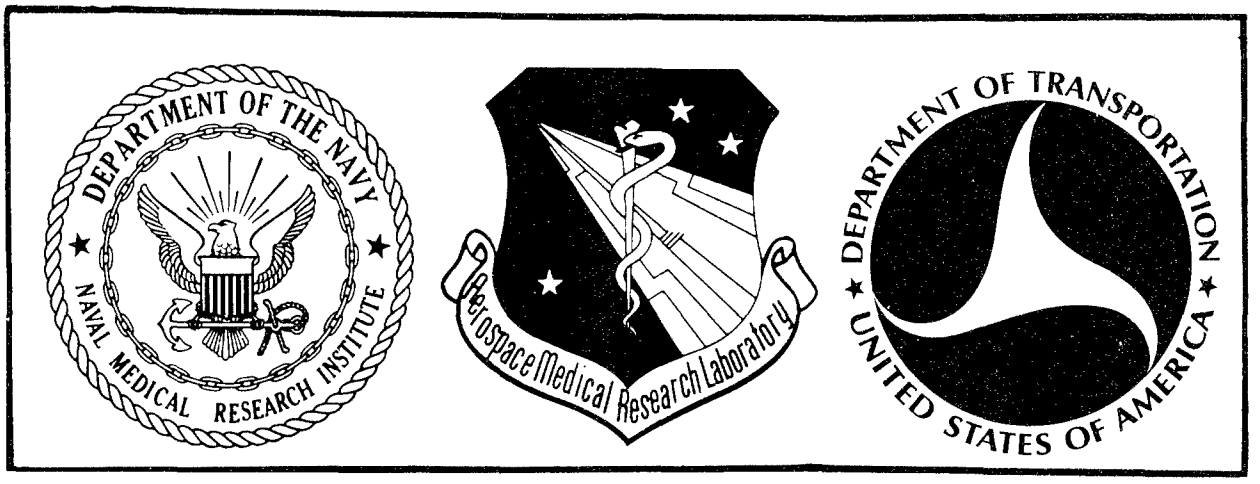
DECEMBER 1981



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TECHNICAL REVIEW AND APPROVAL

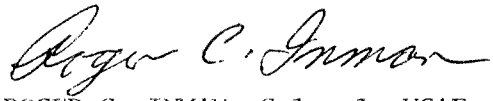
AFAMRL-TR-81-126

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals, "Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER



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AIR FORCE/56780/22 January 1982 — 150

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER AFAMRL-TR-81-126	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE *(and Subtitle)* TOXIC HAZARDS RESEARCH UNIT ANNUAL TECHNICAL REPORT: 1981		5. TYPE OF REPORT & PERIOD COVERED Annual, June 1980 through May 1981 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) J. D. MacEwen, E. H. Vernot		8. CONTRACT OR GRANT NUMBER(s) In part under Contract F33615-80-C-0512
9. PERFORMING ORGANIZATION NAME AND ADDRESS University of California, Irvine P.O. Box 3067, Overlook Branch Dayton, Ohio 45431		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62202F,6302,01,15 MF 58524025.4012
11. CONTROLLING OFFICE NAME AND ADDRESS Air Force Aerospace Medical Research Laboratory, Aerospace Medical Division, Air Force Systems Command, Wright-Patterson AFB, Ohio 45433		12. REPORT DATE December, 1981 13. NUMBER OF PAGES 140
14. MONITORING AGENCY NAME & ADDRESS*(if different from Controlling Office)*		15. SECURITY CLASS. *(of this report)* UNCLASSIFIED 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT *(of this Report)*

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT *(of the abstract entered in Block 20, if different from Report)*

18. SUPPLEMENTARY NOTES

Funded in part by the Naval Medical Research Institute, Naval Medical Research and Development Command, Bethesda, Md. 20014. Naval Medical Research Institute Technical Report No. NMRI-81-52.

19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*

Methylhydrazine	Jet Fuels	Shale Oil
Hydrazine	JP-5	Hydraulic Fluid
1,1-Dimethylhydrazine	JP-4	PGDN
Otto Fuel II	JP-10	Antifouling Paints
Methylcyclohexane	Ferrocene	Diesel Fuel (CONT'D)

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

The research programs of the Toxic Hazards Research Unit (THRU) for the period of June 1980 through May 1981 are reviewed in this report. Chronic toxicity or oncogenic studies were carried out with methylcyclohexane, purified 1,1-dimethylhydrazine, Otto Fuel II, JP-10, RJ-5, and JP-4. A subchronic inhalation study was conducted with shale derived JP-5 and Decalin fuels. Acute toxicity studies were conducted on a variety of chemical agents used by the Air Force and Navy.

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BLOCK 19.

Toxicity
Carcinogenesis
Oncogenesis
Irritation
Skin
Percutaneous
Oral
Inhalation
Sensitization
Metabolites
Dermal

PREFACE

This is the eighteenth annual report of the Toxic Hazards Research Unit (THRU) and concerns work performed by the Department of Community and Environmental Medicine of the University of California, Irvine on behalf of the Air Force under Contract Number F33615-80-C-0512. This document constitutes the first report under the current contract and describes the accomplishments of the THRU from June 1980 through May 1981.

The current contract for operation of the Laboratory was initiated in 1980 under Project 6302, "Occupational and Environmental Toxic Hazards in Air Force Operations", Task 01, "Toxicology of Conventional Propellants, Industrial Chemicals, and Materials", Work Unit Number 63020115. K. C. Back, Ph.D., Chief of the Toxicology Branch and M. K. Pinkerton served as the technical contract monitors for the Air Force Aerospace Medical Research Laboratory.

This is a co-sponsored U. S. Air Force/U. S. Navy research effort. That portion of the work effort sponsored by the U. S. Navy was under the direction of LCDR Morris J. Cowan, Jr., MSC, USN, and identified as Work Unit Number MF58524025.4012 "Toxicity Evaluation and Validation of Exposure Guidelines for Use in Naval Operational Environments".

J. D. MacEwen, Ph.D., served as Laboratory Director for the THRU of the University of California, Irvine and as co-principal investigator with T. T. Crocker, M.D., Professor and Chairman, Department of Community and Environmental Medicine. Acknowledgement is made to A. K. Roychowdhury, Ph.D., C. E. Johnson, C. C. Haun, G. L. Fogle and J. C. Welch for their significant contributions and assistance in the preparation of this report. Partial support for this program was provided by the U. S. Naval Medical Research Institute and the Department of Transportation.

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SECTION I
INTRODUCTION

The research activity of the Toxic Hazards Research Unit (THRU) is a continuing program independent of contract years, with several studies in progress at the beginning and end of each report period. Experiments that were initiated and completed during the past year and were of sufficient magnitude to merit separate technical reports may only be summarized in this document. Unpublished letter reports are given in detail herein. This year's inhalation research program was conducted on a variety of fuels used for powering rockets, ships, torpedoes and aircraft. The results or current status of these studies will be discussed in the body of this report. Acute oral and dermal toxicity studies on a variety of materials were also conducted.

This document constitutes the 18th annual report of the Toxic Hazards Research Unit, a research team which operates a dedicated inhalation toxicology laboratory to investigate potentially hazardous chemicals and materials of interest to the U. S. Air Force, U. S. Navy, and other governmental agencies. The THRU research team is an interdisciplinary group of University of California, Irvine, toxicologists, chemists, statisticians and engineers. Support services in pathology, Veterinary Medicine and medical technology are provided to the contract by the Air Force.

The research facilities used by the THRU consist of animal exposure chambers and supporting laboratories which have previously been described by MacEwen (1965), Fairchild (1967), and Thomas (1968).

During the first six years of operation, the primary research efforts of the THRU were directed to obtaining information on health hazards of spacecraft flight, and the biological data obtained have been used as criteria for setting continuous exposure limits and for engineering design factors. The primary research efforts have in recent years focused more on problems of aircraft environments, chronic occupational health problems, and the potential oncogenicity of chemicals used in military and civilian activities. To this end, many of the current research programs serve the mutual interests of the U. S. Air Force, U. S. Navy, and other governmental agencies.

ANNUAL CONFERENCE

As part of its contractual responsibilities, UCI/THRU presents an annual technical conference to disseminate new toxicologic information to the U. S. Air Force and other governmental and industrial scientists. This year's conference was chaired by Donald L. Dungworth, Ph.D., Professor and Chairman, Department of Pathology, School of Veterinary Medicine, University of California, Davis.

Twenty-seven technical platform papers and 6 poster papers were presented covering a wide variety of occupational and environmental toxicology problems. Six papers were presented by University of California faculty and staff members. The open forum discussions following each session resulted in significant contributions of additional technical information and scientific exchange. The conference, held 18 November through 20 November, 1980, drew 162 participants including speakers.

The papers presented at the conference were prepared for publication as the Proceedings of the 11th Conference on Environmental Toxicology which is a separate technical report (AFAMRL-TR-80-125).

Our next conference, currently in the development stage, will be held in November 1981 at the Holiday Inn Dayton Mall, Dayton, Ohio.

SECTION II
RESEARCH PROGRAM

Toxicology research conducted by the THRU during the past year was primarily concerned with the long-term tumorigenic or combined chronic toxic and tumorigenic effects of inhaled fuels. The exposure phase of studies on two fuels, JP-4 and RJ-5, were completed during the last year and daily exposures to animals were initiated with JP-7, JP-TS, and Otto Fuel II.

ROCKET FUELS

Final pathology information was received for animals exposed to hydrazine 6-hours daily for 1-year and held for 12-18 months postexposure observation. A technical report on the findings of that study has been prepared for Air Force review and was published as AFAMRL-TR-81-56.

A STUDY OF THE ONCOGENIC POTENTIAL OF INHALED MONOMETHYLHYDRAZINE

Inhalation exposures of 4 animal species to monomethylhydrazine (MMH) began in March of 1977 to evaluate the oncogenic potential at or around the Threshold Limit Value (TLV). The exposures were conducted 6-hours daily on an industrial work schedule for 1-year and the animals were held for 1 additional year of observation. The experimental protocol was described in the annual report for that year (MacEwen and Vernot, 1977) and clinical findings have been reported in subsequent annual reports (MacEwen and Vernot, 1978-1980). At the time of the last report, tissue pathology on MMH exposed Golden Syrian hamsters had just been received but not analyzed. In the current year the results of histologic examination of tissues from MMH exposed rats and mice were received and

the incidences of pathologic lesions were analyzed statistically for all three rodent species. A technical report of the experimental findings for the rodents is in preparation. Dogs exposed to MMH in these studies are still being observed and hematologic and growth studies are still underway. This portion of the study will be completed in March 1983 and termination findings will be presented in an appropriate annual report.

Background

Hydrazines administered in the drinking water of Swiss mice and Golden Syrian hamsters have been reported by Toth (1972, 1973) to have carcinogenic activity. In the first of these studies, solutions of 0.001% methylhydrazine sulfate were given daily ad libitum to 5 and 6 week old randomly bred Swiss mice for their entire lifetimes. Hydrazine and methylhydrazine sulfate significantly increased incidence of lung tumors in the mice, while methylhydrazine enhanced the development of neoplasms by shortening the latent period. In the second of Toth's studies, Golden Syrian hamsters received 0.01% methylhydrazine in drinking water daily ad libitum for life. Malignant histiocytomas (Kupffer cell sarcomas) were observed in the livers of 54% of the male hamsters treated, while none was observed in the control groups.

Earlier studies of MMH carcinogenicity by Kelly et al. (1969) and Roe et al. (1967) did not demonstrate any increase in tumor incidence over control animals. Roe administered 0.5 mg MMH per day by mouth to Swiss mice on a 5 day/week for 40 weeks schedule and found a lower incidence of tumor bearing mice (pulmonary adenomas) compared to untreated controls. Kelly reported per os administration of 0.2 ml MMH solution/mouse to female CDF_1 mice, and i.p. administration of 0.1 ml MMH solution/mouse in male mice of the same strain produced no more lung adenomas or leukemias than were found in untreated controls after 8 weeks of treatment. The MMH was given as a 2% aqueous solution.

MacEwen and Vernot (1975) reported the results of a two-year drinking water study in which hamsters were given untreated and acidified drinking water (pH 3.5) containing 0.01% MMH. A third group of hamsters was given acidified water as unexposed controls. Neither the incidence, degree of severity, nor age of onset of non-neoplastic pathologic changes was markedly different between animals drinking MMH in water and control animals. The presence of 23% incidence of adrenocortical tumors in untreated control animals versus 4% in the group given MMH in tap water and 12% in the hamsters receiving MMH in acidified water argues against MMH as a cause of these tumors. The remaining neoplasms, one hemangioendothelioma of the liver, two hepatocellular carcinomas, one cutaneous melanoma, occurred only in the experimental groups. They were derived from four different cell types and as such constitute a 4% incidence for each tumor in their respective groups of animals,

except for an 8% incidence of hepatocellular carcinoma. The overall tumor incidence for the hamsters receiving MMH + tap water was 16%, for those treated with MMH in acidified water was 24%, and for the unexposed control was 31%. These findings are in contrast to the findings of Toth and Shimizu (1973).

The reported investigations presented some evidence that MMH may be carcinogenic and therefore may pose a hazard to man. The case for carcinogenicity of MMH was, however, inconclusive at this point and for this reason, the comprehensive inhalation exposure study described herein was undertaken to evaluate the suitability of the current TLV and estimate the oncogenic risk of inhaled MMH at low concentrations.

Methods

Rats, mice, hamsters and dogs were exposed to MMH by the inhalation route in chambers for one year using an industrial work week schedule of 6 hours/day, 5 days/week with holidays and weekends off to simulate an industrial exposure regimen for man. The highest exposure level tested for each species was based on previous studies in this laboratory to establish maximum tolerated repeated doses.

All rodents were held for an additional year of observation at which time necropsies were performed on survivors and approximately 33 tissues were taken for histopathologic evaluation of tumorigenesis following the National Cancer Institute protocol.

Results

Previous annual reports (MacEwen and Vernot, 1977-1980) contain experimental data including mortality and body weight measurements for all species and clinical chemistry results of dogs tested during the 12 months of MMH exposure and through 26 months postexposure. Significant signs of MMH toxicity during exposure were evidenced in reduced growth and hemolytic effects.

A dose dependent effect on growth was most evident at 2 and 5 ppm MMH for both male and female rats and for hamsters. These differences persisted throughout the 1-year exposure period and diminished during the postexposure observation phase with near normal body weights in 1 month for hamsters, 2 months for female rats and about 6 months for male rats.

Red blood cell counts, hemoglobin levels and hematocrit values were significantly decreased in MMH exposed beagle dogs in a dose dependent manner. Serum glutamic pyruvic transaminase (SGPT) levels were slightly elevated in the 0.2 ppm MMH exposed dogs and were significantly elevated in the dogs exposed at 2.0 ppm. The

hematologic and SGPT changes were reversed on cessation of exposure, returning to unexposed control levels within 4 weeks. Elevated bromsulphalein (BSP) values in these same dogs also returned to control levels in the first postexposure evaluation.

Rodent Pathology

A large variety of pathologic lesions commonly seen in aged hamsters were observed in both MMH exposed and unexposed control animals. Incidence of lesions in a few tissues was, however, significantly increased over control values and lesions not usually seen in hamsters were observed in the MMH exposed hamsters. These lesions are listed in Table 1.

TABLE 1. LESIONS FOUND IN GOLDEN SYRIAN HAMSTERS FOLLOWING INHALATION OF MMH VAPOR FOR ONE YEAR

	Unexposed Controls	0.2 ppm Exposed	2.0 ppm Exposed	5.0 ppm Exposed
LIVER:				
Hepatitis	20/194	15/175	24/177	31/174[a]
Biliary Cysts	41/194	67/175[b]	73/177[b]	76/174[b]
NARES:				
Submucosal Cysts	35/90	52/177[a]	56/180[b]	46/177
Rhinitis	12/190	21/177[a]	25/180	28/177[b]
Adenoma	1/190	0/177	0/180	7/177[a]
Polyps	0/190	0/177	9/180[b]	11/177[b]
Hyperplasia	0/190	0/177	2/180	4/177
LUNG:				
Atelectasis	0/189	2/177	5/174[a]	7/174[b]
Bronchogenic Carcinoma	0/189	0/177	0/174	1/174
Alveolar Adenoma	0/189	0/177	0/174	1/174
KIDNEYS:				
Interstitial Fibrosis	75/195	83/179	105/176[b]	96/177[a]
TOTAL ANIMALS WITH MALIGNANT TUMOR	22/195	27/186	23/184	19/184
TOTAL ANIMALS WITH ANY TUMOR	45/195	48/196	47/184	59/184

[a] Significantly different from controls at 0.05 level.
[b] Significantly different from controls at 0.01 level.

Hepatitis was increased in the highest exposure group while biliary cysts were increased in all groups of exposed hamsters. Both non-neoplastic and neoplastic changes were increased in the nasal cavity of the exposed hamsters. Submucosal cysts, rhinitis and epithelial hyperplasia had increased incidence in the exposed

animals. More importantly, the presence of nasal tumors (adenomas and polyps) in the high level exposed animals is significant as nasal tumors are rare in aged hamsters.

There was a modest but statistically significant increase in the incidence of focal collapse of the lung seen in hamsters exposed to the higher concentration of MMH.

Similar lesions were seen in mice (Table 2). There was a significant increase in lesions observed that indicated irritation of the nasal cavity as shown by nasal inflammation. This was substantiated by a statistically significant increase in plasmacytosis and hemorrhage in the mandibular lymph nodes.

TABLE 2. NON-NEOPLASTIC LESIONS FOUND IN C57BL/6 MICE FOLLOWING INHALATION OF MMH VAPOR FOR ONE YEAR

	Unexposed Controls	0.02 ppm Exposed	0.2 ppm Exposed	2.0 ppm Exposed
NASAL INFLAMMATION	10/367	35/354[b]	17/349	28/355[b]
MANDIBULAR LYMPH NODE:				
Plasmacytosis	17/322	50/334[b]	46/330[b]	31/329
Hemorrhage	2/332	7/334	7/330	10/329[a]
LIVER CYSTS	3/373	4/357	13/357[a]	39/363[b]
BILE DUCT HYPERPLASIA	2/373	2/357	1/357	17/363[b]
HEPATOCYTE PLEOMORPHISM	11/373	6/357	11/357	33/363[b]
GALL BLADDER CRYSTALS	10/303	7/295	8/315	53/312[b]
ANGIECTASIS	16/387	26/371	29/368[a]	59/371[b]
KIDNEY:				
Hydronephrosis	4/374	11/362	6/353	14/365[a]
Cysts	2/374	4/362	10/353[a]	7/365

[a] Significantly different from controls at 0.05 level.
[b] Significantly different from controls at 0.01 level.

There were non-neoplastic changes seen in the liver with marked increase in incidence in the high exposure level. These included cysts, bile duct hyperplasia, and hepatocellular pleomorphism. Gall bladder crystals were also increased significantly at the highest exposure level. There were changes in the microvascular system seen in numerous body locations. Statistically significant increases in angiectasis were seen in the high exposure group of mice.

Nasal adenomas and adenomatous polyps were seen in a few mice at the highest exposure level (Table 3). Although the numbers were not large, this was considered a significant finding since none were found in the control group and we rarely see this lesion in untreated mice. There were statistically significant increases in lung adenomas seen in mice exposed to 2.0 ppm MMH.

TABLE 3. NEOPLASTIC LESIONS FOUND IN C57BL/6 MICE FOLLOWING INHALATION OF MMH VAPOR FOR ONE YEAR

	Unexposed Controls	0.02 ppm Exposed	0.2 ppm Exposed	2.0 ppm Exposed
NASAL MUCOSA:				
Adenoma	0/367	1/354	0/349	1/355
Adenomatous polyps	0/367	0/354	0/349	3/355
LUNG:				
Adenomas	13/364	16/354	23/347	56/360[b]
Carcinomas	0/364	1/354	2/347	3/360
LIVER:				
Adenomas	6/373	2/357	5/357	20/363[b]
Carcinomas	2/373	4/357	4/357	14/363[b]
DUODENUM ADENOMA	1/310	5/303	7/309[a]	5/308
HEMANGIOMA	5/387	9/371	5/368	22/371[b]
HEMANGIOSARCOMA	1/387	4/371	4/368	5/371

[a] Significantly different from controls at 0.05 level.
[b] Significantly different from controls at 0.01 level.

There were also statistically significant increases in hepatocellular adenomas and carcinomas in the 2.0 ppm MMH exposed mice. Neoplastic vascular lesions were markedly increased in the high exposure level. Increases in these types of tumors have been reported in animals following exposure to other hydrazine compounds.

Lesions most frequently seen in male and female rats are shown in Tables 4 and 5, respectively. There were no adverse MMH exposure related lesions in either male or female rats but, as frequently happens with exposure of experimental animals to hydrazine compounds, there were dose related decreases in the incidence of leukemia and pituitary adenomas. The overall tumor incidence (both benign and malignant) was comparable in all groups of rats.

TABLE 4. LESIONS FOUND IN FISCHER 344 MALE RATS FOLLOWING INHALATION OF MMH VAPOR FOR ONE YEAR

	Unexposed Controls	0.02 ppm Exposed	0.2 ppm Exposed	2.0 ppm Exposed	5.0 ppm Exposed
LUNG:					
Hyperplasia	1/150	0/100	0/100	0/99	0/99
Carcinomas	7/150	6/100	0/100	3/99	1/99
LEUKEMIA	8/150	6/100	1/100	1/99	1/99
MALIGNANT LYMPHOMA	10/150	3/100	2/100	2/99	3/99
PITUITARY ADENOMAS	44/150	34/100	32/100	23/99	18/99
KIDNEY NEPHROPATHY	113/150	57/100	82/100	60/99	40/99
TESTICULAR CARCINOMA	113/150	86/100	89/100	74/99	77/99
THYROID "C" CELL ADENOMA	22/150	17/100	18/100	15/99	3/99
TOTAL ANIMALS WITH TUMORS	138/150	95/100	96/100	84/99	89/99

TABLE 5. LESIONS FOUND IN FISCHER 344 FEMALE RATS FOLLOWING INHALATION OF MMH VAPOR FOR ONE YEAR

	Unexposed Controls	0.02 ppm Exposed	0.2 ppm Exposed	2.0 ppm Exposed	5.0 ppm Exposed
LUNG:					
Adenomas	1/149	1/99	2/100	1/99	1/99
Carcinomas	3/149	5/99	1/100	3/99	0/99
LEUKEMIA	9/149	5/99	2/100	0/99	0/99
MALIGNANT LYMPHOMA	10/149	1/99	3/100	1/99	0/99
PITUITARY ADENOMAS	43/149	45/99	43/100	48/99	26/99
KIDNEY NEPHROPATHY	32/149	12/99	19/100	23/99	15/99
MAMMARY:					
Hyperplasia	10/149	9/99	10/100	18/99	9/99
Adenomas	15/149	10/99	10/100	9/99	9/99
Adenocarcinomas	5/149	1/99	0/100	0/99	2/99
TOTAL ANIMALS WITH TUMORS	100/149	65/99	64/100	70/99	54/99

We conclude from these studies that MMH is weakly tumorigenic for the mouse and hamster, but is not tumorigenic for rats. In the hamster, tumors associated with MMH inhalation were seen only in the respiratory system and were associated with other lesions of inflammatory or irritative nature. These tumors were apparent only at the microscopic level and during the last month of postexposure observation and at terminal examination. Tumor incidence in mice, both benign and malignant, was significantly increased in 2.0 ppm MMH exposed mice in the respiratory system, liver and kidney. The tumorigenic response was greater in mice than in hamsters.

THE EVALUATION OF THE ONCOGENIC POTENTIAL OF INHALED HYDRAZINE IN RATS AND HAMSTERS AFTER A SERIES OF WEEKLY ONE-HOUR EXPOSURES

One of the uses of hydrazine is as a fuel in standby power systems of operational aircraft. Maintenance of the systems may result in accidental human exposure to high concentrations for brief periods. The specific concern and purpose of this study was to assess the oncogenic risk of this type of exposure to maintenance personnel. The design and conduct of this study simulated severe human exposure utilizing the total doses of hydrazine that had caused pulmonary tumors and nasal polyps in rats and hamsters in previous chronic inhalation exposure studies.

Background

Hydrazine was shown to be a weak oncogen in rats and hamsters exposed to 5.0 ppm and in rats and mice exposed to 1.0 ppm hydrazine 6 hours/day, 5 days/week for a one-year period (MacEwen et al., 1981). The calculated CT or dose equivalent values (concentration x time) for these exposures was 7500 ppm-hours. In order to closely simulate possible accidental human exposure, the present study utilized exposure periods of one hour and maximum nonlethal concentrations of hydrazine. Since the tumors induced by hydrazine were only seen in the respiratory system where direct contact occurred and were always associated with other lesions produced by the irritative effects of hydrazine on nose epithelial surfaces we believe that the compressed exposure of 7500 ppm hours is a suitable test for the comparison of short versus long-term exposure at the same CT values. Single weekly one-hour exposures permitted recovery from the acute effects of hydrazine. A sufficient number of one-hour exposures to the maximum nonlethal concentrations was utilized to reach a CT of 7500 ppm-hours. Rats and hamsters were selected as the test species since a 7500 ppm-hour CT of hydrazine has already been demonstrated to produce nasal tumors in these species.

Methods

A brief description of the protocol for preliminary studies was presented in the 1980 annual report (MacEwen and Vernot, 1980). Animals used in this experiment consisted of male and female Fischer 344 rats and male Golden Syrian hamsters obtained from Charles River Breeding Laboratory. Modifications to the original protocol have been made and are presented in this report.

An LC_{50} for hamsters was previously determined in our laboratory (MacEwen and Vernot, 1975). Therefore Phase I was designed to determine the 1-hour LC_{50} values for male and female rats. Preliminary exposures demonstrated, however, that it was impossible to generate sufficiently high vapor concentrations of hydrazine for LC_{50} determinations without aerosol formation. Nevertheless, preliminary exposures indicated the maximum non-lethal level was approximately 750 ppm, for repeated 1-hour exposures. The experimental approach was, therefore, modified so that Phase I consisted of exposing 10 male rats, 10 female rats and 20 male hamsters to a concentration of 750 ppm hydrazine twice per week for 5 weeks.

The 10 exposures were conducted in a 1 m^3 Rochester Chamber. The chamber concentration of 750 ppm hydrazine was first established and stabilized. The rats and hamsters were then rapidly inserted into the chamber by means of sliding cage drawers. At the end of one hour the animals were rapidly removed. A total of 4 cage drawers were used. The animals were exposed in groups of 10.

All animals were weighed prior to each exposure and again after a 2-week postexposure observation period. At the end of the observation period all animals were sacrificed. Since the intent of this portion of the study was to establish a maximum non-lethal concentration, pathologic examination was not required. Mean body weights are summarized in Table 6.

TABLE 6. MEAN BODY WEIGHTS OF ANIMALS EXPOSED WEEKLY TO INHALED HYDRAZINE (PHASE I)

Exposure No.	Male Rats Wt. (grams)	Female Rats Wt. (grams)	Male Hamsters Wt. (grams)
1	279	161	126
2	282	165	122
3	285	164	116
4	279	163	112
5	274	161	109
6	271	161	111
7	276	163	107
8	271	164	109
9	271	162	109
10	267	163	111
2 Weeks Post	312	176	122

In the absence of a nonexposed control group, statistical evaluation of the data was not conducted. However, even in the absence of statistical comparisons it was apparent that the body weight gains of all animal groups exposed to the 750 ppm concentration of hydrazine were adversely affected over the entire exposure period. Weight loss by the 10th exposure was seen for male rats and hamsters, while female rats showed minimal weight gains. Recovery was seen for all groups at 2-weeks postexposure. The stress of exposure was reflected in a general unthrifty appearance of the animals, but there was no mortality in any group.

Phase II exposures were conducted in the same manner (sliding cage drawers) and utilized the same chamber as Phase I. Slightly younger animals were used and matched chamber control groups were included. The 1-hour exposures were conducted once per week. A total of 10 male rats, 10 female rats and 20 hamsters as well as equivalent numbers of controls were utilized. From these groups 5 male rats, 5 female rats, 10 hamsters and an equal number of controls were sacrificed after the first 1-hour exposure for gross and histologic examination. The remaining animals were sacrificed and examined 24 hours after the final exposure.

Other than the scheduled sacrifice there was no mortality in any group.

The body weight gain of male rats exposed to the 750 ppm concentration of hydrazine was adversely affected over the entire exposure period (Figure 1). Female body weight gain was slightly reduced in exposed animals for the first part of the exposures (Figure 1). Male hamster body weight gain appeared to be slightly reduced in treated animals by the end of the exposure period (Figure 2).

The mean hydrazine concentrations for the individual Phase II exposures are shown in Table 7.

TABLE 7. MEAN HYDRAZINE CONCENTRATIONS (ppm) RECEIVED BY ANIMALS IN PHASE II HYDRAZINE EXPOSURES (NOMINAL 750 ppm)

Animal Group Numbers									
1	2	3	4	5	6	7	8	9	10
780	768	769	769	771	729	748	754	736	749

CT = 7573 ppm-hours.

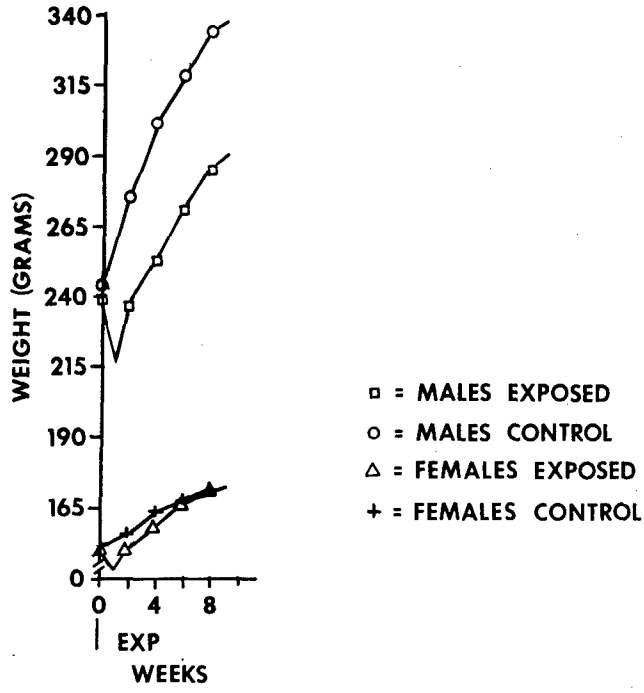


Figure 1. Mean body weights of male and female rats exposed to 750 ppm hydrazine vapor one hour weekly.

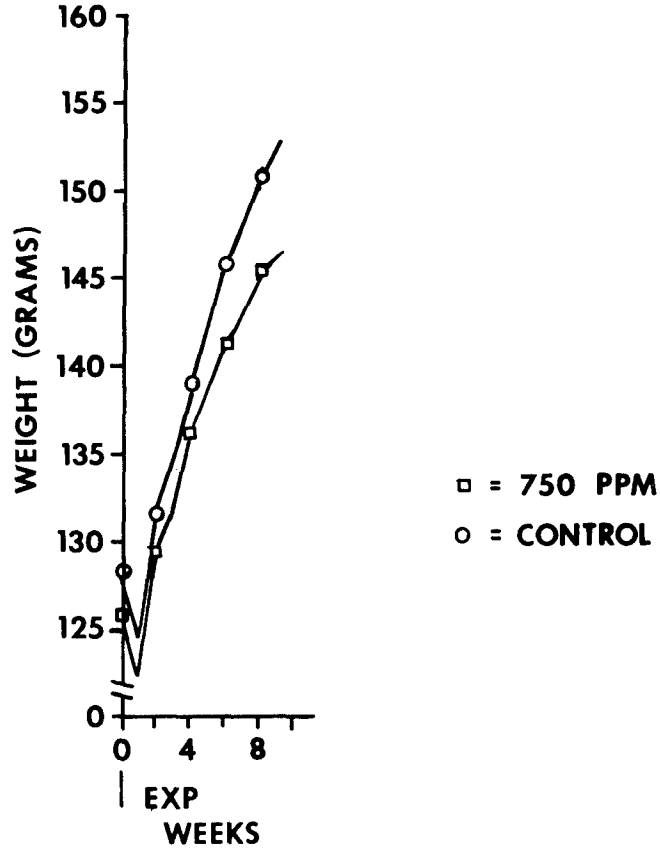


Figure 2. Mean body weights of male hamsters exposed to 750 ppm hydrazine vapor one hour weekly.

There were no treatment related effects noted during gross necropsy. Approximately 33 tissues/animal were taken and submitted for histologic examination. No histologic report has been generated to date and this information was not essential to the initiation of the last phase of the study.

The results of Phase II served adequately as a pilot study for Phase III in that it demonstrated that repeated weekly one-hour exposures to 750 ppm hydrazine were tolerated by rats and hamsters with no mortality. A total of 900 rodents were used in Phase III to evaluate the oncogenic potential of hydrazine following exposure to the selected concentrations of 750 or 75 ppm. The latter dose was chosen in an attempt to establish a no-effect level. The exposure regimen was the same as established in Phase II: one hour per week for 10 weeks (total CT values 7500 and 750 ppm hours). The two exposure groups as well as an unexposed control group each consisted of 100 male rats, 100 female rats and 100 male hamsters.

Because of the large number of animals in each experimental group it was impractical to expose all of the animals in a single one-hour exposure. Therefore, the exposures were conducted with a single sex and species of rodents. A large Rochester Chamber (2 m^3) was used for these purposes. The first exposure was conducted in December 1980 and consisted of 100 male rats. Considerable difficulty was experienced in obtaining and maintaining the desired 750 ppm hydrazine concentration. There was also an extremely high aerosol formation in the chamber. After 1 hour, the animals were removed. They appeared wet and a few were convulsing. The cages were also coated with hydrazine condensate. The female rat group, followed by the hamsters, were then exposed. The conditions of these groups after the one-hour exposures appeared similar to that of the male rats. Close examination of the chemical analysis revealed concentration excursions above the desired level. Within 24 hours, 4 male rats and 14 female rats were dead. At 48 hours there was additional mortality. All surviving animals displayed severe symptoms: lethargy, dyspnea, ataxia, bloody discharge of the nose and eyes.

Due to the mortality and condition of the surviving animals after exposure to the aerosols and/or high hydrazine concentrations, the decision was made to sacrifice the remaining animals and re-initiate Phase III. Accordingly, new animals were purchased and received the same quality control and randomization procedures as the original group of animals.

Additional experimentation indicated that better control of the 750 ppm hydrazine concentration and reduced aerosol formation could be obtained by reducing the number of animals per exposure to 50. It was also advantageous to bring the chamber concentration of hydrazine up to slightly over the desired point and then rapidly insert the animals. A Rochester Chamber ($2m^3$) was modified by the addition of a large hinged portal for rapid insertion and withdrawal of the cages.

The 750 ppm hydrazine exposures were conducted on Thursdays. The animals were caged 12 or 13 per cage (total of 4 cages) during the exposure period. The 75 ppm hydrazine exposures were conducted on Tuesdays. Aerosol formation was not a problem at this concentration and the animals were exposed in groups of 100 (25 per cage) with 3 consecutive exposures conducted.

Immediately following the one-hour exposure periods the animals were transferred to other Rochester Chambers for a degassing period. Food and water were available ad libitum during the degassing and non-exposure periods. The sequence of exposure (i.e., male rat, female rat, male hamsters) was rotated weekly since there was a slight decline in the concentration and aerosol variability during the course of the daily consecutive exposures.

Phase III exposures were started during the week of 23 February 1981 and concluded during the week of 27 April 1981.

The animals were weighed weekly prior to exposure during the course of the exposure period. The results of these weighings are shown in Figures 3 through 5 for male rats, female rats and male hamsters, respectively. Subnormal body weight gains were evident in all groups of animals exposed to 750 ppm hydrazine. After one exposure the body weights of the animals were statistically different ($p \leq 0.05$) from corresponding unexposed control animals. These differences remained throughout the course of the exposure period. Exposure to 75 ppm hydrazine produced only transient body weight effects in rats. The exposed hamsters actually gained more weight than the unexposed controls.

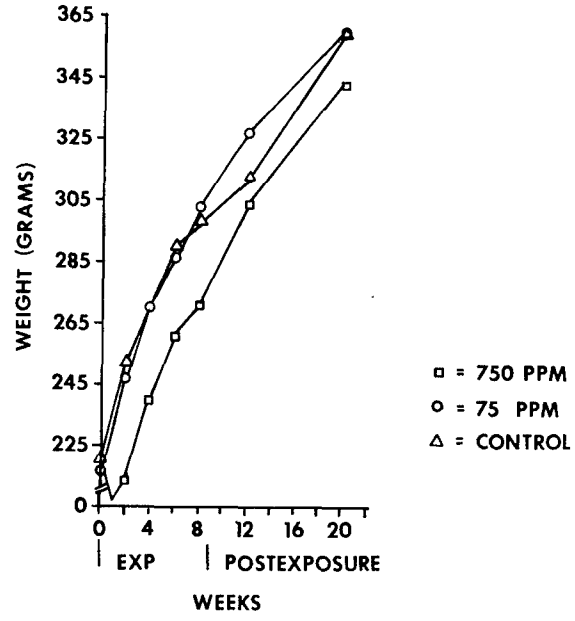


Figure 3. Mean body weights of male rats during 10 weekly one-hour exposures to inhaled hydrazine (Phase III).

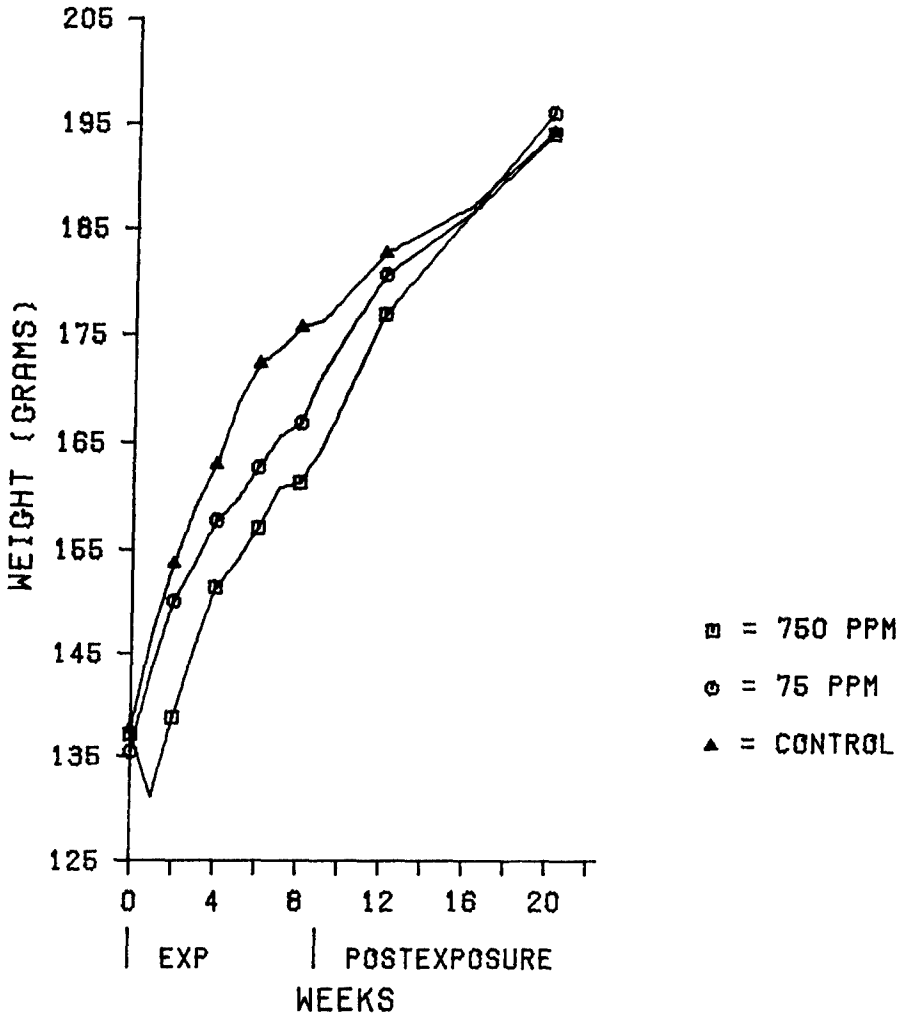


Figure 4. Mean body weights of female rats during 10 weekly one-hour exposures to inhaled hydrazine (Phase III).

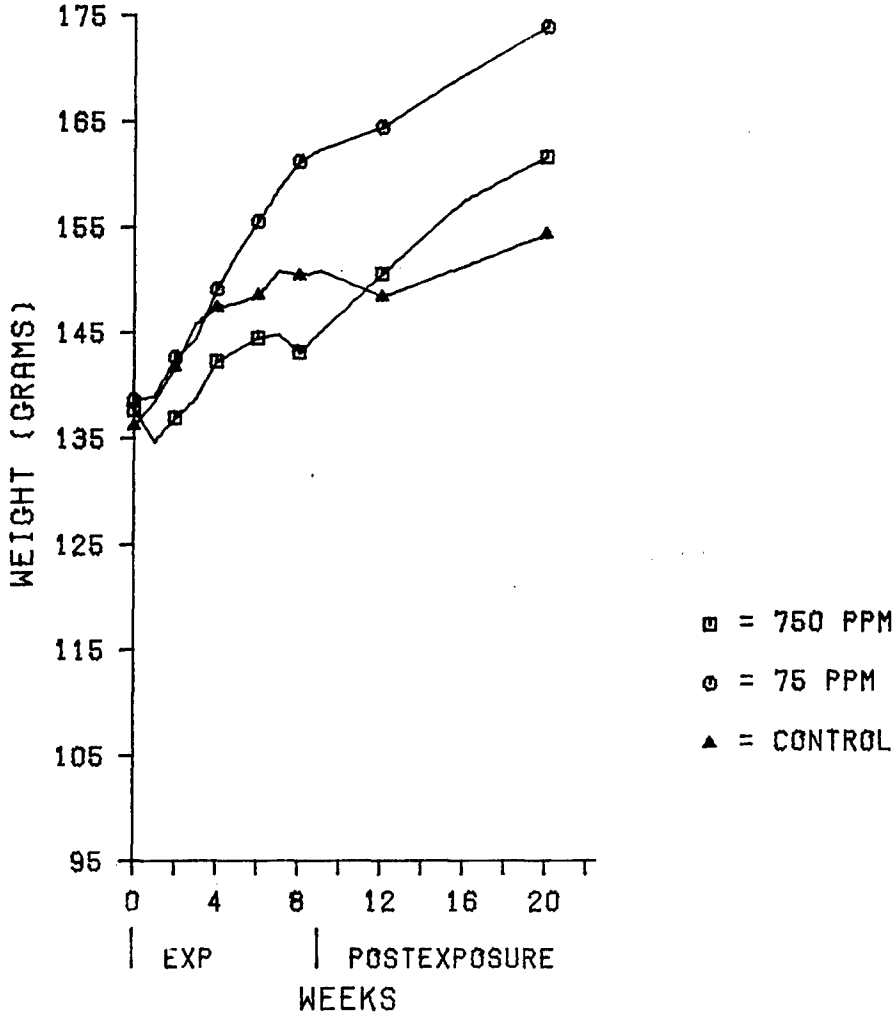


Figure 5. Mean body weights of male hamsters during 10 weekly one-hour exposures to inhaled hydrazine (Phase III).

No significant mortality occurred during Phase III exposures. Mortality ratios are presented in Table 8.

TABLE 8. MORTALITY IN RODENTS AFTER 10 WEEKLY ONE-HOUR EXPOSURES TO 7500 PPM OF HYDRAZINE

Species	Sex	Nominal Conc. (ppm)	Mortality Ratio
Rats	Male	0	0/100
		75	0/100
		750	0/100
	Female	0	0/100
		75	0/100
		750	1/100
Hamsters	Male	0	0/100
		75	1/100
		750	1/100

Histopathologic examination of the tissues from the dead rats and hamsters in the 750 ppm hydrazine exposures failed to reveal a definite cause of death.

The concentration means for the series of 10 exposures are shown in Table 9 along with the calculated CT values. The CT values obtained were, in all cases, within 3% of the desired CT value and should be adequate for the assessment of the oncogenic potential of a few accidental exposures to inhaled hydrazine.

TABLE 9. MEAN CONCENTRATIONS AND TOTAL CT FOR ANIMALS IN PHASE III HYDRAZINE EXPOSURES

Species	Sex	Nominal Conc., (ppm)	Groups 1	2	3	4	5	6	7	8	9	10	ppm/hours CT
Rat	Male	750[a]	733	705	719	693	762	725	736	717	763	745	7298
		750	726	695	727	720	756	750	737	739	783	740	7373
Rat	Female	750[a]	739	764	746	761	713	716	747	752	709	755	7402
		750	740	739	755	764	751	752	739	757	720	707	7424
Hamster	Male	750[a]	747	746	765	764	764	776	730	728	725	759	7504
		750	746	750	758	737	733	760	728	740	748	762	7452
Rat	Male	75	76	72	77	77	74	73	75	77	76	76	753
Rat	Female	75	75	74	77	77	76	75	73	77	75	75	754
Hamster	Male	75	75	76	77	77	77	75	78	75	74	75	758

[a]50 animals per exposure, 2 consecutive exposures.

The postexposure observation period began in April 1981. The surviving male and female rats will be maintained for 24 months after the 10th exposure. At that time 10% of the male and female rats will be sacrificed for tissue examination. The remaining animals will be held for 30 months postexposure or until cumulative

mortality reaches 90%. The hamsters will be maintained for 24 months postexposure or until cumulative mortality reaches 90%. All animals will be weighed monthly during the postexposure observation period.

All animals that die or are sacrificed will be necropsied and all tissues listed in Table 10 will be taken. However, histologic examinations will be routinely conducted only on rodents from the high dose level and restricted to those tissues listed in Table 11 which were the tissues in which tumors were produced in a long-term chronic exposure. Histologic examinations of the selected tissues from the 750 CT rodents will occur if hydrazine-related pathologic alterations are noted in animals from the 7500 ppm hour CT groups. All other tissues taken will be stored and examined if necessary.

TABLE 10. TISSUES TO BE SAMPLED FROM ANIMALS EXPOSED TO HYDRAZINE VAPOR

Gross lesions	Skin
Tissue masses or suspect tumors and regional lymph nodes	Mandibular lymph node
Larynx	Mammary gland
Trachea	Salivary gland
Lungs and bronchi	Stomach
Heart	Duodenum
Thyroids	Ileum
Parathyroids	Colon
Esophagus	Anus
Liver	Mesenteric lymph node
Sternebrae, vertebrae or femur (plus marrow)	Thigh muscle
Kidneys	Sciatic nerve
Bladder	Thymus
Nasal cavity	Gall bladder
Brain	Pancreas
	Adrenals
	Seminal vesicles
	Prostate
	Testes
	Uterus
	Pituitary

TABLE 11. TISSUES SELECTED FOR HISTOPATHOLOGIC EXAMINATION PHASE III HYDRAZINE EXPOSED ANIMALS

Male and Female Fischer 344 Rats	Male Golden Syrian Hamsters
Tissue masses or suspect tumors and regional lymph nodes	Tissue masses or suspect tumors and regional lymph nodes
Nasal cavity	Nasal cavity
Larynx	Larynx
Trachea	Trachea
Lungs and bronchi	Lungs and bronchi
Thyroid	Colon

A STUDY OF THE ONCOGENIC POTENTIAL OF PURIFIED UDMH IN MICE

Repeated inhalation exposures of mice to purified unsymmetrical dimethylhydrazine (UDMH) were conducted to determine whether tumors induced in a previous study with this chemical were caused by the UDMH or a minor contaminant dimethylnitrosamine (DMNA), a well-known carcinogen present in the UDMH used in that study at a concentration of 0.12%.

The mice were exposed 6 hours daily on an industrial type schedule to 5.0 ppm UDMH in Rochester chambers of 2 m^3 volume from June 1978 to June 1979. They were held for postexposure observation for 1 year with survivors being necropsied in June 1980 for histologic examination. The results presented confirm that purified UDMH is a carcinogen in mice. The tumor incidence was greater and more significant than in the previous study when the total exposure time was only 6 months duration and the total dose received was lower.

Background

UDMH (1,1-dimethylhydrazine) is a missile fuel which has been reported to be carcinogenic in animals (Roe, 1967; Toth, 1972 and 1973) when administered orally or by gavage.

The indication that UDMH is carcinogenic led to studies involving the inhalation of UDMH vapor. MacEwen and Vernot (1976) described a study involving the exposure of four animal species to UDMH vapor. Dogs exposed to 5.0 ppm UDMH for a period of six months on an intermittent (5 day/week, 6 hours/day) schedule exhibited hepatotoxic symptoms characterized by increased SGPT levels and increased BSP retention time. Histologic examination of the tissues of rodents exposed to UDMH indicated that mice were the most susceptible species, with significant increases in circulatory and liver tumors in exposed animals.

The UDMH used for the study described by MacEwen and Vernot (1976) contained 0.12% dimethylnitrosamine (DMNA) which was used as a starting material in the manufacturing of UDMH. Haun (1976) suggested that the hepatotoxic effects described by MacEwen and Vernot (1976) were due to the presence of the DMNA in the sample of UDMH used for the inhalation exposure. He conducted inhalation studies in dogs using either purified UDMH or UDMH spiked to contain 0.12% DMNA. He concluded from the results that the hepatotoxic effects observed by MacEwen and Vernot (1976) were related to the DMNA present in the UDMH.

The incidence of tumors in the mice exposed to UDMH by MacEwen and Vernot could not be conclusively associated with UDMH since there was simultaneous exposure to DMNA. DMNA is a potent liver toxin which suggests that it, rather than UDMH, may have been the agent responsible for the tumors.

In an attempt to resolve the uncertainty in interpretation of the previous chronic inhalation results, the present study was designed to use UDMH of as high a purity as possible in a year-long exposure to the highest concentration used previously, 5.0 ppm. The UDMH was redistilled from propellant grade UDMH and contained a DMNA concentration of less than 5 µg/L. Since mice had been most susceptible to the oncogenic effects of exposure, they were the only species tested.

Method

Two hundred female mice (C57BL/6) were exposed for one year to 5.0 ppm purified UDMH in Rochester inhalation exposure chambers 2 m^3 in volume. The exposures were conducted following an industrial work week type schedule of 5 days/week, 6 hours/day. An equal number of mice serving as controls was maintained in similar inhalation exposure chambers. At the conclusion of the exposure, all mice were removed from the chambers and housed in laminar air flow facilities for one year of postexposure observation. At the end of the observation period, all remaining mice were sacrificed. The tissues from these mice as well as the tissues from mice dying during the observation period were taken for histologic examination. Approximately 33 tissues were sampled in each animal using the National Cancer Institute protocol.

The UDMH used in this experiment was redistilled from rocket propellant grade UDMH to remove the dimethylnitrosamine. The redistilled UDMH was stored in teflon-lined, capped, brown 100 ml glass bottles under nitrogen. This storage method has been shown to prevent oxidative degradation of monomethylhydrazine for periods greater than one year.

Each bottle of UDMH was analyzed for DMNA content before and during use with a gas chromatographic method developed in our laboratory. The DMNA concentration in the UDMH used in the study was less than the lower detection limit of 5 µg/L.

A Sage Model 355 syringe pump with a 10 ml glass syringe set up in a contaminant introduction hood was used for chamber UDMH introduction, one for each chamber. The UDMH was evaporated in a 500 ml/minute sample air stream without additional heat and then introduced in the chamber air supply line where the air flow quantity was maintained at 20 cfm. Reaction of UDMH with chamber ducts and walls resulted in a loss of about 10% at equilibrium.

The chamber UDMH concentrations were sampled from a spot just above the breathing zone of the animals. The sample was pulled through polyethylene tubes to an electric two-way valve which sampled for 10 minutes from one chamber and then 10 minutes from the other. The samples were analyzed with an MDA Model 7020 hydrazine analyzer using a sample flow of 31 cc/minute. The switch and analyzer were mounted between the chambers to reduce the distance of sample travel. This was necessary with low sample flow to reduce the switching equilibrium time. Each MDA tape was calibrated before use, and at least one calibration point was run each week thereafter.

Results

The exposure phase of the study was completed in June of 1979. The effect of UDMH exposure on mouse body weight is shown in Figure 6. Differences in mean body weights between 5.0 ppm UDMH exposed mice and unexposed control mice were evident at 7 months of exposure. These differences continued through the postexposure observation period.

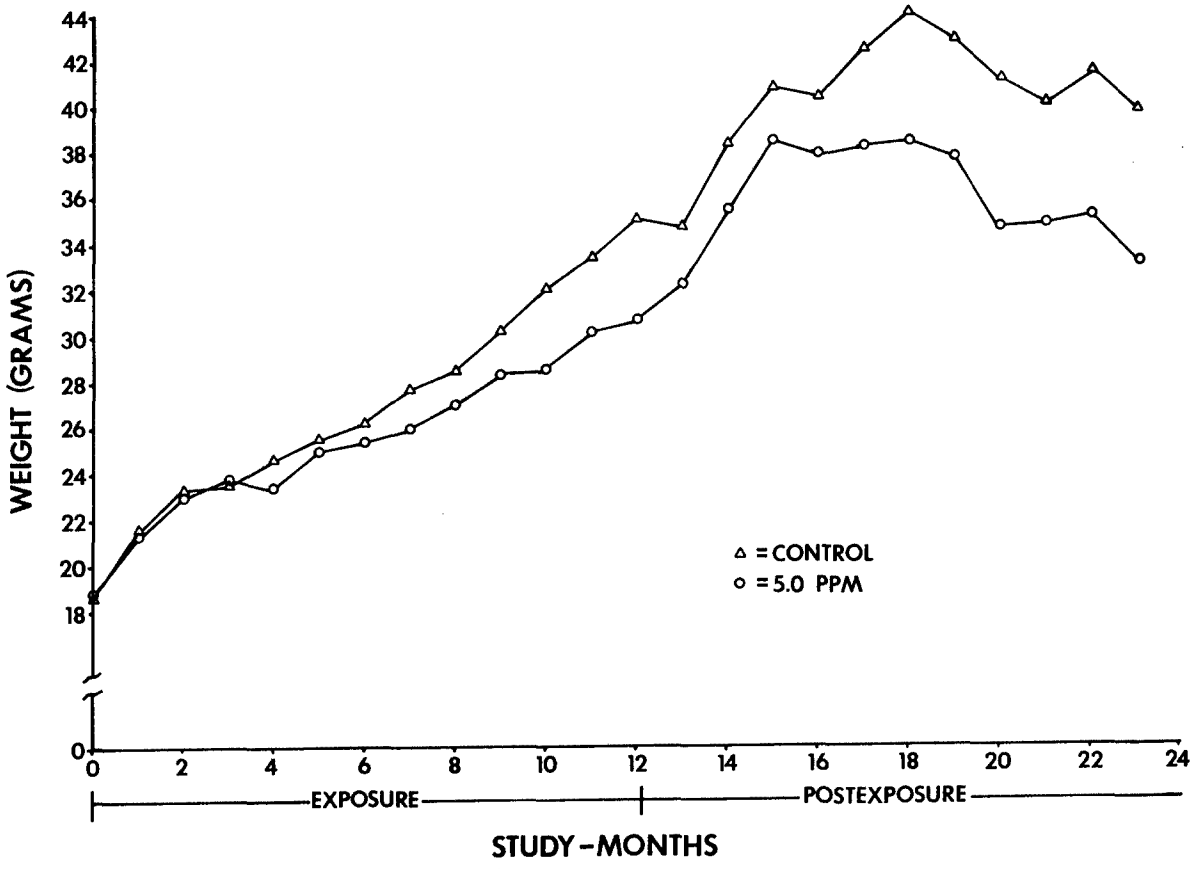


Figure 6. Effect of inhaled purified UDMH on female mouse body weight.

The cumulative mortality of the mice is shown in Figure 7. Exposure to 5.0 ppm UDMH had a negligible effect on mouse mortality.

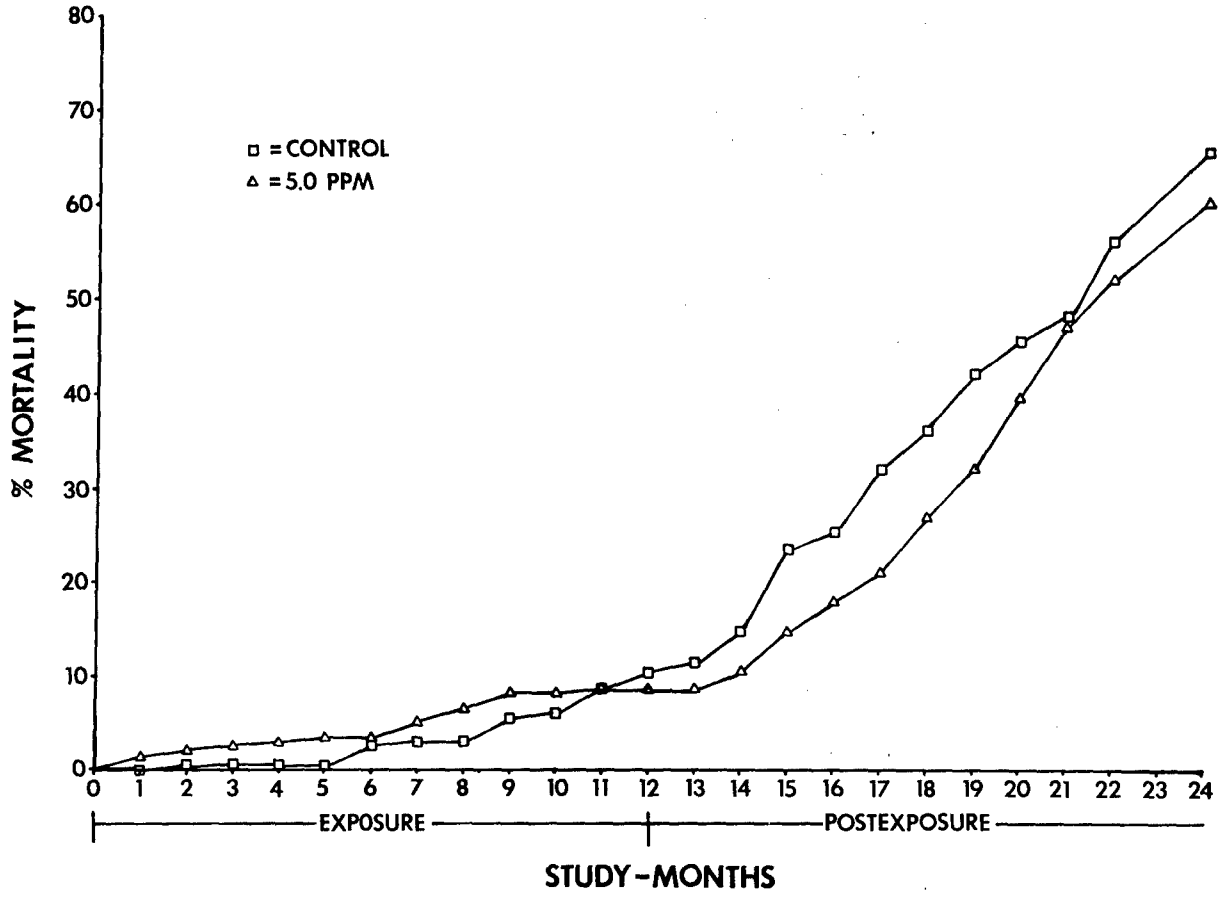


Figure 7. Mortality of female mice exposed to purified UDMH for one year.

Two extra mice were included in the exposed and control groups for electron microscopic examination midway through exposure. These were removed from the study after six months exposure and lungs and livers were collected from each animal. The bronchi, respiratory bronchioles and alveoli were examined using a scanning electron microscope while the liver sections were examined using transmission electron microscopy. All specimens of lung or liver tissue were morphologically normal and there were no differences observed between the 5.0 ppm UDMH exposed and the unexposed control mice after 6 months on study.

Histologic examination of tissues of animals that died during the study or at termination were conducted, and the significance of differences in lesion incidence between test and control groups was evaluated using the Fisher Exact Test. Most lesion types seen were the result of normal aging processes and occurred equally in the UDMH exposed and control group. Lesions for which differences in incidence were observed are presented in Table 12. Most significant are the changes seen in the upper respiratory system of the UDMH exposed mice. There was a much greater incidence of inflammatory changes of nasal mucosa with increased hyperplasia, metaplasia and dysplasia. These signs of increased nasal irritation were accompanied by a variety of benign and malignant tumors, mostly arising from the epithelial lining of the nasal cavity which is a rare site of tumors in aged mice. These nasal tumors included papillomas, papillary carcinomas, adenomatous polyps, and nasal turbinate osteomas. None of these tumor types was observed in the sham exposed control mice.

TABLE 12. LESIONS SEEN IN MICE EXPOSED TO PURIFIED UDMH FOR ONE YEAR AND THEIR UNEXPOSED CONTROLS

	Unexposed Controls	5.0 ppm Exposed
Nasal Mucosa		
Suppurative inflammation	8/183	31/179[b]
Hyperplasia	1/183	7/179[a]
Squamous metaplasia	1/183	19/179[b]
Dysplasia	2/183	14/179[b]
Papilloma	0/183	5/173[a]
Papillary carcinoma	0/183	4/179
Adenomatous polyp	0/183	17/179[b]
Nasal Turbinate Osteoma	0/183	4/179
Lung		
Adenoma	4/187	20/179[b]
Carcinoma	0/187	3/179
Liver		
Adenoma	4/187	20/186[b]
Carcinoma	0/187	1/186
Hemangioma	0/187	9/186[b]
Pituitary Adenoma	88/153[a]	65/144
Malignant Lymphoma	64/191	84/190[a]
TOTAL ANIMALS WITH TUMORS	124/191	145/190

[a] Significant at the 0.05 level.
[b] Significant at the 0.01 level.

While some pulmonary carcinomas were seen in mice exposed in the earlier UDMH inhalation study, there was no statistically significant difference noted between the incidences of pulmonary adenomas of control and exposed groups. The longer exposure of mice to UDMH produced a significant increase in lung adenomas and again showed an increase in pulmonary carcinomas. Six months exposure to UDMH had produced increased hepatic tumors but not a statistically significant difference from controls. The longer exposure to UDMH (12 vs. 6 months) caused significant increases in liver adenomas and hemangiomas. Other hemangiomatous tumors were observed in the livers of exposed animals but are not listed in Table 12. In all, there were 15 hemangiomatous tumors, both benign and malignant, seen in the livers of the UDMH exposed mice compared to none in their sham treated controls. Hemangiomatous tumors occurring in other organ systems were comparable in both groups of mice. Malignant lymphoma incidence was increased in the UDMH exposed mice as has been observed in the earlier study.

Conclusions

UDMH is a tumorigen for mice when inhaled at a 5.0 ppm concentration in repeated daily exposures. This study has shown that the tumor incidence observed in mice exposed to UDMH for a shorter (6 month) period of time was definitely a direct response to the UDMH and not the result of contamination by nitrosodimethylamine as previously suspected. The effect of increasing the exposure phase of the study from 6 to 12 months duration resulted in a significantly increased oncogenic response to the purified UDMH. This was particularly true in the induction of lung adenomas and carcinomas which were not significantly elevated above controls after the 6-month exposure and of nasal tumors which were not seen at all previously.

AIRCRAFT AND MARINE FUELS

The use of jet engines in military and commercial aircraft has led to the development of a number of petroleum distillate fuels with special properties. These fuels are generally less volatile than gasoline fractions used in conventional internal combustion engines.

In 1977 the Toxic Hazards Research Unit began a series of subchronic inhalation exposure studies with the hydrocarbon fuels. The first in the series was jet fuel JP-5 followed by Diesel Fuel Marine, the standard fuel for U.S. naval vessels. These fuels were derived from petroleum sources and the samples delivered for evaluation were considered to be typical batches of fuel meeting military specifications. The first set of studies on fuels were subchronic 90-day continuous exposures of rodents and dogs to determine: 1) target organs for chronic toxic effects; 2) differences

in the toxicities of the inhaled vapor of fuels having different boiling ranges, and; 3) a comparison of the toxic effects and health hazards of fuels having this same boiling range but derived from either petroleum or shale oil sources.

Subchronic inhalation studies were conducted on petroleum and shale oil derived diesel fuel marine in previous contract years. They are not discussed in this report since no active work was conducted during the current year and we are awaiting information on tissue pathology for preparation of final reports.

EVALUATION OF THE TOXIC EFFECTS OF 90-DAY CONTINUOUS EXPOSURE TO PETROLEUM JP-5 JET FUEL

Background

The THRU has conducted studies on a number of jet fuels to determine the toxic effects of prolonged inhalation of the fuel vapor. In 1977, a 90-day continuous inhalation exposure of animals to the petroleum jet fuel JP-5 was conducted. The study was conducted at the request of the U. S. Navy and was designed to determine the toxic effects as well as oncogenic potential of JP-5. Conditions were chosen for the experiment to simulate exposure conditions peculiar to the Navy and to permit comparison with previous exposures to petroleum distillate fuels and future exposures to fuels derived from shale oil sources.

A detailed discussion of the protocol, methodology of the contaminant generation and monitoring system, and available results at the conclusion of the 90-day exposure were presented in an earlier annual report (MacEwen and Vernot, 1978). The present report will update and summarize the results of the study which were not available for previous annual reports.

Male and female beagle dogs, male and female Fischer 344 rats, and female C57BL/6 mice were continuously exposed to concentrations of 150 mg/m^3 or 750 mg/m^3 JP-5 vapor for 90 days in Thomas Dome inhalation chambers. Unexposed controls were held in a separate facility. At the conclusion of the exposures, all dogs and 1/3 of the rodents were killed for gross and histopathologic tissue examination to detect any pathologic lesions caused by exposure to JP-5.

Results

JP-5 related toxic effects noted during the 90-day continuous exposure and immediately after were decreased growth in male rats at both exposure levels compared with unexposed controls. This effect was not observed in female rats or mice. Statistically significant increases in blood urea nitrogen (BUN) and creatinine

levels were measured in both male and female rats from the 750 mg/m^3 exposure group at the conclusion of the 90-day exposure period. Increased levels of BUN and creatinine were also noted in the 150 mg/m^3 exposed groups of rats but at a lower level of statistical significance. These increases were still present in male rats at 19 months postexposure but were not as significant and were absent in the female rats. Significantly lower serum albumin levels and albumin to globulin (A/G) ratios were seen in both the female and male rats exposed to both petroleum JP-5 vapor concentrations and this effect persisted in the male rats exposed at the 750 mg/m^3 JP-5 concentration for 19 months postexposure but was absent in the lower exposure groups of male rats and in the female rats.

Although not included in the original protocol and therefore not measured at the conclusion of the 90-day continuous exposure phase, organ weights were determined for the rats killed at the scheduled interim 19 month postexposure sacrifice. The only difference observed between the petroleum JP-5 exposed rats and their unexposed controls was an increase in the gross kidney weights of male rats exposed at the 750 mg/m^3 concentration. This change in kidney weight appeared to be consistent with the observed clinical chemistry measurements of higher BUN and creatinine levels in the same male rats.

Rodent Pathology

The most significant pathologic changes observed in male Fischer 344 rats immediately following the 90-day continuous exposure to petroleum JP-5 vapor are listed in Table 13.

TABLE 13. PATHOLOGIC RENAL CHANGES IN MALE FISCHER 344 RATS IMMEDIATELY FOLLOWING 90-DAY EXPOSURE TO PETROLEUM JP-5

	N	Kidney Nephrosis	Renal Tubular Necrosis
Unexposed Controls	25	0	0
Exposed 150 mg/m^3	25	19	22
Exposed 750 mg/m^3	25	18	25

Kidney injury was seen in both exposed groups of male rats consisting of early progressive renal nephropathy characterized by multifocal tubular atrophy and of focal tubular necrosis at the cortico-medullary junction. Both lesions were more severe in the 750 mg/m^3 JP-5 exposure group and appeared to be related effects in that the more severe the nephropathy was, the more severe the tubular necrotic lesions. These changes were not seen in unexposed controls or in female rats examined following the 90-day continuous exposure.

Histologic examination of other rat tissues revealed some alveolar pneumonia and pulmonary congestion in female control rats which may have been related to the transient weight loss observed in this group of animals. Three female rats exposed to 150 mg/m^3 JP-5 for 90 days showed atypical hepatic hyperplasia but there were no remarkable changes noted in the higher exposure level group.

In mice, the lesions that appeared to be related to JP-5 exposure were seen in liver and kidney. Mild focal fatty change, consisting of numerous small cytoplasmic vacuoles within the hepatocytes was seen in one (2.7%) of the controls, 24 (72.7%) of the low dose, and eight (23.5%) of the high dose animals. The lesion was of approximately the same mild character in most animals. Representative samples of this lesion were positive for fat with special stains. Mild, diffuse cytoplasmic vacuolization, consisting of "foamyness" or the presence of numerous minute, poorly defined vacuoles within the hepatocytes was present in seven (18.9%) of the controls, five (15.2%) of the low dose, and 15 (44.1%) of the high dose groups. Samples of this lesion were negative for both fat and glycogen with special stains.

Transmission electron microscopic examination of these liver lesions showed that the foamy appearing vacuoles contained fat which may have been lost in the tissue processing for light microscopy.

Tissues from rats that died during the postexposure observation period or were killed at 19 or 21 months postexposure were examined for pathologic lesions. Changes seen in the tissues of the male Fischer 344 rats are listed in Table 14. The most notable effects of continuous petroleum JP-5 vapor inhalation for 90-days had been the early progressive renal nephropathy with some tubular necrosis. This lesion progressed in both exposure groups of male rats and was most severe in the 750 mg/m^3 exposure group. Varying degrees of renal tubular degeneration were present in approximately 80% of both petroleum JP-5 exposed groups. Eighty-eight percent of the high concentration exposure group of male rats and 62% of the lower dose group exhibited diffuse cellular debris of necrotic renal tubular cells with dilatation of the remaining degenerated areas. This lesion was not seen in the unexposed controls of which only 40% have early nephropathy and only 24% of the aging male control rats exhibited mild tubular degeneration.

TABLE 14. PATHOLOGIC CHANGES SEEN IN MALE FISCHER 344 RATS HELD FOR POSTEXPOSURE OBSERVATION AFTER 90-DAY CONTINUOUS EXPOSURE TO INHALED VAPOR OF PETROLEUM JP-5

	Unexposed Controls	150 mg/m^3 Exposed	750 mg/m^3 Exposed
Liver:			
Fatty Change	6	0	2
Hyperplasia	0	20	23
Necrosis	0	1	1
Adenoma	1	2	0
Kidney:			
Nephrosis	0	2	0
Nephropathy	20	1	1
Tubular Degeneration	12	42	40
Dilatation	0	11	2
Dilatation with Medullary Mineralization	0	31	44
Leukemia	14	5	3
Thyroid Cell Adenoma	1	1	6
Pituitary Adenoma	8	18	13
Adrenal Phaeochromocytoma	3	4	5
Testicular Tumors	45	43	41
Preputial Gland Adenoma	0	2	2
Preputial Gland Carcinoma	0	0	1
TOTAL ANIMALS WITH TUMORS	46	49	49
NUMBER OF ANIMALS EXAMINED	50	50	50

Mild hepatic hyperplasia was observed in a significant number of petroleum JP-5 exposed rats but was not sufficient to produce a measurable change in liver weights of these animals.

The total incidence of all tumor types was not significantly greater in the petroleum JP-5 exposed male Fischer 344 rats, but adenomas of the preputial gland, pituitary gland and thyroid C-cells were elevated in a non dose related manner in both groups while their incidence was either very low or absent in the unexposed controls.

The pathologic changes seen in female Fischer 344 rats held for postexposure observation are noted in Table 15. Hepatic hyperplasia was present to a greater degree in the exposed groups of rats as was seen in the male rats and again did not produce any

measurable change in liver weights of these animals. As with the male rats exposed to petroleum JP-5 vapor, the females showed an increased incidence of pituitary and thyroid C-cell adenomas without any significant increase of total tumor incidence. There were also small but apparently dose related increased incidences in mammary fibroadenomas and adenocarcinomas.

TABLE 15. PATHOLOGIC CHANGES SEEN IN FEMALE FISCHER 344 RATS HELD FOR POSTEXPOSURE OBSERVATION AFTER 90-DAY CONTINUOUS EXPOSURE TO INHALED VAPOR OF PETROLEUM JP-5

	Unexposed Controls	150 mg/m^3 Exposed	750 mg/m^3 Exposed
Liver:			
Fatty Change	5	4	5
Hyperplasia	12	20	23
Uterus:			
Endometrial Stromal Sclerosis	28	11	11
Polyps	11	12	6
Adenocarcinoma	1	0	0
Mammary Glands:			
Adenocarcinoma	0	3	4
Fibroadenomas	2	4	7
Leukemia	6	4	6
Pituitary Adenoma	15	23	21
Thyroid C-Cell Adenoma	3	3	6
TOTAL ANIMALS WITH TUMORS	35	39	34
NUMBER OF ANIMALS EXAMINED	44	46	47

There were no lesions in the female C57BL/6 mice exposed to petroleum JP-5 vapor that could be attributed to exposure. The fatty liver lesions seen in these mice immediately after 90-day continuous exposure were not different in degree or incidence from the unexposed control at 2 years of age (Table 16). The small increased incidence of kidney casts and glomerulonephritis seen in exposed mice was not statistically significant.

TABLE 16. PATHOLOGIC CHANGES SEEN IN FEMALE C57BL/6 MICE HELD FOR POSTEXPOSURE OBSERVATION AFTER 90-DAY CONTINUOUS EXPOSURE TO INHALED VAPOR OF PETROLEUM JP-5

	Unexposed Controls	150 mg/m^3 Exposed	750 mg/m^3 Exposed
Liver:			
Fatty Change	34	30	32
Necrosis	5	2	0
Adenoma	1	4	0
Hemangiosarcoma	1	0	0
Kidney:			
Casts	2	3	7
Glomerulonephritis	5	7	8
Leukemia	0	0	1
Malignant Lymphoma	7	14	10
Bone Marrow Hyperplasia	20	27	22
Ovarian Cysts	12	13	6
Pituitary Adenoma	28	24	20
Lung Adenoma	3	2	1
TOTAL ANIMALS WITH TUMORS	39	38	32
NUMBER OF ANIMALS EXAMINED	71	70	71

Summary

Subchronic 90-day continuous inhalation exposure to 750 mg/m^3 petroleum JP-5 vapor produced irreversible renal tubular injury in male rats. Male rats exposed to a 150 mg/m^3 JP-5 concentration also showed kidney lesions to a lesser degree with no apparent dysfunction. Female rats did not show any toxic response during exposure and had only a small increase in some tumor types, postexposure. Subchronic exposure of mice to inhaled petroleum JP-5 vapor produced mild fatty liver changes that were reversible upon cessation of exposure and, with the exception of a slight increase in kidney lesions, were not different from unexposed control animals.

A SUBCHRONIC TOXICITY STUDY OF 90-DAY CONTINUOUS INHALATION EXPOSURE TO SHALE JP-5 VAPOR

A 90-day continuous inhalation toxicity study of oil shale derived JP-5 jet fuel was conducted in 1979 to determine if the health hazards of oil shale materials differ from those associated

with petroleum derived products. The protocol, contaminant, generation and monitoring methods used for the study were similar to other 90-day continuous inhalation studies and were detailed in a previous annual report (MacEwen and Vernot, 1980).

Groups of 3 male and female beagle dogs, 75 male and female Fischer 344 rats, and 150 female C57BL/6 mice were continuously exposed to concentrations of 150 mg/m^3 or 750 mg/m^3 shale JP-5 vapor in Thomas Dome inhalation chambers. Unexposed controls were held in laminar air flow rooms in separate facilities. At the conclusion of the exposure, all dogs and 1/3 of the rodents were sacrificed for gross and histopathologic tissue examination to detect any pathologic lesions caused by exposure to shale JP-5. This sacrifice occurred in October 1979.

The remaining rodents were held for postexposure observation for 19 months, at which time one-half of the animals were killed for tissue collection and examination. This interim sacrifice occurred in May 1981. Animals remaining from the interim sacrifice were held until the 24th month of the study at which time they were killed for tissue comparison with unexposed controls and with petroleum JP-5 exposed animals.

Results that were available through the completion of the 90-day exposure including animal body weights, organ weights, hematology and clinical chemistry results were discussed in the last annual report (MacEwen and Vernot, 1980). The observed toxic effects of greatly increased gross kidney weights in male rats exposed to 750 mg/m^3 shale JP-5 along with significantly elevated blood urea nitrogen and creatinine levels were similar to the changes seen after 90-day continuous inhalation exposure of rats to petroleum JP-5 vapor.

The body weights of both groups of male rats exposed to shale JP-5 vapor were statistically different ($p \leq 0.05$) from the unexposed control groups after 20 months of the study (Figure 8). The general decline in body weights evident in all groups of male rats at 18-20 months on study was also evident in the male rats exposed to petroleum JP-5.

Of the female rats, only those exposed to 750 mg/m^3 shale JP-5 exhibited any body weight effects (Figure 9). At 9 months after termination of the exposure the body weights of female rats in that group were statistically less ($p \leq 0.05$) than the unexposed control female rat body weights. Subsequent mean body weights for the 750 mg/m^3 female rat exposed group were not different from unexposed control rats. Body weights of female rats exposed to 150 mg/m^3 shale JP-5 vapor were not statistically different from unexposed female control rats through the 20th month of the study.

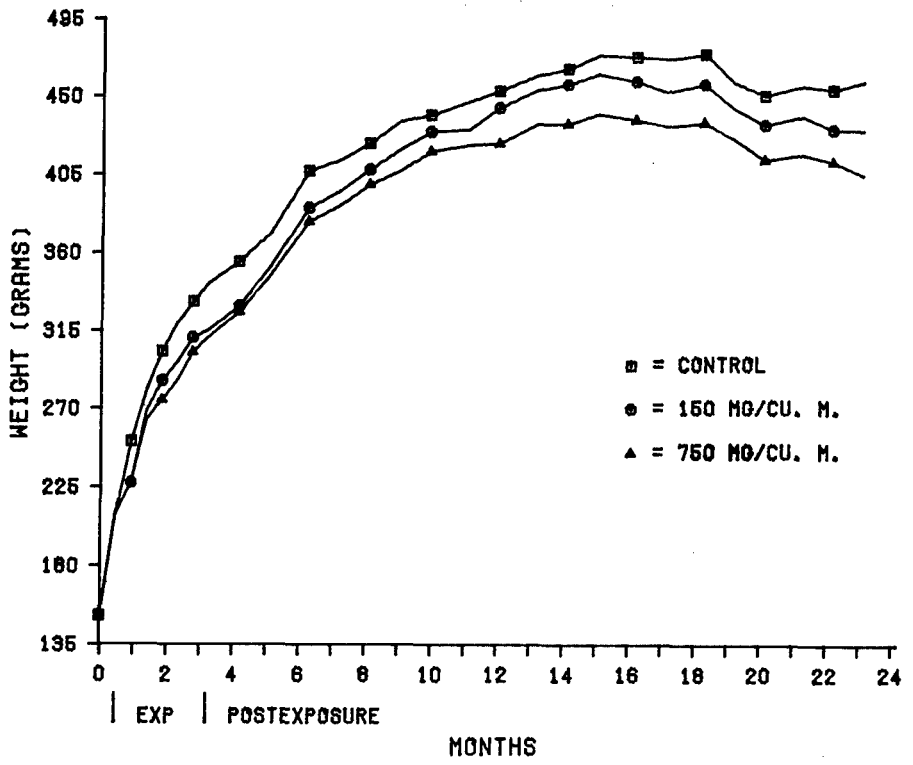


Figure 8. Effect of 90-day continuous exposure to shale JP-5 on male rat body weight.

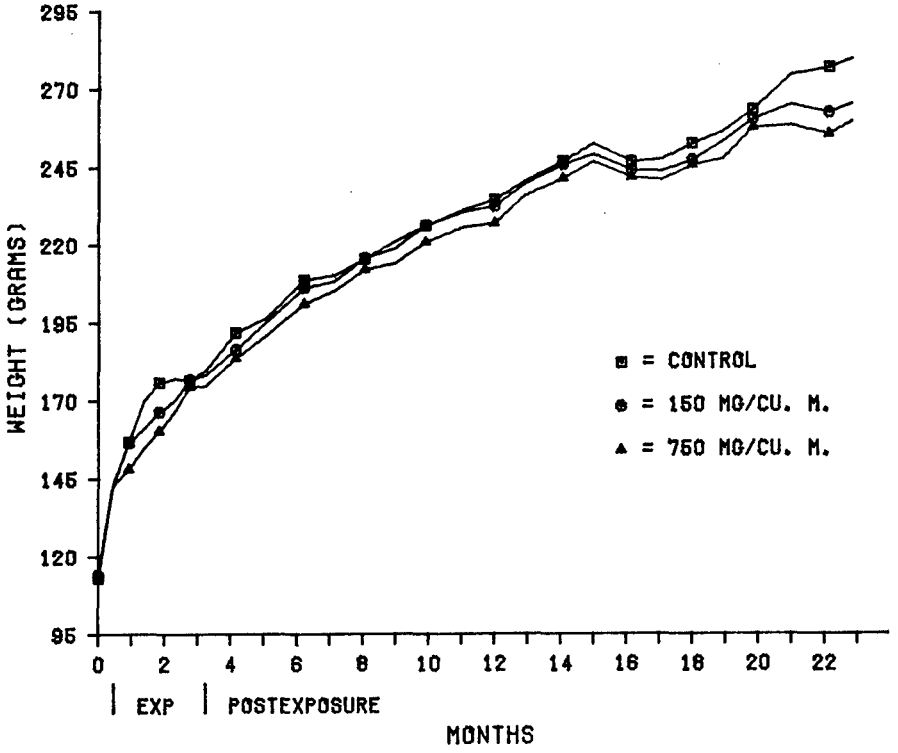


Figure 9. Effect of 90-day continuous exposure to shale JP-5 on female rat body weight.

Histopathologic examination of the tissues obtained from the animals sacrificed at the conclusion of the exposure period is complete and observed lesions noted in male and female rats are listed in Table 17. As was seen with petroleum JP-5 exposure, there was a high incidence of renal tubular necrosis in the exposed male rats but not in females. Again, the degree of injury was greater and the lesion was more diffuse in the 750 mg/m^3 shale JP-5 exposed animals.

TABLE 17. PATHOLOGIC CHANGES IN MALE AND FEMALE FISCHER 344 RATS IMMEDIATELY FOLLOWING 90-DAY CONTINUOUS EXPOSURE TO INHALED SHALE DERIVED JP-5 VAPOR

	Nasal Inflammation	Renal Tubular Necrosis	Hepatocyte Cytoplasmic Vacuolization
Males:			
Unexposed Controls	0	0	0
150 mg/m^3 Exposed	21	24	0
750 mg/m^3 Exposed	12	25	7
Females:			
Unexposed Controls	2	0	0
150 mg/m^3 Exposed	14	0	12
750 mg/m^3 Exposed	10	0	9

N = 25 rats/group

Nasal inflammation was present in both exposed groups of both sexes of rats but was not dose-related. This effect may be a generalized response to inhaled hydrocarbon materials as is probably the case for the increased incidence of a mild, foamy appearing, cytoplasmic vacuolization which was diffuse in nature in hepatocytes of the 750 mg/m^3 exposed male rats and of both groups of exposed females.

In mice the only indication of a toxic effect from inhaled shale JP-5 was a fatty liver change (listed in Table 18) that was diffuse but mild and was not dose related. No other lesions noted in the mice killed immediately following the completion of the 90-day continuous exposure to shale JP-5 could be related to the exposure.

TABLE 18. PATHOLOGIC CHANGES SEEN IN C57BL/6 FEMALE MICE IMMEDIATELY FOLLOWING 90-DAY CONTINUOUS EXPOSURE TO INHALED SHALE DERIVED JP-5 VAPOR

	Unexposed Controls	150 mg/m^3 Exposed	750 mg/m^3 Exposed
Liver:			
Fatty Change	1	45	40
Cytoplasmic Vacuolization	1	0	2
Kidney:			
Tubular Fatty Change	1	10	1
Hydronephrosis	3	0	1
Bone Marrow Hyperplasia	3	9	3
NUMBER OF ANIMALS EXAMINED	50	46	49

The subchronic toxic effects of inhaled JP-5 vapor in mice and rats were similar for both petroleum and shale derived samples of this jet aircraft fuel immediately after 90-day continuous exposure and consisted primarily of mild kidney tubule injury in male rats only. Mild fatty liver changes were seen in both rats and mice exposed to both fuels but the changes were not dose related.

Examination of the tissues from animals held for postexposure observation is still in process and results will be described in a future annual report.

A SUBCHRONIC TOXICITY STUDY OF 90-DAY CONTINUOUS INHALATION EXPOSURE TO DECALIN VAPOR

In 1978 the Toxic Hazards Research Unit conducted a 90-day continuous inhalation exposure to the alicyclic hydrocarbon decalin to determine by comparison if threshold limit values (TLV's) for hydrocarbon fuel mixture were appropriate for this purified solvent. The protocol, experimental methods and clinical findings during and immediately following the conclusion of the 90-day exposure phase of this study have been described in previous annual reports (MacEwen and Vernot, 1979; 1980).

Background

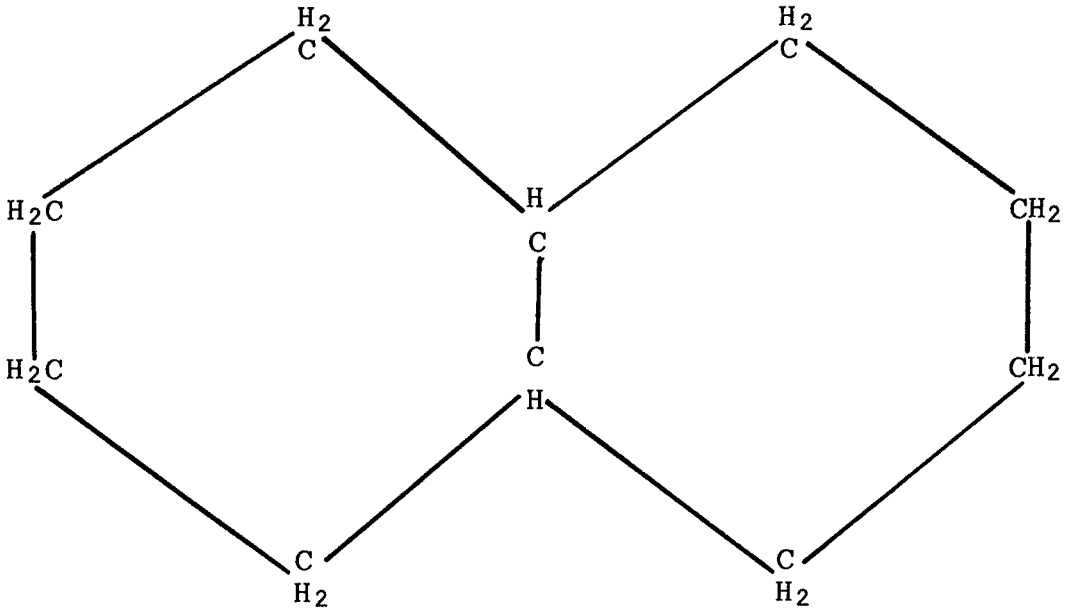
Decalin (decahydronaphthalene) is an alicyclic hydrocarbon commonly used as a solvent. No threshold limit value exists for decalin, and the experimental data available are insufficient for establishing a limit. Gage (1970) described the exposure of 8 rats to 200 ppm decalin for 20 days on a 6 hour/day schedule with no

toxic signs and grossly normal visceral organs at necropsy. Cardini (1942) reported lung congestion, kidney and liver damage in guinea pigs exposed to 319 ppm decalin for up to 23 days.

The THRU conducted a one-month inhalation exposure study of rats, mice, and guinea pigs to decalin vapor (MacEwen and Vernot, 1978). The exposures were 6 hours/day on 22 consecutive working days to 50 or 250 ppm decalin. Respiratory tract irritation was evident in the decalin exposed rats. Hydropic change in the hepatocytes and hyalin droplet formation within the proximal tubular epithelial cytoplasm were seen with increased incidence and severity in the rats exposed to decalin vapor. Mice and guinea pigs exposed to decalin also had signs of respiratory tract irritation.

Because of the incidence of the pathologic lesions noted in animals exposed to decalin vapor intermittently for one month at the THRU, it was deemed necessary that a long-term study with clinical tests and observations be developed. The present study was designed to determine the toxic effects, including oncogenesis, of a 90-day continuous exposure of the animals to decalin vapor.

Decalin is a colorless liquid with a mild characteristic odor. It is typically 99.9% pure with a cis/trans ratio of 54%/46%. Its chemical structure and physical properties are as follows:



Molecular Weight	-	138
Boiling Point Range	-	188-195°C
Flash Point	-	57°C
Specific Gravity	-	0.885-0.890

Results

The toxic effects of exposure to inhaled decalin were similar to those seen after 90-day continuous exposure to JP-5 and other hydrocarbon fuels in male Fischer 344 rats. Body weight gains of the exposed males were significantly lower than unexposed controls at both exposure levels. The mean gross weight of excised male rat kidneys from the 50 ppm decalin exposure group was heavier than those of the 5.0 ppm group or their unexposed controls and this difference was statistically significant. At 19 months postexposure, the body weight differences were still present but kidney weights, although still heavier, were not statistically significant and clinical measurements related to kidney function such as blood urea nitrogen (BUN) and creatinine were not different from unexposed controls.

Pathologic Changes

Clearly dose-related lesions were not observed either grossly or microscopically in dogs exposed to decalin vapor. Of moderate significance, however, was the finding of pulmonary inflammatory lesions in both control and exposed groups. These inflammatory changes ranged from mild, focal, chronic lesions in some dogs to diffuse, chronic active bronchopneumonia in others. In most cases, inflammation was attended by abundant eosinophil infiltrates.

Lesions in rats necropsied at the conclusion of the 90-day exposure that were considered to be exposure-related occurred only in male rats and were restricted to the kidneys where 100% of the 5.0 ppm decalin exposure group and 96% of the 50 ppm decalin exposure group exhibited changes compatible with toxic tubular nephrosis as shown in Table 19. The lesions were dose-related in severity. Lesions of this type were not observed in female rats or in the control animals. With light microscopy, the most striking lesions consisted of mild to moderate focal necrosis of tubular epithelial cells at the level of the corticomedullary junction with mild, cystic tubular dilatation and intraluminal casts of granular, amorphous cellular debris. The tubule retained a lining of flattened, stretched-out epithelium where persisting cells attempted to resurface the denuded tubular basement membrane. These tubular changes were usually accompanied by the presence of moderately abundant cytoplasmic hyalin droplets in the proximal tubular epithelial cells. Hyalin droplets are regarded as microscopically visible aggregates of protein. Their presence in tubular epithelium indicates an inability to efficiently transport resorbed proteins from the glomerular filtrate to the capillary blood at the abluminal surface. Two pathogenic mechanisms, either alone or in combination, may be responsible for droplet formation. The first mechanism involves direct toxic injury to the tubular epithelial cells, causing obstruction of protein transport and increased cytoplasmic accumulations. The other process results from glomerular disease in which excessive proteins leak into the glomerular

filtrate and subsequently overwhelm the transport capacity of the tubular cells. To differentiate between these two possible mechanisms, electron microscopic studies were conducted in an effort to demonstrate glomerular lesions which might promote excess proteinuria. All structures, including the basement membrane, endothelial lining, and epithelial cell foot processes were normal in appearance. Over 190 ultrastructural photomicrographs of renal tissue were examined. Although these photographs confirmed the presence of increased cytoplasmic protein droplets in the proximal tubular epithelium and focal necrosis of tubular epithelium at the corticomedullary junction, there were no distinct morphologic changes observed in glomeruli.

TABLE 19. PATHOLOGIC CHANGES IN MALE AND FEMALE FISCHER 344 RATS IMMEDIATELY FOLLOWING 90-DAY EXPOSURE TO DECALIN (N = 25)

	Kidney Nephrosis	Nasal Inflammation
Males:		
Unexposed Controls	0	0
5.0 ppm Exposed	25	1
50 ppm Exposed	24	0
Females:		
Unexposed Controls	0	1
5.0 ppm Exposed	0	1
50 ppm Exposed	0	4

The occurrence of renal lesions is quite high in the Fischer 344 male rat strain. Coleman et al. (1977) detailed the incidence of pathologic changes during aging of Fischer 344 male rats. In all but one of 144 rats studied, some sort of renal pathology was observed. There was a high correlation between increasing age and increasing severity of chronic nephropathy centered mainly on changes in the glomeruli. In the present study of decalin, there was an absence of glomerular involvement in the renal lesions observed in the male rats exposed to decalin, and the lesions appeared to be distinctly different from those seen in cases of chronic nephropathy. This finding suggests that hyalin droplet formation and tubular epithelial cell necrosis observed at the conclusion of the 90-day exposure period were probably the result of the direct toxic effect of decalin or one of its metabolites.

In mice necropsied at the conclusion of the 90-day exposure, lesions that were considered to be dose-dependent, as shown in Table 20, were limited to the liver where 85% of the 5.0 ppm decalin exposure group and 88% of the 50 ppm decalin exposure group exhibited varying degrees of hepatocellular cytoplasmic vacuolization (fatty change). This lesion was present in only 4% of the unexposed control animals. Electron microscopic examination of the

hepatocytes further indicated that increased cytoplasmic lipids were present in exposed animals and that these changes were accompanied by slight increases in smooth endoplasmic reticulum. Interpretation of ultrastructural findings, however, was largely subjective due to the relatively small sample size (3 exposed and 3 control mice) and the limited quantity of photomicrographs (33). It should be emphasized that fatty changes and increases in smooth encoplasmic reticulum are alterations which may result from a variety of toxic or metabolic insults.

TABLE 20. PATHOLOGIC CHANGES SEEN IN FEMALE C57BL/6 MICE IMMEDIATELY FOLLOWING 90-DAY EXPOSURE TO INHALED DECALIN VAPOR

	Unexposed Controls	5.0 ppm Exposed	50 ppm Exposed
Liver:			
Fatty Change	0	0	3
Cytoplasmic Vacuolization	2	40	44
Renal Hyperplasia	0	2	0
Ovarian Cysts	2	0	1
Lung Adenoma	1	0	0
NUMBER OF ANIMALS EXAMINED	50	47	50

Rats that died during postexposure holding or were sacrificed at 22 or 24 months postexposure were necropsied and the results of histologic examination are enumerated in Tables 21 and 22 for male and female rats, respectively. Lesions that occurred infrequently and appeared unrelated to decalin exposure are not included in these tables. Renal nephropathy was increased in both male and female rats when compared with unexposed controls, but was not as significant as that seen with exposure to 750 mg/m^3 exposure to JP-5 shale or petroleum derived fuel. This lesser response may be related to the differences in concentration of hydrocarbon to which the rats were exposed. The 50 ppm decalin concentration is equivalent to 280 mg/m^3 which may be compared with the 750 mg/m^3 JP-5 concentrations.

TABLE 21. PATHOLOGIC CHANGES SEEN IN MALE FISCHER 344 RATS HELD FOR POSTEXPOSURE OBSERVATION AFTER 90-DAY CONTINUOUS INHALATION EXPOSURE TO DECALIN

	Unexposed Controls	Exposed 5.0 ppm	Exposed 50 ppm
Kidney:			
Nephrosis	2	2	2
Nephropathy	40	45	48
Pelvic Hyperplasia	0	0	27
Liver:			
Fatty Change	8	3	8
Necrosis	4	4	3
Hepatocyte Vacuolization	1	5	2
Bile Duct Hyperplasia	41	37	35
Leukemia	7	8	7
Malignant Lymphoma	0	1	1
Thyroid C-Cell Adenoma	7	7	16
Pituitary Adenoma	5	16	16
Testicular Tumor	45	45	42
Adrenal Adenoma	2	1	1
Pancreas Islet Cell Adenoma	2	0	1
Liver Adenoma	0	2	2
TOTAL ANIMALS WITH TUMORS	48	45	49
NUMBER OF ANIMALS EXAMINED	50	49	50

TABLE 22. PATHOLOGIC CHANGES SEEN IN FEMALE FISCHER 344 RATS HELD FOR POSTEXPOSURE OBSERVATION AFTER 90-DAY CONTINUOUS INHALATION EXPOSURE TO DECALIN

	Unexposed Controls	Exposed 5.0 ppm	Exposed 50 ppm
Kidney:			
Nephrosis	3	4	7
Nephropathy	12	12	24
Liver:			
Fatty Change	6	4	7
Necrosis	0	2	5
Bile Duct Hyperplasia	7	7	6
Leukemia	3	3	2
Malignant Lymphoma	1	1	3
Thyroid C-Cell Adenoma	7	3	6
Pituitary Adenoma	11	17	16
Adrenal Adenoma	0	2	0
Pancreas Islet Cell Adenoma	0	1	1
Uterus:			
Polyps	14	9	10
Adenocarcinoma	5	1	4
Sarcoma	1	3	1
Mammary:			
Adenocarcinoma	1	0	1
Fibroadenoma	0	4	2
TOTAL ANIMALS WITH TUMORS	37	37	41
NUMBER OF ANIMALS EXAMINED	50	50	50

Total incidence of rats with tumors is similar for unexposed controls and both exposure groups of male and female rats but in male rats the incidence of pituitary adenomas is increased in both decalin exposed groups. Thyroid C-cell adenoma incidence was increased in the 50 ppm decalin exposed group of male rats and a 4% incidence of liver adenomas was seen in both exposed groups while none were seen in their control group.

Examination of tissues collected from mice held for postexposure observation after the 90-day continuous decalin exposure is not complete. They will be described in our next annual report and recommendations for exposure limits will be given at that time.

THE EXPERIMENTAL DETERMINATION OF THE ONCOGENIC EFFECTS OF ONE-YEAR EXPOSURE TO PETROLEUM JP-4 VAPOR

A study was designed to compare the tumorigenic potential of inhaled petroluem JP-4 fuel vapor with that from shale oil derived JP-4 fuels. Shale oil has been reported to be a more potent carcinogen than petroleum oils when painted on mouse skin.

Beginning in February 1980, mice and rats were exposed to JP-4 concentrations of 500 mg/m^3 and 1000 mg/m^3 by the inhalation route in Thomas Dome inhalation chambers for one year using a work week schedule of 6 hours/day, 5 days/week with holidays and weekends excluded to simulate a human industrial exposure regimen. Each exposure group consisted of 100 male and 100 female Fischer 344 rats and 100 male and 100 female C57BL/6 mice. Another group with the same numbers of animals was held at the Veterinary Sciences Division Building (Vivarium) to serve as controls. Animals were caged in conformance with ILAR standards for laboratory animal care.

Following the exposure period, 10% of the rodents from each group were sacrificed while the remaining rodents will be held for postexposure observation for one additional year or until cumulative mortality reaches 90%.

The experimental protocol for the one-year inhalation exposure of rats and mice to petroleum JP-4 vapor can be found in a previous annual report (MacEwen and Vernot, 1980).

Results

Except for the male mice, mortality during the exposure phase was insignificant and independent of exposure concentration. Due to the aggressive nature of the male mice, many were lost due to excessive fighting when regrouped (25 per cage) in the exposure chamber. One cage lost 24 of the 25 mice within the first two weeks of the study. After the first month, the pecking order was

established and mortality among male mice was similar to that seen in female mice and rats of both sexes. The mortality ratios at exposure termination are shown in Table 23. Although mortality in male mice appeared dose-related, the difference occurred during the first month after which death rates were similar for all three groups.

TABLE 23. MORTALITY RATIOS FOR GROUPS OF JP-4 EXPOSED AND CONTROL RODENTS AT EXPOSURE TERMINATION

	Controls	1000 mg/m^3	5000 mg/m^3
Mice, Male	18/100	35/100	47/100
Mice, Female	5/100	9/100	9/100
Rats, Male	3/100	4/100	2/100
Rats, Female	4/100	3/100	4/100

Mean body weights for the rat groups obtained on a biweekly schedule through 12 months of exposure are shown in Figures 10 and 11. Both test groups of male rats showed a significant depression in mean body weight for the extent of the study. The female control group failed to gain weight at a rate equal to the test group during the 12-month exposed period. Group mouse weights revealed no exposure-related effects.

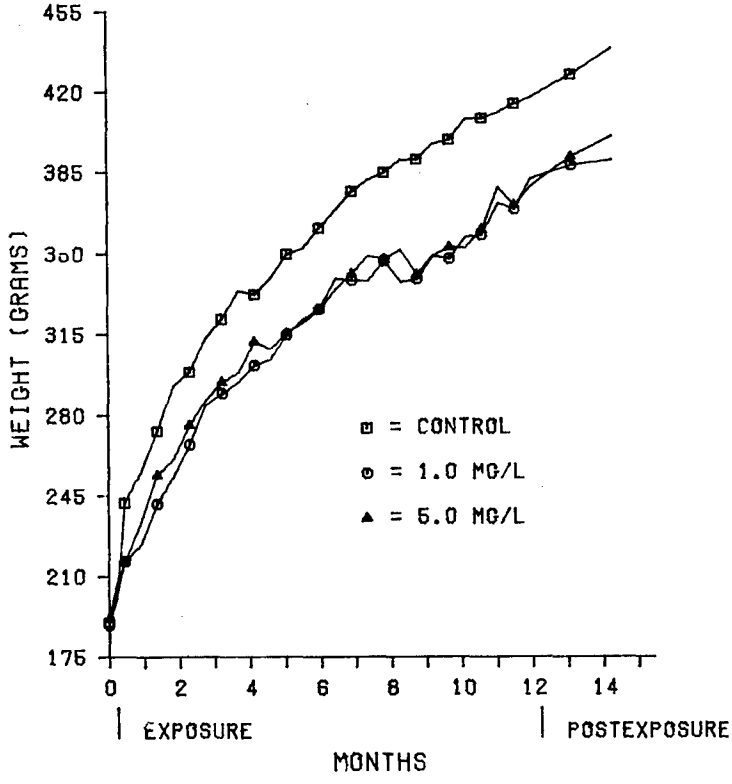


Figure 10. Effect of intermittent exposure to JP-4 on male rat body weight.

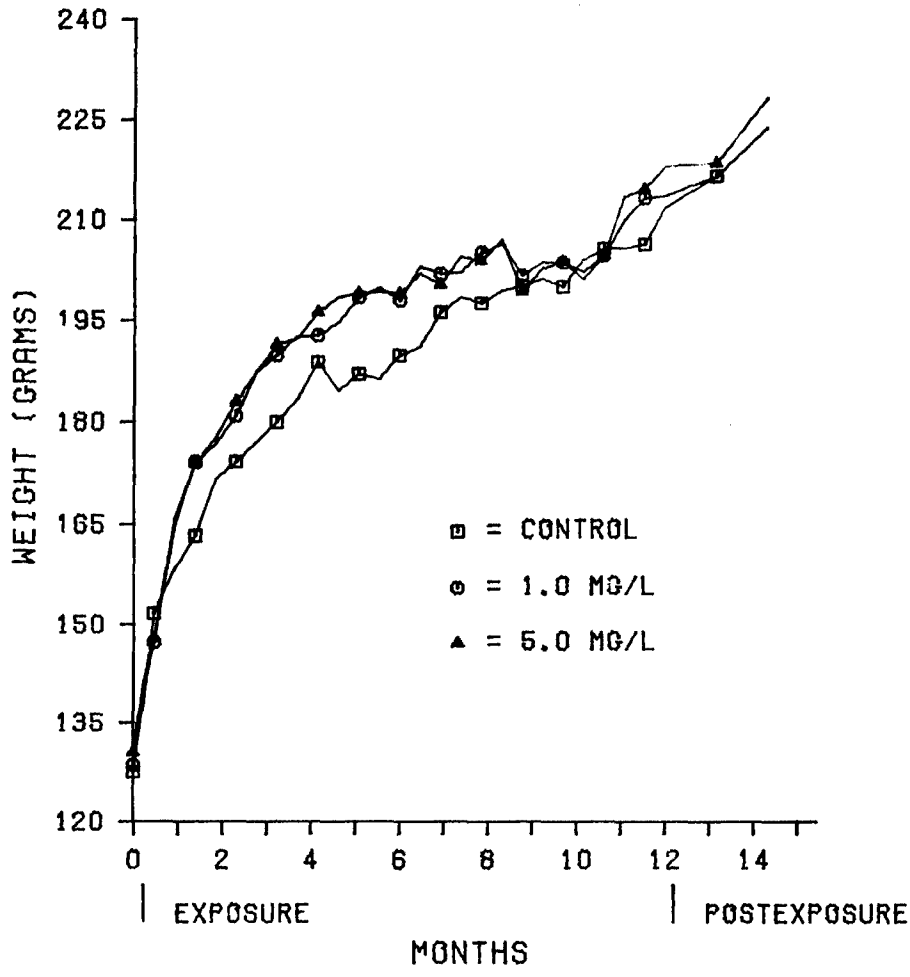


Figure 11. Effect of intermittent exposure to JP-4 on female rat body weight.

Organ weights for male and female rats sacrificed at exposure termination are shown in Tables 24 and 25, respectively. Slight, but statistically significant increases were found in the ratios of kidney and liver to body weights of the male rats. Significant differences noted in the female rat organ weights included high kidney weight and low spleen to body weight ratio in the high level group only.

TABLE 24. THE EFFECT OF ONE-YEAR EXPOSURE TO PETROLEUM JP-4 JET FUEL ON MALE RAT ORGAN WEIGHTS

	Control	1000 mg/m^3	5000 mg/m^3
Body Weight, gm	385.0 ± 31.6	375.0 ± 35.7	363.8 ± 30.1
Liver Weight, gm	9.03 ± 1.15	8.57 ± 0.99	9.01 ± 1.09
Liver/100 gm body wt	2.34 ± 0.13	2.29 ± 0.13	2.47[a] ± 0.16
Spleen Weight, gm	0.62 ± 0.10	0.60 ± 0.09	0.56 ± 0.06
Spleen/100 gm body wt	0.16 ± 0.02	0.16 ± 0.01	0.15 ± 0.02
Kidney Weight, gm	2.34 ± 0.19	2.37 ± 0.28	2.41 ± 0.26
Kidney/100 gm body wt	0.61 ± 0.04	0.63 ± 0.05	0.66[b] ± 0.03

[a] Different from controls at 0.05 level of significance.
[b] Different from controls at 0.01 level of significance.

TABLE 25. THE EFFECT OF ONE-YEAR EXPOSURE TO PETROLEUM JP-4 JET FUEL ON FEMALE RAT ORGAN WEIGHTS

	Control	1000 mg/m^3	5000 mg/m^3
Body Weight, gm	203.7 ± 10.9	201.9 ± 9.3	200.6 ± 15.3
Liver Weight, gm	5.22 ± 0.35	4.82 ± 0.39	4.89 ± 0.65
Liver/100 gm body wt	2.56 ± 0.23	2.39 ± 0.19	2.43 ± 0.23
Spleen Weight, gm	0.41 ± 0.03	0.40 ± 0.05	0.36 ± 0.04
Spleen/100 gm body wt	0.20 ± 0.12	0.20 ± 0.02	0.18[a] ± 0.01
Kidney Weight, gm	1.46 ± 0.08	1.47 ± 0.08	1.48 ± 0.16
Kidney/100 gm body wt	0.72 ± 0.05	0.73 ± 0.05	0.74 ± 0.06

[a] Different from controls at 0.05 level of significance.

The hematologic and clinical chemistry values of the male and female rats sacrificed at the conclusion of the one-year exposure are shown in Tables 26 and 27. Although statistically significant differences were seen in various parameters, they were not consistent between sexes and appeared sporadic. Most values are within normal ranges and the elevated blood urea nitrogen (BUN) and creatinine levels seen in 90-day continuous hydrocarbon exposures were not seen.

TABLE 26. MEAN HEMATOLOGIC AND CLINICAL CHEMISTRY VALUES OF MALE RATS OBTAINED AT THE CONCLUSION OF ONE-YEAR EXPOSURE TO PETROLEUM JP-4 JET FUEL

	Control	N	1000 mg/m^3	N	5000 mg/m^3	N
RBC (10^6)	11.0	11	12.0	11	11.9	11
WBC (10^3)	4.9	11	3.7[b]	11	3.7[b]	11
HCT (%)	45.1	11	45.5	11	44.5	11
HGB (gm/dl)	14.6	11	15.1	11	14.7	11
Total Protein (gm/dl)	6.9	11	6.8	11	6.9	11
Albumin (gm/dl)	4.2	10	4.2	11	4.1	11
Globulin (gm/dl)	2.7	10	2.6	11	2.7	11
A/G Ratio	1.6	10	1.6	11	1.5	11
Glucose (mg/dl)	152.5	11	135.9	11	113.7[b]	11
Potassium (mEq/L)	6.0	10	5.4	11	4.8[b]	11
Calcium (mg/dl)	10.8	11	10.3[a]	11	10.0[b]	11
Sodium (mEq/L)	162.2	10	159.4[a]	11	156.2[b]	11
Bilirubin (mg/dl)	0.38	11	0.34	11	0.33	11
Creatinine (mg/dl)	0.44	11	0.46	11	0.47	11
SGPT (IU/L)	62.9	11	54.5	11	44.0[b]	11
SGOT (IU/L)	100.9	11	114.7[a]	11	103.7	11
Alk. Phos. (IU/L)	8.1	11	7.4[a]	11	7.4	11
BUN (mg/dl)	11.8	11	12.0	11	12.0	11
MCV	41.8	11	38.6	11	57.9	11
MCH	13.5	11	12.8	11	12.6	11
MCHC	32.4	11	33.1	11	33.1	11

[a] Different from controls at 0.05 level of significance.

[b] Different from controls at 0.01 level of significance.

TABLE 27. MEAN HEMATOLOGIC AND CLINICAL CHEMISTRY VALUES OF FEMALE RATS OBTAINED AT THE CONCLUSION OF ONE-YEAR EXPOSURE TO PETROLEUM JP-4 JET FUEL VAPOR

	Control	N	1000 mg/m^3	N	5000 mg/m^3	N
RBC (10^6)	9.9	10	10.4	10	11.0[a]	10
WBC (10^3)	3.5	10	2.7[b]	10	2.6[b]	10
HCT (%)	41.8	10	43.0	10	43.7	10
HGB (gm/dl)	14.2	10	14.4	10	14.5	10
Total Protein (gm/dl)	7.5	9	7.1	10	7.2	10
Albumin (gm/dl)	4.7	9	4.3[b]	10	4.4[a]	10
Globulin (gm/dl)	2.7	9	2.8	10	2.8	10
A/G Ratio	1.8	9	1.5[b]	10	1.6[b]	10
Glucose (mg/dl)	144.0	9	117.8[b]	10	106.6[b]	10
Potassium (mEq/L)	5.3	7	4.9	10	5.0	9
Calcium (mg/dl)	10.0	9	9.2[b]	10	9.5[b]	10
Sodium (mEq/L)	151.0	7	150.1	10	149.9	9
Bilirubin (mg/dl)	0.36	9	0.45[a]	10	0.57[b]	10
Creatinine (mg/dl)	0.42	9	0.38	10	0.34[a]	10
SGPT (IU/L)	58.4	9	45.6[a]	10	47.2[a]	10
SGOT (IU/L)	121.4	9	117.4	10	100.8	10
Alk. Phos. (IU/L)	6.3	9	5.3	10	5.5	10
BUN (mg/dl)	12.5	9	12.0	10	11.3[a]	10
MCV	42.6	10	41.3	10	39.8[a]	10
MCH	14.5	10	13.9	10	13.2[a]	10
MCHC	33.9	10	33.6	10	33.3	10

[a] Different from controls at 0.05 level of significance.

[b] Different from controls at 0.01 level of significance.

THE EXPERIMENTAL DETERMINATION OF SAFE ATMOSPHERIC EXPOSURE CONCENTRATIONS OF JP-10 VAPOR

A 12-month industrial type chronic exposure of four animal species to 100 ppm JP-10 was conducted from June 1978 to June 1979. This experiment was designed to provide information to establish safe exposure levels and identify the oncogenic potential of JP-10 in rodents. Previous annual reports (MacEwen and Vernot, 1979; 1980) contain the experimental protocol and clinical information collected from the start of the exposure through 10 months postexposure.

Background

JP-10 is a synthetic saturated polycyclic hydrocarbon. It is being utilized as a jet fuel either alone or as a major constituent (70%) of JP-9 fuel because of its high density and other desirable properties. In the latter application, it has been substituted for RJ-4 which is a reduced dimer of methylcyclopentadiene. The chronic inhalation toxicity of RJ-4, and RJ-4 in combination with RJ-5, another constituent of JP-9 fuel, was detailed in previous annual reports (MacEwen and Vernot, 1974; 1975; 1976).

JP-10 is a single chemical entity identified as tricyclo-$(5.2.10^{2,6})$ decane.

Since no information appears in the literature concerning the toxicologic properties of JP-10, a series of studies was planned beginning with acute studies including emergency exposure limit tests and culminating in a long-term chronic study. The experiments were conducted to develop the necessary data for hazard evaluation and establishment of safe exposure limits as well as to identify the oncogenic potential of JP-10 fuel.

Preliminary acute inhalation experiments had shown that mice were the most sensitive species to JP-10 with all of 6 animals exposed to 1000 ppm dying within 4 hours. To aid in selection of a concentration of JP-10 suitable for use in a year long, 6 hours/day, 5 days/week exposure regimen, groups of 5 female rats and 5 female mice were exposed to 250 ppm for five 6-hour exposure days. The coordination of the mice appeared slightly affected on the first day of exposure. Respiration rates of both rats and mice were more rapid than normal during the second day's exposure. One mouse had a slight convulsion early in the second exposure day but recovered and appeared normal thereafter. For the rest of the exposure, no further signs of toxic stress were noted in either species. Mean body weights of the mice did not increase during the week following termination of exposure.

As a result of the toxic effects shown in mice in the short-term inhalation tests, an exposure concentration of 100 ppm JP-10 was selected for chronic exposure of animals to determine safe exposure limits.

The animal species, sex, strain and numbers used in this study are shown below.

Species	Sex	Strain	100 ppm JP-10 Chamber 1	100 ppm JP-10 Chamber 2	Unexposed Controls
Rats	M	Fischer 344	---	50	50
Rats	F	Fischer 344	---	50	50
Mice	F	C57BL/6	200	--	200
Hamsters	M	Golden Syrian	100	--	100
Dogs	M	Beagle	---	4	4
Dogs	F	Beagle	---	4	4

Results

The mortality ratios at the end of the 12 months of exposure and at 12 months postexposure immediately prior to the sacrifice of all surviving rodents in June 1980 are shown in Table 28. This table includes the 20 exposed, 20 control mice, 10 exposed hamsters and 10 control hamsters which were sacrificed at the end of exposure and submitted for necropsy to determine if tissue changes were present at that time.

TABLE 28. MORTALITY RATIOS FOR GROUPS OF JP-10 EXPOSED AND CONTROL ANIMALS AT EXPOSURE CONCLUSION AND AT 12 MONTHS POSTEXPOSURE

Species, Sex	Unexposed Controls Exposure Conclusion	Unexposed Controls 12-Months Postexposure	100 ppm JP-10 Exposed Exposure Conclusion	100 ppm JP-10 Exposed 12-Months Postexposure
Mice, Female	30/200	148/200	20/200	143/200
Rats, Male	0/50	16/50	0/50	8/50
Rats, Female	4/50	21/50	0/50	20/50
Hamsters, Male	5/100	47/100	9/100	43/100
Dogs, Male	0/4	0/4	0/4	0/4
Dogs, Female	0/4	0/4	0/4	0/4

The similarity of mortality ratios and absence of CNS changes during the study for exposed and control rodents indicate safety of the 100 ppm JP-10 concentration.

Mean body weights for groups of exposed and control male rats, female rats, and male hamsters obtained on a biweekly schedule throughout 12 months of exposure and monthly through 12 months postexposure are shown in Figure 12. Weights of male rats and hamsters showed depression relative to controls as a result of JP-10 exposure. Values for male rats were statistically different from control values at all times during exposure and postexposure. Values for exposed hamsters were also statistically different from controls at all weighing periods during exposure. This pattern was

continued during the postexposure phase. Exposed female rat weights were not significantly different from controls at any phase of the study. An examination of exposed dog and mouse weights taken during and after exposure revealed no effect of JP-10 exposure.

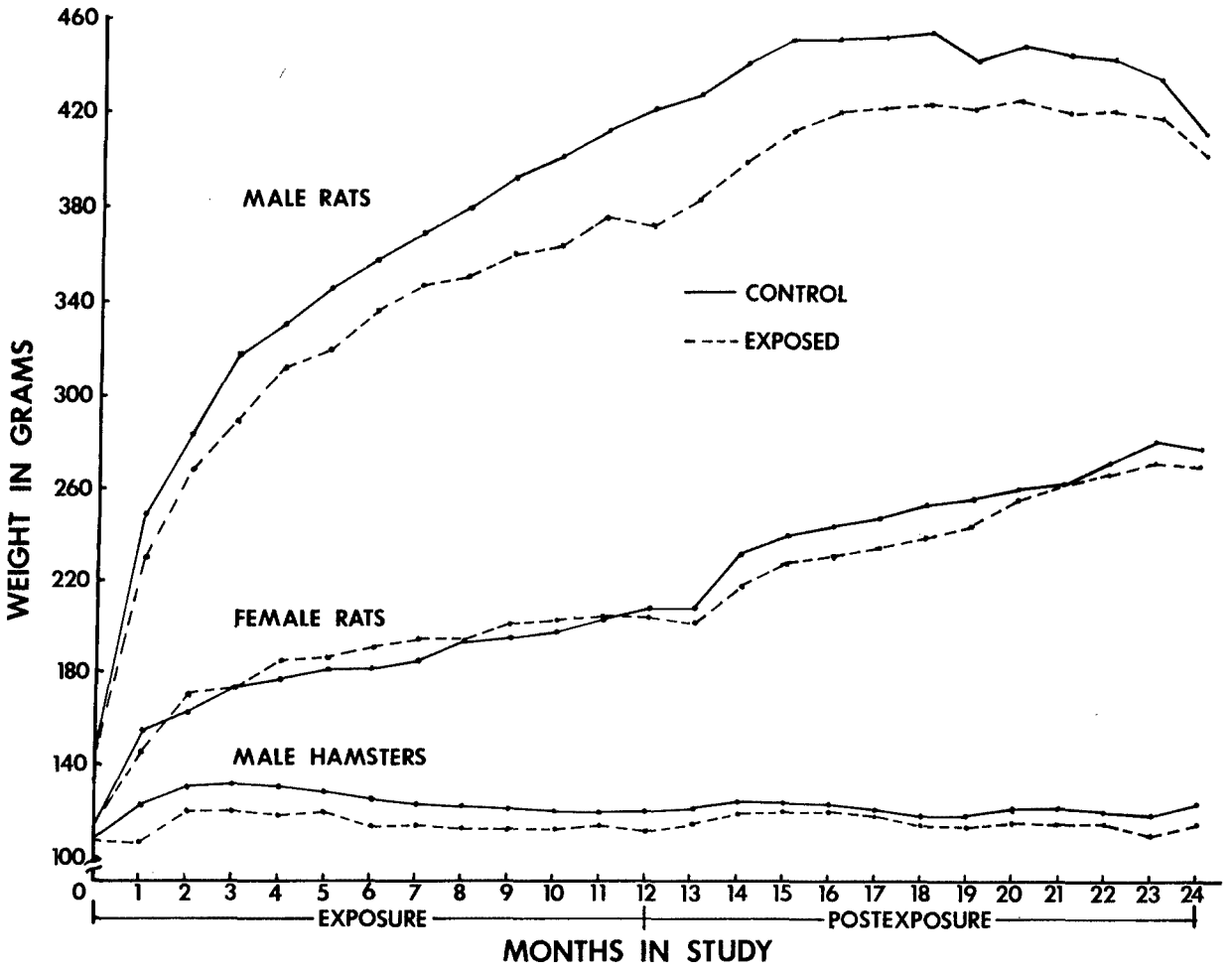


Figure 12. Mean body weights of rats and hamsters exposed intermittently to 100 ppm JP-10 for one-year.

Maintenance of the dogs will continue for 5 years postexposure (June 1984). A review of the results of all quarterly physical examinations and semiannual clinical chemistry measurements indicate that all of the dogs used in this study are healthy at this time.

Histopathologic examination of tissue collected from mice sampled at the end of the 1-year intermittent exposure to 100 ppm JP-10 showed no lesions that could be attributed to exposure (Table 29). The overall incidence of the various lesions observed appeared to be lower in exposed mice when compared with their controls. Examination of tissues from 10% of the hamsters and rats necropsied at the termination of the 12-month exposure period has not been completed. The analysis of the results of these examinations and those of the animals held for postexposure evaluation will be described in a future annual report at which time recommendations for exposure limits will be discussed.

TABLE 29. PATHOLOGIC CHANGES SEEN IN C57BL/6 FEMALE MICE DURING AND AFTER 1-YEAR INHALATION EXPOSURE TO JP-10 VAPOR

	Unexposed Controls	Exposed
Liver:		
Degeneration	1/49	0/29
Cytoplasmic Vacuolization	30/49	20/29
Kidney:		
Glomerulonephritis	1/49	3/29
Hydronephrosis	0/49	1/29
Hyperplasia	1/49	0/29
Endometrial Stromal Sclerosis	29/49	5/29
Bone Marrow Hyperplasia	22/49	8/29

THE EXPERIMENTAL DETERMINATION OF SAFE ATMOSPHERIC CONCENTRATIONS OF RJ-5

A study of the oncogenic potential of inhaled RJ-5, (norbornane dimer) was initiated in October, 1979 for four animal species. The exposure phase of the study was concluded in October 1980 and most of the animals were transferred to postexposure holding facilities. The animals of each species were divided into three groups, unexposed controls and those exposed to either 30 or 150 mg/m^3 RJ-5.

Twenty mice, 10 male and 10 female rats, and 10 hamsters from each group were sacrificed at the termination of exposure and submitted for gross and histopathologic examination to determine if tissue changes were present at that time.

The experimental protocol designed to identify toxic effects and establish safe exposure limits as well as to identify the oncogenic potential of RJ-5 fuel can be found in a previous annual report (MacEwen and Vernot, 1980) which also contains information on the first five months of the 12-month industrial type exposure of the four animal species.

The numbers of animals of each species used are listed in Table 30 which also shows the distribution of animals in the Thomas Dome exposure chambers along with RJ-5 concentrations.

TABLE 30. ANIMAL DISTRIBUTION IN RJ-5 EXPOSURE CHAMBERS

	Concentration, mg/m^3				
Chamber Number	30 1	30 2	150 3	150 4	Unexposed Controls
Species and Sex					
Rats, Male	---	65	65	---	65
Rats, Female	---	65	65	---	65
Mice, Female	200	--	--	200	200
Hamsters, Male	100	--	--	100	100
Dogs, Male	---	4	4	---	4
Dogs, Female	---	4	4	---	4

Background

A six-month chronic inhalation toxicity exposure to 0.15 mg/liter RJ-5 was conducted with animals in our laboratory and reported by MacEwen and Vernot in 1975. A subnormal weight gain was noted in rats and particularly in dogs during the course of the experiment. Dogs and rats (CFE strain) sacrificed immediately following the conclusion of the exposure showed acute inflammation of the lungs as well as several cases of bronchopneumonia in the test groups.

A high incidence of alveolargenic carcinomas was seen in the mice held one-year postexposure following the six-month exposure to 0.15 mg/liter RJ-5. The mice used in that study were of the CF-1 strain which is predisposed to this type of tumor. To determine if this compound truly possesses oncogenic properties, it was decided to do a more in-depth study for a longer time and to maintain a greater number of animals during the postexposure observation period. The rats and mice being used in this study are the strains which have been used in all of our recent oncological studies, Fischer 344 and C57BL/6, respectively.

RJ-5 is a mixture of stereoisomers of the reduced dimer of bicycloheptadiene containing six major components. Some of the physical properties are listed below:

Empirical Formula:	$C_{14}H_{20}$
Molecular Weight:	188
Boiling Range (°F):	500-525
Vapor Pressure (70°F):	0.25 mm Hg
Density (70°F):	1.0813 g/ml

Results

Mean body weights for groups of rats and hamsters obtained on a biweekly schedule through 12 months of exposure and monthly thereafter are shown in Figures 13 through 15. Weights of male rats and hamsters showed a depression in mean body weights as a result of RJ-5 exposure. The mean body weights of these groups continued in this pattern through the postexposure period to date. The mean body weights of expired female rats outgained the control group. An examination of exposed dog and mouse weights, during and after exposure, revealed no effect which could be attributed to RJ-5 exposure.

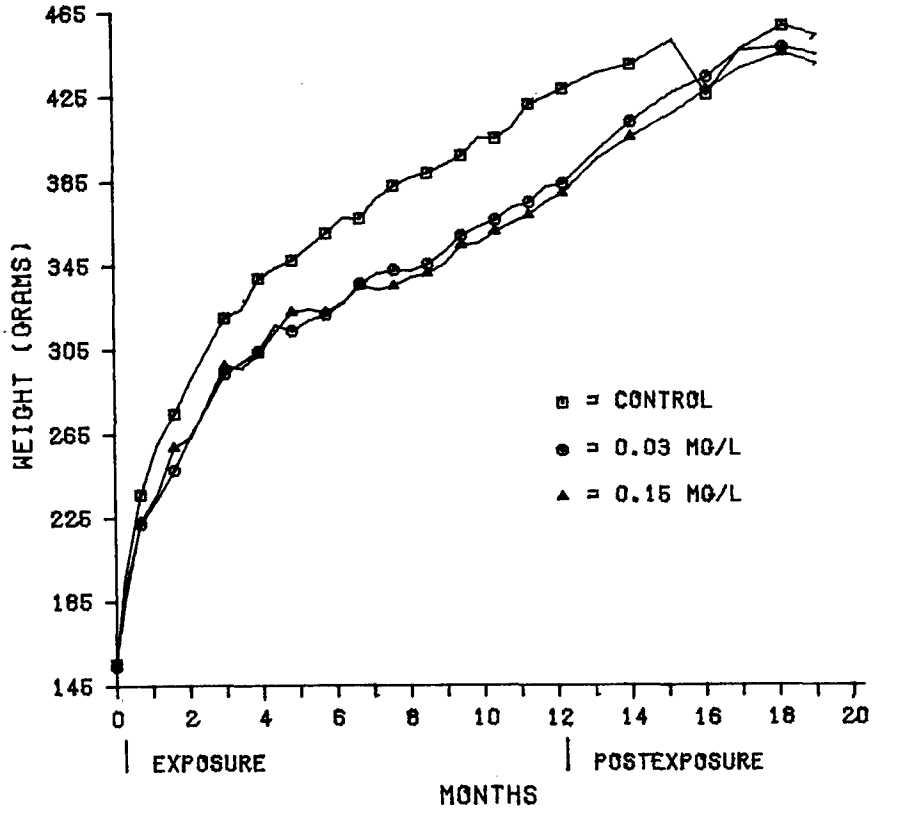


Figure 13. Effect of a one-year intermittent exposure to RJ-5 on male rat body weight.

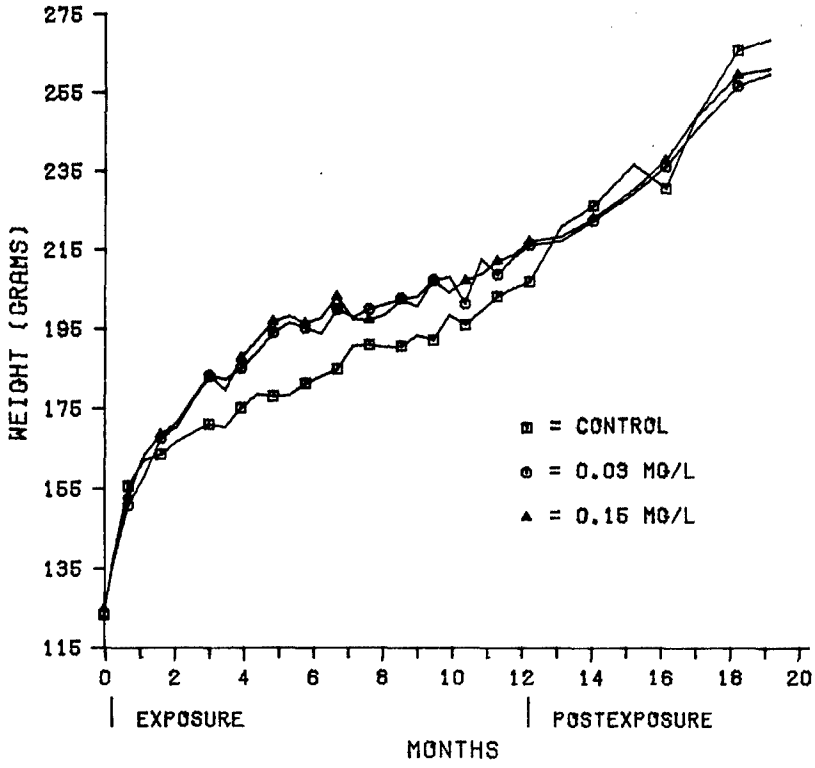


Figure 14. Effect of a one-year intermittent exposure to RJ-5 on female rat body weight.

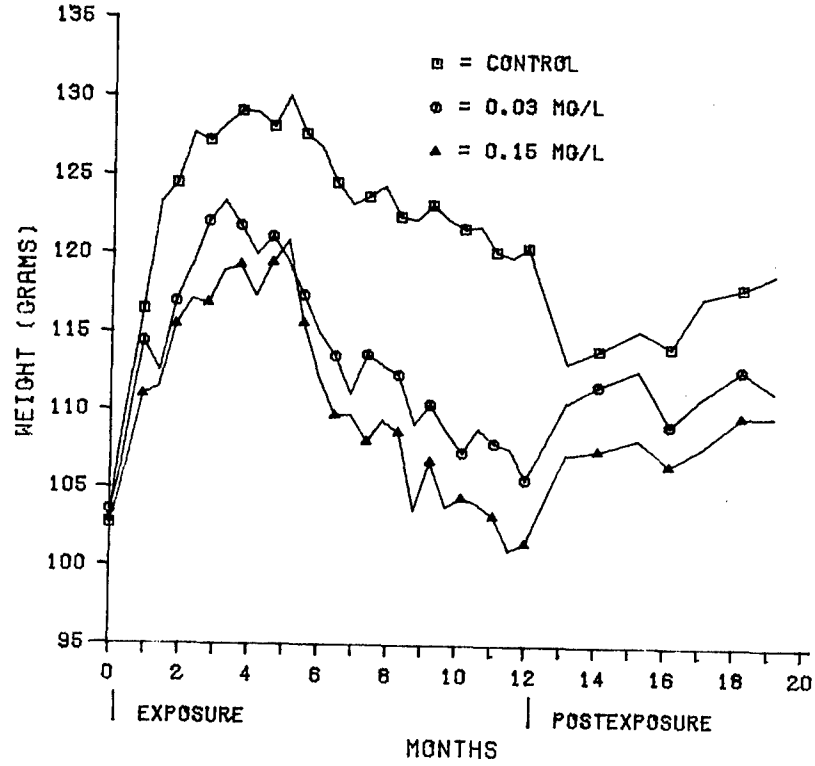


Figure 15. Effect of one-year intermittent exposure to RJ-5 on hamster body weight.

The number of animal deaths that occurred during the exposure phase was insignificant and independent of exposure concentration. The mortality ratios at exposure termination are shown in Table 31.

TABLE 31. MORTALITY RATIOS FOR GROUPS OF RJ-5 EXPOSED AND CONTROL ANIMALS AT EXPOSURE TERMINATION

	Controls	30 mg/m^3	150 mg/m^3
Mice	29/200	9/200	10/200
Rats, Male	3/65	3/65	3/65
Rats, Female	9/65	5/65	7/65
Hamsters	7/100	9/100	8/100
Dogs	0/8	1/8	0/8

Organ weights for male and female rats sacrificed at exposure termination are shown in Tables 32 and 33, respectively. Statistically significant differences were found in mean body weights of the male rats exposed to 150 mg/m^3 RJ-5, as well as several mean organ weights of both male and female rats in this exposure group. When organ/body weight ratios were analyzed statistically, most differences disappeared.

TABLE 32. THE EFFECT OF ONE-YEAR EXPOSURE TO RJ-5 JET FUEL ON MALE RAT ORGAN WEIGHTS

	Control	30 mg/m^3	150 mg/m^3
Body Weight, gm	409.7 ± 39.1	388.4 ± 39.5	362.3^b ± 26.7
Liver Weight, gm	11.01 ± 1.44	9.44^b ± 1.30	9.21^b ± 0.69
Liver/100 gm body wt	2.70 ± 0.35	2.43 ± 0.21	2.55 ± 0.14
Spleen Weight, gm	0.65 ± 0.08	0.60 ± 0.08	0.55^a ± 0.13
Spleen/100 gm body wt	0.16 ± 0.02	0.16 ± 0.02	0.15 ± 0.03
Kidney Weight, gm	2.49 ± 0.23	2.23^a ± 0.21	2.12^b ± 0.22
Kidney/100 gm body wt	0.61 ± 0.07	0.58 ± 0.04	0.61 ± 0.04
Heart Weight, gm	1.05 ± 0.10	0.92^b ± 0.11	0.90^b ± 0.07
Heart/100 gm body wt	0.26 ± 0.03	0.24^a ± 0.02	0.25 ± 0.02
Lung Weight, gm	3.10 ± 0.40	2.72^a ± 0.31	2.63^b ± 0.17
Lung/100 gm body wt	0.76 ± 0.10	0.70 ± 0.05	0.73 ± 0.05

^{a} Different from controls at 0.05 level of significance.
^{b} Different from controls at 0.01 level of significance.

TABLE 33. THE EFFECT OF ONE-YEAR EXPOSURE TO RJ-5 JET FUEL ON FEMALE RAT ORGAN WEIGHTS

	Control	30 mg/m^3	150 mg/m^3
Body Weight, gm	201.8 ± 14.8	212.6 ± 13.5	207.6 ± 9.0
Liver Weight, gm	5.29 ± 0.50	5.32 ± 0.32	5.18 ± 0.52
Liver/100 gm body wt	2.63 ± 0.19	2.52 ± 0.25	2.50 ± 0.26
Spleen Weight, gm	0.37 ± 0.03	0.40[a] ± 0.03	0.41[a] ± 0.05
Spleen/100 gm body wt	0.18 ± 0.01	0.19 ± 0.02	0.20 ± 0.02
Kidney Weight, gm	1.42 ± 0.09	1.48 ± 0.06	1.43 ± 0.13
Kidney/100 gm body wt	0.71 ± 0.03	0.70 ± 0.05	0.69 ± 0.06
Heart Weight, gm	0.64 ± 0.07	0.61 ± 0.06	0.62 ± 0.06
Heart/100 gm body wt	0.32 ± 0.03	0.29[a] ± 0.03	0.30 ± 0.03
Lung Weight, gm	2.10 ± 0.77	1.92 ± 0.11	1.77 ± 0.19
Lung/100 gm body wt	1.06 ± 0.47	0.91 ± 0.04	0.85 ± 0.07

[a] Different from controls at 0.05 level of significance.

The mean hematologic and clinical chemistry values determined for the male and female rats sacrificed at the conclusion of the one-year exposure are shown in Tables 34 and 35. The RJ-5 exposed male rats showed statistically significant differences in potassium and sodium values when compared to their respective controls. This is probably because control values are at the low end of the normal range for these parameters. Scattered differences were noted in various blood parameters of the female rats. Most were sporadic and within normal limits. No blood abnormalities appeared to be a result of exposure to the contaminant.

TABLE 34. MEAN HEMATOLOGIC AND CLINICAL CHEMISTRY VALUES OF MALE RATS OBTAINED AT THE CONCLUSION OF ONE-YEAR EXPOSURE TO RJ-5 (NORBORNANE DIMER)

	Control	30 mg/m^3	150 mg/m^3
RBC (10^6)	8.7	8.8	8.7
WBC (10^3)	4.5	4.1	3.6
HCT (%)	43.8	43.9	43.5
HGB (gm/dl)	14.0	14.2	14.1
Total Protein (gm/dl)	7.0	7.0	6.7
Albumin (gm/dl)	4.2	4.2	4.0
Globulin (gm/dl)	2.8	2.9	2.8
A/G Ratio	1.5	1.5	1.4
Glucose (mg/dl)	159.5	143.6[a]	142.9[a]
Potassium (mEq/L)	4.6[c]	5.3[a]	5.3[a,c]
Calcium (mg/dl)	10.4	10.5	10.2
Sodium (mEq/L)	139.6[c]	146.9[b]	146.1[b,c]
Bilirubin (mg/dl)	0.52[c]	0.52	0.47[a]
Creatinine (mg/dl)	0.38[c]	0.36	0.34
SGPT (IU/L)	62.7[c]	60.0	54.4
SGOT (IU/L)	134.2[c]	155.9	147.8
Alk. Phos. (IU/L)	7.7[c]	7.8	7.4
BUN (mg/dl)	14.5[c]	12.8	12.4[a]
MCV	50.2	50.2	49.8
MCH	16.0	16.2	16.2
MCHC	31.9	32.3	32.5
NUMBER OF SAMPLES	10		

[a] Different from controls at 0.05 level of significance.
[b] Different from controls at 0.01 level of significance.
[c] Number of samples is 9.

TABLE 35. MEAN HEMATOLOGIC AND CLINICAL CHEMISTRY VALUES OF FEMALE RATS OBTAINED AT THE CONCLUSION OF ONE-YEAR EXPOSURE TO RJ-5 (NORBORNANE DIMER)

	Control	30 mg/m^3	150 mg/m^3
RBC (10^6)	8.9[c]	9.6[b]	9.6[b]
WBC (10^3)	5.7[c]	4.2[a]	4.6
HCT (%)	46.0[c]	45.7	44.8
HGB (gm/dl)	14.6[c]	14.2	14.2
Total Protein (gm/dl)	7.2	7.1	7.5[a]
Albumin (gm/dl)	4.3	4.0[b]	4.2
Globulin (gm/dl)	2.9	3.1[a]	3.3[b]
A/G Ratio	1.5	1.3[b]	1.3[b]
Glucose (mg/dl)	168.2	156.9	155.5
Potassium (mEq/L)	5.2	4.3[a]	4.9
Calcium (mg/dl)	10.9	10.5[b]	10.8
Sodium (mEq/L)	145.0	140.6[b]	150.7[b]
Bilirubin (mg/dl)	0.46[c]	0.38[b]	0.36[b]
Creatinine (mg/dl)	0.51[c]	0.46	0.55
SGPT (IU/L)	79.3[c]	53.6[b]	64.6
SGOT (IU/L)	132.5	104.4[a]	122.4
Alk. Phos. (IU/L)	11.1[c]	11.7	11.9
BUN (mg/dl)	15.7[c]	15.4	17.6[a]
MCV	51.9[c]	47.9[b]	46.8[b]
MCH	16.5[c]	14.9[b]	14.8[b]
MCHC	31.8[c]	31.2	31.7
NUMBER OF SAMPLES	10	10	10

[a] Different from controls at 0.05 level of significance.
[b] Different from controls at 0.01 level of significance.
[c] Number of samples is 9.

The concentrations in the four exposure chambers were monitored sequentially with Beckman Model 400 hydrocarbon analyzers throughout the 12-month exposure period. The final mean concentration of RJ-5 fuel of each chamber for the 251 exposure days in the year long study is given in Table 36.

TABLE 36. RJ-5 JET FUEL EXPOSURE CONCENTRATIONS PROVIDED FOR EXPERIMENTAL ANIMALS (mg/m^3)

Chamber #	Nominal Concentration	Measured Mean Concentration	Standard Deviation
1	30	29.97	0.42
2	30	30.12	0.41
3	150	149.12	7.15
4	150	149.47	5.63

Examination of the animal tissues collected for histopathologic evaluation at the termination of the one-year exposure to RJ-5 vapor is incomplete. The animals are continuing to be held for postexposure observation and will be terminated in October 1981. Additional information will be available in the next annual report.

A 12-MONTH CHRONIC INHALATION EXPOSURE OF ANIMALS TO METHYLCYCLOHEXANE TO DETERMINE ITS ONCOGENIC POTENTIAL

The exposure portion of this study began in August 1978 and continued for one year after which 20 mice, 10 rats, and 10 hamsters from each group were necropsied to assess chronic toxicity effects in primary tissues. The remaining rodents were held for an additional year of observation and the dogs will continue to be held through 1984. Each exposure and control group of animals consisted of 65 male and 65 female rats, 200 female mice, 100 male hamsters, and 8 dogs equally divided by sex. The numbers of rodents used were selected to provide a statistically valid number of each sex and species which had reached the required age for tumor induction allowing for natural and toxicologic attrition.

Information on the experimental protocol, methodology of inhalation exposure and clinical data obtained during the 12-month exposure phase was given in the two latest annual reports for the operation of the THRU. This report updates clinical observations during the postexposure observation period and presents histopathologic evaluation of the exposed rodents necropsied for this purpose at the end of the 12-month long intermittent exposure phase.

Background

Methylcyclohexane (MCH) is a constituent of jet aircraft fuel JP-9. This fuel is a mixture of three primary ingredients, JP-10, RJ-5, and MCH. JP-10 and RJ-5 are high density hydrocarbons yielding a greater BTU output than conventional aircraft fuels. They also have high viscosities which cause pumping and flow problems at low temperature that are corrected by the addition of MCH to the mixture.

In 1976, the American Conference of Governmental Industrial Hygienists lowered the threshold limit value (TLV) for MCH from 500 ppm to 400 ppm or 1600 mg/m^3. The recommended short-term exposure limit (STEL) is 500 ppm or 2000 mg/m^3. These values are based on analogy to the toxicity of heptane and are identical to the TLV and STEL of heptane.

The scarcity of chronic exposure data for animals with the consequent use of analogy to other solvents make the setting of human exposure limits risky. Prolonged exposures to methylbutylketone and n-hexane have been shown to cause peripheral polyneuropathy in man (Billmaier, 1974; Allen, 1975). A TLV of 500 ppm had been set for n-hexane based solely on acute toxicity data and comparison with other petroleum solvents such as pentane. Reports of neuropathy in workers exposed to hexane resulted in the lowering of the American Conference of Governmental Industrial Hygienists TLV to 100 ppm in 1977.

These studies were undertaken to obtain the data needed to assess the safety margin of current exposure limits for methylcyclohexane. The design of the study also provides for the identification of the oncogenic potential of methylcyclohexane.

Animal exposure concentrations of MCH for this study were selected on the basis of the current TLV (400 ppm) and the maximum tolerated level for repeated exposures which appeared to be 2000 ppm.

Results

Mean body weights of MCH exposed and control rats are shown in Figures 16 and 17. The female rat weights were unaffected during exposure as well as during the postexposure observation period. MCH exposed male rats showed a growth depression during the exposure portion of the study and although they gained weight after removal from the exposure chambers, they still did not attain the mean weight of the unexposed control group. A definite depression in mean body weights was seen in the exposed hamster groups (Figure 18). Immediately following exposure, both exposed groups gained weight and became equivalent to the control group.

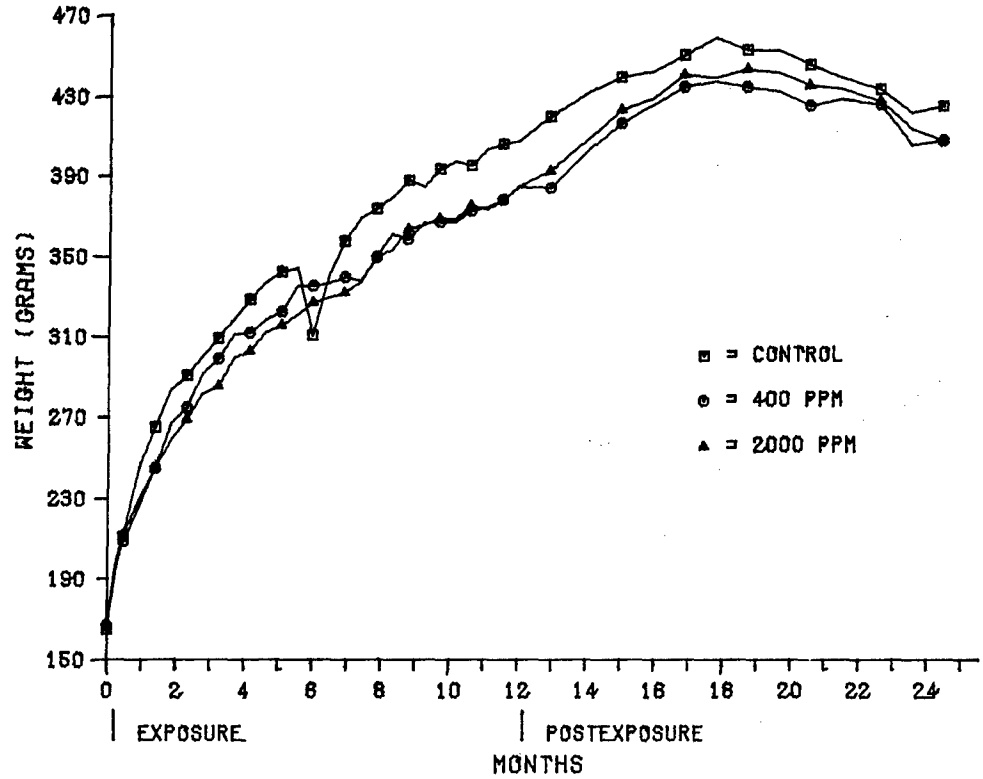


Figure 16. Mean body weights of male rats exposed to methylcyclohexane vapor for 12 months.

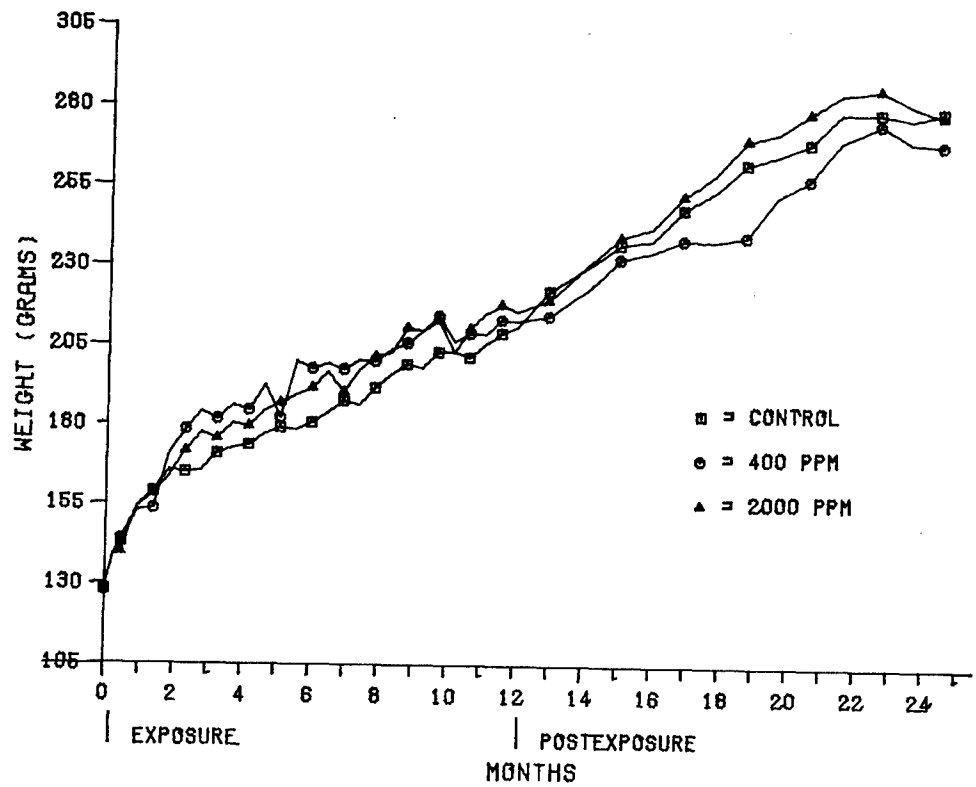


Figure 17. Mean body weights of female rats exposed to methylcyclohexane vapor for 12 months.

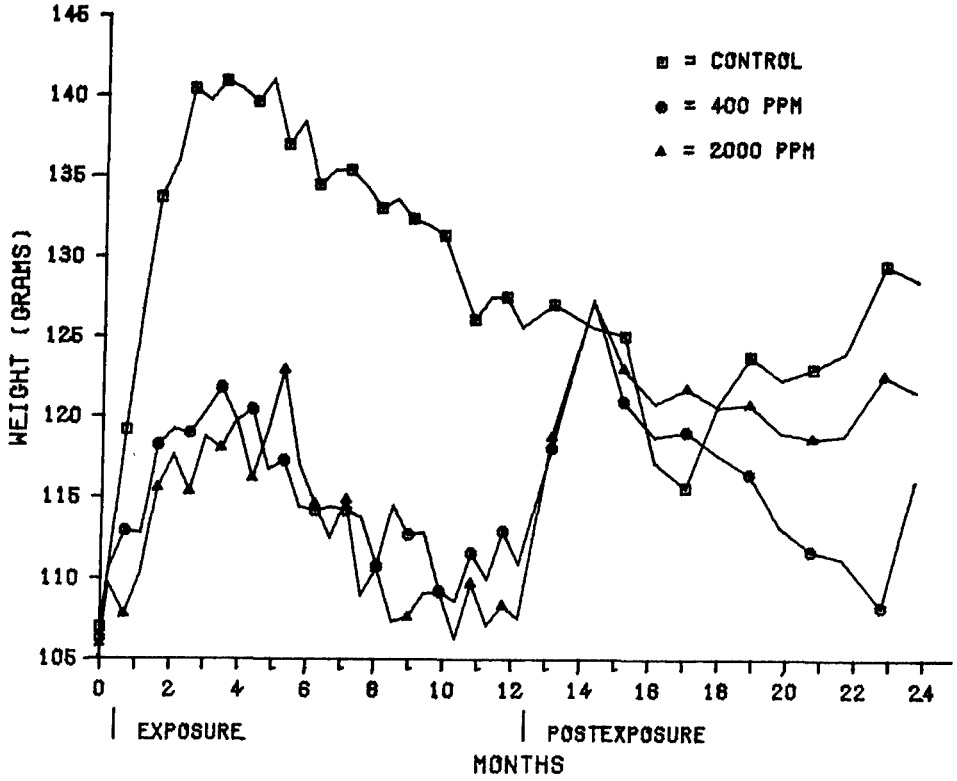


Figure 18. Mean body weights of male golden syrian hamsters exposed to methylcyclohexane vapor for 12 months.

Clinical laboratory determinations on dog blood taken at bi-weekly intervals gave variable but non-MCH related results. Post-exposure blood values were within the expected normal limits.

All rodents were killed in August 1980 following one year of postexposure observation. The dogs will continue to be held for observation, including scheduled physical examinations, until July 1984. The final mortality ratios are shown in Table 37.

TABLE 37. MORTALITY IN MCH EXPOSED AND CONTROL ANIMALS AT STUDY CONCLUSION

Species and Sex	Controls	400 ppm	2000 ppm
Mice, Female	126/200[a]	149/200[a]	149/200[a]
Rats, Male	25/65[b]	24/65[b]	24/65[b]
Rats, Female	31/65[b]	26/65[b]	28/65[b]
Hamsters, Male	48/100[a]	55/100[a]	61/100[a]
Dogs, Male and Female	0/8	1/8	0/8

[a] Includes a 10% sacrifice within a few days of exposure termination.

[b] Includes 10 animals sacrificed within a few days of exposure termination.

Micropathologic tissue changes seen in the rodents sacrificed at the end of 12-months intermittent exposure to MCH are listed in Tables 38-40 for rats, mice and hamsters, respectively. The numbers of animals examined include those that died during the exposure period as well as those from the planned sampling group.

TABLE 38. TISSUE CHANGES SEEN IN MALE AND FEMALE FISCHER 344 RATS AT THE END OF 12-MONTHS INTERMITTENT EXPOSURE TO INHALED METHYLCYCLOHEXANE

	Unexposed Controls	400 ppm Exposed	2000 ppm Exposed
Female Rats			
Pulmonary Hyperplasia	7	0	3
Ovarian Cyst	1	4	2
Endometrial Stromal Polyp	0	1	0
NUMBER OF ANIMALS EXAMINED	12	11	10
Male Rats			
Pituitary Adenoma	2	0	0
Testicular Tumor	0	5	1
Adrenal Pheochromocytoma	1	1	0
Bile Duct Hyperplasia	1	2	0
Renal Tubular Dilatation	1	3	4
NUMBER OF ANIMALS EXAMINED	12	10	10

TABLE 39. TISSUE CHANGES SEEN IN FEMALE C57BL/6 MICE AT THE END OF 12-MONTHS INTERMITTENT EXPOSURE TO INHALED METHYLCYCLOHEXANE

	Unexposed Controls	400 ppm Exposed	2000 ppm Exposed
Lung:			
Lymphoid Hyperplasia	1	4	3
Alveolar Bronchiolar Adenoma	1	0	0
Liver:			
Fatty Change	10	7	6
Hyperplasia	3	4	1
Malignant Lymphoma	3	4	4
Pituitary Adenoma	3	0	0
Uterine Cysts	18	16	14
Kidney Hyperplasia	5	4	2
NUMBER OF ANIMALS EXAMINED	29	35	40

TABLE 40. TISSUE CHANGES SEEN IN MALE GOLDEN SYRIAN HAMSTERS AT THE END OF 12-MONTHS INTERMITTENT EXPOSURE TO INHALED METHYLCYCLOHEXANE

	Unexposed Controls	400 ppm Exposed	2000 ppm Exposed
Kidney Mineralization	5	4	1
Renal Tubular Dilatation	6	1	3
Pituitary Fatty Change	2	4	3
Adrenal Adenoma	0	0	1
NUMBER OF ANIMALS EXAMINED	23	20	17

Since only a small number of lesions, both tumor and nontumorous, were observed in these animals, they are tabulated together. Only one tumor was noted in all female rats - a benign endometrial stromal polyp found in the 400 ppm MCH exposure group. The other lesions noted were of a nonsignificant nature.

The tumors seen in the male rats are commonly found in this strain, although the onset of interstitial cell tumors of the testicles may be advanced. Incidence and onset of this tumor type will be examined carefully when final results are available. There appeared to be a slight but statistically insignificant increase in dilatation of renal tubules in the 2000 ppm MCH exposed rats but no other indication of kidney injury was seen at this time.

Only one tumor, a benign tumor of an adrenal gland, was seen in all hamsters examined. This tumor was seen in the 4000 ppm MCH exposure group. The other incidental lesions seen were commonly found changes that were fairly uniform in all groups and not related to MCH exposure.

The examination of tissue from the animals that died during the postexposure observation period or were killed at the study termination is not yet complete and will be discussed in a future report.

THE EVALUATION OF THE ONCOGENIC POTENTIAL OF OTTO FUEL II

In September 1980 the THRU began a one-year industrial type exposure of laboratory animals to Otto Fuel II. Chronic industrial type inhalation exposures have not previously been conducted, and no information is available pertaining to the toxic effects resulting from this type of exposure. It is the purpose of this study to provide this information.

Background

Otto Fuel II is used by the U. S. Navy as a liquid propellant in torpedoes and other weapon systems. The chief component (≈75%) of Otto Fuel II is the nitrate ester 1,2-propylene glycol dinitrate (PGDN). The balance of Otto Fuel II is comprised of 2-nitrodiphenylamine (2%) added as a stabilizer and di-n-butyl sebacate (23%) added as a desensitizer.

The constituent of Otto Fuel II that presents the major health concern is the nitrate ester, PGDN. Nitrate esters are known to produce vasodilation, headaches, nasal congestion, dizziness, nausea and methemoglobinemia. These symptoms can result from either inhalation of vapor or skin absorption.

A ceiling threshold limt value of 1.4 mg/m^3 PGDN vapor has been established. In 1974, Stewart et al. reported a series of experimental inhalation exposures of short duration to humans with PGDN vapor. Headaches occurred in a majority of the individuals exposed to 1.4 mg/m^3 PGDN for 4 hours or more. Repetitive exposure to this concentration produced a tolerance to the headaches. The development of tolerance has previously been described for other organic nitrate compounds. No alteration in blood clinical chemistry values occurred in humans after a single 8-hour exposure or a few repeated 8-hour exposures to 1.4 mg/m^3 PGDN vapor. Increasing the concentration of PGDN to 3.4 mg/m^3 increased the severity of the headaches. After 8 hours of exposure to this concentration, all three subjects had abnormal modified Romberg tests. One individual was unable to perform a heel-to-toe test with his eyes open. Blood clinical chemistry values in individuals exposed to 3.4 mg/m^3 PGDN were unchanged.

Acute and subchronic experimental studies of PGDN using laboratory animals have also been conducted. An acute oral LD_{50} of 250 mg/kg in male Sprague-Dawley rats has been reported by Andersen and Mehl (1973).

Primary skin irritation tests in rabbits conducted by Jones et al. (1972) were negative, while applications of PGDN to rabbit eyes produced conjunctival erythema 5 minutes after application. The erythema subsided within 24 hours. The iris and cornea were not involved. PGDN is readily absorbed through the skin. Rabbits dosed repeatedly with 4 g/kg PGDN via dermal application were weak and cyanotic after the second application; 13 of 14 animals were dead after the fifth application.

Inhalation studies involving animals were also conducted by Jones et al. (1972). Male Sprague-Dawley rats were exposed to 65 mg/m^3 PGDN vapor, 7 hours/day, 5 days/week for a total of 30 exposures. No toxic effects were seen. Hematologic parameters were unchanged and histopathologic examination of tissue did not show any exposure related lesions. Continuous 90-day inhalation exposures of rats, guinea pigs, monkeys and dogs were conducted at

three PGDN concentrations: 67 mg/m^3, 108 mg/m^3 and 236 mg/m^3. Rate of body weight gain was unaffected by PGDN exposure. Decreases in hemoglobin (63%) and hematocrit (37%) were observed in dogs exposed to 236 mg/m^3 PGDN. Methemoglobin levels were elevated in all species with dogs and monkeys showing the most dramatic increase. Iron-positive deposits were present in the livers, spleens and kidneys of dogs and monkeys exposed to 236 mg/m^3 PGDN. Fatty changes in the livers and kidneys were also noted in the animals exposed to PGDN vapor.

Subcutaneous injections of an LD_{50} dose (≈400 mg/kg) of PGDN in rats caused almost complete conversion of hemoglobin to methemoglobin suggesting that the destruction of the oxygen carrying capacity of the blood was the cause of death (Clark and Litchfield, 1969). Following injection of PGDN in rats, a marked decrease in blood pressure occurred. The maximum decrease in blood pressure coincided with the maximum level of PGDN in the blood. Inorganic nitrate was found to be the major urinary metabolite with only very small amounts of inorganic nitrite and propylene glycol mononitrate (PGMN) excreted. In vitro incubation of red blood cells with PGDN produced PGMN, inorganic nitrite and inorganic nitrate. The concentration of inorganic nitrate rose steadily through the incubation period, while the concentration of inorganic nitrite increased to a low level, remained unchanged through the reaction and ultimately decreased at the end of the incubation period. Andersen and Smith (1973) have suggested that the metabolism of PGDN involves inorganic nitrite as a reactive intermediate which is eventually converted to inorganic nitrate. A similar type of process has been suggested for ethylene glycol dinitrate (EGDN) by Clark and Litchfield (1967).

There is no direct evidence that PGDN is a tumorigenic agent; however, a recent publication indirectly suggests this possibility. The report describes a two year feeding study of trinitroglycerin (TNG) to rats, mice and dogs (Dacre et al., 1979). Cholangiofibrosis and hepatocellular carcinomas were found in all of the rats surviving two years of exposure to food containing 1.0% TNG. These same lesions were observed to a lesser degree, in the surviving rats fed 0.1% TNG. PGDN and TNG are closely related in structure.

The principal route of exposure of individuals working with Otto Fuel II is via skin and/or inhalation absorption. For this reason inhalation exposure was chosen as the route of administration for this study. While Otto Fuel II is used as the contaminant for the exposure, it is the nitrate ester PGDN that presents the major health problem for personnel working with Otto Fuel II. Therefore, the concentrations chosen were based on levels of PGDN produced in the exposure chambers.

Otto Fuel II is a red-orange, free flowing liquid with a distinctive odor. The physical properties are listed in Table 41.

TABLE 41. PHYSICAL PROPERTIES OF OTTO FUEL II*

Density:	1.232 g/ml (25°C)
Vapor Pressure:	0.0877 mm Hg (25°C)
Freezing Point:	-27.7°C
Boiling Point:	Decomposes above 121°C
Flash Point (Cleveland Open Cup):	130°C
Viscosity:	4.4 cp (25°C)
Surface Tension:	34.45 dynes/cm
Water Saturation:	0.31% (25°C)
Heat Capacity:	0.445 BTU/lb F (25°C)
Maximum Air Saturation Conc. (Calculated)	781 mg/m^3
Solubilities:	
Insoluble:	Water, ethylene glycol, propylene glycol
Very Slightly Soluble:	Heptane, petroleum ether
Very Soluble:	Alcohols, benzene, carbon tetrachloride, hexane, chloroform, toluene, dibutyl phthalate, trichloroethylene, acetone

* From NAVORD OP-3368 and NAVMED P-5112.

The Otto Fuel II supplied for this study was contained in 10 drums, each weighing approximately 45 lbs. Each drum of Otto Fuel II was analyzed by HPLC to insure uniformity of the entire supply. The results of these analyses indicated the PGDN content of the 10 individual drums varied less than 2%.

Methods

Dogs, rats and mice are being exposed to Otto Fuel II vapor at a concentration of 1.4 mg/m^3 PGDN. This concentration was chosen based on the work of Jones et al. (1972). Exposures are conducted in a Thomas Dome inhalation chamber on an industrial type work schedule of 6 hours/day, 5 days/week and will continue for a period of one year. Exposures began in September 1981.

A second Thomas Dome chamber is used for the exposure of rats and mice to Otto Fuel II vapor at a concentration of 240 mg/m^3 PGDN. This concentration was chosen based on the work of Jones et al. (1972). These exposures are conducted on the same type of intermittent schedule described above.

A third group of dogs, rats and mice are held at the Veterinary Sciences Division Building (Vivarium) to serve as unexposed controls.

At the completion of the one-year exposure 10 male and 10 female rats, 10 male and 10 female mice from each experimental group will be randomly chosen for necropsy and tissue collection. The exposed and control dogs will also be necropsied at this time. The remaining rodents will be held for one year of postexposure observation or until the cumulative mortality reaches 90%.

All animals that die or are sacrificed in this study will be necropsied. The tissues listed in Table 42 will be fixed for histopathologic examination. Liver, kidney and spleen weights will be obtained for all sacrificed dogs and rats at the time of the exposure termination for statistical comparison.

TABLE 42. TISSUES SAMPLED FROM ANIMALS EXPOSED TO OTTO FUEL II VAPOR

Gross lesions	Skin
Tissue masses or suspect tumors and regional lymph nodes	Mandibular lymph node
Larynx	Mammary gland
Trachea	Salivary gland
Lungs and bronchi	Stomach
Heart	Duodenum
Thyroids	Ileum
Parathyroids	Colon
Esophagus	Anus
Liver	Mesenteric lymph node
Sternebrae, vertebrae or femur (plus marrow)	Thigh muscle
	Sciatic nerve
Spleen	Thymus
Kidneys	Gall bladder
Bladder	Pancreas
Nasal cavity	Seminal vesicles
Brain	Prostate
Bone marrow smear	Testes
	Ovaries
	Uterus
	Pituitary

Chamber concentration assignments and animal load are shown below:

		Dogs Beagle		Rats Fischer 344		Mice C57BL/6	
Chamber No.	PGDN Concentration	M	F	M	F	M	F
Vivarium	0	3	3	100	100	100	100
8	1.4 mg/m^3	3	3	75	75	75	75
7	240 mg/m^3			100	100	100	100

The great difference in contaminant concentrations between the two dome atmospheres, as well as safety considerations necessitated separate generation systems.

The contaminant introduction system for the 1.4 mg/m^3 PGDN exposure consists of an agitated supply of Otto Fuel II maintained at a constant temperature (45°C). A controlled air sweep carries the necessary PGDN vapor (approximately 3 mg/min) to the chamber air input line. Chamber air flow is maintained at about 40 CFM. Concentration is controlled by a combination of Otto Fuel II temperature and chamber air flow rate.

Continuous concentration monitoring of the 1.4 mg/m^3 PGDN exposure is accomplished by a Miran IA infrared analyzer equipped with a 20 meter path length cell using the 12 micron band with a 2 mm slit.

The contaminant introduction system for the 240 mg/m^3 exposures consists of three large electrically heated evaporator towers. Each tower has a fuel flow of approximately 0.33 ml/min with a counter-current air flow of 5 CFM. The vapor passes through 1" stainless steel lines to the dome input air line. Waste fuel is pumped from the bottom of the towers to a container for disposal.

Dome concentration is maintained by input heat as well as evaporator tower air flow rate. Fine control of concentration is by use of total chamber air flow.

A Beckman 400 hydrocarbon analyzer modified to act as a loop injected isothermal (120°F) gas chromatograph is used to analyze the 240 mg/m^3 PGDN exposure chamber atmosphere. The automatic injection system provides a sample every 5 minutes. An 8 cm x 1/8" nickel column packed with 10% UCW-98 on Chromosorb W-HP separates PGDN with peak elution 2 minutes after injection.

Animals are being observed hourly during the course of the exposure. Overt signs of toxicity have not been observed in the Otto Fuel II exposed animals. The numbers of deaths occurring in the Otto Fuel II exposed groups are comparable to those occurring in the unexposed control group through 6 months of exposure.

Results

Dogs are individually weighed at biweekly intervals during the exposure period. Mice are weighed in groups on a monthly basis. Body weights in both the dogs and mice have been unaffected by exposure to Otto Fuel II vapor. Rats are individually weighed at biweekly intervals during the exposure period and will be weighed monthly during the postexposure period. There has been a marked effect of exposure to Otto Fuel II vapor on the body weight of the Fischer 344 male rats (Figure 19). Body weights of male rats at

either exposure level have been statistically different ($p < 0.01$) from unexposed control rats through the first six months of the exposure period. A dose related effect is also apparent. The mean body weights of female rats are shown in Figure 20. Otto Fuel II exposure has not had as great an effect on this sex as is seen in the male rats. Only transient differences between the 240 mg/m^3 PGDN exposure groups and unexposed controls are seen. Exposures to 1.4 mg/m^3 PGDN have had no effect on female rat body weight.

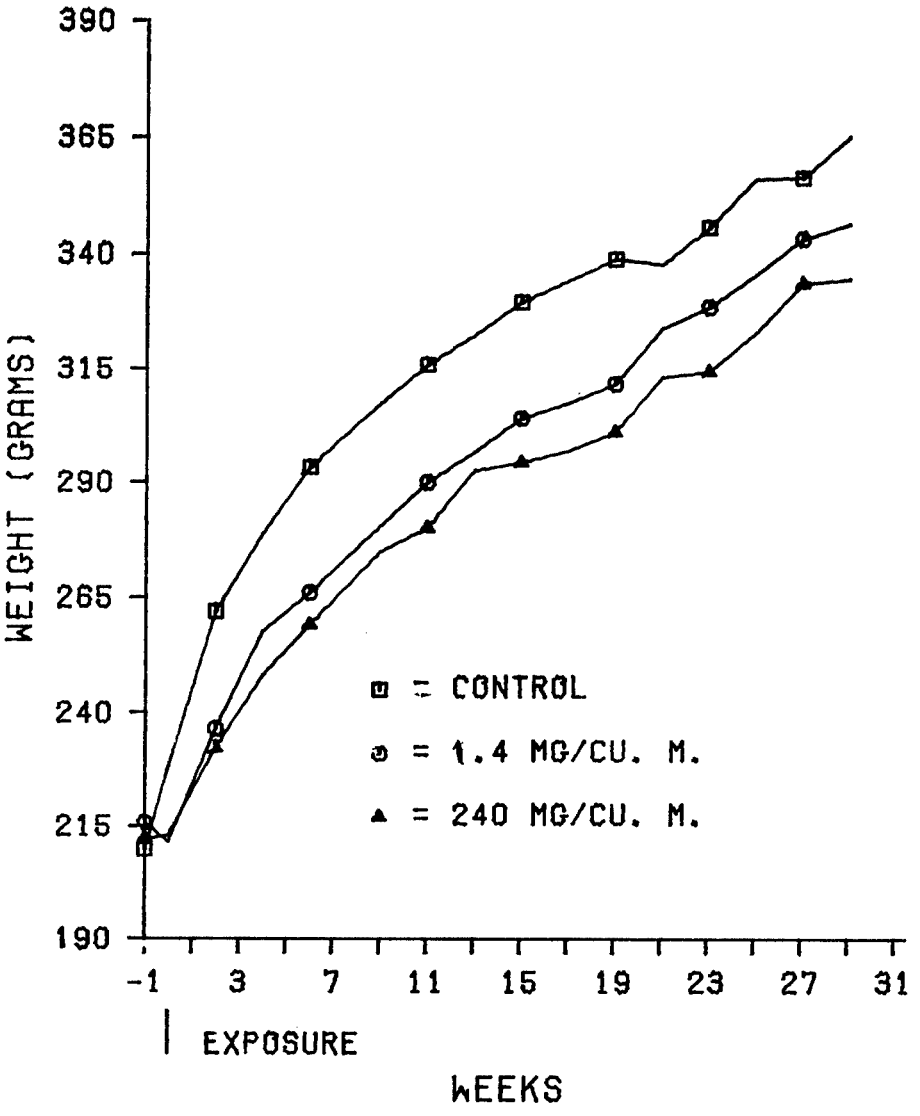


Figure 19. Effect of Otto Fuel II exposure on male rat body weight.

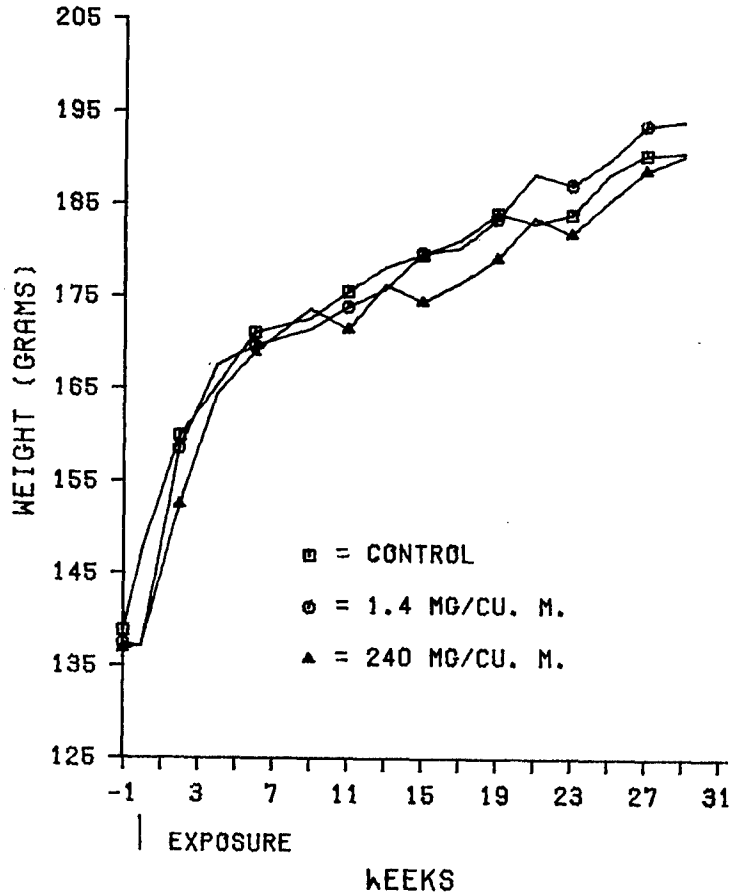


Figure 20. Effect of Otto Fuel II exposure on female rat body weight.

Blood samples are obtained from the dogs at biweekly intervals for clinical chemistry and hematology. These same tests will be performed on blood collected from the rats sacrificed at the conclusion of the exposure phase of the study. After 2 weeks of exposure, decreased hematocrit (Figure 21) and hemoglobin (Figure 22) values were evident in the dogs exposed to 1.4 mg/m^3 PGDN when compared to the unexposed control dogs. Red blood cell counts in exposed dogs were also depressed after 4 weeks of exposure (Figure 23). However, the anemia present in the Otto Fuel II exposed dogs has evinced no increase in reticulocyte counts; rather, a progressive decrease in reticulocytes appears in the exposed dogs (Figure 24). All other blood parameters have remained normal. Examination of dog RBC's after 6 months of exposure failed to reveal the presence of Heinz bodies.

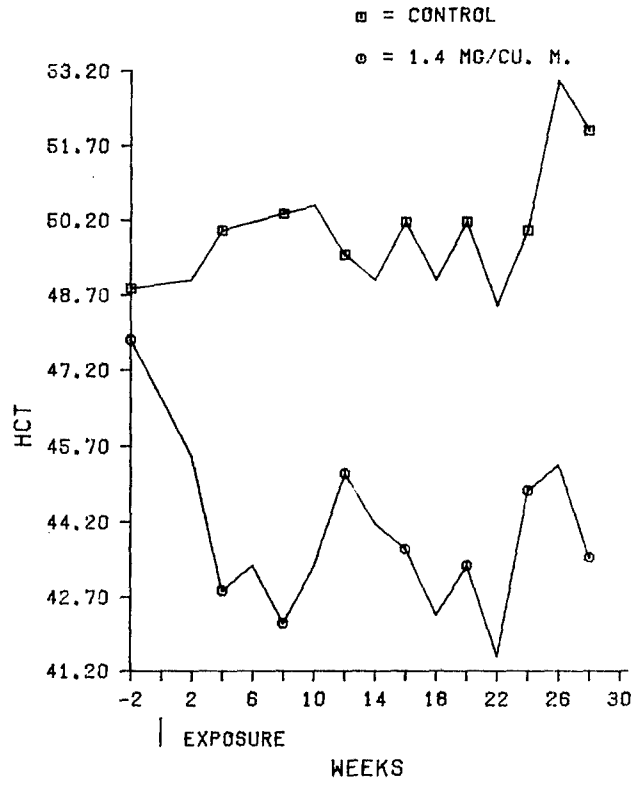


Figure 21. Effect of Otto Fuel II exposure on dog hematocrit.

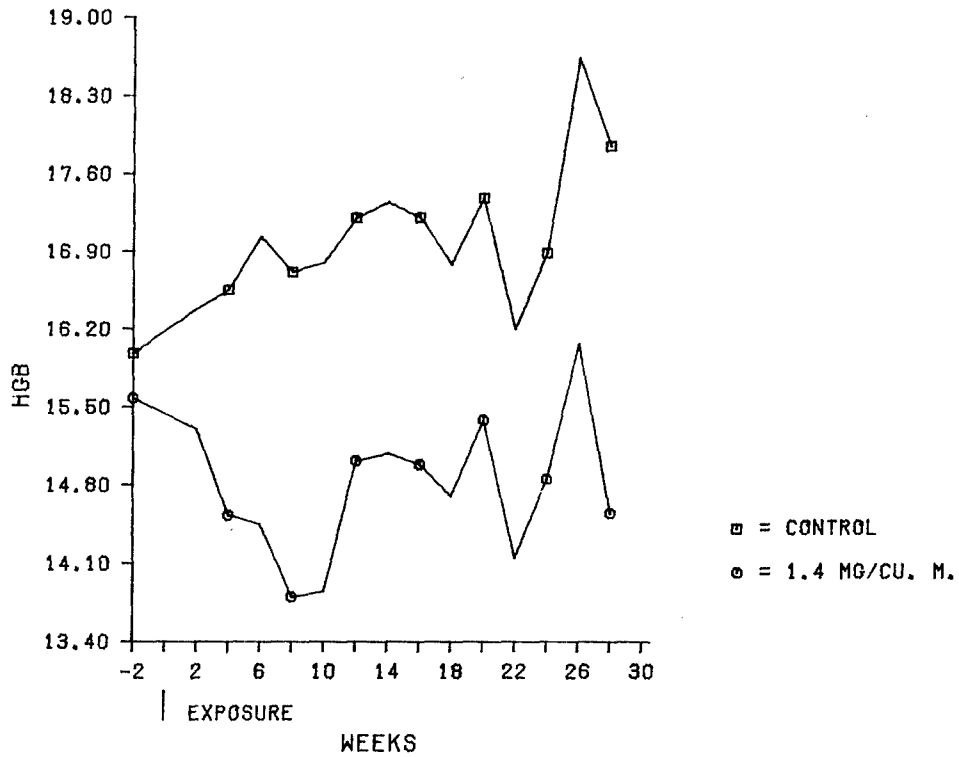


Figure 22. Effect of Otto Fuel II exposure on dog hemoglobin.

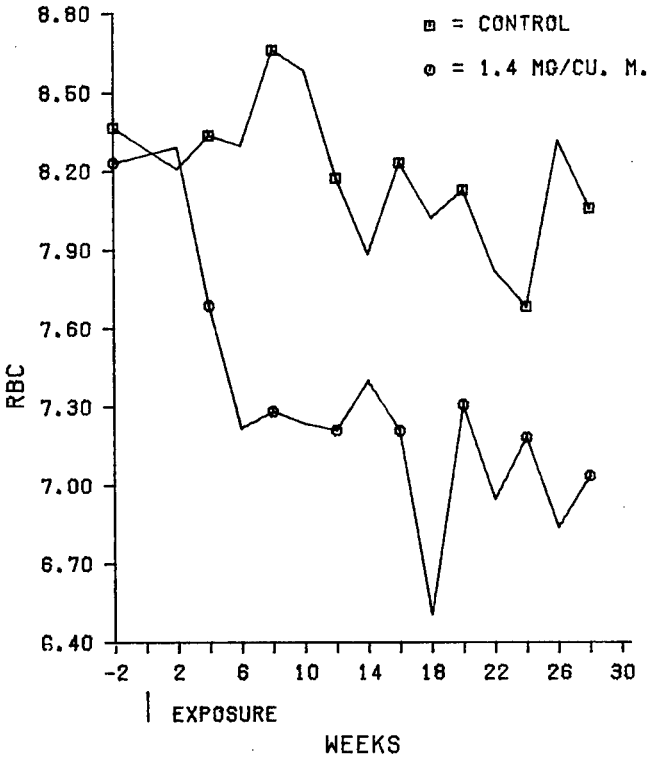


Figure 23. Effect of Otto Fuel II exposure on dog red blood cell count.

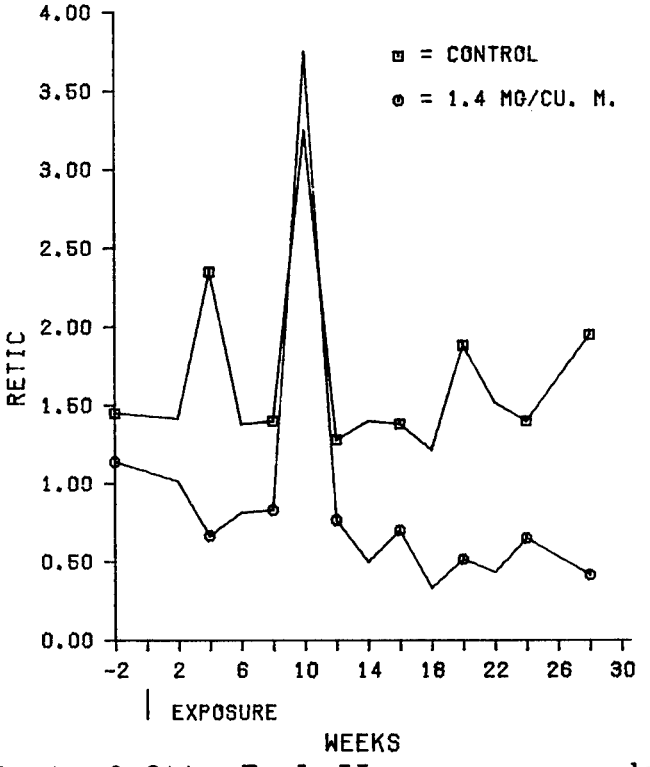


Figure 24. Effect of Otto Fuel II exposure on dog reticulocytes.

Because of the blood changes seen in the Otto Fuel II exposed dogs, blood samples were obtained from the rats to determine if the effects were also present in that species. The samples were obtained after approximately 6 months of exposure. Approximately 1.0 ml of blood was collected via the orbital sinus of 10 male and 10 female rats from each study group for hematology. The results of these examinations are shown in Table 43. Although there were statistical differences noted between the exposed and unexposed groups, all values were within normal biological variability for the species. While there was an indication of lower RBC counts in both male and female rats exposed to 1.4 mg/m^3 PGDN, the mean values were not statistically different from unexposed control rat mean values. Decreased hemoglobin was noted only in the male rats exposed to 1.4 mg/m^3 PGDN, while hematocrit and hemoglobin were actually increased in the female rats exposed to this concentration of Otto Fuel II. Exposure to a higher Otto Fuel II concentration decreased RBC counts in both male and female rats, but the red cells were larger and contained more hemoglobin per cell than the unexposed controls. The WBC counts of the Otto Fuel II exposed rats were well within the normal range, even though there was an indication of a statistical difference between the test and control groups. Examination of the RBC's failed to reveal the presence of Heinz bodies.

TABLE 43. EFFECT OF OTTO FUEL II EXPOSURE ON RAT BLOOD[a,b]

	Male Rats		
	Control	1.4 mg/m^3	240 mg/m^3
RBC (10^6)	8.4	8.2	7.5[d]
WBC (10^3)	4.1	5.2[d]	5.7[d]
HCT (%)	49.9	49.4	50.0
HGB (gm/dl)	17.3	16.8[c]	17.2
Retic (%)	2.2	1.9	2.8[c]
MCV	59.7	60.2	67.3[d]
MCH	20.7	20.5	23.2[c]
MCHC	34.6	33.9	34.4

	Female Rats		
	Control	1.4 mg/m^3	240 mg/m^3
RBC (10^6)	9.3	8.5	7.8[c]
WBC (10^3)	3.7	4.9[d]	5.8[d]
HCT (%)	47.9	49.8[c]	48.3
HGB (gm/dl)	16.6	17.4[c]	16.9
Retic (%)	2.6	2.1	1.7[d]
MCV	52.1	59.3[c]	65.6[c]
MCH	18.1	20.7[c]	22.9[c]
MCHC	34.7	34.9	35.0

[a] N = 10
[b] 6 months of exposure
[c] Statistically different from controls at $p \leq 0.05$.
[d] Statistically different from controls at $p \leq 0.01$.

The results of the rat hematologic examination after 5 months of exposure suggest that this species is more resistant than dogs to the blood effects of Otto Fuel II vapor.

Since it had previously been reported that exposure to PGDN produced methemoglobin, it was decided in the original study protocol to measure methemoglobin in rats during the first two months of exposure. The method chosen for methemoglobin determination was developed by Rodkey et al. in 1979. It has the advantage of being a rapid technique using very small quantities of blood, thus allowing the sampling of rats without terminal sacrifice. It does, however, have the disadvantage of being fairly insensitive at very low methemoglobin concentrations. Blood was collected from the tail vein from male and female rats in the unexposed control and high concentration exposure group. Different rats were selected for each bleeding to minimize stress and tissue damage. Measurements occurred near the end of the exposure week at the conclusion of a day's exposure. The results of these tests are shown in Table 44. Exposure to 240 mg/m^3 Otto Fuel II vapor has resulted in a slightly higher methemoglobin concentration when compared to unexposed controls.

TABLE 44. METHEMOGLOBIN DETERMINATIONS ON RATS EXPOSED TO 240 mg/m^3 OTTO FUEL II

		Methemoglobin % Unexposed Control			Methemoglobin % 240 mg/m^3		
Exposure Day	Sex	X	S.D.	N	X	S.D.	N
3	M	0.97	0.92	7	1.84	1.10	10
8	F	0.81	0.70	7	2.00	1.70	9
13	M	0.40	0.31	8	1.59[a]	0.68	10
18	F	0.71	0.51	10	1.58[a]	0.64	9
23	M	0.62	0.35	10	2.9[a]	0.69	10
28	F	1.19	0.71	10	2.31[a]	0.95	10
33	M	1.20	0.71	10	2.49[a]	1.33	10

[a] Statistically different from controls, $p \leq 0.05$.

Because of the increased methemoglobin concentration in rats it was decided to also examine dog blood for methemoglobin content. The results of these tests are shown in Table 45. Exposure to Otto Fuel II vapor has caused a slight but statistically significant increase in methemoglobin concentration in dogs. The apparent resistance of rats to the blood effects of Otto Fuel II exposure is also indicated by the results of the methemoglobin analysis. The mean methemoglobin percentages for rats and dogs are approximately in the same range even though the rats are sampled from the 240 mg/m^3 exposure while the dogs are sampled from the 1.4 mg/m^3 exposure. Methemoglobin determinations in rats and dogs will continue to be periodically conducted through the exposure phase of the study.

TABLE 45. METHEMOGLOBIN DETERMINATIONS ON DOGS EXPOSED TO 1.4 mg/m^3 OTTO FUEL II

Exposure Day	Methemoglobin % Unexposed Controls X	S.D.	N	Methemoglobin % 1.4 mg/m^3 X	S.D.	N
44	0.38	0.15	6	1.21	0.48	6
136	1.79	0.87	6	4.6	1.51	6

Further information on the progress of this study will appear in future annual reports.

EVALUATION OF THE IRRITATION AND SENSITIZATION POTENTIAL OF ANTIFOULING PAINT FORMULATIONS

Introduction

The Toxic Hazards Research Unit of the Department of Community and Environmental Medicine, University of California, Irvine, was requested to evaluate the eye and skin irritation and sensitization potential of antifouling paint formulations. The paint formulations contain organometallic polymers, either singly or in combination, as the active ingredients.

The skin and eye irritation and sensitization properties of these organometallic polymers (OMP), as well as cuprous oxide, had been previously investigated in this laboratory with the results shown in Table 46. Dyckman et al. (1973) have described the environmental compatibility of these highly effective agents against barnacles, tubeworms, algae, hydroids, sponges, and bacteria.

TABLE 46. IRRITATION PROPERTIES OF OMP COMPOUNDS

Compound	Skin Irritation	Eye Irritation	Sensitization
OMP-1	-----	-----	Negative
OMP-2	-----	-----	Negative
OMP-4	Positive	Positive	Negative
OMP-5	Negative	Positive	Positive
Cuprous Oxide	Negative	Negative	-----

Miller et al. (1976) have found OMP-1 to have an oral LD_{50} in rats of 230 mg/kg and a 4-hour inhalation LC_{50} of 64 mg/m^3. OMP-1 was also irritating to the skin and eyes of rabbits. OMP-2 has an oral LD_{50} in rats of 280 mg/kg and an LC_{50} of 51 mg/m^3 (Naval Medical Research Institute Letter Report, 1976). OMP-2 was also irritating to the skin and eyes of rabbits.

Materials and Methods

Animals

Female New Zealand albino rabbits, weighing approximately five pounds, purchased from Willoughby's Rabbitry, Sabina, Ohio, were used for the eye and skin irritation studies. Male, Hartley derived, albino guinea pigs, weighing between 400 and 600 grams, purchased from Murphy Breeding Labs., Plainfield, Indiana, were used for the sensitization studies.

Materials

The following paint formulations were all supplied by the Naval Medical Research Institute/Toxicology Detachment, Wright-Patterson Air Force Base, Dayton, Ohio. The formulations are classified and will not be presented in this report.

Paint Formulation MRI25
Paint Formulation RO72X05
Paint Formulation S115Ce08

Primary Skin Irritation

The primary skin irritation potential of the paint formulations was measured by a patch test technique on intact and abraded skin areas of albino rabbits. Six rabbits were used for the evaluation of each formulation. The dorsal area of each rabbit was clipped free of hair 24 hours prior to administration of the compound, thus allowing irritation from the clipping process to heal. Equal numbers of exposures were made on the intact and abraded skin. Abrasions were minor incisions through the stratum corneum which were not deep enough to disturb the derma or to produce bleeding. The paints were applied in quantities of 0.5 milliliters. Each site was covered with a 1 x 1 inch piece of surgical gauze two layers thick followed by dental dam and a 4 x 4 inch piece of elastoplast adhesive tape. The rabbits were then fitted with leather restraining collars to prevent disturbance of the patch area. After 24 hours, the collars, dental dam, and patches were removed and the test areas evaluated for irritation using the Draize et al. (1959) scoring method (Table 47) as a reference standard.

TABLE 47. EVALUATION OF SKIN REACTIONS

	Value
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Edema formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extended beyond exposed area)	4

Acute Eye Irritation

A 0.1 ml sample of the paint formulation was applied to one eye of each of six albino rabbits. The opposite eye was untreated and served as a control. Examinations for gross signs of eye irritation were made at 24, 48, and 72 hours following application. Scoring of irritative effects was according to the methods of Draize (1959), Table 48, in which corneal, iris, and conjunctival effects are scored separately.

Skin Sensitization

Groups of test animals consisted of 20 male albino guinea pigs. All formulations were applied topically as a suspension in spectrographic grade acetone. The sensitization tests were started on a Monday when the guinea pigs were weighed and closely clipped on the scapular areas. A volume of 0.05 ml of a 0.1% dilution of the test material was administered onto the upper right scapular area of each guinea pig. A similar control administration of acetone was made into the upper left scapular area. Readings were taken 24 hours and 48 hours later and recorded.

TABLE 48. SCALE FOR SCORING OCULAR LESIONS

1. Cornea	
A. Opacity-degree of density	
No opacity	0
Scattered or diffuse area, details of iris clearly visible	1
Easily discernible translucent areas, details of iris slightly obscured	2
Opalescent areas, no details of iris visible, size of pupil barely discernible	3
Opaque, iris invisible	4
B. Area of cornea involved	
One quarter (or less) but not zero	1
Greater than one quarter, but less than half	2
Greater than half, but less than three quarters	3
Greater than three quarters, up to whole area	4
SCORE EQUALS A x B x 5 TOTAL MAXIMUM -	80
2. Iris	
A. Values	
Normal	0
Folds above normal, congestion, swelling, circumcorneal injection, iris still reacting to light	1
No reaction to light, hemorrhage, gross destruction	2
SCORE EQUALS A x 5 TOTAL MAXIMUM -	10
3. Conjunctivae	
A. Redness	0
Vessels normal	0
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2
Diffuse beefy red	3
B. Chemosis	
No swelling	0
Any swelling above normal	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half closed	3
Swelling with lids about half closed to completely closed	4
C. Discharge	
No discharge	0
Any amount different from normal	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3
SCORE EQUALS (A - B - C) x 2 TOTAL MAXIMUM -	20

The maximum total score is the sum of all scores obtained for the cornea, iris, and conjunctivae. Total maximum score possible - 110.

Doses of 0.1 ml of the same dilutions (freshly prepared) were then applied onto the clipped dorsal lumbo-sacral areas of the guinea pigs on the following Wednesday, Friday, Monday, etc., until seven doses had been administered. The guinea pigs were rested for three weeks (incubation period), weighed and given a challenge application of 0.05 ml of the test material onto the lower right scapular area. The left scapular area was again used for vehicle control tests. The reactions were read after 24 and 48 hours and recorded.

The grading system was designed so that the intensity of the skin reaction is represented by a proportionate numerical value and also that any reaction elicited by the vehicle is subtracted from the reaction elicited by the test material and vehicle combined.

The product of the width and length of the reaction (in mm) is multiplied by the following reaction scores:

1 = very faint pink ("vfp") - no value for this reaction
2 = faint pink ("fp")
3 = pink ("p")
4 = red ("r")
5 = bright red ("R")
6 = edema - <1 mm in height ("e")
7 = edema - >1 mm in height ("E")
*8 = necrosis - <1 sq. mm ("n")
*9 = necrosis - >1 sq. mm ("N")

*The product of width and length of the necrotic area multiplied by 8 or 9 is added to the numerical value of the foregoing reactions that are present - calculated in the same manner.

A final grade of 25 or less indicated no sensitizing potential, and a final grade of 100 indicated a moderate sensitization potential.

Results

Primary Skin Irritation

Paint formulations MRI25 and RO72X05, when applied undiluted to intact and abraded skin, produced a primary irritation score of zero. These scores indicate that neither formulation is a primary skin irritant.

Paint formulation S115Ce08 produced erythematous reactions ranging from very slight on one rabbit to moderate to severe on two rabbits (Table 49). In most cases, the erythema persisted through 72 hours with equal reactions between the intact and the abraded areas. This paint formulation is considered to be a moderate skin irritant and dermal contact should be avoided.

TABLE 49. RABBIT PRIMARY SKIN IRRITATION RESULTS AFTER APPLICATION OF PAINT FORMULATION S115Ce08

	Reading Time (Hours)	Rabbit Number 1		2		3		4		5		6	
		I	A	I	A	I	A	I	A	I	A	I	A
Erythema	24	2	2	2	2	1	1	3	3	3	3	2	2
	72	2	2	2	2	0	0	2	2	2	2	2	2
Edema	24	0	0	0	0	0	0	0	0	0	0	0	0
	72	0	0	0	0	0	0	0	0	0	0	0	0
Necrosis	24	0	0	0	0	0	0	0	0	0	0	0	0
	72	0	0	0	0	0	0	0	0	0	0	0	0

I - Intact Skin
A - Abraded Skin

Primary Irritation Score 1.9

Eye Irritation

Paint formulation MRI25 caused a slight swelling of the conjunctivae and moderate discharge in all six rabbits at 24 hours (Table 50). The 48 and 72 hour effects were a result of a continual, slight discharge from the treated eyes. No corneal or iris damage was seen in any of the six rabbits tested with this formulation.

Instillation of paint formulation RO72X05 in rabbit eyes resulted in injection of the blood vessels of the palpebral and bulbar conjunctivae with obvious swelling and partial eversion of the eyelids in all six rabbits (Table 50). No corneal or iris damage was seen in any of the six rabbits treated with this paint formulation.

Paint formulation C115Ce08 caused slight reddening and swelling of the conjunctivae as well as a discharge sufficient to moisten the fur surrounding the eyes of the six rabbits (Table 50). No corneal or iris damage was noted, and the eyes of four of the rabbits were normal at 72 hours. The fifth rabbit showed only minimal redness and chemosis at 72 hours. The sixth rabbit (number B07) had easily discernible translucent areas involving more than 75% of the entire corneal area. The lesion persisted throughout the 72-hour scoring period and was still obvious at 7 days. The reason for the vast difference in the response of this rabbit was that the eye sealed closed after instillation of the paint, and the eye did not clear itself as was the case with the eyes of the other five rabbits. As a result, the material was in contact with the surface of the eye for 24 hours and greater damage was incurred.

TABLE 50. RABBIT EYE IRRITATION RESULTS AFTER APPLICATION OF ANTIFOULING PAINT FORMULATIONS

FORMULATION MRI25:

Rabbit No.	Numerical Score		
	24 Hours	48 Hours	72 Hours
A09	6	1	1
A15	6	4	1
A17	6	1	1
A13	6	1	1
A19	6	1	1
A11	8	1	1
MEAN	6	1.5	1

FORMULATION RO72X05:

Rabbit No.	Numerical Score		
	24 Hours	48 Hours	72 Hours
A25	10	6	4
A21	10	10	8
A23	10	6	6
A35	10	8	4
A29	10	10	8
A27	10	6	4
MEAN	10	7.7	5.7

FORMULATION S115Ce08:

Rabbit No.	Numerical Score		
	24 Hours	48 Hours	72 Hours
B07	66	60	60
B09	4	4	0
B11	10	0	0
B13	6	0	0
B15	6	4	0
A37	8	6	4
MEAN	16.7	12.3	10.7

Skin Sensitization

The following table is a summary of the results of the skin sensitization tests. Each group of guinea pigs originally consisted of twenty animals. However, one guinea pig treated with

paint formulation MRI25 died of an extraneous infection during the experiment and was not replaced. The response for that compound is based on 19 animals.

Paint Formulation	Sensitizing N	Sensitizing Response	Potential
MRI25	19	None	None
R072X05	20	None	None
S115Ce08	20	None	None

Discussion

Application of two of the paint formulations (MRI25 and R072X05) to the intact and abraded skin of rabbits produced a primary irritation score of zero. Neither of these paint formulations is regarded as a primary skin irritant and would not require precautionary labelling. Application of formulation S115Ce08 to the intact and abraded skin resulted in moderate to severe erythema on two rabbits which persisted through 72 hours. The primary irritation score of 1.9 would classify this compound as a moderate irritant and appropriate protective measurements would be necessary.

Application of paint formulation MRI25 resulted in slight eye irritation and minimal discharge and would be considered negative for this test. Paint formulation S115Ce08 caused a severe reaction in the one rabbit when the eye sealed closed for 24 hours. However, the other five rabbits showed only slight reddening and swelling which would classify this paint as a negative eye irritant. The third formulation, R072X05, caused definite conjunctival swelling with partial eversion of the eyelids in all six rabbits which indicates that this formulation is a positive eye irritant.

The skin sensitization test is designed to evaluate the potential of a material to act as an antigen. Applications of small quantities of the material over a period of time induce antibody synthesis. The induction potential is then evaluated by grading the irritation of a single challenge administration. None of the three paint formulations showed a sensitizing potential by this method of testing.

These results indicate that paint formulation MRI25 is not an eye or skin irritant nor is it a potential sensitizer. Paint formulation S115Ce08 is negative for eye irritation and sensitization but is considered a skin irritant, and care must be taken to avoid skin contact. Paint formulation R072X05 is not a skin irritant nor is it a potential sensitizer. However, the Draize test results show it to be an eye irritant and precaution against eye contamination should be taken during handling procedures.

EVALUATION OF THE IRRITATION AND SENSITIZATION POTENTIAL OF THREE FERROCENE SOLUTIONS

Introduction

Ferrocene (dicyclopentadienyliron), in mixed xylene isomers solvent, is being used by the U. S. Navy as a fuel additive to reduce jet engine exhaust smoke. The manufacturer of the fuel additive, Arapahoe Chemicals, Incorporated, has proposed changing the solvent from a xylene mixture to a coal tar naphtha blend.

Eye and skin irritation tests with ferrocene were conducted by the Navy Toxicology Unit in 1974. The ferrocene was tested as a dry powder and in solution with either toluene or JP-5. The results of these tests indicated that ferrocene was not irritating to eyes or skin. The toluene solvent, however, caused minor eye irritation. Information from the material safety data sheet supplied by the manufacturer indicates that skin irritation has not been observed in workers associated with the production of ferrocene. Skin sensitization studies with ferrocene or ferrocene/solvent mixtures have not been reported.

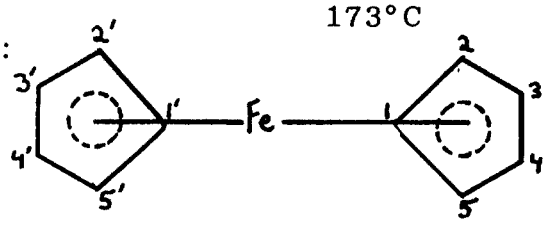
The Navy requested that the Toxic Hazards Research Unit of the University of California, Irvine, conduct an evaluation of the irritation and sensitization potential of three materials containing ferrocene. Two of the materials were coal tar naphtha solvent based. The third was xylene solvent based.

Materials and Methods

Test Materials

The three ferrocene mixtures described below were supplied by the Naval Medical Research Institute/Toxicology Detachment (NMRI/TD), Wright-Patterson Air Force Base, Ohio. Testing was performed at the Toxic Hazards Research Unit of the University of California, Irvine, located at Wright-Patterson Air Force Base, Ohio under contract number F33615-80-C-0512.

Ferrocene - Fe-55®

Appearance:	Bright orange crystals
Solubility:	
H_2O:	Insoluble
Solvents:	19 g/100 g benzene
MW:	186.04
MP:	173°C
Structure:	

1.	Arapahoe Chemical No.	PD-1475 with Fe55
	NMRI/TD Log No.	0162-2
	Material Composition	10% ferrocene in mixed xylene isomers
	Physical State	Liquid
2.	Arapahoe Chemical No.	PD-1651
	NMRI/TD Log No.	0162-5
	Material Composition	10% ferrocene in coal tar naphtha solvent PD-165A
	Physical State	Liquid
3.	Arapahoe Chemical No.	PD-1652
	NMRI/TD Log No.	0162-7
	Material Composition	10% ferrocene in coal tar naphtha solvent PD-165B
	Physical State	Liquid

Animals

Species	Weight Range	Strain and Sex	Source
Rabbits	4-5 lbs.	New Zealand White, Female	Willoughby's Rabbitry, Sabina, Ohio
Rabbits	4-5 lbs.	New Zealand White, Female	J & J Research Farms, Hamilton, Ohio
Guinea Pigs	400-500 gms	Hartley, Albino, Male	Murphy Breeding Labs, Plainfield, Indiana

A total of 27 rabbits were used for the primary eye irritation tests.

Primary Eye Irritation

One-tenth milliliter of the liquid was applied to one eye of each of nine albino rabbits. The opposite eye was untreated and served as a control. The treated eye of six rabbits remained unwashed. The remaining three rabbits received test material and then the treated eye was flushed for one minute with lukewarm water 20-30 seconds after instillation. Examinations for gross signs of eye irritation were made at 24, 48, and 72 hours following application. Scoring of irritative effects was according to the method of

Draize (1959) in which corneal, iris, and conjunctival effects are scored separately. In this scoring system, injuries to the cornea and iris may represent as much as 80% of the total score. Cornea and iris scores are heavily emphasized because of the essential role of these tissues in vision.

Primary Skin Irritation

A patch-test method was conducted to measure the degree of primary skin irritation of intact and abraded skin of albino rabbits.

Six rabbits were used for each test material and they were clipped of all possible hair on the back and flanks 24 hours prior to exposure to allow for recovery of the skin from any abrasion resulting from the clipping. Two areas on the back, one on each side, were designated as patch test areas. One of the areas was abraded by making minor incisions through the stratum corneum. These abrasions were not sufficiently deep to disturb the dermis or to produce bleeding.

Each test material (0.5 ml) was applied to the designated patch areas and was covered by a 1-inch square of surgical gauze two single layers thick. The gauze patches were held in place with strips of elastoplast tape. The entire area was covered with a rubber dental dam strip and secured with more elastoplast tape. The patches remained in place on the rabbits for 24 hours. During that time, the rabbits were fitted with restraining collars to prevent disturbance of the patch area, while allowing the rabbits freedom of movement and access to food and water. After 24 hours, the wrap and patches were carefully removed, and the test areas evaluated for irritation using the Draize (1959) table as a reference standard. Readings were also made at 72 hours (48 hours after the first reading).

Skin Sensitization

The guinea pig sensitization method used is a modification of the method of Maguire. Prior to initiation of the sensitization routine, primary irritation tests were conducted using three guinea pigs for each material. A 0.1 ml aliquot of the undiluted test substance was applied to the clipped flanks of the animals. Observations made at 24 hours post application showed no signs of irritation. The sensitization tests, therefore, proceeded using the ferrocene solutions as supplied by the Navy, without further dilution.

Ten male albino Hartley strain guinea pigs were used for each test material. An area on the back of each animal directly above the forelegs was clipped with electric clippers and chemically

depilated with a commercial depilatory the morning of the first insult exposure. Test solutions, 0.1 ml at each application, were applied to this area on a 1/2 x 1/2 inch cotton square, covered with dental dam and held in place with adhesive tape. The first insult patch was allowed to remain in place for two days, then removed and a second application of 0.1 ml made. Two days later this patch was removed, a total of 0.2 ml of Freund's* adjuvant per animal was injected intradermally using 2-3 points adjacent to the insult site. A new patch of 0.1 ml of the test material was then applied. On the third day after this application, the patch was removed and a fresh patch of 0.1 ml of the material was applied. The last patch was removed two days later and the animals allowed to rest for two weeks. Each time the insult patches were removed, the condition of the skin at the insult site was evaluated and recorded.

After the two week rest period, the flanks of the animals were clipped. An 0.1 ml aliquot of the test material was applied to one flank while the other remained untreated and served as a control site. The challenge applications were not occluded.

The skin responses at the challenge sites were evaluated for erythema and edema 24 and 48 hours after application according to the following grading system.

Erythema
0 - None
1 - Very slight pink
2 - Slight pink
3 - Moderate red
4 - Very red

Edema
0 - None
1 - Very slight
2 - Slight
3 - Moderate
4 - Marked

Animals showing any degree of erythema and/or edema at the test solution challenge site were rated as positive responders.

* Bacto Adjuvant Complete, Freund, Difco Laboratories, Detroit, Michigan.

Results

Primary Eye Irritation

The results of the primary eye irritation tests are shown in Table 51. With the exception of one rabbit, no signs of primary eye irritation were evident in the animals treated with PD1475 W/FE55. The rabbit that exhibited signs of irritation had minor redness and chemosis.

All rabbits with unwashed eyes treated with either PD1651 or PD1652 exhibited signs of moderate eye irritation 24 hours after application. The irritation was limited to the conjunctivae in most of the rabbits. Three of the six rabbits treated with PD1652 had eyelid swelling (either partial eversion of the lids or swelling with lids half closed). Two of these rabbits also had circumcorneal injection. Signs of irritation were reduced in all of the rabbits at 48 or 72 hours post application. Washing the eyes 30 seconds after application alleviated the irritation. The presence of irritation in the single rabbit with the washed eye treated with PD1652 possibly resulted from incomplete washing.

Primary Skin Irritation

The results of the primary skin irritation (P.I.) tests are shown in Table 52. Very mild erythema was observed in two of the six rabbits treated with PD1475 W/FE55. The P.I. score is very low and this material would probably not be considered a skin irritant. Both PD1651 and PD1652 produced mild skin irritation. The irritation consisted of mild erythema at both intact and abraded sites in all rabbits tested with the two coal tar naphtha solvent materials.

TABLE 51. EFFECTS OF THREE FERROCENE SOLVENT MIXTURES ON RABBIT EYES

Material	Eye Washed	Rabbit No.	Numerical Score 24 hr.	48 hr.	72 hr.
PD1475 W/FE55	No	C11	0	0	0
	No	C13	2	4	2
	No	C15	0	0	0
	No	C17	0	0	0
	No	C19	0	0	0
	No	C21	0	0	0
		Mean	0.3	0.7	0.3
PD1475 W/FE55	Yes	B75	0	0	0
	Yes	B77	0	0	0
	Yes	B79	0	0	0
		Mean	0	0	0
PD1651	No	C01	2	2	0
	No	C03	4	2	0
	No	C05	12	10	0
	No	C07	6	0	0
	No	C09	6	0	0
	No	C23	6	0	0
		Mean	6.0	4.0	0
	Yes	C25	0	0	0
	Yes	C27	0	0	0
	Yes	B93	0	0	0
		Mean	0	0	0
PD1652	No	B95	14	2	2
	No	B97	4	0	0
	No	B99	6	0	0
	No	B81	6	2	2
	No	B83	15	2	0
	No	B85	21	6	2
		Mean	11.0	2.0	1.0
	Yes	B87	0	0	0
	Yes	B89	0	0	0
	Yes	B91	6	4	2
		Mean	2.0	1.3	0.7

TABLE 52. PRIMARY SKIN IRRITATION TEST RESULTS OF THREE FERROCENE SOLUTIONS

Material	Primary Irritation Scores*
PD1475 W/FE55 (10% Ferrocene in mixed xylene isomers)	0.33
PD1651 (10% Ferrocene in coal tar naphtha solvent PD-165A)	1.08
PD1652 (10% Ferrocene in coal tar naphtha solvent PD-165B)	0.83

*Primary Irritation Score =

$$\frac{\text{Total Reaction Score (Erythema + Edema)}}{\text{(No. of Animals x No. of Observation x No. of Test Sites)}}$$

Skin Sensitization

None of the guinea pigs showed a sensitization response to the challenge application of PD1475 W/FE55.

Both of the coal tar naphtha based materials demonstrated sensitization potential in guinea pigs. Six of the ten guinea pigs treated with PD1651 responded to the challenge application. Evaluation of the test areas 24 hours after application revealed areas of mild erythema in the six animals. Evaluation 48 hours post application showed erythema still present in four of these animals. Edema was not seen in any of the animals.

Six of the ten guinea pigs treated with PD1652 responded to the challenge application. Mild erythema was present at the challenge site in these six animals 24 hours post application. At 48 hours post application, five of the animals still showed mild erythema. Edema was not evident in any of the animals.

Inhalation Hazard

A comparison of inhalation hazards of the three solvents was made based on the TLV values of the important constituents of the ferrocene solvents. The steps involved in estimation of the relative inhalation hazards of the presently used mixed xylene and proposed coal tar naphtha solvents are detailed below:

1. Determination of present TLV values for the important components of the solvents from the latest ACGIH values.

2. Calculation of room temperature (22°C) saturated vapor pressures of these components using Clapeyron-Clausius (log P vs 1/T) plots.

3. Calculation of mole % concentrations of the components utilizing GC/MS data.

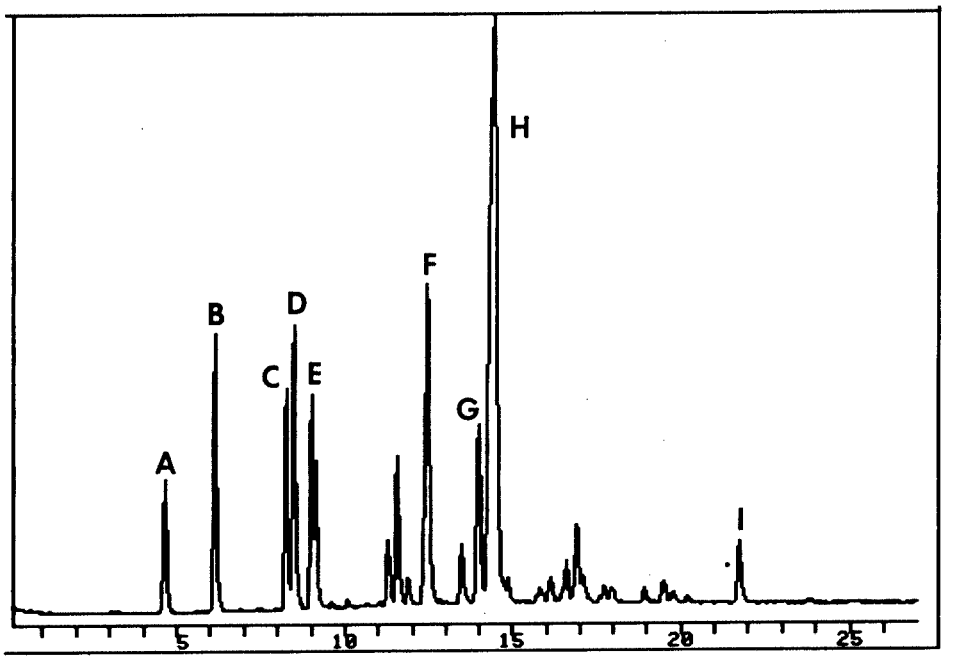
4. Calculation of the saturated partial pressures of these components over the solvents, assuming Raoult's Law holds.

5. Calculation of a relative hazard index by dividing the saturated VP's of these components by the TLV's.

GC/MS analyses of both candidate solvents, PD-165A and PD-165B, were performed and major peaks identified as shown in Figures 25 and 26. Quantitation was achieved using peak areas of the gas chromatograms assuming constant detector response. Table 53 is a listing of the major constituents of both candidate solvents and their concentrations. Meta-xylene was selected as being representative of the mixed-xylene solvent. A GC of the mixed xylene solvent showed only 3 peaks indicative of the 3 xylene isomers (Figure 27).

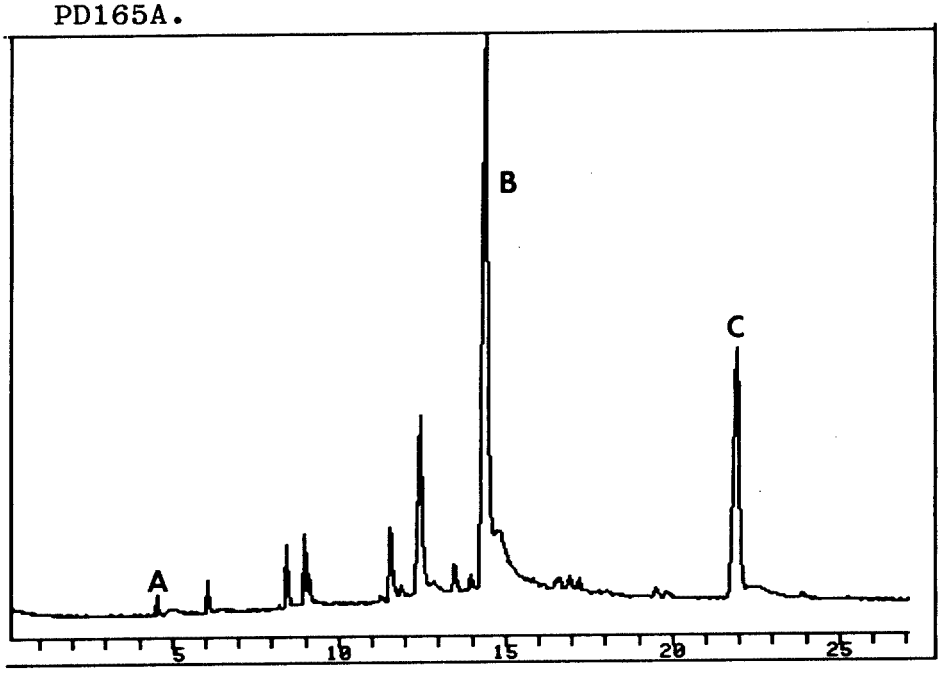
TABLE 53. CONCENTRATIONS OF MAJOR COMPONENTS IN CANDIDATE FERROCENE SOLVENTS

Component	PD-165A Concentration, %	PD-165B Concentration, %
Benzene	1.0	0.7
Toluene	1.9	
Ethyl Benzene	1.8	
Xylene	3.0	
Styrene and Xylene	3.1	
Indene	59.1	56.6
Naphthalene	5.0	19.2
TOTAL	74.8	76.5



A - BENZENE
B - TOLUENE
C - ETHYLBENZENE
D - XYLENE
E - STYRENE
F - TRIMETHYLBENZENE
G - INDANE
H - INDENE
I - NAPHTHALENE

Figure 25. Total ion gas chromatogram of coal tar naphtha sample PD165A.



A - BENZENE
B - INDENE
C - NAPHTHALENE

Figure 26. Total ion gas chromatogram of coal tar naphtha sample PD165B.

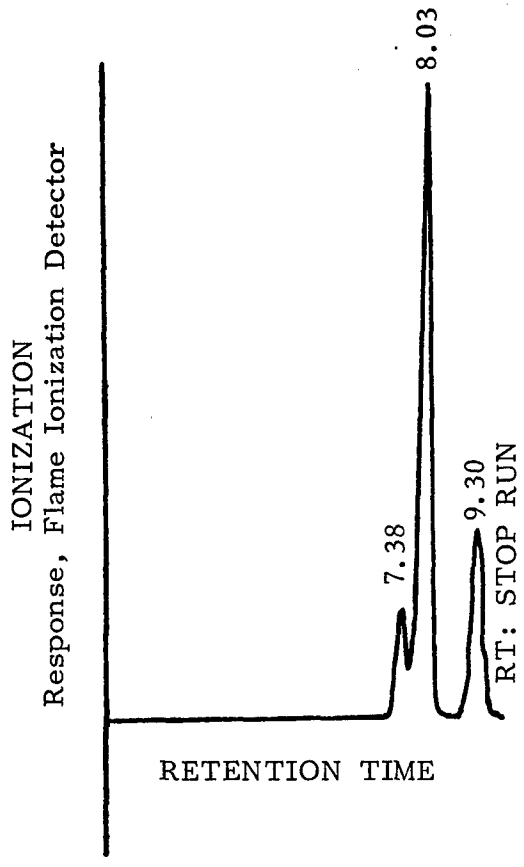


Figure 27. Gas chromatogram of mixed xylene solvent.

Benzene, indene, and naphthalene were selected as the important components of the mixture since these have TLV's of 10 ppm while all the others have TLV's of 100 ppm (50 ppm for styrene). The following calculations were made under two assumptions. One was that trimethylbenzene (TLV = 25 ppm) was not a significant component of the mixture; the other included trimethylbenzene in this calculation assuming a maximum concentration of 10 mole %. The concentration of trimethylbenzene could not be calculated from the GC since its peak contained other components.

A. TLV Values from 1980 ACGIH Handbooks

1. m-Xylene (assume representative of mixed xylenes) - 100 ppm.
2. Benzene, indene, naphthalene - 10 ppm.
3. Trimethylbenzene - 25 ppm

B. Room Temperature Saturated Vapor Concentrations

Benzene	-	109,000 ppm
m-Xylene	-	9,350 ppm
Indene	-	1,970 ppm
Naphthalene	-	197 ppm
Trimethylbenzene	-	3,000 ppm

C. Mole % Concentrations

Component	PD-165A	PD-165B
Benzene	1.5	1.0
Indene	58.0	55.0
Naphthalene	4.5	17.0
Trimethylbenzene	10.0	10.0

D. Partial Vapor Pressures of Components

Component	PD-165A	PD-165B
Benzene	1635 ppm	1090 ppm
Indene	1142 ppm	1085 ppm
Naphthalene	9 ppm	34 ppm
Trimethylbenzene	300 ppm	300 ppm
Total Vapor Concentration of Components		
With Trimethylbenzene	3089 ppm	2509 ppm
Without Trimethylbenzene	2789 ppm	2209 ppm

E. Calculation of TLVs of Vapor Mixtures Containing Trimethylbenzene

$$TLV = \frac{1}{\frac{\text{Mole Fraction A}}{TLV_A} + \frac{\text{Mole Fraction B}}{TLV_B}}$$

For PD-165A With Benzene

Mole Fraction of Components with TLV 10 $= \frac{2789}{3089} = 0.90$

Mole Fraction of Trimethylbenzene = 0.10

$$TLV = \frac{1}{\frac{0.9}{10} + \frac{0.1}{25}} = \frac{1}{0.094} = 10.6$$

For PD-165B With Benzene
Mole Fraction of Components with TLV 10 = $\frac{2209}{2509} = 0.88$

Mole Fraction of Trimethylbenzene = 0.12

$$TLV = \frac{1}{\frac{0.88}{10} + \frac{0.12}{25}} = \frac{1}{0.093} = 10.7$$

For PD-165A Without Benzene
Mole Fraction of Components with TLV 10 = $\frac{1154}{1454} = 0.794$

Mole Fraction of Trimethylbenzene = 0.206

$$TLV = \frac{1}{\frac{0.794}{10} + \frac{0.206}{25}} = \frac{1}{0.0876} = 11.4$$

For PD-165B Without Benzene
Mole Fraction of Components with TLV 10 = $\frac{1119}{1419} = 0.79$

Mole Fraction of Trimethylbenzene = 0.21

$$TLV = \frac{1}{\frac{0.790}{10} + \frac{0.21}{25}} = \frac{1}{0.0874} = 11.5$$

F. Hazard Indices

m-Xylene - $\frac{9350}{100} = 94$

PD-165A	With Benzene	Without Benzene
With Trimethylbenezene	$\frac{3089}{10.6} = 292$	$\frac{1454}{11.4} = 128$
Without Trimethylbenzene	$\frac{2509}{10} = 279$	$\frac{1154}{10} = 115$

PD-165B	With Benzene	Without Benzene
With Trimethylbenzene	$\frac{2509}{10.7} = 236$	$\frac{1419}{11.5} = 123$
Without Trimethylbenzene	$\frac{2209}{10} = 221$	$\frac{1119}{10} = 112$

The data in "E" show that the inhalation hazard indices of the candidate solvents are 2 - 3 times that of the mixed xylene solvent. However, the partial pressure data in "D" indicate that benzene is responsible for half of the total pressure of the components while its concentration is only approximately 1%. If benzene were eliminated, there would be practically no increased hazard posed by use of the candidate solvents. Even if an unrealistically high concentration of trimethylbenzene is assumed to be present, the conclusion still holds that benzene is the major contributor to the hazard. This is demonstrated by the data showing this high level of trimethylbenzene has practically no effect on the hazard ratios.

Conclusions

Of the three ferrocene solutions tested, the 10% ferrocene in mixed xylene isomers produced the least amount of irritative effects. The eye and skin irritation test scores are very low while the sensitization test was negative. These results indicate that irritation or sensitization should not be a hazard associated with this material.

The other two ferrocene solutions, 10% ferrocene in coal tar naphtha solvent PD165A (PD1651) and 10% ferrocene in coal tar naphtha solvent PD165B (PD1652), produced similar irritative effects. Moderate eye irritation developed after exposure to either material. A slightly more severe response was produced by PD1652. Washing the eyes shortly after exposure alleviated the irritation. Skin irritation and sensitization test results indicate that personnel working with these materials should use appropriate protective measures.

Chemical analysis of the candidate solvents indicates that the inhalation hazards for both solvents is 2 - 3 times higher than for this mixed xylene solvent, primarily because of the presence of benzene in the coal tar naphtha.

EVALUATION OF THE IRRITATION POTENTIAL OF THE HYDRAULIC FLUID HF-20

Introduction

The Toxic Hazards Research Unit of the Department of Community and Environmental Medicine, University of California, Irvine, was requested to examine a hydraulic fluid by the U.S. Navy to evaluate the potential to produce eye or skin irritation in rabbits and skin sensitization in guinea pigs. The compound, HF-20, is produced by Chem-Trend Incorporated and is one of several water-ethylene glycol compounds being considered for use as fire resistant hydraulic fluids. This compound is a candidate replacement for the hydraulic fluid presently in use under the military specifications for fire resistant hydraulic fluids (MIL-H-22072A).

Materials and Methods

Animals

Female New Zealand albino rabbits weighing approximately 5 pounds were purchased from Willoughbys Rabbitry, Sabina, Ohio. Male, Hartley derived, albino guinea pigs weighing between 400 to 600 grams were purchased from Murphy Breeding Labs, Plainfield, Indiana.

Material

The sample of HF-20 was supplied by the Navy Medical Research Institute/Toxicology Detachment, Wright-Patterson Air Force Base, Dayton, Ohio.

The physical properties of HF-20 supplied by the manufacturer are listed below:

NMRI/TD Sample No.	0247-1
Boiling Point (°F):	212
Specific Gravity:	1.07*
pH:	10.7
Physical State:	Liquid
Chemical Composition:	49% Water
	34% Ethylene Glycol
	12.8% Thickeners
	4.2% Additives

* Measured in our laboratory

The sample of HF-20 received from the U. S. Navy to be used in this study also contains approximately 0.7% di-n-butylamine and 0.2% morpholine, among the additives.

Primary Dermal Irritation

A patch-test method was conducted to measure the degree of primary dermal irritation of intact and abraded skin of albino rabbits.

Six rabbits were clipped of all possible hair on the back and flanks, 24 hours prior to exposure to allow for recovery of the skin from any abrasion resulting from the clipping. Two areas on the back, one on each side, were designated as patch-test areas. One of the two areas was abraded by making minor incisions through the stratum corneum. These abrasions were not sufficiently deep to disturb the derma or to produce bleeding.

HF-20 was applied in the amount of 0.5 ml to the designated patch-test area and was covered by a one-inch square of surgical gauze two single layers thick. The gauze patches were held in place with strips of elastoplast tape. The entire area was covered with a rubber dental strip and secured with more elastoplast tape. The patches remained in place on the rabbits for 24 hours. During that time, the rabbits wore leather restraining collars to prevent disturbance of the patch area while allowing the rabbits freedom of movement and access to food and water.

After 24 hours, the wrap and patches were carefully removed, and the test areas were evaluated for irritation using the Draize table (1959) as a reference standard. Readings were also made at 72 hours (48 hours after the first reading).

Primary Eye Irritation

A 0.1 ml sample of HF-20 was applied to one eye of each of nine albino rabbits. The opposite eye was untreated and served as a control. The treated eyes of six of the rabbits were unwashed. The treated eyes of the remaining three rabbits were flushed with lukewarm water approximately 30 seconds after instillation of the dose. Examinations for gross signs of eye irritation were made at 24 and 72 hours following application. Scoring of the irritative effects was according to the method of Draize (1959). In this scoring system, injuries to the cornea and iris may represent as much as 80% of the total score because of their essential role in vision.

Skin Sensitization

The guinea pig sensitization method used is a modification of the method of Maguire (1973). Ten male albino guinea pigs, Hartley strain, six to eight weeks of age, were used. The test materials were tested for primary irritation on three guinea pigs by application to the clipped flank. Observation made at 24 hours showed no signs of irritation and therefore, the material was tested undiluted.

An area on the back of each animal directly above the forelegs was clipped with electric clippers and chemically depilated with a commercial depilatory on the morning of the first insult exposure. Test solutions, 0.1 ml at each application, were applied to this area on a 1/2 x 1/2 inch cotton gauze square, covered with dental dam, and held in place with adhesive tape. The first insult patch was allowed to remain in place for two days, then removed, and a second application of 0.1 ml made. Two days later, this patch was removed and a total of 0.2 ml of Fruend's adjuvant per animal injected intradermally, using 2 points adjacent to the insult site, then a new patch of 0.1 ml of the test material was applied. On the third day after application, the patch was removed and a fresh patch of 0.1 ml of the material applied. The last patch was removed two days later, and the animals were allowed to rest for two weeks. Each time the insult patches were removed, the condition of the skin at the application site was evaluated and recorded. When the last patch was removed, the toes of the hind feet of each animal were taped to prevent the animal from scratching the treated area.

After the two-week rest period, the flanks of the animals were clipped and challenged, but were not covered. The skin response at this site was recorded at 24 and 48 hours after application. Any animal showing measurable erythema and/or edema at the test solution challenge site was rated as a positive responder.

Results

Primary Dermal Irritation

The hydraulic fluid, HF-20, when applied undiluted to intact and abraded skin, produced a primary irritation score of zero. HF-20 is not a primary skin irritant.

Eye Irritation

The hydraulic fluid did not cause any occular irritation in the rabbits. No differences could be noticed when comparing the test eyes with the respective control eyes at 24 or 72 hours.

Skin Sensitization

None of the guinea pigs showed a response to the challenge application of HF-20. Examination of the guinea pigs 24 and 48 hours after application of the compound revealed no erythema or edema at any of the test sites.

Conclusions

Under the conditions of these tests, HF-20 was determined not to be a primary skin or eye irritant. This hydraulic fluid does not cause a sensitization reaction in guinea pigs. Under the condition of use in these tests HF-20 is an acceptable hydraulic fluid from an industrial hygiene standpoint.

SECTION III
FACILITIES

The support activities of the THRU essential to the operation of a research activity are usually not of sufficient magnitude to merit separate technical reports. Therefore, these activities are grouped together under the general heading "Facilities" to describe their contributions to the overall program of the laboratory.

MEASUREMENT OF ENVIRONMENTAL DISCHARGE OF PROPYLENE GLYCOL DINITRATE (PGDN) DURING OTTO FUEL II STUDIES

Air exhausted from the Thomas Dome chambers is intimately mixed with water in the vacuum pumps. Therefore it is possible for both effluent water and air to contain the contaminants generated into the chambers. In the case of Otto Fuel II, the active ingredient is PGDN, which appears to be the only compound vaporized into the exposure chambers during exposure. In the two exposure chambers, the concentrations of PGDN are 1.4 mg/m^3 and 240 mg/m^3, respectively. At flows of 40 cfm in the chambers, 273 mg/min PGDN is being introduced and presumably exhausted.

Valves had previously been placed in the pump exhaust water lines to permit sampling, and a stainless steel tube had been installed from the chamber air exhaust stack to a gas chromatograph in the Chemistry Laboratory so that exhaust air samples could be analyzed. For both air and water, a gas chromatographic analytical procedure was developed utilizing the following conditions:

Column	-	50 cm x 1/8 in. SS
Packing	-	5% OV - 101 on chromosorb G-HP
Carrier	-	Helium ~30 ml/min
Detector	-	Flame Ionization
Oven	-	Water - Isothermal 65°C
	-	Air - Isothermal 90°C
Sample Volume	-	Water - 1µl
	-	Air - 10 ml

The concentrations of PGDN in effluent pump water over the course of a day are listed in Table 54 and in effluent air in Table 55.

TABLE 54. CONCENTRATIONS OF PGDN IN EFFLUENT WATER FROM VACUUM PUMPS

Time	Pump 2 Concentrations, mg/L	Pump 3
0750	None Detected	None Detected
0855	8.4	8.4
1010	17.0	14.0
1130	17.5	15.0
1315	18.0	16.0

TABLE 55. CONCENTRATIONS OF PGDN IN EFFLUENT AIR FROM VACUUM PUMPS

Time	Conc. mg/m^3	Time	Conc. mg/m^3	Time	Conc. mg/m^3
0805	2.0	0915	17.9	1300	20.3
0812	2.0	0930	20.8	1345	17.9
0820	2.0	0936	21.9	1405	18.7
0830	1.6	0950	21.3	1425	18.9
0835	1.3	1035	20.7	1450	18.8
0845	3.2	1105	21.6	1515	10.4
0850	5.6	1135	19.5	1620	4.0
0900	12.3	1210	21.7		

Effluent water from both pumps contains approximately 16 mg/L or 60.6 mg/gal. For two pumps, the water flow rate is about 2 gal/min. Therefore, approximately 121 mg/min PGDN is being exhausted in the water. The average exhaust air concentration is 20 mg/m^3. Since each pump has a capacity of 200 cfm, the air flow in the effluent stacks approximates 400 cfm or 11.3 m^3/min, and 226 mg/min is being exhausted in the air.

For both air and water:

121 mg/min - water exhaust
226 mg/min - air exhaust
347 mg/min total exhaust

This compares with 273 mg/min being generated. This material balance is acceptable in view of the uncertainties in estimating air and water flow. The PGDN generated into the chambers has been satisfactorily accounted for in vacuum pump effluent air and water. The analyzed effluent rates can, therefore, be used for calculations in air and water diffusion models.

PHYSIOLOGIC FLUIDS - DETERMINATION OF METHYLCYCLOHEXANE AND JP-4 METABOLITES BY GAS CHROMATOGRAPHY

Methylcyclohexane (MCH)

Identification of the cis and trans isomers of 2, 3 and 4-methylcyclohexanol in the urine of rats exposed to methylcyclohexane was presented in the last annual report (MacEwen and Vernot, 1980). We also noted a large unidentified peak that was present in the gas chromatogram (GC) of urine of rats exposed to MCH. The parent peak in the mass spectrum of this compound was 114, the same mass number as that of methylcyclohexanol. The only isomer which had not been obtained and subjected to GC/MS analysis was 1-methylcyclohexanol. This compound was purchased and injected into the GC/MS. It proved to have the same retention time, 16.8 minutes, and mass spectrum as that of the unknown compound.

On the basis of GC/MS analysis, all positional and geometric isomers of methylcyclohexanol have been identified in the urine of rats exposed to 400 ppm methylcyclohexane. It is possible that cis- trans isomerization took place in the urine or during analysis. We have not investigated the effects of standing or of GC injection on geometric isomerism of methylcyclohexanols. No evidence of the presence of any methylcyclohexanone in the urine of exposed rats was obtained indicating that further oxidative metabolism of methylcyclohexanols did not occur. Martis et al. (1980) examined the pharmacokinetics of cyclohexanone and showed that less than 1% of an IV dose was excreted in urine as the parent compound while at least 60% was excreted as the glucuronide of cyclohexanol. This indicates that cyclic alcohols are metabolically favored for excretion over cyclic ketones and might explain the lack of methylcyclohexanones in the urine of rats exposed to methylcyclohexane.

JP-4

As in the case of MCH, urine was sampled from rats being exposed to 5000 ppm JP-4 on an industrial schedule by overnight collection. Gas chromatographic conditions were as follows:

Column:	50 m x 0.28 mm WCOT, coated with Carbowax 20M
Carrier:	He; 1.0 ml/min
Injector Temperature:	210°C

Urine samples from control rats and those exposed to 5000 ppm JP-4 were injected into the GC/MS. The total ion chromatograms of these samples are compared in Figure 28 along with identifications made using the disk library of compounds available in the GC/MS computer files. The chromatogram of the control sample consisted primarily of peaks due to odd-carbon 2- and 4- substituted ketones. These peaks appeared in the urine from exposed animals which also

contained at least 10 new peaks. Using the GC/MS library, 3 of these new peaks were identified as benzene, 2,5-dimethylfuran and 2-hexanone. 2,5-Dimethylfuran has been identified by DiVincenzo et al. (1977) as a urinary metabolite of 2-hexanone and 2-hexanone has been shown to be a metabolite of n-hexane in rats. Tentatively, therefore, both 2-hexanone and 2,5-dimethylfuran may be considered to be urinary metabolites of the inhaled n-hexane fraction of JP-4 vapor. Benzene is present in liquid JP-4 at a concentration of about 0.4% and in the chamber at about 0.8% of the fuel vapor equivalent to 38 mg/m^3. Benzene has some slight solubility in water, and it might be expected that excretion of the unmetabolized compound through glomerular filtration would be a factor in its elimination.

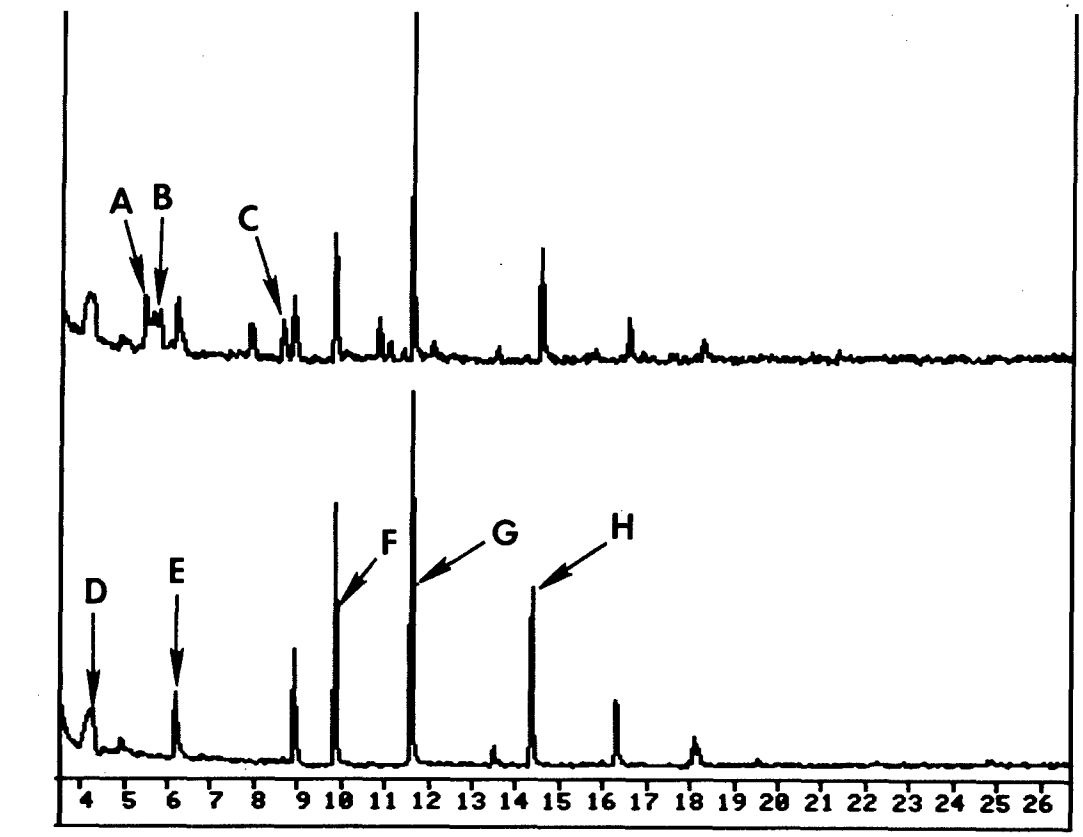
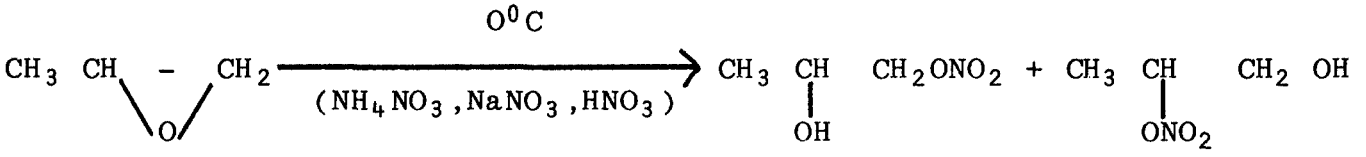


Figure 28. Total ion gas chromatograms of urine sampled from rats exposed to 5000 mg/m^3 of JP-4 intermittently and controls.

Development of procedures for analysis of urine metabolites is continuing. Concentration of the excreted materials by extraction in ether and evaporation is being investigated with the view of increasing the sensitivity of the GC/MS procedure. We are also examining the hydrolysis of glucuronides and sulfates to optimize our detection of hydroxylated metabolites. Finally, we intend to examine the formation of trimethylsilyl derivatives of metabolites containing active hydrogen, e.g. alcohols, acids and amines.

SYNTHESIS OF PGDN METABOLITES

In a supporting effort to the investigation of the toxicokinetics of PGDN, a synthesis of the putative metabolites, 1-nitrato-2-propanol and 2-nitrato-1-propanol, was attempted. A method was outlined in a German patent[1] for synthesis of ethylene glycol mononitrate. Theoretically the isomers of propylene glycol mononitrate would be formed according to the following scheme:



The reactants were mixed for the time specified in the patent and then distilled in an effort to purify the mononitrates. A chloroform extract of the distillate was injected into the GC/MS using an OV-101 capillary column to give the chromatogram shown in Figure 29. The assignments shown for the peaks were made using the GC/MS library and indicate considerable reaction between propylene oxide and the solvent.

The spectra of the two peaks marked A and B are shown in Figure 30. They could not be matched to any library mass spectrum. The spectra were analyzed and assignments made for the significant ions as shown in Figure 30. From this analysis, an assignment of 1-nitrato-2-propanol was made for A and 2-nitrato-1-propanol for B.

Consideration of the concentrations of the mononitrates and that of the solvent chloroform suggests that distillation of the reaction mixture may have decreased rather than increased the concentration of the desired products. In addition, the identification by GC/MS of 1,1-dichloro-2-propanone, 1,3-dichloro-2-propanone and 1-chloro-2-propanone indicated that distilling the solution may have caused the solvent to react either with propylene oxide or with the expected reaction products, 1- and 2-propylene glycol mononitrate.

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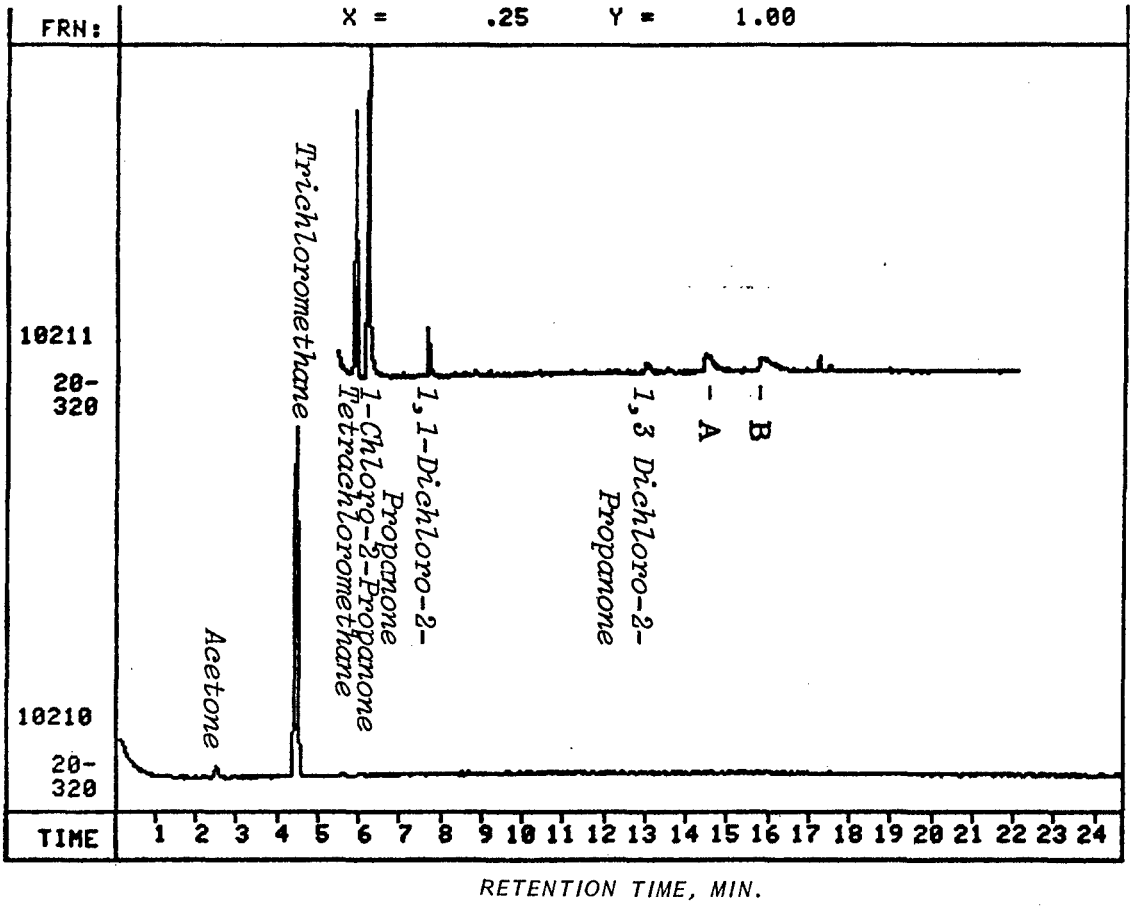
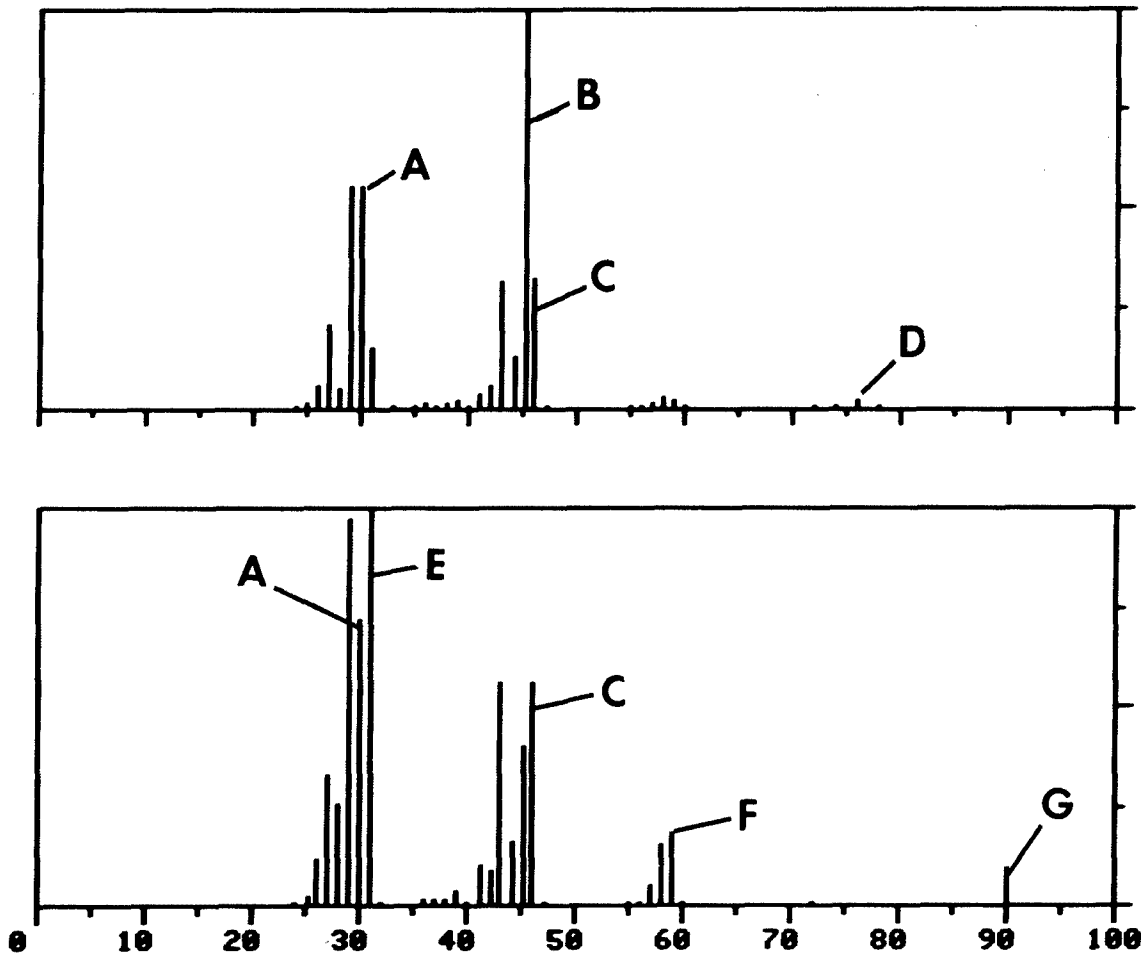
(1) Ger. Offen. 2, 258, 771.



[a]*Lower curve is an attenuated scan of the upper to show solvent peaks.*

Figure 29. Total ionization chromatogram of distilled propylene oxide reaction extract[a].

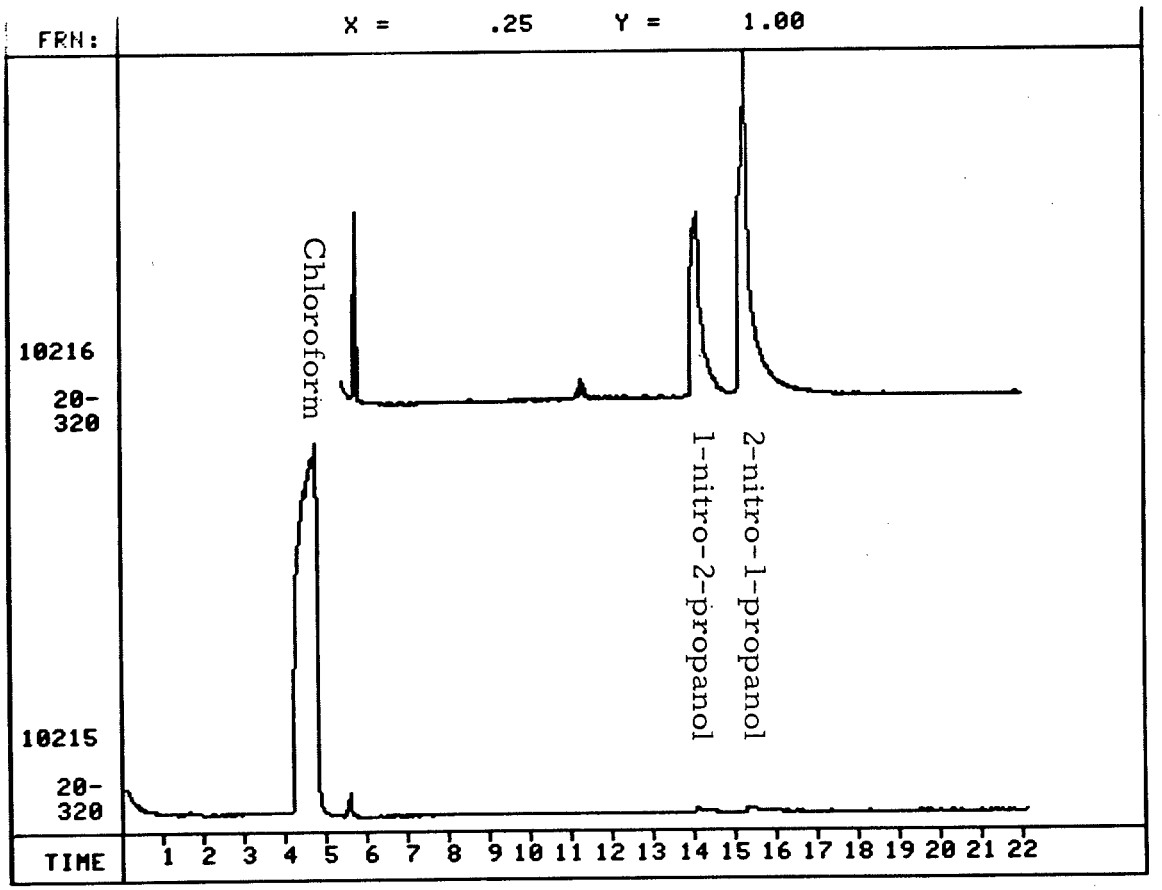


FRAGMENT ION ASSIGNMENT

A	–	NO^+	E	–	CH_2OH^+
B	–	CH_3CHOH^+	F	–	$CH_3CHCH_2OH^+$
C	–	NO_2^+	G	–	$CH_3CHONO_2^+$
D	–	$CH_2ONO_2^+$			

Figure 30. Mass spectra of peaks A and B in Figure 29.

Therefore, the synthesis was repeated using the same conditions as previously with the exception that the chloroform extract was not distilled. When the extract was injected directly into the GC/MS, the total ionization chromatogram shown in Figure 31 resulted.



[a]Lower curve is an attenuated scan of upper to show solvent peaks.

Figure 31. Total ionization chromatogram of undistilled propylene oxide reaction extract[a].

In this chromatogram, the concentrations of the two mononitrates have increased significantly over those in the distilled product. Moreover, there are no peaks due to chlorinated acetones or to acetone itself which was present in the chromatogram of the distillate. Therefore, the oxidation and chlorine substitution which occurred on distillation was avoided. The mass spectra obtained for the mononitrates were essentially identical to those obtained previously.

CHAMBER EXHAUST AIR SCRUBBERS

The effluent air scrubbers for our Rochester and Longley exposure chambers have been in use for almost 18 years. During this time, the ceramic packing elements have deteriorated through abrasion and deposition of scale. This has caused large pressure drops across the scrubbers which limit the flowrate of air in the ambient chambers. Recent legislation on air and water effluents requires that efficient systems be installed to remove toxic materials from air and water effluents. Additionally, technologic advances in design of scrubber packing elements have made it possible to design scrubbers with much lower resistance and pressure drops than formerly. A prototype scrubber was designed and constructed which included the following concepts and components:

1. Use of plastic spherical packing elements with large mesh perforations.

2. Inclusion of a recirculating mode of operation with a corrosion resistant pump for chemical neutralization of materials too toxic to release into effluent water.

3. Provision for removing samples from the system in the recirculating mode. This would permit periodic sampling of the solution to determine when the neutralizing agent is nearing depletion and requires recharging.

4. Building of an inspection window into the scrubber tower to detect scale buildup on the packing elements so they may be replaced before significant deterioration of the scrubber operation occurs.

The scrubber was tested in the recirculating mode during exposures to 750 ppm hydrazine in the ambient chambers. A gallon of commercial sodium hypochlorite solution was added to the scrubber water. Analysis of effluent scrubber air and water showed that hydrazine could not be detected until 2 1/2 hours of operation when it was obvious that all hypochlorite had been reacted. Addition of another gallon of hypochlorite brought hydrazine concentration in air and water below detectable levels.

In both the recirculating and the non-recirculating modes with maximum water flows, the air flows were found to be the same as through the empty tower. Because all of the operating specifications for the new towers were attained by the prototype, plans are being made to replace the three remaining old towers with ones of the new design which is illustrated in Figure 32.

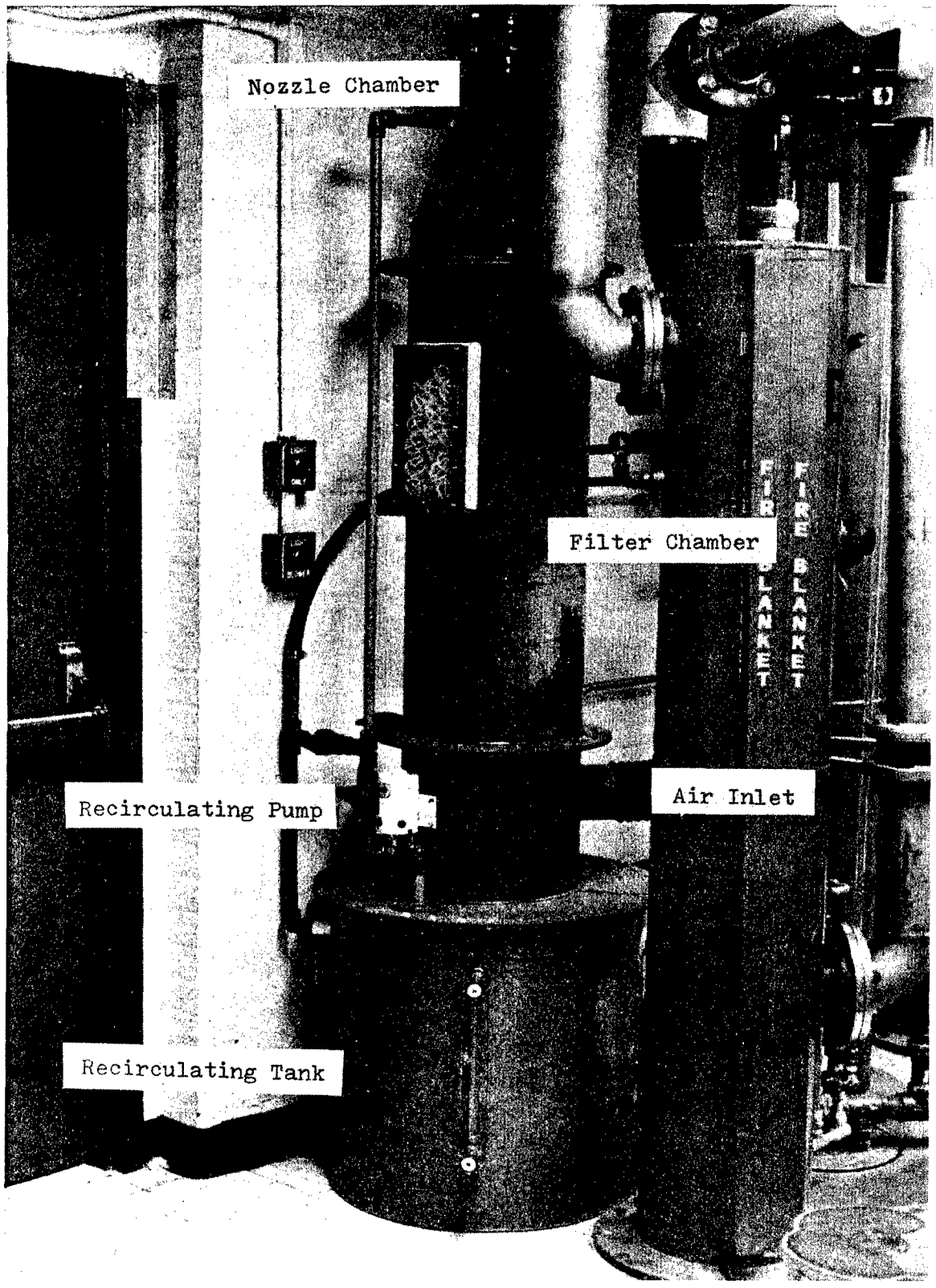


Figure 32. Chamber effluent air scrubber.

BREATHING HOODS AND COMMUNICATION SYSTEMS

From the inception of the Toxic Hazards Research Unit, chamber technicians entering the Thomas Dome Inhalation Exposure Chambers for animal maintenance and cleaning had worn U. S. Air Force aviator breathing masks and helmets with built-in earphones and speakers. Fitting the masks so they were leak-free inwardly was a continuing problem, and movement while wearing the equipment was restricted because it was designed for use while seated in an aircraft. Recently, lightweight breathing equipment became commercially available which consists of a plastic helmet from which a light hood is suspended extending to the shoulders. It has a clear plastic face for easy viewing (Figure 33). Air passes through the hood at the rate of 10 cfm insuring that air containing the exposure agent cannot leak in. One available hood was tested and we found that it gave chamber entrants better protection, better vision, more freedom of movement and greater comfort than the face masks.

Figure 33. Breathing hood used during large chamber entry.

The use of the air supply hood system required the use of different types of headsets and several were evaluated. The Plantronics Starset communication system was selected because it had the lowest signal to noise ratio, was lightest in weight, and was most suitable for interface with existing amplifiers in the Dome Communication System and required only volume adjustments on the voice amplifiers. Figure 34 is an illustration of the communications system worn beneath the hood. Modification to the existing breathing air system consisted of installing triplicate air outlets on a single manifold both inside the Thomas Domes and in the entry airlocks. Installation of a filter system with a high air flow capacity was also required. Special connectors were used for this system to avoid accidental hook up of the breathing hood to high pressure air, negating the chance of personal injury or damage to the air regulating valve.

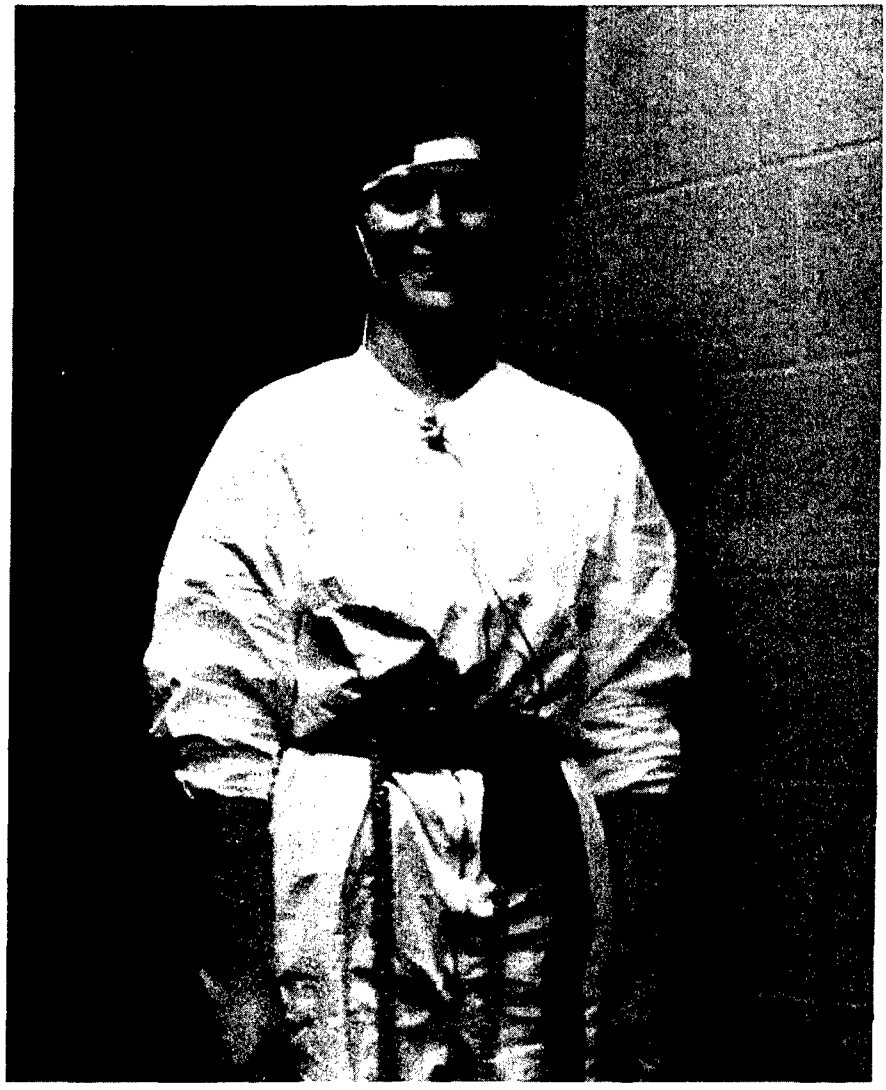


Figure 34. Technician wearing large chamber communications equipment.

The breathing hood and communication modifications have been installed and have been operational since July 1980 and there have not been any electronic or mechanical failures. Routine replacement of normal batteries for the amplifiers and shrouds for the breathing hoods have been made. The system is both reliable and durable.

ELECTRONIC CONTROL AND DATA ACQUISITION SYSTEMS FOR INHALATION EXPOSURE

The control system originally designed for the Thomas Domes utilized pneumatic signals to operate flow recorder-controllers, pressure recorder-controllers and chamber isolation valves. After 16 years of continuous use, it became increasingly difficult to maintain the pneumatic system because replacement parts were being phased out of production and inventory by the manufacturers. Additionally, electronic advances in the years since installation of the facility made systems available which had greatly superior noise, sensitivity and response characteristics and also had capabilities for data recording and integration. Experience with the original system had also revealed certain weaknesses in control which were changed in the new design. One of these areas of concern was chamber pressure control which was attained by maintaining absolute chamber pressure at 720 mm Hg. This is 20 mm below the average atmospheric pressure for our laboratory and was selected to insure that any leaks occurring in the chambers would be inboard so that no contaminant being tested in the chambers would escape into the laboratory. The drawback to this approach was that low atmospheric pressure systems passing through the area would occasionally drop external pressure below that in the chambers and external leaks could develop. The problem was solved in the new system by using differential rather than absolute pressure as the controlling variable. The chambers are now maintained at a constant 20 mm below atmospheric pressure so that low pressure systems no longer affect operation.

Another data element for which measurement and readout parameters were altered was relative humidity (RH). We had found the most satisfactory method for RH determination was measurement of wet and dry bulb temperatures. In the old system, calculation of RH required use of a nomograph by chamber technicians after obtaining wet and dry bulb values from the console. This was unsatisfactory because the probability of human error in reading the nomograph was significant. In order to overcome this in the present system, the datalogger was programmed to compute RH directly from electronic signals for wet and dry bulb temperatures that it received. Relative humidity is now recorded directly along with temperature, flow and differential pressure.

The data acquisition portion of the Exposure Chamber Control System was designed to monitor and store the following parameters:

1. Chamber Flow (0-100 cfm)
2. Chamber Differential Pressure - (0-50 mmHg)
3. Chamber Dry Bulb Temperature - (32°-212°F)
4. Chamber Wet Bulb Temperature - (32°-212°F)
5. Chamber Relative Humidity - (0-100% RH)

The system consists of a United Systems Corporation Model 3000 datalogger with associated input devices and an Intelligent Systems Corporation Graphics Terminal with disc-storage system. The datalogger monitors chamber parameters, provides signal conditioning where required, transforms the input analog voltage signals to digital information and then transmits the digital information to the chamber operator's graphic console.

The datalogger is the central control point for all exposure chamber control system-generated signals. The first phase of the installation, which is nearly complete, provides data acquisition for chambers one through four. However, the internal programming of the datalogger allows for signals from all eight chambers plus 20 additional signals. Additional output functions of the datalogger will provide high and low alarms for each point, local video display, local print capability, signal transmission to the operator console for each point and remote video display of each point. The data unit has a maximum capacity of 250 channels for which the current functional assignment of these channels by the datalogger internal program is; Channels 0 to 99 as external signal input channels, Channels 100 to 199 as external computational channels, and Channels 200 to 249 as internal computational channels. Channel allocations are shown in the following table:

FUNCTION	SCANNER CHANNEL	INPUT CHANNELS	DISPLAY CHANNELS	ALARM CHANNELS
Future	240	000-009	100-104	105-109
Chamber 1	241	010-019	110-114	115-119
Chamber 2	242	020-029	120-124	125-129
Chamber 3	243	030-039	130-134	135-139
Chamber 4	244	040-049	140-144	145-149
Chamber 5	245	050-059	150-154	155-159
Chamber 6	246	060-069	160-164	165-169
Chamber 7	247	070-079	170-174	175-179
Chamber 8	248	080-089	180-184	185-189
Future	249	090-099	190-194	195-199
Internal Computational				200-239

Each functional block of channels is assigned to inhalation exposure chamber parameters as exemplified by the Chamber 1 assignments shown below:

Dome 1

Channel	Function	Range
010	Flow	0-100 cfm
011	Pressure	0-50 mm Hg diff.
012	Dry Bulb Temp.	32°-212°F
013	Wet Bulb Temp.	32°-212°F
014	% Relative Humidity	0-100% RH
015	Spares	
016	Spares	
017	Spares	
018	Spares	
019	Spares	
110	Flow Display	0-100 cfm
111	Pressure Display	0-50 mm Hg diff.
112	Dry Bulb Temp. Disp.	0-100°F
113	Wet Bulb Temp. Disp.	0-100°F
114	% RH Display	0-100% RH
115	Flow Alarm Output (Hi & Low)	adj.
116	Pressure Alarm Output (Hi & Low)	adj.
117	Dry Bulb Temp. Alarm output (Hi & Low)	adj.
118	Wet Bulb Temp. Alarm output (Hi & Low)	adj.
119	% RH Alarm output (Hi & Low)	adj.

Datalogger capabilities are extensive enough to allow for programming of individual channels for future needs which may arise. Typical items available for assignment to each channel are: 10mV to 10VDC input range, variable dwell time with 100 μ sec minimum, 7 character labels, 25 character messages, conversion equations, output alarms and hard-copy printouts. These capabilities may be programmed individually for any channel.

The datalogger has been programmed to scan each set of functional channel blocks in succession once every 20 seconds, average each set of channel readings over a time period of one hour and output these averages to the Chamber Operator's Console for printout and storage. When control limits are exceeded it actuates an alarm and relevant data are transmitted to the operator's console. The capability of reprogramming individual channels allows for efficient reconfiguration of datalogger functions as a result of changes in experimental requirements.

The data acquisition system was designed to provide the following functions at the chamber operator's console:

a. Operator entry of messages;
b. Display and storage of alarm parameters;
c. Display and storage of hourly means;
d. Printout of daily summaries.

Accomplishment of these functions required a microprocessor link to the datalogger and operator console. The microprocessor collects, stores and performs calculations on data received from the datalogger and transmits them to the operator console in the appropriate form.

The link between the datalogger and the microprocessor is a standard bidirectional serial data link. Handshaking is utilized which lets the microprocessor shut off the data stream from the datalogger while data is being written to the micro's floppy disk. By running a terminal emulator program at the microprocessor, the datalogger can be programmed remotely.

The software is divided into two major programs. One is the "data acquisition" program and the other the "report generator". Both programs were written in BASIC and have been compiled. Compilation was necessary in order to gain required speed.

The data acquisition program serves several functions. First, it writes the data coming from the datalogger to a disk file. Second, it writes any comments or observations typed at the console keyboard to a separate disk file. Once an hour, the datalogger transmits the time to the microprocessor which stores it in the comments file on the disk so that any information typed on the console by the chamber operator will be associated with a particular time.

One of the features of this program is that it has provisions for power failure recovery with minimal loss of data. This is accomplished by taking advantage of Microsoft BASIC's random access record scheme. Each data record is actually assigned its own random record. After each record is written to disk, the record number of this last record is stored and updated in a special place at the beginning of the file, and the disk file directory is also updated. If power fails, then the program will have to be manually restarted. This "last record number" will be read from the disk and new data will be appended to the file with little or no loss of data. This procedure is impossible with ordinary sequential files. Every hour, averages of flow, differential pressure, dry bulb temperature and RH are printed at the operator console. Current instantaneous conditions will be printed in case of an alarm and may be printed on operator demand. Every day at 1600 hours a report will be generated and printed of all the hourly means obtained during the previous 24 hours.

The report generator first reads the data at the beginning of the data file finding the record number of the last data collected just prior to the generation of the previous report. This is needed because data from previous days are still in the data file, and we do not wish to receive another report on that information.

Data are then scanned sequentially for the special characters provided by the datalogger. The program determines which exposure chambers are in use and to be reported on. An array is then constructed containing the records for each chamber for each hour. Another array contains the records for the alarms for each active chamber. After completion of data scanning and construction of the record number arrays, the actual printing of the report is begun. For each chamber, first the operating parameters and then the alarm messages are printed on an hour-by-hour basis. After the data for each chamber have been printed, the "operator comments and observations" file is printed without modification.

Finally, the record number of the last data reported is filed so that only new data will be used in the next day's report. The "data acquisition" program is called, and data collection resumes. Eventually, the disk will be full of data. For this reason, a special program must be periodically run in order to clear old data from the disk and to re-initiate the data collection file.

Regulation of flow and pressure is attained through the same controllers which transmit the signals to the datalogger (Figures 34 and 35). Since the system control valves operate using a pneumatic signal, the 4-20 ma current from the controller is transformed into a 3-15 psig signal using an electronic to pneumatic transmitter. The outputs of the resistance thermometers measuring wet and dry bulb temperatures are directed to the datalogger for disk entry and calculation of RH. Figures 35, 36, 37 and 38 are schematics of the dome control and temperature and RH indicating systems and the isolation and containment vent systems. Large digital displays of the flow rates in the domes are presented over the control panels. On the panels themselves, digital meters display wet and dry bulb temperatures and vertical scale meters indicate differential pressures.

Control System alarms were installed to provide for audible and visual alarms in case of process excursions outside of preset limits. These signals are activated by either the datalogger system or by pressure switches installed at the output of the process electrical transducers. The most critical operating parameters are flow and pressure and these parameter alarms are activated by the pressure switches.

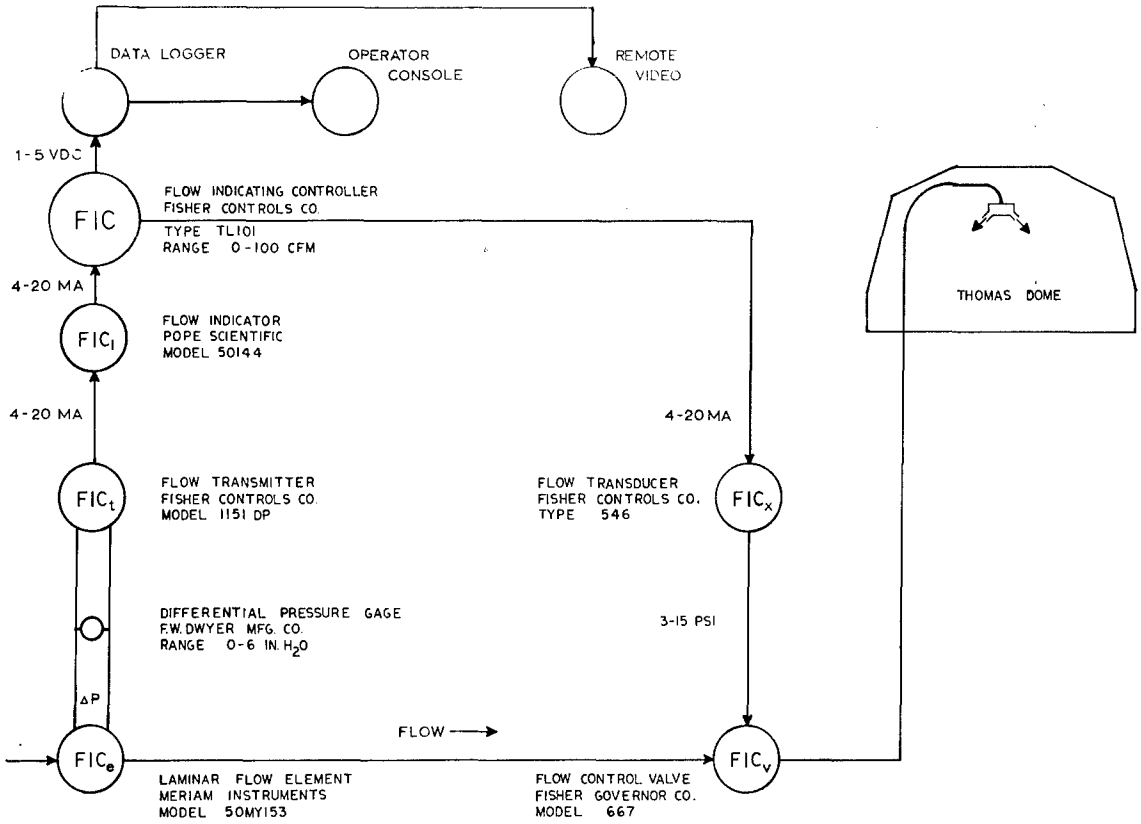


Figure 35. Large chamber flow control system.

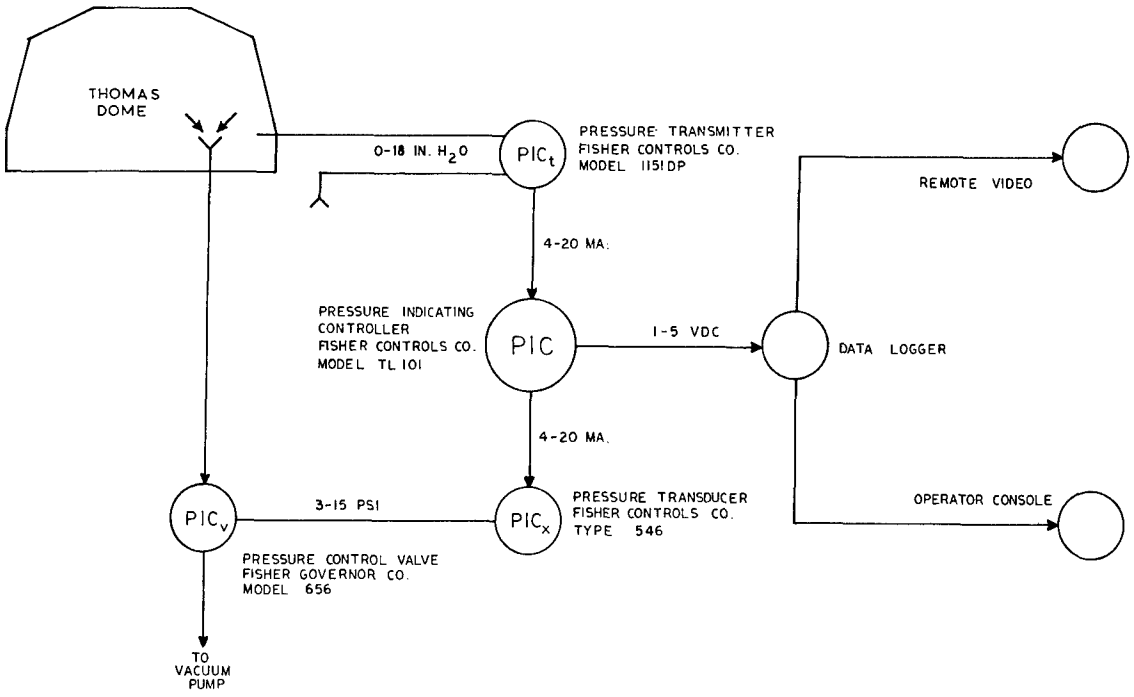


Figure 36. Large chamber pressure control system.

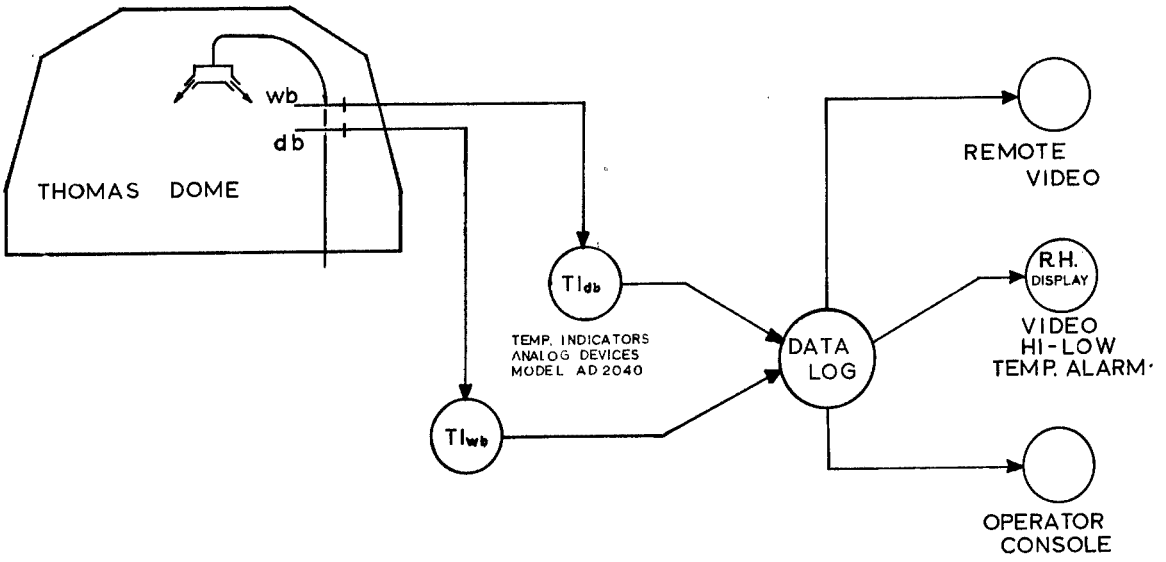


Figure 37. Large chamber temperature and RH indicating systems.

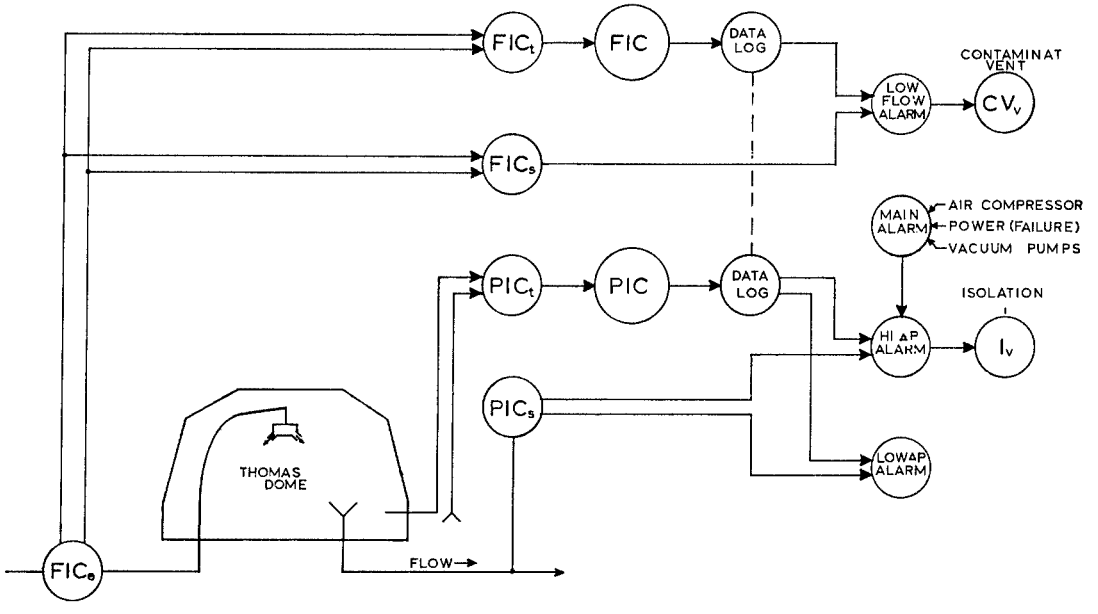


Figure 38. Large chamber isolation and contaminant vent system.

Alarm limits are set as follows:

Parameter	Range	Pressure Switch	Datalogger
Flow	0-100 cfm	15 cfm	25 cfm Low 35 cfm High
Pressure	0-50 mm Hg Neg	-10 mm Hg Neg -80 mm Hg Neg	-15 mm Hg Neg Low -50 mm Hg Neg High
Dry Bulb Temp.			66F Low 78F High
Wet Bulb Temp.			66F Low 78F High
% Relative Humidity			40% RH Low 60% RH High

Pressure switch alarms are hard-wired into the control systems and are set outside of the limits of the datalogger alarms. This performs a dual function of providing a redundant alarm capability for the critical chamber parameters of flow and pressure and additionally provides alarm protection for the system in case of datalogger malfunction. The output of the controls alarm system is connected to an existing alarm system which provides audible and visual alarms throughout the laboratory areas.

CHAMBER TECHNICIAN TRAINING PROGRAM

Since the last annual report, three technicians have been hired to replace personnel leaving the THRU. One of the newly hired technicians was certified by AALAS as an Assistant Laboratory Technician and another had vocational school training in animal care. All new technicians have been given the training program covering operations of the large exposure chambers. During this year, three more chamber technicians received AALAS certification at each level of competence. The number of chamber technicians AALAS certified at each level is:

1 Laboratory Animal Technologist
5 Laboratory Animal Technicians
2 Assistant Laboratory Animal Technicians

The Thomas Dome Standard Operating Procedures training program for new technicians and the routine monthly emergency training procedures have been revised since the last annual report. The program for new technicians is as follows:

I. Orientation
   A. Laboratory Mission
   B. Job Responsibilities
      1. Introduction to SOP's
      2. Introduction to Lab Operations
   C. Personnel Responsibilities

II. Standard Operation of Chambers
   A. Observer A Normal Routine
      1. Dome Start-Up
      2. Establish Flow
      3. Normal Readings
      4. Dome Entry Operation
   B. Observer B Checklist
   C. Dome Entrant Duties
      1. Dome Entry Operation
      2. Dome Cleaning and Cage Changes
   D. Dome Cap Raising and Lowering

III. Mechanical Equipment
   A. Vacuum Pump Failure
      1. Facility A Pump
      2. Facility B Pump
      3. Observer Duties
   B. Air Compressor Failure
      1. Main Air Compressor
      2. Back-up Air Compressors
      3. Air Dryers
      4. Observer Duties
   C. Complete Power Failure
      1. Facility A Procedures
      2. Facility B Procedures
      3. Observer Duties
   D. Air Supply Fan Failure
      1. Main Supply Fan
      2. Back-up Supply Fan
      3. Observer Duties
   E. Waste Catch Tank Draining
      1. Transfer Dome to Tank
      2. Emptying of Tank

IV. Emergencies
   A. Fire in Dome During Entry
      1. Observer A Duties and Options
      2. Observer B Duties and Options
      3. Dome Entrant Duties
   B. Fire in Dome - No Entrant
      1. Observer A Duties and Options
      2. Observer B Duties and Options
   C. Fire in Airlock During Entry
      1. Observer A Duties and Options
      2. Observer B Duties and Options
      3. Dome Entrant Duties

D. Fire in Exposure Laboratory
   1. Observer A Duties and Options
   2. Observer B Duties and Options
E. Rescue of Incapacitated Dome Entrant
   1. Rescue Criteria
   2. Observer A Duties
   3. Observer B Duties
F. Operation of Scott Air Pak (SCBA)
   1. Criteria for Use
   2. Procedures

The audiotutorial course on laboratory animal medicine (MacEwen and Vernot, 1980) was also utilized in the chamber technician training this year.

The monthly emergency training procedures program has also been revised. Training in the procedures is accomplished by the senior technicians on each shift. Periodic written examinations are given by the principal technician to all chamber technicians. Revisions of any procedure and/or retraining is made by the principal technician as the need arises. Listed below is the schedule for the training procedures and examinations given during the past year. Documentation of all practical, oral, and written examinations is also maintained.

Date		Procedure
		1980
May	-	Vacuum Pump Failure
*June	-	Rescue of Incapacitated Dome Entrant
July	-	Supply Air Fan Failure
*August	-	Fire in Exposure Laboratory During Entry
September	-	Fire in Dome During Entry
*October	-	Complete Power Failure
November	-	Air Compressor Failure
*December	-	Vacuum Pump Failure
		1981
January	-	Fire in Airlock During Entry
*February	-	Operation of Scott Air Pak (SCBA)
March	-	Fire in Dome During Entry
*April	-	Air Compressor Failure

*Written examinations given.

ANIMAL TECHNICIAN TRAINING PROGRAM

Since last year's annual report, four technicians have become certified in the AALAS program. Two have been certified at the highest level, Laboratory Animal Technologist, and two were certified at the second level, Laboratory Animal Technician. All UCI animal technicians are certified by the AALAS board. The present status of the group is as follows:

5 Laboratory Animal Technologists
4 Laboratory Animal Technicians
2 Assistant Animal Technicians

The basic course outline of certification by AALAS was described in detail in a previous report (MacEwen and Vernot, 1975). All references listed by AALAS utilization in preparing for examinations are now available through the UCI and Air Force libraries.

The Biotech small animal series has been purchased from the American Institute of Biological Sciences by the Air Force Veterinary Medicine Division and is available to UCI animal personnel. The following titles are covered in the Biotech training program:

Handling, Restraint, and Gavage of the Mouse
Handling, Restraint, and Gavage of the Rabbit and Guinea Pig
Handling, Restraint, and Gavage of the Hamster and Gerbil
Housing of Laboratory Animals
Sanitation of Housing for Laboratory Animals

Additional training programs are available for training new animal care personnel and as refresher courses for experienced technicians. The following is a list of programs being utilized:

Purina Animal Care
Animal Care Training Videotapes
Advanced Practical Training
Laboratory Animal Medicine and Science Audiotutorial Series

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☆U.S.Government Printing Office: 1981 — 559-007/6014