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TOXIC HAZARDS RESEARCH UNIT ANNUAL TECHNICAL REPORT: 1985

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TECHNICAL REVIEW AND APPROVAL

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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

BRUCE O. STUART, PhD
Director Toxic Hazards Division
Air Force Aerospace Medical Research Laboratory

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The research program of the Toxic Hazards Research Unit (THRU) for the period of June 1984 through May 1985 is reviewed in this report. Chronic toxicity and oncogenic studies were carried out with hydrazine, JP-4, and JP-8. Results of histopathologic examination became available for a number of studies including chronic inhalation exposures to monomethylhydrazine, methylcyclohexane, and Otto Fuel II; and subchronic to petroleum and oil shale diesel fuel marine. These studies are now complete. Other investigations are complete except for histopathologic results. These include chronic exposures to petroleum JP-4, RJ-5, JP-7, JP-TS, and JP-10; subchronic exposures to petroleum JP-4 and JP-8; and weekly exposures to hydrazine. Three studies have concluded the exposure phases and are now being held postexposure - 90 day continuous exposures to shale JP-4 and dimethyl methylphosphonate and rat strain susceptibility to shale JP-4. A series of short-term cont'd

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Oral
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Dermal
Shale Oil Fuels
Petroleum Fuels
Neurotoxicity
Intraperitoneal
Antimony Thioantimonate
O-ethyl-O'-(2-diisopropylaminoethyl)methylphosphonite
Dimethyl Methylphosphonate
Methylcyclohexane
Alveolar Clearance
Metabolites
Thionyl Chloride
Chlorotrifluoroethylene
Cyclotriphosphazene
JP-7
JP-10
RJ-5
Monomethylhydrazine
Otto Fuel II
Diesel Fuel Marine

19. Abstract cont'd

toxicity studies was conducted on a variety of chemicals and chemical agents used by the Army, Air Force, and Navy.

PREFACE

This is the 22nd 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 fifth report under the current contract and describes the accomplishments of the THRU from June 1984 through June 1985.

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 Aerospace Chemicals and Materials," Work Unit Number 63020115. M. K. Pinkerton served as the technical contract monitor 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 Captain David E. Uddin, MSC, USN, and identified as Navy Task Area Number MF58524001 "Chemical Hazards/Exposure Limits."

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, Department of Community and Environmental Medicine. Acknowledgement is made to C. L. Gaworski, C. C. Haun, J. R. Horton, C. E. Johnson, E. R. Kinkead, P. E. Newton, Ph.D., A. K. Roychowdhury, Ph.D., R. K. Blasingame, and J. L. Monroe 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, Toxicology Detachment, Wright-Patterson Air Force Base, Ohio, and the United States Army.

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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 research program was conducted on gas simulants, binary compounds, and a variety of chemical mixtures used as hydraulic fluids. 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. Pathology reports were received for several outstanding studies and final reports have been prepared and are either published as AFAMRL Technical Reports or are in process.

This document constitutes the 22nd 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 (1965).

During the first 6 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, the current research programs serve the mutual interests of the U. S. Air Force, Navy, Army, 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 Bruce O. Stuart, Ph.D., Director of the AFAMRL Toxic Hazards Division. Twenty-two technical platform papers were presented. This year's conference did not have a specific theme and consisted of five interesting and topical sessions as listed below:

I Factors Influencing Lung Toxicity of Atmospheres
II Issues Raised by The National Toxicology Program Ad Hoc Panel on Chemical Carcinogenesis Testing and Evaluation
III In Vitro Approaches to In Vivo Toxicity: General Principles
IV In Vitro Approaches to In Vivo Toxicity: Liver and Lung
V The Skin as a Route of Entry

The open forum discussions following each session resulted in significant contributions of additional technical information and scientific exchange. The conference, held 30 October through 1 November 1984, drew 187 participants including speakers.

The welcoming remarks were presented by Col. Warren L. Carpenter, Vice Commander, USAF Aerospace Medical Division.

The conference program was submitted to the American Board of Industrial Hygiene and to the University of California, Irvine Continuing Education organization for evaluation. The ABIH awarded 2-1/2 points for recertification and 16 C.E.U.'s were awarded to attending physicians.

The papers presented at the conference were prepared for publication as the Proceedings of the 15th Conference on Environmental Toxicology which is a separate technical report (AFAMRL-TR-84-002).

SECTION II

RESEARCH PROGRAM

Toxicology research conducted by the THRU during the past year was primarily concerned with continuing studies of toxic and

tumorigenic effects of inhaled aircraft and rocket fuels. Inhalation studies on a binary compound, EDMP, were also conducted. Histologic examinations of animal tissues from several studies on the chronic effects of inhaled aircraft fuels were completed and are described in this report. Subchronic inhalation toxicity studies of dimethyl methylphosphonate, a simulant test gas, were initiated in the previous report year and the animals were tested and observed during the current year. Studies on the metabolism of this compound are continuing.

Other research activities of the THRU during the past year included a series of acute toxicity tests on O-ethyl-O'-(2-diisopropylaminoethyl)methylphosphonite (EDMP) and a series of by-product chemicals produced in the manufacture of EDMP. Hydraulic fluids of various chemical composition were studied for acute toxic effects.

The current status of these ongoing studies is detailed in this report.

THE EVALUATION OF THE ONCOGENIC POTENTIAL OF INHALED HYDRAZINE IN RATS AND HAMSTERS AFTER A SERIES OF WEEKLY 1-HOUR EXPOSURES

Hydrazine is a strong reducing agent with numerous applications in the military and in industry. One of the important military uses of hydrazine is as a fuel in the standby power systems of aircraft. During the maintenance of these systems workers could be subjected to occasional brief exposures of high concentrations of hydrazine vapors. The purpose of this study was to assess the oncogenic risk of several short, high concentration exposures to hydrazine, utilizing the same total dose of hydrazine (concentration x time) that induced pulmonary tumors and nasal polyps in rats and hamsters in previous chronic inhalation studies (MacEwen et al., 1981; Vernot et al., 1985). Description of the methods and partial results from this study have been presented in previous THRU Annual Reports (MacEwen and Vernot, 1982, 1983, and 1984).

This study was divided into 3 phases where phases I and II were range-finding studies for the 3rd phase. In phase III, groups of male hamsters and male and female rats were exposed weekly for 1 hour periods to hydrazine concentrations of either 750 ppm (maximum non-lethal level) or 75 ppm for a 10-week period. These exposures resulted in total CT (concentration x time) values of 7500 and 750 ppm hours.

Male and female rats in both exposure groups demonstrated reduced body weight gains during the exposure period. However, postexposure body weight gains were not significantly different ($p < 0.05$) than control group weight gains for either male or female rats at either exposure level.

Hamsters in the higher level exposure group demonstrated reduced weight gain during the exposure period when compared to the control hamsters. During the remainder of the test, however, both treatment groups exhibited higher mean body weights than did the control group.

Hamster mortality reached a point that necessitated their final sacrifice after 22 months postexposure (approximately 30 months of age). The male rat final sacrifice was conducted at 28 months postexposure and the female rat final sacrifice was conducted as originally scheduled at 30 months postexposure.

Results of the histologic examination of animals in this study are not yet available. They will be presented in a future annual report.

A 2-YEAR STUDY ON THE CARCINOGENICITY OF HYDRAZINE ADMINISTERED IN DRINKING WATER TO MALE GOLDEN SYRIAN HAMSTERS

Groups of male Golden Syrian hamsters are being exposed to aqueous solutions of hydrazine sulfate as their sole source of drinking water. The hydrazine sulfate concentrations are 170, 340, and 510 mg/L. A negative control group is also being maintained on distilled water. A positive control group, exposed to 10 mg/L dimethyl nitrosoamine, was also initiated in October, 1983 but discontinued after approximately 7 months due to high mortality. Each exposure group initially consisted of 40 male hamsters. The experimental protocol and summaries of body weight, mortality and water consumption data were presented in a previous annual report (MacEwen and Vernot, 1984). This study is being conducted in collaboration with Dr. Ronald Shank at the Irvine Campus who is analyzing tissues for DNA constituents and examining the tissues.

Background

Hydrazine is a strong reducing agent that is widely used for a number of diverse applications by the military and in industry. Under appropriate conditions, hydrazine has been shown to cause an increased tumor incidence in rats and mice.

Hydrazine administration has also been demonstrated to induce the formation of methylated guanines in the livers of test animals. These methylated guanines, thought to be relevant to carcinogenesis, have been shown to persist in hamster liver DNA much longer than in rat liver. However, in previous studies, hydrazine exposure failed to induce liver cancer in hamsters (Toth, 1972 and MacEwen et al., 1981). Therefore this study was developed to expose hamsters to high concentrations of hydrazine over prolonged periods of time in order to study the relationship between O^6-methyl guanine in DNA and chemical carcinogenesis.

Results

Water consumption was measured for 4-week intervals at the study initiation, and after 5, 9, and 16 months of exposure. These water consumption data are summarized in Table 1. A repeated measured analysis of variance was conducted, the results of which indicated that the effect of dose was parallel with respect to time ($P = 0.5109$) and that the dose effect on water consumption was not significant ($p < 0.05$). The measured water consumption values were used to estimate daily doses of 19, 32, and 40 mg hydrazine sulfate/kg of body weight. These values are somewhat lower than the projected doses of 23, 46, and 69 mg hydrazine sulfate/kg of body weight.

TABLE 1. WATER CONSUMPTION[a] (mL/ANIMAL/DAY) OF MALE GOLDEN SYRIAN HAMSTERS

Period	Control	Hydrazine Sulfate 170 mg/L	340 mg/L	510 mg/L
4 weeks	9.1 ± 0.2	9.2 ± 0.2	8.1 ± 0.2	7.2 ± 0.1
5 months	15.4 ± 1.1	15.4 ± 1.4	12.0 ± 1.2	9.4 ± 0.4
9 months	14.0 ± 1.6	13.5 ± 1.1	11.3 ± 1.0	8.7 ± 0.3
16 months	18.6 ± 2.1	16.9 ± 1.4	16.5 ± 1.1[b]	14.0 ± 1.2
Average	14.3 ± 0.4	13.7 ± 0.8	11.9 ± 0.6	9.8 ± 0.3

[a] Mean ± SE, N = 10.
[b] N = 9.

Body weight data are presented in Figure 1. The body weights of all groups were statistically ($p < 0.05$) equal at the study initiation. During the first month of exposure both the

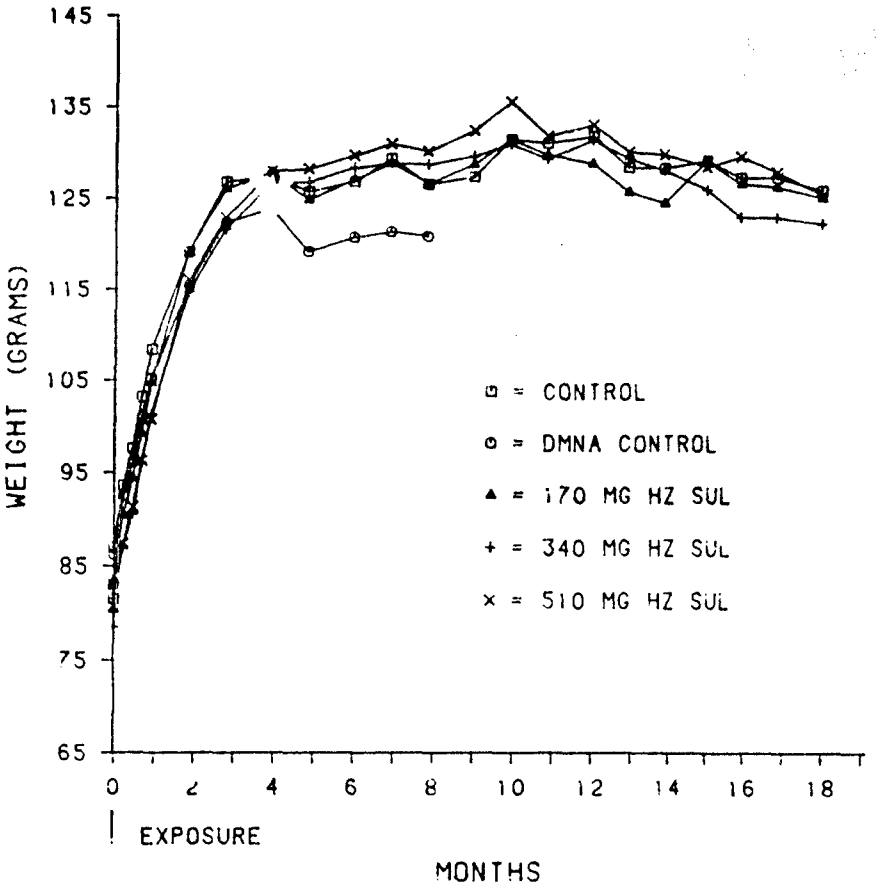


Figure 1. Body weights of male Golden Syrian hamsters exposed to hydrazine sulfate in drinking water.

340 and 510 mg/L groups had significantly ($p < 0.05$) reduced rates of growth. However, after that first month all groups have been essentially the same.

DNA analysis and pathology are being conducted at U. C. Irvine and are not available for inclusion in this report.

A CHRONIC INHALATION TOXICITY STUDY ON MONOMETHYLHYDRAZINE

Monomethylhydrazine (MMH) is a highly reactive chemical used as a rocket fuel component as well as a chemical intermediate. The acute health hazards from handling MMH and its analogs, hydrazine and unsymmetrical dimethylhydrazine (UDMH), have been well defined (Comstock et al., 1954; Shook and Cowart, 1957; Rinehart et al., 1960; Weeks et al., 1963; Jacobson et al., 1966; Haun et al., 1970; Darmer and MacEwen, 1973). The symptoms of

acute exposure were irritation, emesis, ataxia, and convulsions. MMH was also found to cause a dose related hemolytic anemia which was reversible within a few weeks postexposure (Haun et al., 1970). The hemolytic anemia was characterized by significant decreases in hematocrit, red blood cells, and hemoglobin which continued for several days postexposure. The destruction of red blood cells was accompanied by an increase in reticulocytes during the period of maximum decline of hematocrit levels. Persistent renal damage was also seen in dogs and monkeys.

Exposures of 90 days to 6 months have been evaluated extensively in our laboratory to assess the safety factor and appropriateness of the current TLV for health of workmen. These studies have included both continuous and intermittent exposures to rats, mice, dogs, and monkeys. The weekly dose of MMH in ppm hours for the previous experiments is shown in Table 2.

TABLE 2. COMPARISON OF WEEKLY DOSE EXPOSURE EQUIVALENTS OF MMH IN CHRONIC STUDIES CONDUCTED AT THE THRU

Chamber Conc. (ppm)	Length of Exposure	Type of Exposure	Weekly Dose (ppm-hours)
0.2	6 Months	Intermittent[a]	6
0.04	90 Days	Continuous	6.72
0.1	90 Days	Continuous	17
1.0	6 Months	Intermittent[a]	30
0.2	6 Months	Continuous	33.6
2.0	6 Months	Intermittent[a]	60
5.0	6 Months	Intermittent[a]	150

[a] 6 hours/day - 5 days/week.

Numbers of reports have been written detailing results of these studies. Haun (1970) and MacEwen and Haun (1971) reported chronic studies which included four 6-month intermittent exposures (6 hours/day/5 days/week) of rats, mice, dogs, and monkeys to 0.2, 1.0, 2.0, and 5.0 ppm MMH, and continuous exposure of the same 4 species to 0.2 ppm MMH. The MMH-induced blood dyscrasias following acute exposures (Haun et al., 1970) were dose-related effects with intermittent exposures as well. The effects occurred in both species examined, dogs and monkeys, but to a greater extent in dogs. MMH produced increases in methemoglobin formation, reticulocytes, serum alkaline phosphatase, total phosphorus, and serum bilirubin levels. Decreases were found in

hematocrit, hemoglobin, and red blood cell counts. There was a definite shift in dog erythrocyte fragility with increasing MMH dosage. Further indications of MMH induced changes were dose related depression of the ratio of myeloid to erythroid elements of bone marrow. Intermittent exposures at the concentrations tested failed to establish a no-effect level.

Darmer and MacEwen (1973) reported the results of a 6-month continuous exposure of dogs, monkeys, and rats to concentrations of 0.10 and 0.04 ppm MMH. The hematologic effects of continuous exposure to 0.10 ppm MMH were consistent with the dose response previously reported at higher exposure levels (MacEwen and Haun, 1971). Continuous exposure at 0.04 ppm MMH did not significantly alter the hematology of the test animals and had no effect on rat growth.

Another common finding following chronic exposure to MMH is renal damage. In the intermittent exposures (MacEwen and Haun, 1971) the dogs exhibited hematuria, hemoglobinuria, methemoglobinuria, and cast formation. Sopher et al. (1967) reported kidney effects in dogs which included proteinaceous precipitates in the proximal tubules with occasional hemoglobin casts. Degeneration of the proximal tubules with tubular necrosis was present in many cases. Van Stee (1965) reported a decrease in tubular excretion in dogs with a decrease in the glomerular filtration rate which he attributed to the renal plasma flow rate. Kroe (1971) found periportal hepatic hemosiderosis and cholestasis in dogs and mice exposed to 5.0 and 2.0 ppm MMH for 6 months. Renal tubular hemosiderosis of the proximal convoluted tubules was also reported for the same species.

Hydrazines administered in the drinking water of Swiss mice and Golden Syrian hamsters were reported by Toth (1972, 1973) to have carcinogenic activity. In the first of these studies, solutions of 0.001% hydrazine and methylhydrazine sulfate were given 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. Toth and Shimizu (1973) report a study in which Golden Syrian hamsters received 0.01% methylhydrazine in drinking water ad libitum for life. Malignant histiocytomas (Kupffer cell sarcomas) were observed in the livers of 54% of the male hamsters treated, while none were 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/day by mouth to Swiss mice on a 5 day/week schedule for 40 weeks and found a lower incidence of pulmonary adenomas compared to untreated controls. Kelly reported that oral administration of 2% aqueous solutions of MMH at a dose of 0.2 mL/mouse to female CDF_1 mice, and i.p. administration of 0.1 mL/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.

MacEwen and Vernot (1975) reported the results of a 2-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 at onset of non-neoplastic pathologic changes was markedly different in animals drinking aqueous MMH and control animals. Neoplasms occurring only in the experimental groups included one hemangioendothelioma of the liver, two hepatocellular carcinomas, and one cutaneous melanoma. 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 reported investigations presented some evidence that MMH may be carcinogenic and therefore may pose a hazard to man. This study was undertaken to determine oncogenic effects in large enough populations to reveal reasonably small increases in tumor incidence.

MATERIALS AND METHODS

Test Agent

Monomethylhydrazine for use in this study was prepared by Olin Corporation. The batch of MMH was purified at Olin by bubbling nitrogen gas through it to drive off the more volatile contaminants. Five liters were delivered in two containers and rebottled at the THRU in 100 mL units under nitrogen. This was done to minimize oxidative degradation during use and also to reduce hazards of handling large volumes routinely.

Analysis of Chamber Concentration

The analytical procedure for monitoring MMH concentrations in the chambers was a modification of the method reported by

Geiger and Vernot (1967). A chamber air sample was drawn through a glass scrubber column filled with glass beads to mix the MMH vapor with a potassium iodide absorber solution. The chamber MMH contaminated air reduced the iodine to colorless iodide ion proportional to the amount of MMH present in accord with Beer's Law. The air and liquid were separated and the liquid was pumped to a Technicon AutoAnalyzer® to determine MMH concentration. Over the year-long series of daily exposures, the MMH concentrations were maintained with daily relative standard deviations of 10% or less in all chambers.

Animals

Fischer 344 rats (CDF[F344]/CrlBR)[1], Golden Syrian hamsters (Lak:LVG[SYR])[1], C57BL/6J mice[2], and beagle dogs[3] were exposed to MMH by the inhalation route in 840 cubic foot chambers described by Thomas (1965) for 1 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. Rats and mice were 10 weeks of age, hamsters 12 weeks of age, and dogs 11 to 20 months of age at the onset of the study. Food was available only during nonexposure periods while water was available ad libitum. Animal assignments and exposure concentrations (ppm) selected were:

Species	Sex	0.0	0.02	0.2	2.0	5.0
Rats	Male	150	100	100	100	100
	Female	150	100	100	100	100
Mice	Female	400	400	400	400	---
Hamsters	Male	200	---	200	200	200
Dogs	Male	4	---	4	4	---
	Female	4	---	4	4	---

[1] Charles River Breeding Laboratory, Wilmington, Massachusetts.
[2] Jackson Laboratories, Bar Harbor, Maine.
[3] Ridglan Farms, Inc., 301 W. Main Street, Mt. Horeb, Wisconsin.

EXPERIMENTAL RESULTS

Growth

MMH exposure caused a dose related depression of growth rate in male rats throughout the entire study. This was particularly evident at the 5 ppm concentration where growth was inhibited through the exposure period. The effects were less noticeable at the 0.2 and 0.02 ppm MMH exposure levels, but weights were statistically different from their unexposed controls throughout the treatment period. Rats at all levels of MMH exposure continued to show statistically significant depression in mean weights when compared to their respective control groups for the entire 52 weeks postexposure observation period. Mean body weights of female rats fluctuated more than those of males, but the weights of the two highest exposure concentration groups remained significantly below the control group for the duration of the exposure. After 1-year only the 5.0 ppm exposure group was significantly lower in weight than controls. Thereafter, all exposed groups gained at a significantly slower rate than controls.

All groups of hamsters showed the bimodal weight distribution seen in previous studies with this species (MacEwen et al., 1981). The mean weight of the 5 ppm MMH exposure group of hamsters showed a definite depression when compared to the unexposed control group. The two intermediate concentration levels remained below control values in most cases, but did not show a clear dose response as was seen in the male rats. In contrast to the rats, hamsters exposed to 5 ppm MMH were able to gain weight and finally overtake the control group during the postexposure phase of the study.

Clinical Laboratory Measurements

The hemolytic effects seen in the exposed dog groups were similar to those seen in a previous long-term MMH study done in our laboratory (Haun, 1970). The mean red blood cell count, hemoglobin and hematocrit values were depressed, starting after 2 weeks of exposure and continuing through to the conclusion of the exposure. Statistical analyses of these measurements revealed significant differences between the test groups and their controls at almost every sampling point.

The SGPT values increased significantly in the 2 ppm MMH exposed group at the first biweekly sampling and continued to increase for 12 weeks. This was followed by a slight decline, but values remained approximately 5-fold greater than controls

for the remainder of the exposure. Twice during the study, at 18 and 46 weeks, one dog exposed at the 0.2 ppm MMH concentration level exhibited an extremely high SGPT value which returned to a normal level at the next sampling period while the rest of the dogs in that group remained marginally elevated over controls. Concurrent with high SGPT values, increases in alkaline phosphatase and bilirubin values were most compatible with liver stress for the 2 ppm MMH exposed dogs. Dogs exposed to 0.2 ppm were not different from controls in these parameters.

Methemoglobin values determined for the 2 ppm exposed dogs were statistically higher than their unexposed controls. Each of the measurements made during the course of the exposure as well as at the conclusion showed higher values for these dogs with group mean methemoglobin values ranging from 0.97 to 1.83%. Methemoglobin values in dogs exposed to 0.2 ppm MMH were different from their unexposed controls only at the 6-month sampling period. The increase in methemoglobin in the 2 ppm MMH exposed dogs confirmed the methemoglobin effects seen in the dogs in previous studies.

BSP retention in the 2.0 ppm exposed dogs was significantly higher than that measured in unexposed controls at exposure termination, but by 2 weeks postexposure BSP measurements in exposed dogs had returned to control values. SGPT values in dogs exposed to 2 ppm returned to normal levels within 4 weeks postexposure. Biannual examinations of the dogs during the 5-year postexposure period showed all blood parameters to be within normal limits.

Pathology

Rats - Lesions most frequently seen in male and female rats are shown in Tables 3 and 4, respectively. There were no adverse MMH exposure-related lesions in either male or female rats but, as frequently happens with stressed rodents, there were dose related decreases in the incidence of leukemia and in pituitary adenomas at the highest dose. The overall tumor incidence (both benign and malignant) was comparable in all groups of rats.

Hamsters - Histologic examination of hamster tissues (Table 5) revealed some modest but statistically significant increases in non-neoplastic liver lesions in the exposed groups of hamsters indicating a chronic hepatotoxic effect. Hepatitis was increased in the high exposure group while biliary cysts were increased in all groups of exposed hamsters.

TABLE 3. LESIONS FOUND IN FISCHER 344 MALE RATS FOLLOWING INHALATION OF MMH VAPOR FOR 1 YEAR (INCIDENCE RATIO)

	Unexposed Controls	0.02 ppm Exposed	0.2 ppm Exposed	2.0 ppm Exposed	5.0 ppm Exposed
Lung Carcinoma	7/150	6/100	0/100	3/99	1/99
Mononuclear Cell Leukemia	18/150	9/100	3/100[b]	3/99[b]	4/99[b]
Pituitary Adenoma	44/150	34/100	32/100	23/99	18/99
Kidney Nephropathy	113/150	57/100[a]	82/100[b]	60/99[a]	40/99[a]
Testicular Interstitial Cell Tumor	125/149	86/100[a]	89/100[a]	73/95	80/96
Thyroid "C" Cell Adenoma	22/150	17/100	18/100	15/99	3/99[a]

[a] Different from controls, $p < 0.01$.
[b] Different from controls, $p < 0.05$.

TABLE 4. LESIONS FOUND IN FISCHER 344 FEMALE RATS FOLLOWING INHALATION OF MMH VAPOR FOR 1 YEAR (INCIDENCE RATIO)

	Unexposed Controls	0.02 ppm Exposed	0.2 ppm Exposed	2.0 ppm Exposed	5.0 ppm Exposed
Lung:					
Adenoma	1/149	1/99	2/100	1/99	1/99
Carcinoma	3/149	5/99	1/100	3/99	0/99
Mononuclear Cell Leukemia	19/149	6/99	5/100[b]	1/99[a]	0/99[a]
Pituitary Adenoma	43/149	45/99[a]	43/100[a]	48/99[a]	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
Adenoma	15/149	10/99	10/100	9/99	9/99
Adenocarcinoma	5/149	1/99	0/100	0/99	2/99

[a] Different from controls, $p < 0.01$.
[b] Different from controls, $p < 0.05$.

TABLE 5. LESIONS FOUND IN GOLDEN SYRIAN HAMSTERS FOLLOWING INHALATION OF MMH VAPOR FOR 1 YEAR (INCIDENCE RATIO)

	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	35/190	52/177[a]	56/180[b]	46/177
Rhinitis	12/190	21/177[a]	25/180[a]	28/177[b]
Adenoma	1/190	0/177	0/180	7/177[a]
Polyp	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 Adenoma	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]
Lymph Nodes:				
Reticuloendothelial tumors	7/192	5/167	2/170	6/168
Lymphoid hyperplasia	26/192	12/167	15/170	6/168
Adrenals:				
Cortical adenoma (Benign)	16/191	16/173	10/172	23/176[b]
Cortical adenoma (Malignant)	11/191	14/173	11/172	10/176
Bone:				
Osteoma	0/190	0/180	2/181	1/182

[a] Different from controls, $p < 0.05$.
[b] Different from controls, $p < 0.01$.

Both non-neoplastic and neoplastic changes were increased in the nasal cavity of exposed hamsters. Submucosal cysts, rhinitis and epithelial hyperplasia increased in incidence in the exposed

animals. More importantly, the presence of nasal tumors (adenomas and polyps) in the animals exposed to the higher levels is significant as nasal tumors are rare in aged hamsters. Significant numbers of adenomatous polyps also appeared in the noses of hamsters exposed to 5 ppm hydrazine for 1 year on an industrial 6 hour/day, 5 day/week schedule (MacEwen and Vernot, 1979).

There is a modest but statistically significant increase in the incidence of focal collapse of the lung seen in hamsters exposed to the two highest concentrations of MMH.

Mice - Non-neoplastic lesions found in mice are listed in Table 6. There were significant increases in irritation of the nasal cavity such as nasal inflammation, plasmacytosis, and hemorrhage in the mandibular lymph nodes. A number of changes were seen in the liver with marked increases in incidence of cysts, bile duct hyperplasia, hepatocellular pleomorphism and gallbladder crystals in the high exposure group. Statistically significant increases in angiectasis were also seen in the highest MMH exposure group of mice.

TABLE 6. NON-NEOPLASTIC LESIONS FOUND IN C57BL/6 MICE FOLLOWING INHALATION OF MMH VAPOR FOR 1 YEAR (INCIDENCE RATIO)

	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	50/344[b]	46/330[b]	31/329
Hemorrhage	2/322	7/344	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 Plemorphism	11/373	6/357	11/357	33/363[b]
Gallbladder 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] Different from controls, $p < 0.05$.
[b] Different from controls, $p < 0.01$.

Neoplastic lesions found in mice after exposure to MMH are shown in Table 7. Adenomas and adenomatous polyps were seen in the nasal mucosa of a few mice at the highest MMH exposure level. Although the numbers are not large, they are considered significant since none were found in the control group, and we rarely see these lesions in untreated mice. There were marked, dose dependent increases in lung tumors seen in mice exposed to 0.2 and 2 ppm MMH. A small number of unusual neoplasms (osteomas) were observed in nasal tissue of the 2.0 ppm exposed mice.

TABLE 7. NEOPLASTIC LESIONS FOUND IN C57BL/6 MICE FOLLOWING INHALATION OF MMH VAPOR FOR 1 YEAR (INCIDENCE RATIO)

	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 polyp	0/367	0/354	0/349	4/355
Osteoma	0/367	0/354	0/349	3/355
Epithelial neoplasms (nasal and respiratory mucosa)	0/367	2/354	1/349	4/355
Lung:				
Adenoma	13/364	16/354	23/347	56/360[b]
Carcinoma	0/364	1/354	2/347	3/360
Liver:				
Adenoma	6/373	2/357	5/357	20/363[b]
Carcinoma	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] Different from controls, $p < 0.05$.
[b] Different from controls, $p < 0.01$.

Statistically significant increases in liver adenomas and carcinomas were also seen in mice exposed to 2 ppm MMH, and parallel pleomorphic changes were seen in hepatocytes with a significant increase at the highest dose level. Neoplastic vascular

lesions (hemangiomas) were markedly increased in the high exposure level.

Dogs - No MMH induced lesions were found in any of the MMH exposed dogs. The lesions seen were those normally found in aged beagle dogs.

DISCUSSION

Chronic exposure to MMH vapors resulted in depressed growth in rats and hamsters. Although the rats and hamsters showed an accelerated weight gain postexposure, they remained significantly smaller than their respective unexposed control groups.

Hematologic effects of MMH exposure were seen in dogs after 2 weeks and continued throughout the treatment period. Liver insult, as indicated by increases in SGPT, alkaline phosphatase, and bilirubin levels, was present during exposure; however, the SGPT values of the 2 ppm exposed dogs returned to normal levels 4 weeks postexposure. This was a surprising and much more rapid recovery than seen in dogs exposed to 5.0 ppm UDMH where it required more than 11 weeks to return to control levels (MacEwen and Vernot, 1979). The return of BSP and SGPT measurements in MMH exposed dogs to unexposed control levels indicated that the MMH induced liver injury was reversible.

Oncogenic changes were noted in the respiratory, hepatic, and vascular systems of rodents. Significant increases were noted in nasal tumors in the hamsters exposed to 5.0 ppm and, although not statistically significant, hyperplastic lesions were also found in the nasal mucosa of the mice exposed to 2 ppm MMH. Alveolar/bronchiolar and hepatocellular adenomas and carcinomas, and vascular hemangiomas were all found in increased numbers in the mice exposed to this MMH concentration.

Rats showed no exposure related lesions at necropsy. Decreases in pituitary adenomas and leukemia incidence have been noted previously in experimental animals exposed to hydrazine compounds (MacEwen and Vernot, 1980, 1981) as well as other toxic compounds (Young and Gries, 1984). Histopathologic changes in dogs were typical of aging and unrelated to MMH exposure.

The ACGIH adopted a TLV ceiling concentration of 0.2 ppm (0.35 mg/m^3) for MMH in 1966 that has remained unchanged. That MMH exposure to skin, mucous membranes, and eyes could contribute to the overall exposure to MMH has also been noted (U. S. Department HEW, 1978). The ceiling concentration of 0.2 ppm was based

on a comparison of the acute toxicity of MMH with that of 1,1-dimethylhydrazine (Jacobson et al., 1966; Smyth, 1956). This was largely based on the observation by Jacobson et al. that MMH resembled 1,1-dimethylhydrazine and hydrazine in its toxic effects and that the acute toxicity of MMH was approximately three times that of 1,1-dimethylhydrazine (TLV = 0.5 ppm).

The American Conference of Governmental Industrial Hygienists (ACGIH, 1982) has proposed a classification system for experimental animal carcinogens. Substances occurring in the occupational environment found carcinogenic for animals may be grouped into three classifications - those of high, intermediate, and low potency.

To qualify as a carcinogen of intermediate potency, a substance must fulfill one of the following conditions in 2 animal species: 1) Elicit cancer from dosages between 1 and 10 mg/m^3 via the respiratory tract in 6-7 hour daily, repeated inhalation exposures throughout lifetime. 2) Elicit cancer by daily intake via the gastrointestinal tract, within a 6 month holding period, at a dosage between 1 and 50 mg/kg body weight/day.

The A2 designation (Industrial Substances Suspect of Carcinogenic Potential to Man) was first assigned to this compound in 1980. The designation was assigned as a result of the studies by Toth and Shimizu (1973). Although there were conflicting results of carcinogenicity in hamsters, the committee felt the classification of A2 suspected carcinogen was in order.

CONCLUSIONS

Malignant histiocytomas were observed in the livers of hamsters (Toth and Shimizu, 1973) following a lifetime intake of 0.01% MMH in drinking water, a dose of approximately 16 mg/kg per day based on an average body weight of 120 grams. This study has produced alveolar/bronchiolar adenomas, hepatocellular adenomas and carcinomas, and hemangiomas in female mice exposed to 2 ppm (3.5 mg/m^3) of MMH. In addition, nasal adenomatous polyps were found in the female mice exposed to 2 ppm and in the male hamsters exposed to 5 ppm (9.4 mg/m^3) MMH. The neoplastic response in both mice and hamsters inhaling 2 and 5 ppm MMH, respectively, and the incidence of hepatic tumors in hamsters receiving 15 mg/kg MMH per day in their drinking water, classifies MMH as an intermediate carcinogen and justifies the A2 designation recommended by ACGIH.

CHRONIC INHALATION EXPOSURE OF EXPERIMENTAL ANIMALS TO METHYLCYCLOHEXANE

INTRODUCTION

Methylcyclohexane (MCH) is a liquid hydrocarbon used as a solvent for cellulose ethers and has been used in the aircraft fuel designated JP-9. Acute toxicity studies of MCH were reported by Treon et al. (1943). Six-hour acute exposures of rabbits to inhaled concentrations of MCH above 10,000 ppm caused convulsions, light narcosis, labored breathing, salivation, and conjunctival congestion. Between 5500 ppm and 7300 ppm, lethargy and impaired coordination were the only signs. Lazarew (1929) reported that 7500 to 10,000 ppm vapor for 2 hours produced narcosis in mice while 10,000 to 12,000 ppm caused death. Lehman and Flury (1943) indicated that the acute toxicity of MCH was greater than that of heptane, but less than that of octane. Similar high level narcotic effects were reported when mice were exposed to heptane vapors between 10,000 and 15,000 ppm (Fuehner, 1921). In addition, Patty and Yant (1929) reported slight dizziness in man after exposure to 1000 ppm for 6 minutes. Concentrations of 2000 to 5000 ppm resulted in marked vertigo, hilarity, incoordination, and nausea which persisted for several hours after exposure. The American Conference of Governmental Industrial Hygienists lowered the threshold limit value (TLV) for MCH in 1976 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 based on analogy to the toxicity of heptane, and the TLV and STEL values are identical to the TLV and STEL of heptane.

The use of analogy with other solvents for setting human exposure limits has resulted in serious occupational disease outbreaks in exposed workers. A TLV of 500 ppm had been set for n-hexane based solely on acute toxicity data in animals for other petroleum solvents such as pentane. Reports of peripheral neuropathy in workers exposed to hexane resulted in the lowering of the American Conference of Governmental Industrial Hygienists TLV to 100 ppm in 1974 and to 50 ppm in 1980.

Since methylcyclohexane is a highly volatile liquid, chronic exposure would be expected to be fairly high vapor concentrations. Consequently, 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 provided for the detection of 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.

MATERIALS AND METHODS

Test Agent

The methylcyclohexane used in this study was manufactured and obtained from Eastman Organic Chemical Corporation. The MCH supplied came in steel drums and consisted of 3 different lots designated A8, A9, and B8. Purity analyses were conducted on the MCH in each drum. The results are tabulated below:

Lot No.	% Purity	% N-Heptane	% Toluene
A8	98.57	0.86	0.56
A9	98.50	0.97	0.52
B8	98.66	0.74	0.60

Only two impurities, n-heptane and toluene, were identified and the relative purity of the MCH was consistent from drum to drum within lots and between lots used in the study.

Generation and Analysis

The generation of desired chamber concentrations of MCH was accomplished by metering liquid MCH directly into the chamber inlet air supply stream where vaporization was accomplished in sufficient air volume to prevent formation of an explosive vapor mixture.

Air samples were continuously drawn from the chambers during animal exposures for analysis using a total hydrocarbon analyzer. Each pair of chambers with the same nominal concentration was sampled alternately on a 15 minute cycle with a single analyzer.

Animals

Fischer 344 rats (CDF [F344]/CrlBR), Golden Syrian hamsters (Lak:LVG ([SYR]), C57BL/6J mice and purebred beagle dogs were used in this study. Rats were 10 weeks of age, mice 8 weeks of age, and hamsters 12 weeks of age at the onset of the study. The

beagle dogs ranged in age between 8 and 13 months. Food was available only during nonexposure periods while water was available ad libitum.

Exposure Conditions

The animals were exposed to MCH by the inhalation route in dome shaped, 840 cubic foot chambers described by Thomas (1965) for 1 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. Each exposure and control group 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 species after 2 years on study for comparison of exposure group and control incidence of histologic changes. Dogs were included in the study to provide indices of non-tumor effects, mainly from analyses of blood.

The exposure portion of this study continued for 1 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 and the dogs for 5 years of postexposure observation.

EXPERIMENTAL RESULTS

Exposure Measurements

The exposure concentrations achieved during the 12-month exposure period are shown in Table 8. Exposure chamber concentration control was excellent after the first few weeks and is reflected in the relatively small standard deviations of the means.

TABLE 8. METHYLCYCLOHEXANE CONCENTRATIONS MEASURED IN ANIMAL EXPOSURE CHAMBERS

	Chamber 1	Chamber 2	Chamber 3	Chamber 4
No. of Sampling Days	243	243	243	243
Nominal Conc., ppm	400	400	2000	2000
Mean Measured Conc., ppm	401.5	398.9	2009	1998
Standard Deviations, ppm	± 4.5	± 2.5	± 46.6	± 52.4
Concentration Range, ppm	393-412	395-402	1878-2080	1847-2047

Growth

Male rats exposed to both levels of MCH showed depressed growth throughout the study. Although the male rats showed an increase in weight gain after removal from the exposure chambers, they still did not attain the mean weight of the unexposed control group. The female rat weights were unaffected during exposure as well as during the postexposure observation period. A definite depression in mean body weights was seen in the exposed hamster groups but was not dose related. Immediately following exposure, both exposed hamster groups gained weight and became equivalent to the control group.

Clinical Laboratory Measurements

Hematology and clinical chemistry determinations were made on male and female rats sacrificed after 1 year of exposure. Although there were no biologically significant differences between MCH exposed and control rats, a significant increase in creatinine along with an increase in BUN was seen in the male group exposed to 400 ppm MCH but was not evident in the high exposure group. Low WBC's were found in all exposed groups, both male and female. Because of hemolysis in most samples of female rat blood, no clinical chemistry comparisons could be made.

Clinical determinations on dog blood taken at biweekly intervals gave variable but non-MCH related results. The only parameter affected by MCH exposure was a transient increase in the mean SGPT level of the dogs exposed to 2000 ppm which was caused by a single dog exhibiting a high SGPT level during the seventh exposure week while the other animals of the group were normal. The increase in SGPT values occurred again between 39 and 43 weeks in the same dog from the 2000 ppm group as well as in 2 dogs from the 400 ppm group. Significant differences in SGPT values of dogs were not seen during the 5-year postexposure period.

Pathology

All of the animals used in this study were necropsied at death with a battery of approximately 33 tissues sampled from each animal for histopathology examination following the protocol used by the National Cancer Institute (Sontag et al., 1976).

At the end of the 12-month intermittent exposure to MCH 15 to 20% of the animals of each sex and species were killed for

histologic examination to determine immediate effects of chronic inhalation exposure to this agent. The remaining animals were held for an additional year to determine if delayed effects of the MCH exposure would be seen.

There were no histologic lesions seen in the animals killed immediately following the 12-month exposure period that could be attributed to MCH. The incidence of lesions of any type was low and was comparable among exposed and control animals.

The results of examination of tissue from the animals that died during the postexposure observation period or were killed at the study termination are listed in Tables 9 through 16. The tables of non-neoplastic lesions of the 4 species have been abbreviated to exclude lesions of very low incidence.

Rats

In male rats, the major target organ was the kidney where two types of lesions were associated with exposure. Virtually all of the male rats had lesions consistent with progressive renal nephropathy, common in older male rats. In the male rats exposed to the higher level, there was a statistically significant increase in the occurrence of medullary mineralization and epithelial hyperplasia of the renal papilla. However, no increase of these lesions over controls was seen in the group exposed to 400 ppm. Interstitial cell tumors of the testes, seen at study termination, appeared to be equally distributed between the test and control groups and not related to exposure. No dose related lesions were noted in the exposed female rats when compared to the control group.

Mice, Hamsters, and Dogs

No significant lesions were found in female mice, male hamsters, or beagle dogs when compared to their respective control groups. Lesions noted were those commonly seen in older animals of these groups.

Neoplastic changes seen in rats, mice, and hamsters were those expected in aging animals of these species. No neoplastic lesions were found in dogs. Statistical analysis of the data failed to indicate any significant increase in tumor formation in the MCH exposed animals when compared to their respective controls.

TABLE 9. SELECTED NON-NEOPLASTIC LESIONS[a] SEEN IN RATS HELD FOR POSTEXPOSURE OBSERVATION AFTER 12-MONTH INTERMITTENT INHALATION EXPOSURE TO METHYLCYCLOHEXANE

	Controls	400 ppm	2000 ppm
Males			
Liver			
Bile Duct Hyperplasia	32/53	22/55	19/52
Necrosis	2/53	0/55	1/52
Circulatory System			
Myocardial Fibrosis	11/53	3/55	14/52
Pulmonary Artery Mineralization	6/53	3/55	0/52
Kidney			
Medullary Mineralization	1/53	2/55	19/52[b]
Nephropathy	49/53	52/55	52/52
Papillary Hyperplasia	1/53	1/55	23/52[b]
Tubular Degeneration	1/53	0/55	2/52
Testes			
Atrophy	4/53	2/55	1/52
Lungs			
Adenomatosis	1/53	2/55	0/52
Females			
Liver			
Bile Duct Hyperplasia	5/52	2/50	3/54
Necrosis	4/52	0/50	1/54
Circulatory System			
Myocardial Fibrosis	1/52	3/51	4/53
Pulmonary Artery Mineralization	6/52	2/51	3/54
Kidney			
Medullary Mineralization	4/52	0/51	1/54
Nephropathy	15/52	7/51	15/54
Reproductive			
Ovarian Cysts	6/50	2/51	3/52
Uterine Dilatation	5/52	9/51	4/52
Mammary Gland			
Cystic Hyperplasia	10/47	17/53	14/48
Lungs			
Adenomatosis	2/52	0/51	1/54

[a] Number observed/Number examined.
[b] Different from control, $p < 0.01$.

TABLE 10. NEOPLASTIC LESIONS[a] SEEN IN MALE RATS HELD FOR POSTEXPOSURE OBSERVATION AFTER 12-MONTH INTERMITTENT INHALATION EXPOSURE TO METHYLCYCLOHEXANE

	Control	400 ppm	2000 ppm
Skin/Subcutaneous			
Keratoacanthoma	0/51	1/55	3/52
Fibroma	3/53	4/55	0/52
Fibroadenoma	0/53	1/55	0/52
Osteosarcoma	1/53	0/55	0/52
Basal Cell Tumor	0/53	1/55	1/52
Mammary Gland Fibroadenoma	0/46	0/47	2/52
Myxoma	1/53	0/55	0/52
Lungs			
Squamous Cell Carcinoma	0/54	1/55	0/52
Nasal			
Squamous Cell Carcinoma	1/53	0/55	0/52
Liver			
Mononuclear Cell Leukemia	0/53	0/55	0/52
Pituitary			
Adenoma	17/51	11/54	16/48
Carcinoma	2/51	1/54	0/48
Neoplasm	1/51	0/54	0/48
Thyroid			
Adenoma	4/52	5/54	5/51
Carcinoma	0/52	1/54	2/51
Kidney			
Renal Cell Adenoma	0/54	0/55	1/52
Renal Cell Carcinoma	0/54	1/55	0/52
Adrenals			
Adenoma	1/54	1/55	5/52
Carcinoma	0/54	1/55	0/52
Pheochromocytoma	3/54	0/55	2/52
Stomach			
Leiomyoma	0/53	0/54	1/52
Pancreas			
Islet Cell Adenoma	1/53	1/54	1/51
Testis			
Interstitial Cell Tumor	49/54	49/55	50/52
Zymbals Gland			
Squamous Cell Carcinoma	0/54	0/55	1/52
Preputial Gland			
Adenocarcinoma	0/54	0/55	1/52
Parathyroid			
Adenoma	1/54	0/55	0/52
Multiple Organ			
Mesothelioma	1/54	1/55	1/52
Malignant Lymphoma	1/54	2/55	0/52
Bronchial Mucous Gland			
Adenoma	0/54	1/55	0/52
Histiocytic Leukemia	0/54	3/55	2/52

[a] Number observed/Number examined.

TABLE 11. NEOPLASTIC LESIONS[a] SEEN IN FEMALE RATS HELD FOR POSTEXPOSURE OBSERVATION AFTER 12-MONTH INTERMITTENT INHALATION EXPOSURE TO METHYLCYCLOHEXANE

	Control	400 ppm	2000 ppm
Skin			
Keratoacanthoma	0/49	0/54	2/51
Fibroma	1/52	0/51	3/51
Trichoepithelioma	1/52	0/51	0/51
Fibroadenoma	1/52	3/51	3/51
Adenoma	1/52	0/51	0/51
Sarcoma, Undifferentiated	0/52	1/51	0/51
Sarcoma	0/52	1/51	0/51
Mammary Gland Fibroadenoma	0/47	4/50	6/48
Lungs			
Alveolar/Bronchiolar Carcinoma	0/52	1/54	0/54
Osteosarcoma	0/52	0/54	1/54
Sarcoma	0/52	1/54	0/54
Pituitary			
Adenoma	11/50	16/50	17/54
Carcinoma	3/50	4/50	5/54
Thyroid			
Adenoma	1/52	1/52	2/51
Carcinoma	2/52	3/52	1/51
Parathyroid			
Adenoma	0/31	1/40	0/35
Mediastinal Lymph Node			
C-Cell Carcinoma	0/52	1/54	0/54
Adrenals			
Adenoma	0/52	1/53	1/54
Adenocarcinoma	1/52	0/53	0/54
Pancreas			
Adenocarcinoma	1/51	0/54	0/50
Uterus			
Endometrial Stromal Polyp	7/52	4/54	0/52
Adenocarcinoma	3/52	0/54	0/52
Leiomyosarcoma	0/52	1/54	0/52
Urinary Bladder			
Adenocarcinoma	1/45	0/53	0/51
Brain			
Astrocytoma	0/52	1/54	0/53
Clitoral Gland			
Adenoma	2/52	0/54	0/53
Abdominal Cavity			
Lipoma	1/52	0/54	1/53
Adenocarcinoma	1/52	0/54	3/53
Mesothelioma	0/52	1/54	0/53
Myxosarcoma	0/52	0/54	1/53
Circulatory System			
Histiocytic Leukemia	2/52	2/54	5/53
Malignant Lymphoma	1/52	0/54	0/53

[a] Number observed/Number examined.

TABLE 12. SELECTED NON-NEOPLASTIC LESIONS[a] SEEN IN FEMALE MICE HELD FOR POSTEXPOSURE OBSERVATION AFTER 12-MONTH INTERMITTENT INHALATION EXPOSURE TO METHYLCYCLOHEXANE

	Control	400 ppm	2000 ppm
Lungs			
Alveolar Crystals	24/170	8/158	6/155
Alveolar Macrophages	31/170	17/158	9/155
Perivascular Cuffing	20/170	25/158	19/155
Lymphoid Hyperplasia	20/170	23/158	25/155
Spleen			
Hematopoiesis	22/164	34/150	23/154
Liver			
Fatty Change	36/171	14/159	16/155
Hematopoiesis	20/171	20/159	24/155
Duodenum			
Mesentery Strangulation	19/167	7/150	10/151
Kidney			
Hydronephrosis	10/171	13/159	7/155
Perivascular Cuffing	13/171	14/159	8/155
Uterus			
Multiple Cysts	10/164	22/158	23/152
Endometrial Dilatation	30/164	38/158	29/152
Ovaries			
Cysts	19/149	20/155	24/135
Hemorrhagic Cysts	12/149	14/155	12/135
Mammary Gland			
Cystic Hyperplasia	26/145	10/118	12/119
Thyroid Gland			
Papillary Hyperplasia	79/164	39/151	44/145

[a] Number observed/Number examined.

Discussion

The only significant toxic effect of chronic exposure to inhaled methylcyclohexane found was renal change in male rats. The exposure related renal injury was not seen in female rats, female mice, male hamsters, or in either sex of beagle dog. Only male rats exposed to 2000 ppm MCH had significantly greater incidence of renal tubular dilation at exposure termination, and progression of renal pathology to papillary hyperplasia and medullary mineralization occurred only in the group exposed to

TABLE 13. NEOPLASTIC LESIONS[a] SEEN IN FEMALE MICE HELD FOR POSTEXPOSURE OBSERVATION AFTER 12-MONTH INTERMITTENT INHALATION EXPOSURE TO METHYLCYCLOHEXANE

	Control	400 ppm	2000 ppm
Skin/Subcutaneous			
Keratoacanthoma	0/167	0/154	1/150
Fibroma	1/167	0/154	0/150
Lung			
Alveolar/Bronchiolar Adenoma	4/170	6/158	0/155
Alveolar/Bronchiolar Carcinoma	3/170	1/158	3/155
Lymph Node			
Hemangiosarcoma	1/166	0/150	0/146
Heart			
Carcinoma	1/170	0/158	0/155
Liver			
Hepatocellular Adenoma	0/171	1/152	0/152
Hemangiosarcoma	0/171	0/152	1/152
Duodenum			
Papilloma	1/167	0/150	0/151
Papillary Adenoma	1/167	1/150	0/151
Uterus			
Neoplasm	1/164	0/158	1/152
Leiomyosarcoma	0/164	1/158	1/152
Ovaries			
Adenoma	1/149	0/155	0/135
Tubular Adenoma	1/149	1/155	4/135
Pituitary			
Adenoma	72/142	40/142	44/118
Carcinoma	4/142	0/142	0/118
Adenocarcinoma	5/142	0/142	2/118
Adrenal			
Adenoma	0/170	0/158	1/149
Thyroid			
Adenoma	0/164	1/151	0/145
Follicular-Cell Adenoma	2/164	1/151	1/145
Lacrimal Gland			
Adenoma	0/171	1/162	0/155
Bone			
Osteosarcoma	0/162	1/159	0/151
Circulatory System			
Malignant Lymphoma	45/171	44/162	56/155
Leukemia	0/171	0/162	1/155

[a] Number observed/Number examined.

TABLE 14. SELECTED NON-NEOPLASTIC LESIONS[a] SEEN IN MALE HAMSTERS HELD FOR POSTEXPOSURE OBSERVATION AFTER 12-MONTH INTERMITTENT INHALATION EXPOSURE TO METHYLCYCLOHEXANE

	Control	400 ppm	2000 ppm
Kidney			
Cortical Fibrosis	4/75	12/76	10/81
Mineralization:			
Collecting Tubules	26/75	19/76	24/81
Convoluted Tubules	14/75	10/76	8/81
Renal Pelvis	10/75	0/76	6/81
Dilatation:			
Convoluted Tubules	17/75	16/76	17/81
Testis			
Atrophy	4/76	3/76	2/81
Aspermatogenesis	3/76	4/76	5/81
Adrenal Gland			
Cortical Hyperplasia	35/75	30/76	29/80

[a] Number observed/Number examined.

the higher level. These findings are consistent with those produced by other paraffinic, cycloparaffinic, and alkylaromatic hydrocarbons. This syndrome now referred to as hydrocarbon nephropathy, is characterized by an increase in the incidence of hyalin droplets and of regenerative tubular epithelia in the cortex. In its most severe form the tubules at the corticomedullary junction become dilated and filled with proteinaceous debris with some necrosis.

The incidence and severity of the hydrocarbon nephropathy seen after chronic MCH inhalation exposure was much less than that reported for gasoline (MacFarland, 1983), decalin (Gaworski et al.. 1979[b]), and for Petroleum and Shale derived JP-5 fuels (Gaworski et al., 1984) even though exposure levels were greater with MCH. Moreover, these previous studies had not shown any level of hydrocarbon exposure that did not cause kidney pathology. In this study, 400 ppm MCH had no pathologic effects on male rat kidney that could be distinguished from controls.

TABLE 15. NEOPLASTIC LESIONS[a] SEEN IN MALE HAMSTERS HELD FOR POSTEXPOSURE OBSERVATION AFTER 12-MONTH INTERMITTENT INHALATION EXPOSURE TO METHYLCYCLOHEXANE

	Control	400 ppm	2000 ppm
Skin/Subcutaneous			
Fibroma	0/74	0/76	1/80
Trachea			
Adenoma	0/72	0/75	2/79
Spleen			
Hemangiosarcoma	0/71	1/75	1/78
Lymph Node			
Neoplasm	1/73	0/74	0/78
Liver			
Carcinoma	0/74	0/77	1/80
Islet-Cell Carcinoma, Metastatic	0/74	1/77	0/80
Hepatocellular Carcinoma	0/74	1/77	0/80
Hemangiosarcoma	0/74	1/77	1/80
Angioma	0/74	1/77	0/80
Pancreas			
Islet-Cell Carcinoma	0/63	1/72	1/75
Duodenum			
Undifferentiated Sarcoma	0/71	0/76	1/79
Kidneys			
Renal-Cell Carcinoma	0/75	1/76	0/81
Adrenal Gland			
Carcinoma	2/75	3/76	7/80
Adenoma	18/75	21/76	12/80
Adenocarcinoma	1/75	0/76	0/80
Pheochromocytoma	0/75	1/76	0/80
Thyroid			
C-Cell Adenoma	0/71	0/67	1/71
C-Cell Carcinoma	2/71	1/67	0/71
Parathyroid			
Adenoma	1/45	0/43	1/47
Multiple Organs			
Carcinoma	0/76	0/77	1/82
Sarcoma	0/76	0/77	1/82
Malignant Lymphoma	2/76	5/77	4/82
Myelogenous Leukemia	0/76	1/77	0/82

[a] Number observed/Number examined.

TABLE 16. SELECTED NON-NEOPLASTIC LESIONS[a] SEEN IN BEAGLE DOGS HELD FOR POSTEXPOSURE OBSERVATION AFTER 12-MONTH INTERMITTENT INHALATION EXPOSURE TO METHYLCYCLOHEXANE

	Control	400 ppm	2000 ppm
Thyroid			
Follicular Hyperplasia	2/8	0/8	3/8
Inflammation, Chronic	2/8	1/8	1/8
Gallbladder			
Microcystic Degeneration	2/8	3/8	3/8
Spleen			
Siderotic Nodule	2/8	1/8	4/8

[a] Number observed/Number examined.

Conclusions

Under the conditions of this inhalation study, exposure to 2000 ppm methylcyclohexane (8000 mg/m^3) produced no tumors. Similar exposure to inhaled JP-10 (MacEwen and Vernot, 1983) was shown to produce severe hydrocarbon nephropathy and renal carcinoma in 18% of the male Fischer 344 rats held 1-year postexposure after 12 months inhalation exposure to 100 ppm JP-10 (tricyclodecane). Inhalation exposure to 2000 ppm gasoline also produced renal carcinomas (Kitchen, 1983) in Fischer 344 rats.

The results of this study indicate no increase in tumor formation in exposed animals when compared to their untreated controls. The tumors seen in all groups were those common to the species.

The results of this study support the selection and safety of the current TLV of 400 ppm for methylcyclohexane.

EVALUATION OF THE 90-DAY INHALATION TOXICITY OF PETROLEUM AND OIL SHALE DIESEL FUEL MARINE (DFM)

A 90-day continuous inhalation toxicity study of diesel fuel marine (DFM) vapor was conducted by the Toxic Hazards Research Unit (THRU). Samples of DFM derived from petroleum as well as oil shale sources were tested to determine if the two fuels presented different health hazards. Petroleum DFM has been used by

the U. S. Navy as the fleet standard fuel for a variety of ships. Oil shale DFM was developed as a possible replacement fuel.

As part of the overall evaluation of the oil shale fuels, it is desirable to assess the toxicity associated with typical use exposure. Data of this type allows for a comparison of the hazards of shale and petroleum derived fuels and is valuable in establishing proper workplace procedures and controls. Since inhalation will be the primary route of exposure for personnel working with DFM, inhalation exposures were conducted to compare the effects at potential exposure levels. A 90-day continuous inhalation exposure period was chosen to simulate conditions where Naval personnel may be exposed during a cruise situation. While this type of exposure is less traditional than a 6 hour/day, 5 day/week regimen, it does create a maximum exposure situation and increases the probability of observing exposure related effects.

DFM is typically a mixture of branched and cyclic hydrocarbons. The fuel contains a small amount of benzene which was considered to be a constituent of major toxicological interest. The reported effects of benzene exposure involve blood disorders with reductions in the number of erythrocytes, leukocytes, and platelets being found in humans after long-term exposure to benzene at high concentrations (Greenberg et al., 1939; Hardy and Elkins, 1948; Aksoy et al., 1972).

A number of recent investigations with hydrocarbon fuels have shown that exposed male Fischer 344 rats often develop hyaline droplets and necrosis of the proximal tubular epithelium (Gaworski et al., 1985; MacNaughton and Uddin, 1984; Parker et al., 1981). MacFarland et al. (1984) found similar kidney lesions in male rats exposed to unleaded gasoline. In addition, renal neoplasia developed in the male rats exposed to unleaded gasoline.

METHODS

Test Material

DFM is described in military specification MIL-F-16884G (7 March 1973), from which the properties shown in Table 17 were selected. Petroleum and Shale DFM were supplied to the THRU by the Naval Medical Research Institute/Toxicology Detachment (NMRI/TD) in clean 55-gallon drums. The Petroleum DFM was obtained from stock supplies. The Shale DFM was refined from Paraho crude oil by the Sohio refinery in Toledo, Ohio, and

designated by Sohio as FIN-DFM. The Shale DFM was shipped via rail tank car to the Naval Toxicology Detachment at Wright-Patterson Air Force Base where it was transferred to clean drums.

TABLE 17. PHYSICAL CHEMICAL SPECIFICATIONS (MIL SPECS) FOR DIESEL FUEL MARINE

Appearance:	Clear bright and free from visible particulate matter
Distillation Temperature (°C)	
Initial Boiling Point:	--
90% Recovery:	357
End Point, Maximum Temperature:	385
Sulfur, Total Weight, % Maximum:	1.00
Flashpoint, °C, Minimum:	60
Pour Point °C, Maximum:	-6.7
Viscosity at 100°F, Kinematic, Centistokes:	1.8-4.5

DFM Generation and Monitoring

The petroleum and oil shale studies were both conducted in a similar manner. The basic design for the DFM generation system was adapted from previous studies of hydrocarbon fuels. Since DFM is a multicomponent material with a wide boiling range, it was necessary to design a closely controlled generation system to produce a well defined vapor. To reduce potential fire hazard, an overheat alarm with a fuel shut-off capability was incorporated into the generation apparatus.

Three solvent evaporator towers were used to generate sufficient Petroleum DFM vapor for the assigned chamber concentrations. Two of the evaporator tower outputs were mixed and split between the 2 exposure chambers. The output of the third tower went directly into the higher concentration chamber. Operating conditions of the evaporator towers were identically maintained to assure similarity in the DFM vapor constituents within the chambers.

A Beckman Model #400 hydrocarbon analyzer was used for mass analysis. Chamber concentrations were analyzed using a single analyzer by dilution of the higher DFM concentration chamber sample to a similar concentration as the low concentration using input chamber air for diluent and as the source of baseline air.

Since the hydrocarbon analyzer response was directly related to the total carbon content of the sample, standardization was

possible using a reliable defined system. Various concentrations of instrument grade propane (99+ % as C_3) diluted in 100 L mylar bags served as standards. Instrumental response was determined to be linear and stable for prolonged periods of time, provided the instrumental parameters were strictly maintained. Twenty-four hourly mean readings were used for daily concentration determinations.

In the Petroleum DFM study a Varian 1200 gas chromatograph (GC) equipped with a FID detector and a Spectra Physics Model I computing integrator was used for quality control checks of each drum of fuel prior to use, for spent fuel, and for generation system operation checks and also chamber atmosphere fingerprint analysis.

A Royco 225 particle counter was used on a daily basis to monitor any condensate aerosol formed by components of the DFM generated into the chambers.

Animals

Young, adult purebred beagle dogs were selected from a colony maintained by the Air Force at Wright-Patterson Air Force Base. CDF (Fischer 344)/Crl/BR rats (9-11 weeks old) were purchased from Charles River Breeding Laboratories (Wilmington, Massachusetts). C57BL/6 mice (9-11 weeks old) were purchased from Jackson Laboratories (Bar Harbor, Maine). Test animals were gang-caged by species in stainless steel, wire-mesh cages during exposure. Animals had access to food (Purina, St. Louis, Missouri) and water ad libitum. All cage areas were cleaned daily during which time food remaining in the feeders was discarded and replaced with a fresh supply.

Exposure Conditions

Groups of 3 male and 3 female dogs, 150 male and 150 female rats, and 150 female mice were exposed via inhalation to 50 mg/m^3 or 300 mg/m^3 Petroleum or Shale DFM vapor continuously for a period of 90 days. Exposures were conducted in 25 m^3 inhalation chambers on a 24-hour basis and personnel servicing the chambers during the exposure were provided with respiratory protection and disposable protective clothing. Control groups were maintained in Bioclean® laminar air flow rooms in a separate facility. The airflow, pressure, relative humidity, and temperature of each chamber were monitored and recorded hourly. Relative humidity was maintained at 50% ± 10% and the temperature at 22°C ± 2.

Because of space limitations, male mice were not included in the exposure.

Upon termination of the 90-day exposure period, all of the dogs and 1/3 of the rodents from each group were killed for detection of pathologic lesions caused by exposure. The remaining rodents were held for long-term postexposure observation. An interim sacrifice was conducted at 19 months postexposure with a final sacrifice during the 24th month of the study.

All animals were carefully observed throughout the exposure and postexposure periods for signs of altered physical condition. Rats and dogs were weighed individually at biweekly intervals during exposure, and rats were weighed monthly during the postexposure period. Mice were weighed monthly throughout the study, and the group mean weights were monitored. All animals that died or were killed were necropsied and approximately 38 tissues were taken for histopathologic examination. Dog red blood cell osmotic fragility tests were conducted at exposure termination. The liver, spleen, and kidneys of individual dogs and rats were weighed at exposure termination and 19 months postexposure. At the conclusion of the Shale DFM study kidney tissue from three male rats in each group was fixed by vascular perfusion with 2.5% glutaraldehyde and 2% paraformaldehyde buffered with 0.1 M cacodylate at pH 7.4. Thin sections were then prepared for Transmission Electron Microscopic examination. Blood samples were drawn from fasted dogs biweekly and from fasted rats at exposure termination and interim necropsy for hematology and clinical chemistry tests: hematocrit (HCT), hemoglobin (HGB), red blood cell (RBC) count, white blood cell (WBC) count, differentials, albumin, alkaline phosphatase, bilirubin, blood urea nitrogen (BUN), calcium, creatinine, glucose, potassium, glutamic-oxaloacetic transaminase (SGOT), glutamic-pyruvic transaminase (SGPT), sodium, and total protein.

Data Analysis

Body weights, blood test results, and organ weights were analyzed by an independent t-test, and a Fisher exact test was used to analyze the incidence of histopathologic lesions (Zar, 1974).

RESULTS

Exposure concentrations presented to the animals were well controlled throughout both studies with hourly mean values within 10% of the target values.

Analysis for condensate aerosol in the exposure chambers indicated negligible aerosol formation in either the Petroleum or Shale DFM study.

Dogs

Dogs exposed to either Petroleum or Shale DFM were generally heavier than unexposed controls. Despite this weight difference, body weights were well within normal limits and the apparent effect was considered incidental. No mortalities occurred in dogs exposed to DFM.

Examination of the RBC osmotic fragility of dogs exposed to Petroleum DFM for 90 days indicated a non-dose related increase. Osmotic fragility curves for dogs exposed to Shale DFM for 90 days were not different from controls. The trend toward increased RBC fragility in dogs exposed to Petroleum DFM was not accompanied by any abnormal changes in other erythrocyte parameters that were periodically measured throughout the exposure.

The only serum chemistry parameter that demonstrated any consistent trend occurred in dogs exposed to Shale DFM, where a slight but statistically significant increase in BUN was seen (Table 18). The elevated BUN in dogs exposed to the Shale DFM was clearly not dose related, however.

TABLE 18. EFFECT OF DFM EXPOSURE ON DOG SERUM BUN LEVELS (IU/L)[a]

Time (wk)	Petroleum DFM Concentration[a] (mg/m^3)			Shale DFM Concentration[a] (mg/m^3)		
	0	5	300	0	50	300
0	14.3 ± 1.4	15.7 ± 1.0	16.1 ± 1.5	17.7 ± 2.0	14.2 ± 1.0[b]	15.0 ± 0.6
2	12.5 ± 1.0	19.5 ± 2.0[c]	16.6 ± 1.2[c]	11.8 ± 0.6	13.7 ± 0.8	14.8 ± 1.3
4	13.6 ± 1.0	14.0 ± 1.2	14.4 ± 1.0	10.5 ± 1.4	18.9 ± 1.9[c]	16.8 ± 1.1[c]
6	14.3 ± 1.1	14.9 ± 1.6	15.6 ± 0.8	12.0 ± 0.6	17.5 ± 2.0[c]	17.6 ± 1.0[c]
8	14.2 ± 0.8	15.6 ± 1.8	13.9 ± 0.4	12.2 ± 0.6	16.5 ± 0.9[c]	17.5 ± 1.1[c]
10	13.2 ± 1.1	16.3 ± 1.6[b]	16.6 ± 1.0[c]	15.7 ± 1.0	19.1 ± 2.0	20.6 ± 3.0[c]
12	14.3 ± 1.0	15.6 ± 2.8	15.6 ± 1.3	13.3 ± 0.4	20.3 ± 2.5[c]	17.9 ± 1.8[c]

[a] Mean ± SE, N = 5 or 6 samples/group.
[b] Different from control, $p < 0.05$.
[c] Different from control, $p < 0.01$.

Cytoplasmic vacuolization of hepatocytes occurred with slightly increased frequency in dogs exposed to Petroleum DFM (control - 2 of 6; 50 mg/m^3 - 4 of 6, 300 mg/m^3 - 5 of 6). This lesion was interpreted as an accumulation by hepatocytes of excessive glycogen. All other remaining lesions noted in the dogs exposed to Petroleum DFM were considered incidental findings unrelated to exposure. No significant exposure or dose related lesions were observed in dogs exposed to Shale DFM.

Mice

The mean survival time of mice exposed to Shale DFM was less than the control group. The death rate in mice exposed to DFM increased after 9 to 10 months on study. Most of the deaths resulted from necrotic dermatitis. No significant differences were noted in mice exposed to petroleum DFM when compared to their control group. In this study the frequency of dermatitis was less.

No body weight effects were seen in mice exposed to either Petroleum or Shale DFM.

Pulmonary inflammatory changes consisting predominantly of mild peribronchiolar and perivascular mononuclear infiltrates were observed in the majority of the mice examined at completion of the 90-day exposure to Shale DFM (Table 19). Mice exposed to Petroleum DFM did not show these inflammatory changes in the respiratory tract. Liver inflammatory changes consisting of multifocal accumulations of chronic inflammatory cells were noted in all groups. These lesions were mild, invariably small, involving only a few hepatocytes and not seen with other manifestations of liver disease. Hepatocellular vacuolization/fatty change was noted more frequently in mice exposed to Shale DFM when compared to their control group. The distribution of the vacuolization was centrilobular in controls, but panlobular in Shale DFM exposed mice. The severity of the change was also considered to be greater in the exposed mice. Mice exposed to Petroleum DFM did not exhibit any increase in liver cell vacuolization or fatty change.

TABLE 19. HISTOPATHOLOGIC LESIONS[a] IN MICE AT TERMINATION OF 90-DAY CONTINUOUS INHALATION EXPOSURE TO DFM

	Petroleum DFM Concentration (mg/m^3)			Shale DFM Concentration (mg/m^3)		
	0	50	300	0	50	300
Lung						
Inflammatory Changes	5/46(11)	9/43(21)	0/42(0)	12/49(24)	34/49(69)[b]	38/48(79)[b]
Liver						
Inflammation	12/45(27)	15/43(35)	25/42(60)[b]	14/51(27)	23/49(47)[b]	9/48(19)
Vacuolization/ Fatty Change	12/45(27)	1/43(2)[b]	4/42(10)	18/51(35)	46/49(94)[b]	41/48(85)[b]

[a] Number observed/Number examined (%).
[b] Different from control, $p < 0.01$.

The vast majority of the pathologic lesions observed in mice held for the postexposure period were common changes attributable to aging processes. Most of these lesions were equally distributed among the exposed and non-exposed groups in both studies. Table 20 presents a list of the major changes noted in mice. Many of the common aging changes as well as lesions occurring with low incidence are not shown. There was an increased incidence of acute and chronic inflammation of the skin in mice exposed to DFM. These were generally regarded as common changes which in some cases were acquired by fighting among cagemates, although the pathogenesis in most instances was unclear. Secondary changes related to the dermatitis were bone marrow hyperplasia and splenic hematopoiesis.

Hyaline degeneration of the respiratory epithelium and crystal deposition are frequently observed in C57BL/6 mice used in this laboratory, and the causes of both are poorly understood. In some instances these entities observed in mice exposed to Shale DFM appeared to be related, in that crystals were observed arising from hyalinized respiratory epithelium. No significant lung tumors were noted in either study.

Liver cell vacuolization and fatty change, which had been increased in mice at termination of exposure to Shale DFM, was found with equal frequency in controls and exposed groups examined postexposure. Liver inflammation was increased at exposure termination in mice exposed to Petroleum DFM. This was not diagnosed in mice held for postexposure observation. The distribution of liver inflammation in mice from the Shale DFM study clearly demonstrates that DFM was not the causative agent. There was no significant increase in liver tumors in mice exposed to DFM.

TABLE 20. HISTOPATHOLOGIC LESIONS[a] IN MICE HELD FOR POSTEXPOSURE OBSERVATION AFTER 90-DAY CONTINUOUS INHALATION EXPOSURE TO DFM

Tissue	Petroleum DFM Concentration (mg/m^3)			Shale DFM Concentration (mg/m^3)		
	0	50	300	0	50	300
Skin						
Inflammation	15/87(17)	20/88(23)	24/91(26)	30/96(31)	41/97(42)	45/99(45)
Bone Marrow						
Hyperplasia	3/82(4)	4/87(5)	9/83(11)	17/90(19)	30/86(35)[b]	31/92(34)[b]
Fibrosis	3/82(4)	5/87(6)	6/83(7)	1/90(1)	0/86(0)	1/92(1)
Respiratory						
Nose -						
Hyaline degeneration	0/92(0)	0/87(0)	0/92(0)	23/96(24)	17/97(18)	13/98(13)
Crystals	1/92(1)	0/87(0)	0/92(0)	25/96(26)	19/97(19)	11/98(11)[c]
Lung -						
Crystals	19/91(21)	22/90(24)	13/94(14)	19/98(19)	11/94(12)	3/98(3)[c]
Alveolar adenoma	4/91(4)	4/90(4)	3/94(3)	5/98(5)	1/94(1)	4/98(4)
Alveolar carcinoma	0/91(0)	0/90(0)	2/90(2)	1/98(1)	1/94(1)	1/98(1)
Liver						
Vacuolization/						
Fatty change	3/93(3)	2/91(2)	6/94(6)	14/97(14)	12/98(12)	16/97(16)
Adenoma	4/93(4)	6/91(7)	9/94(10)	1/97(1)	2/98(2)	0/97(0)
Carcinoma	1/93(1)	3/91(3)	0/94(0)	0/97(0)	0/98(0)	0/97(0)
Inflammation	0/93(0)	0/91(0)	0/94(0)	41/91(45)	17/98(17)[c]	19/97(20)[c]
Urinary						
Kidney -						
Hyaline degeneration	3/92(3)	1/91(1)	0/94(0)	10/94(11)	5/94(5)	2/97(2)[b]
Spleen						
Hematopoiesis	17/91(19)	28/89(31)	24/92(26)	24/95(25)	34/94(36)	38/96(40)
Endocrine						
Pituitary - adenoma	43/71(61)	38/77(49)	41/78(53)	25/81(31)	28/77(36)	14/76(18)
Thyroid -						
Papillary hyperplasia	40/82(49)	38/84(45)	40/82(49)	62/94(66)	51/92(55)	48/96(50)
Adenoma	9/82(11)	7/84(8)	4/82(5)	5/94(5)	2/92(2)	4/92(4)
Lymphoreticular						
Malignant lymphoma	37/93(40)	26/91(29)	29/94(31)	29/98(30)	29/99(29)	22/99(22)

[a] Number observed/Number examined (%).
[b] Different from control, $p < 0.05$.
[c] Different from control, $p < 0.01$.

No evidence of substantial kidney toxicity was observed. Renal tubular cell hyaline degeneration was not a significant finding. Endocrine system tumors and malignant lymphomas were common in all groups, with no relationship to DFM exposure.

Rats

Exposure to Petroleum or Shale DFM did not significantly alter survival time when compared to respective controls. All groups from either study had mean survival times of approximately 22 months.

Exposure to Petroleum DFM resulted in a dose related decrease in weight gain. This effect occurred during the exposure phase and was present throughout most of the postexposure observation period. Shale DFM exposure at 300 mg/m^3 depressed male rat growth in a similar manner, with significant ($p < 0.01$) differences between this group and the control group evident during the entire study. Exposure to 50 mg/m^3 Shale DFM resulted in only transient weight differences from the control group.

Female rats exposed to Petroleum DFM demonstrated reduced body weight gain that became more pronounced during the postexposure period. The effect was not dose related, however. Exposure to Shale DFM did not affect female rat body weight gain.

Acute and chronic inflammatory processes were seen in the nasal mucosa of male and female rats exposed to Petroleum DFM for 90 days (Table 21). Lymphoid hyperplasia of the bronchial submucosa occurred in all groups in the petroleum study. The incidence was slightly increased in exposed female rats compared to controls. No significant lesions were noted in the respiratory tract of rats exposed to Shale DFM.

At exposure termination the most striking lesions seen in rats exposed to DFM occurred in the kidneys of males (Table 21). Renal tubular hyaline degeneration was observed in most of the male rats exposed to either Petroleum or Shale DFM for 90 days. Necrosis of the renal tubular epithelial cells was evident in multifocal regions of the outer and middle cortex in virtually all of the male rats exposed to 300 mg/m^3 DFM, and it was considered to be an irreversible consequence of the hyaline degenerative process. Necrosis was not a distinctive feature of the kidneys of male rats exposed to 50 mg/m^3 DFM. Associated with the necrosis of the tubular epithelium was the presence of multiple, markedly dilated renal tubules at the corticomedullary junction. These dilated tubules were lined by attenuated squamous epithelial cells, and were always impacted with eosinophilic material and detritus of necrotic tubular segments of affected tubular nephrons. Chronic inflammation of the renal cortical interstitial tissue was observed with increased frequency in the male rats exposed to Shale DFM at 300 mg/m^3 and was often associated with degenerative tubular lesions. This inflammatory

TABLE 21. HISTOPATHOLOGIC LESIONS[a] IN RATS OBSERVED AT TERMINATION OF 90-DAY CONTINUOUS INHALATION EXPOSURE TO DFM

	Male Rats					
	Petroleum DFM Concentration (mg/m^3)			Shale DFM Concentration (mg/m^3)		
	0	50	300	0	50	300
Respiratory						
Nasal inflammation	0/25(0)	1/25(4)	3/25(12)	5/25(20)	1/24(4)	0/23(0)[b]
Lung -						
Bronchial lymphoid hyperplasia	12/25(48)	16/25(64)	13/25(52)	0/25(0)	1/23(4)	0/25(0)
Kidney						
Hyaline degeneration	0/25(0)	17/25(68)[c]	21/25(84)[c]	0/26(0)	23/24(96)[c]	23/25(92)[c]
Necrosis	0/15(0)	0/25(0)	24/25(96)[c]	0/25(0)	0/24(0)	25/25(100)[c]
Interstitial tissue inflammation	6/25(24)	1/25(4)	0/25(0)[b]	4/25(16)	4/24(17)	21/25(84)[c]

	Female Rats					
	Petroleum DFM Concentration (mg/m^3)			Shale DFM Concentration (mg/m^3)		
	0	50	300	0	50	300
Respiratory						
Nasal inflammation	0/25(0)	8/25(32)[c]	7/25(28)[c]	5/22(23)	0/25(0)[b]	1/24(4)
Lung -						
Bronchial lymphoid hyperplasia	7/24(28)	15/25(60)	14/25(56)	0/25(0)	0/25(0)	0/25(0)
Kidney						
Hyaline degeneration	0/25(0)	0/25(0)	0/25(0)	0/21(0)	0/25(0)	0/24(0)
Necrosis	0/25(0)	0/25(0)	0/25(0)	0/21(0)	0/25(0)	0/24(0)
Interstitial tissue inflammation	1/25(4)	0/25(0)	0/25(0)	3/21(14)	0/25(0)	1/24(4)

[a] Number observed/Number examined (%).
[b] Different from control, $p < 0.05$.
[c] Different from control, $p < 0.01$.

process was characterized by focal to multifocal aggregations of lymphocytes, plasma cells, macrophages, and occasionally accompanied by the early deposition of fibrous connective tissue.

Transmission electron microscopic examination of the kidney of male rats in the Shale DFM study revealed cytotoxic alterations in the proximal tubular cells attached to the basal lamina. The most striking feature was the presence of large numbers of variably sized, angulated, membrane-bound, osmophilic granules in the cytoplasm. They were observed at all levels in the cell. In some instances the granules appeared as dark crystalline inclusions surrounded by less dense granular matrix in structures thought to be lysosomes. Others showed a slight gradation of electron densities suggesting coalescence of smaller granules to form larger ones. Microvilli were absent on the luminal surfaces of some degenerating cells while others demonstrated club-like swelling of microvillar tips.

Prominent large vacuoles and/or spaces were present extending from the luminal to the basilar surfaces of the tubular epithelial cells. Many of these were interpreted as widened lateral intercellular space between adjacent cells. Occasionally in lytic cells, sequestered crystal and subcellular organelles were present in vacuoles and were undergoing degeneration. Several tubules had voided regions where epithelial cells had been exfoliated exposing the underlying peritubular basal lamina. The necrotic debris present in the associated tubular lumina was thought to be derived in part from such desquamated cells.

Lesions observed in the outer medulla could best be described as medullary tubular cysts. At least some were occurring at the level of the descending loop of Henle (junction of outer and inner stripe of outer medulla). The tubular epithelial cells lining the cysts were ultrastructurally compatible with those of the proximal descending limb with low cuboidal epithelium, absence of a brush border, few microvilli, sparsity of cytoplasmic organelles, and prominent lateral interdigitating membranes. Although an occasional tubular epithelial cell lining the dilated tubules showed some degeneration, most appeared as normal, viable cells. The cells remained firmly attached to the basement membrane; they often had markedly attenuated cytoplasm, but usually the plasmalemma and cytoplasmic organelles appeared essentially normal.

In sharp contrast to the aforementioned tubular cells lining the medullary cysts, the lumina of the cysts were filled with cellular detritus consisting of prominent swollen degenerating mitochondria with flocculent densities and lipid droplets, lysosomes, microbodies, vesicles, granules, and fragmented membranes.

Results of microscopic examination of the tissues collected from male rats maintained for postexposure observation are shown in Table 22. The list has been abbreviated by excluding the lesions that occurred with low frequency and were considered incidental to exposure. The clearest indication of an exposure related effect was again demonstrated in the kidneys of male rats. Virtually all of the male rats, controls included, developed lesions characteristic of chronic progressive renal nephropathy (CPN). These changes were consistent with normal aging lesions; however, CPN was judged to be more severe in the male rats exposed to 300 mg/m^3 DFM when compared to the other groups in each respective study. The most striking difference between DFM exposed rats and unexposed controls was the development of tubular mineralization and epithelial hyperplasia. Furthermore, a clear dose response relationship was demonstrated. Virtually

all of the male rats exposed to 300 mg/m^3 had mineralized deposits in the renal papilla.

The incidence of this lesion in male rats exposed to 50 mg/m^3 Shale DFM was about 50%, while the incidence of mineralization in male rats exposed to 50 mg/m^3 Petroleum DFM was less than the background level in the control group. Associated with the mineralization was the presence of papillary epithelial hyperplasia. As with the mineralization, a dose response relationship was also demonstrated. The mineralized deposits were thought to be calcium impregnated debris shed from the tubule during exposure. The development of hyperplasia was considered to be related to the mechanical irritation of the mineralized debris. Two benign kidney tumors were seen in the studies. One developed in a male rat exposed to 50 mg/m^3 Petroleum DFM, while the other was observed in a male rat exposed to 300 mg/m^3 Shale DFM. No kidney tumors were seen in control male rats.

Tumors and tissue changes occurred in several other organ systems of male rats (Table 22). In general, the frequencies of occurrence were equally distributed among the exposed and control groups for the respective studies. Furthermore, the lesions noted were consistent with changes normally seen in aged rats.

Lesions identified in female rats maintained for postexposure observation are shown in Table 23. Female rats from all groups, including controls, developed renal lesions consistent with CPN. There was no indication of an exposure related increase in severity as was seen with male rats. Female rats in the Shale DFM study demonstrated mineralization of the renal papilla.

The severity of this lesion was characterized as mild, with no indication of a dose related increase in frequency of occurrance. Papillary hyperplasia was not a significant finding in female rats from either study. No kidney tumors developed in female rats exposed to either Petroleum or Shale DFM, while one kidney tumor was found in a female control rat in the Petroleum DFM study. Other lesions noted in female rats exposed to DFM were considered to be normal aging processes. No other organ systems in female rats developed signs of significant DFM related tissue changes.

TABLE 22. HISTOPATHOLOGIC LESIONS[a] IN MALE RATS HELD FOR POSTEXPOSURE OBSERVATION AFTER 90-DAY CONTINUOUS INHALATION EXPOSURE TO DFM

Tissue	Petroleum DFM Concentration (mg/m³) 0	50	300	Shale DFM Concentration (mg/m³) 0	50	300
Skin						
Mammary gland -						
Hyperplasia/dilatation	6/36(17)	1/35(3)	0/35(0)[b]	12/42(29)	7/44(16)	16/45(36)
Fibroadenoma	1/36(3)	0/35(0)	0/35(0)	0/42(0)	3/44(7)	4/45(9)
Cardiovascular						
Myocardial fibrosis/ degeneration	32/50(64)	23/49(47)	32/50(64)	46/49(94)	40/48(83)	45/51(88)
Pulmonary artery mineralization	5/50(10)	6/50(12)	9/50(18)	26/49(53)	16/48(33)	22/51(43)
Respiratory						
Nose - inflammation	4/50(8)	2/48(4)	4/49(8)	12/50(24)	16/47(34)	17/49(35)
Lung -						
Inflammation	23/50(46)	23/50(46)	20/50(40)	3/50(6)	21/49(43)[b]	14/50(28)[b]
Alveolar adenoma	1/50(2)	2/50(4)	1/50(2)	0/50(0)	0/49(0)	1/50(2)
Alveolar carcinoma	1/50(2)	0/50(0)	1/50(2)	0/50(0)	0/49(0)	1/50(2)
Liver						
Focal cell change	28/50(56)	20/50(40)	30/50(60)	30/49(60)	30/50(60)	25/50(50)
Bile duct hyperplasia	44/49(90)	44/50(88)	46/50(92)	49/49(100)	46/50(92)	46/50(92)
Carcinoma	0/50(0)	1/50(2)	2/50(4)	1/49(2)	1/50(2)	0/50(0)
Adenoma	2/50(4)	1/50(2)	0/50(0)	3/49(6)	0/50(0)	0/50(0)
Urinary						
Kidney -						
Nephropathy	50/50(100)	48/49(98)	49/50(98)	50/50(100)	49/49(100)	49/49(100)
Papillary hyperplasia	7/50(14)	2/49(4)	15/50(30)[b]	4/50(8)	15/49(30)[b]	42/49(86)[b]
Mineralization	5/50(10)	2/49(4)	42/50(84)[b]	5/50(10)	26/49(52)[b]	49/49(100)[b]
Adenoma	0/50(0)	1/49(2)	0/50(0)	0/50(0)	0/49(0)	1/49(2)
Reproductive & Endocrine						
Pituitary -						
Adenoma	18/48(38)	13/45(29)	14/47(30)	14/48(29)	8/47(17)	8/45(18)
Carcinoma	0/48(0)	0/45(0)	0/47(0)	0/48(0)	1/47(2)	3/45(7)
Thyroid -						
Follicular cell tumors	0/47(0)	0/46(0)	0/44(0)	4/50(8)	5/47(11)	0/50(0)
C cell tumors	1/47(2)	3/46(7)	3/49(7)	0/50(0)	0/47(0)	0/50(0)
Hyperplasia	5/47(11)	2/46(4)	8/46(17)	24/50(48)	18/47(38)	16/50(32)
Parathyroid -						
Hyperplasia	1/40(3)	1/40(3)	1/38(3)	1/43(2)	1/33(3)	5/34(15)
Adenoma	0/40(0)	0/40(0)	1/38(3)	1/43(2)	1/33(3)	1/34(3)
Testes -						
Interstitial cell tumor	45/49(92)	40/47(85)	46/50(92)	45/49(92)	44/47(94)	46/47(98)
Prostate -						
Inflammation	3/44(7)	2/39(5)	1/40(3)	10/42(24)	6/43(14)	14/45(31)
Hyperplasia	2/44(5)	2/39(5)	2/40(5)	10/42(24)	10/43(23)	14/45(31)
Adrenal -						
Focal cell change	1/50(2)	0/50(0)	0/49(0)	2/50(4)	5/50(10)	10/50(20)[c]
Neoplastic nodule	1/50(2)	1/50(2)	2/49(4)	0/50(0)	1/50(2)	0/50(0)
Pheochromocytoma	4/50(8)	3/50(6)	6/49(24)	5/50(10)	10/50(20)	4/50(8)
Lymphoreticular						
Mononuclear cell leukemia	3/50(6)	4/50(8)	7/50(14)	7/50(14)	8/50(16)	3/50(6)

[a] Number observed/Number examined (%).
[b] Different from control, $p < 0.01$.
[c] Different from control, $p < 0.05$.

TABLE 23. HISTOPATHOLOGIC LESIONS[a] IN FEMALE RATS HELD FOR POSTEXPOSURE OBSERVATION AFTER 90-DAY CONTINUOUS INHALATION EXPOSURE TO DFM

Tissue	Petroleum DFM Concentration (mg/m³) 0	50	300	Shale DFM Concentration (mg/m³) 0	50	300
Skin						
Mammary gland -						
Hyperplasia/dilatation	36/45(8)	34/43(79)	36/43(84)	18/50(36)	14/45(3)	15/46(33)
Adenocarcinoma	1/45(2)	0/43(0)	0/43(0)	1/50(2)	0/45(0)	0/46(0)
Fibroadenoma	3/45(7)	2/43(5)	8/43(19)	3/50(6)	1/45(2)	4/46(9)
Cardiovascular						
Myocardial fibrosis/ degeneration	13/49(27)	8/50(16)	9/49(18)	26/50(52)	31/50(62)	18/50(36)[b]
Pulmonary artery mineralization	3/50(6)	7/50(14)	2/49(4)	11/50(22)	16/50(32)	10/50(20)
Respiratory						
Nose - inflammation	3/50(6)	2/50(4)	2/49(4)	1/50(2)	0/50(0)	3/50(6)
Lung -						
Inflammation	28/49(57)	16/50(32)[b]	17/49(35)	3/50(6)	10/50(20)[b]	4/50(8)
Adenoma	1/49(2)	0/50(0)	0/49(0)	0/50(0)	0/50(0)	0/50(0)
Liver						
Focal cell change	14/50(28)	23/49(47)	22/49(44)	25/50(50)	22/50(44)	18/50(36)
Bile duct hyperplasia	14/50(28)	12/49(24)	12/50(24)	39/50(78)	42/50(84)	38/50(76)
Adenoma	1/50(2)	0/49(0)	1/50(2)	1/50(2)	0/50(0)	0/50(0)
Urinary						
Kidney -						
Nephropathy	38/48(79)	29/50(58)[b]	29/48(60)[b]	29/48(60)	31/46(67)	26/49(53)
Papillary hyperplasia	0/48(0)	0/50(0)	1/48(2)	1/48(2)	1/46(2)	0/49(0)
Mineralization	9/48(19)	3/50(6)	2/48(60)[b]	21/48(44)	20/46(43)	14/49(29)
Adenoma	1/48(2)	0/50(0)	0/48(0)	0/48(0)	0/46(0)	0/49(0)
Reproductive & Endocrine						
Pituitary -						
Adenoma	29/49(59)	24/45(9)	25/48(52)	16/49(32)	14/46(30)	16/46(35)
Carcinoma	0/49(0)	0/45(0)	2/48(4)	0/49(0)	1/46(2)	1/46(2)
Thyroid -						
Follicular cell tumors	0/41(0)	0/46(0)	1/48(2)	1/50(2)	3/49(6)	3/46(7)
Hyperplasia	3/41(7)	3/46(7)	2/48(4)	19/50(38)	21/49(42)	20/46(43)
Parathyroid - adenoma	1/34(3)	0/33(0)	0/36(0)	0/38(0)	0/35(0)	1/33(3)
Adrenal -						
Cell change	2/50(4)	5/50(10)	4/49(8)	9/50(18)	9/49(18)	13/49(27)
Carconoma	1/50(2)	0/50(0)	0/49(0)	0/50(0)	0/49(0)	0/49(0)
Adenoma	0/50(0)	0/50(0)	1/49(2)	1/50(2)	0/49(0)	1/49(2)
Pheochromocytoma	2/50(2)	1/50(2)	3/49(6)	0/50(0)	3/49(6)	2/49(4)
Uterus -						
Carcinoma	2/46(4)	1/48(2)	5/49(10)	0/49(0)	0/49(0)	1/50(2)
Stromal polyps	8/46(4)	14/48(29)	11/49(22)	1/49(2)	1/49(2)	2/50(4)
Lymphoreticular						
Mononuclear cell leukemia	8/50(16)	5/50(10)	8/49(16)	3/50(6)	3/50(6)	4/50(8)

[a] Number observed/Number examined (%).
[b] Different from control, $p < 0.05$.

DISCUSSION

The development of male rat nephrotoxicity in rats exposed to DFM is consistent with the effects reported with a number of other hydrocarbon fuels and solvents (Carpenter et al., 1975a, 1975b; Gaworski et al., 1984, 1985; Parker et al., 1981; Phillips

and Egan, 1984a, 1984b). Nephrotoxic changes consisted primarily of hyaline degeneration with the necrosis noted in male rats exposed to 300 mg/m^3 considered to be the end product of this change. Transmission electron micrographs obtained during the Shale DFM study showed large osmophilic granules that correlated well with the hyaline droplets detected by light microscopy. Most of the granules were thought to be secondary lysosomes formed after fusion of primary lysosomes with endocytotic vacuoles containing electron dense material. The electron micrographs implicate the proximal tubule as the primary location of the hydrocarbon-associated cytotoxic effect on the nephron. There was distinct cytolysis of the tubular epithelium in that nephron segment. It appeared that only a relatively few epithelial cells undergoing degeneration terminated in necrosis. The pattern of necrosis was unique inasmuch as some of the ultrastructural architecture of the cell seemed to be retained. It seemed that complete enzymatic degradation was somehow inhibited. The mechanism of precisely how that effect occurred remains undetermined. This electron microscopic study supported the light microscopic evidence that the medullary cysts in the region of the proximal descending limb of the loop of Henle resulted from cellular debris that was washed downstream from more proximal segments of the nephron. The normal ultrastructure of most cells lining the cysts, with only an occasional cell showing reversible degeneration, suggested a secondary damaging effect, possibly derived from lytic enzymes associated with the necrotic cells in the lumina. If the damage was primary, such as might be caused by a direct toxic insult on the lining cells of the descending limb, a more diffuse pattern of cellular degeneration would be expected. Changes occurring in the kidney subsequent to exposure were mineralization, papillary hyperplasia, and more severe chronic progressive nephropathy.

The results of microscopic examination of kidney tissue suggest that the lower dose utilized in this study may have approached a "no effect" level for the development of hydrocarbon associated renal nephropathy. In a previous 90-day continuous exposure to JP-5, the lowest concentration tested, 150 mg/m^3, produced structural alterations in male rat kidneys including necrosis at exposure termination with mineralization and papillary hyperplasia developing postexposure (Gaworski et al., 1985). In the present study exposure to 50 mg/m^3 produced hyaline degenerative changes without, however, evidence of tubular cell necrosis at exposure termination. Subsequent postexposure examination indicated no substantial mineralization nor papillary hyperplasia in male rats exposed to 50 mg/m^3 Petroleum DFM. Although these changes were seen in male rats exposed to Shale DFM at 50 mg/m^3,

the incidence was considerably less than that occurring in male rats exposed to a higher concentration.

Although there were kidney tissue changes in male rats at the microscopic level, there was no indication of increased kidney weight, nor was there any suggestion of substantial functional changes, indicated by serum BUN and creatinine elevations. Male rats exposed to either Petroleum or Shale DFM had decreased weight gains compared to their respective controls. Erythrocyte parameters of male rats exposed to DFM tended to show slight reductions when compared to controls. This effect was consistent with the trends noted in a previous 90-day continuous exposure to JP-5 jet fuel (Gaworski et al., 1985), although DFM exposure failed to reduce RBC counts, hematocrit, and hemoglobin levels in all cases.

The absence of renal toxicity in female rats, female mice, and male and female dogs exposed to DFM is consistent with the theory that the development of hydrocarbon induced nephrotoxicity is related to the presence of a protein unique to male rats. Alden et al. (1984) has identified α_{2u} globulin as the protein responsible for the formation and accumulation of hyaline droplets.

In general, the effects noted in dogs exposed to DFM were very mild. Changes noted in blood parameters, including red blood cell osmotic fragility, body weights, organ weights, as well as microscopic examination of tissues did not establish any exposure related effect.

Mice exposed to Shale DFM developed lung inflammation and fatty change in the liver. Mice exposed to Petroleum DFM were generally free of these changes. Fatty change in the liver is often regarded as a non-specific tissue alteration. The lung and liver changes in Shale DFM exposed mice were reversible as indicated by the absence of any significant increase in these lesions postexposure. The solvent nature of DFM may have been responsible for the increased occurrence of dermatitis in exposed mice, which ultimately was responsible for the slightly decreased length of survival noted in the Shale DFM exposed mice.

In a recently completed study of the effects of inhaled gasoline vapors, renal neoplasia was reported (MacFarland et al., 1984). In the present study of DFM, kidney tumors were identified in one male rat exposed to Petroleum DFM at 50 mg/m^3 and in one male rat exposed to Shale DFM at 300 mg/m^3. No control male rats from either study developed renal tumors; however, a female control rat in the petroleum study did develop a renal neoplasm.

Although kidney tumors in rats are generally regarded as rare, the development of tumors in the two male rats exposed to DFM is most probably an incidental occurrence. Overall, continuous 90-day exposure to DFM, derived from either Petroleum or Shale DFM did not produce increased incidence of any tumor type in any species tested.

In conclusion, the results of this study are in close agreement with the effects noted in other studies of hydrocarbon fuels. Male rat nephrotoxic changes were evident in animals exposed to either Petroleum or Shale DFM at 300 mg/m^3. A dose response was suggested by the reduced severity of nephrotoxicity in male rats exposed to 50 mg/m^3 Shale DFM. Furthermore, the absence of significant renal changes in male rats exposed to Petroleum DFM at 50 mg/m^3 indicates that this concentration is near a "no effect" level for male rat renal toxicity. Comparison of the effects observed with Petroleum DFM with those observed with Shale DFM suggest only minor differences between the two materials.

THE EVALUATION OF THE ONCOGENIC POTENTIAL OF OTTO FUEL II

In September of 1980 the Toxic Hazards Research Unit initiated a 1-year industrial-type inhalation exposure of laboratory animals to Otto Fuel II. Exposure of rats and mice terminated on schedule in September of 1981. Exposure of dogs terminated in November 1981 after an additional 2 months on study. This additional exposure was conducted to investigate rapid decreases in hematologic values which occurred during the latter portion of the exposure.

Otto Fuel II is used by the U. S. Navy as a liquid propellant in torpedoes and other weapon systems. The chief component (approximately 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 most concern is PGDN, which is a potent vasodilator and has been shown to produce severe headaches in humans at low vapor concentrations (Stewart et al., 1974). This study was undertaken to investigate long-term effects of chronic exposure to low concentrations of inhaled vapors.

Background information for this study was presented in a previous annual report (MacEwen and Vernot, 1981). Pertinent

Otto Fuel II generation and monitoring data are also described in that report. Briefly, male and female beagle dogs, Fischer 344 rats, and C57BL/6 mice were exposed to 1.4 mg/m^3 Otto Fuel II vapor. Additional groups of male and female rats and mice were exposed to 240 mg/m^3 Otto Fuel II vapor. Exposures were conducted in Thomas Dome Inhalation Chambers on a 6 hour/day, 5 day/week basis. Exposures were not conducted on weekends or holidays. At the completion of 1 year of exposure, 10 male and 10 female rats and 10 male and 10 female mice from each experimental group were randomly chosen for necropsy. All dogs were sacrificed at the conclusion of the exposure phase. Remaining rodents were held for a 1-year postexposure observation period. At the time of necropsy, tissues were collected for histopathologic examination. Rat blood was also collected for hematologic and clinical chemistry tests. Liver, kidney, and spleen weights were recorded on all rats and dogs sacrificed. Body weights were recorded regularly through the course of the study. Results of these various observations were presented in previous annual reports (MacEwen and Vernot, 1981, 1982, and 1983).

During the past year the examination of tissues collected from the animals in the study was completed. Because of the large number of rodents in the study, the usual practice of reading all tissues from all animals was altered slightly. All tissues from the control and high dose rodents were examined. In the low dose group, the examination was reduced to include all lungs, livers, kidneys, as well as gross lesions noted at necropsy. In addition, all tissues considered to be demonstrating an exposure related increased incidence, based on examination of the control and high dose groups, were examined in the low dose group.

The discussion for each animal species consists of 2 parts. The first section includes animals which died during the 1-year exposure or were sacrificed immediately following exposure. The second section includes animals that died during the postexposure observation period or were killed for histologic examination after 2 years on the study.

RESULTS

Dogs

Dogs exposed to 1.4 mg/m^3 Otto Fuel II demonstrated no tissue changes that were considered to be exposure related. During the later period of exposure the dogs exposed to 1.4 mg/m^3 Otto Fuel II exhibited reduced red blood cell counts, hematocrit and hemoglobin levels. To further examine this phenomenon, special

bone marrow stains were prepared to determine myeloid:erythroid ratios. Unfortunately, the technical quality of these preparations was poor making the examination of results unreliable. However, examination of the bone marrow taken in routine bone sections did not suggest any unusual effects on the blood forming cells.

Mice

The results of the microscopic examination of tissues collected from mice exposed to Otto Fuel II are presented in Table 24. Only the most frequently observed lesions are noted. At exposure termination, chronic ulcerative dermatitis was recorded in 44% of female control mice and in none of the exposed subjects. These skin lesions are common in adult mice. Hyaline degeneration of the nasal epithelium was also recorded more frequently in control mice than in the high dose subjects. Granulocytic hyperplasia of the bone marrow was also significantly more prevalent in control mice. In C57BL/6 mice, this lesion is often associated with chronic ulcerative dermatitis, a skin disease of obscure etiology. Hyaline degeneration of gallbladder epithelium was noted with increased frequency in control mice. This change may be associated with increased inflammatory disease in the control group, and is also considered a common finding in aging mice. Hepatocellular fatty change was noted with a greater frequency in control mice. Often this change can be associated with chronic ulcerative dermatitis in mice. In contrast, hepatocellular cytoplasmic vacuolization was recorded exclusively in exposed mice. At the light microscopic level, cytoplasmic vacuolization is attributed to increased cytoplasmic glycogen and probably was related to an abbreviated fasting period just prior to sacrifice and not to any pathologic event.

Lesions observed at the conclusion of the 1-year postexposure observation period were generally consistent with those observed at exposure termination (Table 24). In addition, extramedullary hematopoiesis in the spleen was observed more frequently in controls and probably was related to inflammatory lesions elsewhere in the body. Splenic lymphoid hyperplasia was noted to be slightly more common in mice exposed to 240 mg/m^3 Otto Fuel II. This finding may have represented early lymphoma which is very common in older C57BL/6 mice. Both atrophy and focal hyperplasia were common in the adrenal cortex of older male mice assigned to all experimental groups. These lesions were thought to be spontaneous aging changes and unrelated to Otto Fuel II exposure.

All other tissue changes noted in mice examined at either exposure termination or 1-year postexposure demonstrated no relationship to exposure.

TABLE 24. LESIONS OBSERVED IN MICE EXPOSED TO OTTO FUEL II INTERMITTENTLY FOR 1 YEAR[a]

	Exposure Termination					
	Male			Female		
	Control	1.4 mg/m^3	240 mg/m^3	Control	1.4 mg/m^3	240 mg/m^3
Skin						
Ulcerative dermatitis	3/21 (14)	2/16 (13)	2/17 (12)	8/18 (44)	0/18 (0)[b]	0/24 (0)[b]
Nasal						
Hyaline degeneration	11/21 (52)	7/16 (44)	1/14 (7)[b]	13/17 (76)	8/15 (53)	4/18 (22)[b]
Bone Marrow						
Granulocytic hyperplasia	13/20 (65)	3/13 (23)[c]	1/14 (7)[b]	12/16 (67)	0/14 (0)[b]	0/18 (0)[b]
Liver						
Fatty change	12/21 (57)	2/15 (13)[c]	5/15 (33)	10/17 (59)	9/16 (56)	3/20 (15)[b]
Vacuolization	0/21 (0)	3/15 (20)	3/15 (20)	0/17 (0)	3/16 (19)	9/20 (38)[b]
Gall Bladder						
Hyaline degeneration	6/19 (29)	1/12 (8)	0/11 (0)	3/16 (19)	3/12 (25)	1/14 (7)

	One Year Postexposure					
	Male			Female		
	Control	1.4 mg/m^3	240 mg/m^3	Control	1.4 mg/m^3	240 mg/m^3
Skin						
Ulcerative dermatitis	5/79 (6)	3/59 (5)	5/83 (6)	17/82 (21)	9/57 (16)	11/75 (15)
Mammary Gland						
Cystic Hyperplasia	0/79 (0)	[d]	0/75 (0)	28/82 (34)	[d]	4/75 (5)[b]
Bone						
Osteosarcoma	0/70 (0)	[d]	0/82 (0)	0/76 (0)	[d]	1/70 (1)
Osteosclerosis	0/70 (0)	[d]	1/82 (1)	0/76 (0)	[d]	0/70 (0)
Bone Marrow						
Granulocytic Hyperplasia	30/70 (43)	[d]	6/81 (7)[b]	26/74 (35)	[d]	12/69 (17)[c]
Liver						
Fatty Change	39/79 (49)	28/57 (49)	54/82 (66)	52/81 (64)	19/56 (34)[b]	39/75 (52)
Spleen						
Hematopoiesis	24/76 (32)	4/14 (29)	16/79 (20)	49/78 (63)	15/35 (43)	23/73 (32)[b]
Lymphoid Hyperplasia	2/76 (3)	2/14 (14)	15/79 (19)[b]	2/78 (2)	3/35 (9)	7/73 (10)
Adrenal Gland						
Atrophy	12/74 (16)	36/42 (86)[b]	15/72 (21)	0/80 (0)	0/9 (0)	1/72 (1)
Salivary Gland						
Lymphocytic infiltrates	45/73 (62)	[d]	47/81 (57)	38/74 (51)	[d]	35/72 (49)

[a] Number observed/Number examined (%).
[b] Different from control, $p < 0.01$.
[c] Different from control, $p < 0.05$.
[d] Tissues not examined.

Rats

Lesions observed in rats at exposure termination are shown in Table 25. The table has been abbreviated to exclude most of the common spontaneous lesions showing no exposure relationship. At exposure termination, a modest number of rats assigned to both the low and high dose exposure groups exhibited mild hyaline degeneration of the nasal epithelium. This is a common aging change in rats, and it is considered to be of little pathologic significance. Very slight increases in pulmonary inflammatory

TABLE 25. LESIONS OBSERVED IN RATS EXPOSED TO OTTO FUEL II INTERMITTENTLY FOR 1 YEAR[a]

	Exposure Termination					
	Male			Female		
	Control	1.4 mg/m³	240 mg/m³	Control	1.4 mg/m³	240 mg/m³
Nasal						
Hyaline degeneration	0/15 (0)	5/15 (30)[b]	5/11 (45)[c]	0/17 (0)	3/10 (30)	2/13 (15)
Lung						
Perivascular cuffing	1/15 (7)	4/15 (27)	1/11 (9)	1/17 (6)	1/10 (10)	6/13 (46)[b]
Alveolar macrophages	0/15 (0)	1/15 (7)	1/11 (9)	0/17 (0)	0/10 (0)	0/13 (0)
Congestion	3/15 (30)	3/15 (2)	0/15 (0)	3/17 (18)	0/10 (0)	2/13 (15)
Lymphocytic infiltration	0/15 (0)	2/15 (13)	0/15 (0)	0/17 (0)	0/10 (0)	0/13 (0)

	One Year Postexposure					
	Male			Female		
	Control	1.4 mg/m³	240 mg/m³	Control	1.4 mg/m³	240 mg/m³
Nasal						
Hyaline degeneration	18/82 (22)	[d]	23/87 (26)	51/83 (61)	[d]	59/85 (69)
Bone						
Osteosclerosis	0/78 (0)	0/55 (0)	0/85 (0)	10/80 (13)	5/61 (8)	24/83 (29)[b]
Osteosarcoma	0/78 (0)	1/55 (2)	2/85 (2)	0/80 (0)	0/61 (0)	0/83 (0)
Osteoma	0/78 (0)	0/55 (0)	0/85 (0)	0/80 (0)	1/61 (2)	0/83 (0)
Spleen						
Mononuclear cell, leukemia	12/82 (15)	6/58 (10)	5/88 (6)	22/82 (27)	19/65 (29)	6/87 (7)[c]
Hemosiderosis	7/82 (9)	1/58 (2)	8/88 (9)	34/82 (41)	5/65 (8)[c]	53/87 (61)[b]
Uterus						
Adenocarcinoma	-	-	-	24/81 (30)	3/65 (5)[c]	6/85 (7)[c]
Endometrial Stromal Polyp	-	-	-	3/81 (4)	7/65 (11)	12/85 (14)

[a] Number observed/Number examined (%).
[b] Different from control, $p < 0.05$.
[c] Different from control, $p < 0.01$.
[d] Tissues not examined.

changes (including perivascular cuffing, lympocytic infiltrates, chronic interstitial inflammation and alveolar macrophages) were noted in exposed subjects, but not in a dose-related fashion. These minor changes suggest that Otto Fuel II might be a very mild pulmonary irritant or predispose to secondary infectious diseases.

Changes noted in rats 1-year postexposure are also shown in Table 25. Primary bone neoplasms (1 osteoma and 3 osteosarcomas) were observed in exposed rats whereas no skeletal tumors were recorded in control subjects. Osteosclerosis (hyperostosis) of cortical bone was also noted with modest frequency in the high dose females. This change is common in aging Fischer 344 females and probably is related to increased levels of estrogenic hormones. Mononuclear cell leukemia (large granular lymphocyte leukemia) was observed with diminished frequency in both male and female exposed rats. This hematopoietic neoplasm is very common in aging rats, and the decreased incidence in exposed subjects

suggests that Otto Fuel II may have some protective effect. Splenic hemosiderosis was frequently diagnosed in both control and high dose females. This is considered to be a common aging change. Uterine (endometrial) adenocarcinomas were recorded in 30% of the control females whereas only 7% of the high dose females exhibited this neoplasm. Tumor incidences in both groups appeared to be within the range of accepted variability for uterine adenocarcinomas in Fischer 344 rats. In contrast to the decreasing trend for uterine adenocarcinomas in exposed rats, endometrial stromal polyps were slightly increased in exposed females, 14% in high dose subjects compared with 11% in low dose females and 4% in controls. Endometrial stromal polyps are common in older Fischer 344 rats. All other changes noted in male and female rats from the study were considered to be unrelated to Otto Fuel II exposure.

DISCUSSION

The results of the microscopic examination of tissues collected from rodents indicated no clear evidence of substantial non-neoplastic changes. In addition, no strong tumorigenic potential was demonstrated in rats or mice exposed for 1 year to Otto Fuel II vapors at a very high concentration near the saturated vapor value. Primary bone tumors in rats are generally considered rare. The occurrence of two such tumors in both the low dose and high dose rat groups, with none in the controls, suggests a potential relationship with Otto Fuel II exposure. However, the low frequency and absence of a dose response relationship, in view of the drastic difference in exposure concentrations, implies that the development of these neoplasms was probably unrelated to exposure.

A recent publication by Dacre et al. (1979) indicated that rats fed 1% trinitroglycerin (TGN) developed a significantly greater number of liver tumors compared to controls. TGN is closely related in structure to 1-2 propylene glycol dinitrate (PGDN), the major component of Otto Fuel II. This study has demonstrated that despite the similarity in structure of these 2 compounds, 1 year of intermittent inhalation exposure to concentrations of PGDN up to 240 mg/m^3 produced no significant liver tumors in rats or mice.

THE EXPERIMENTAL DETERMINATION OF THE ONCOGENIC EFFECTS OF 1-YEAR EXPOSURE TO PETROLEUM JP-4 VAPOR

A study was designed to compare the tumorigenic potential of inhaled Petroleum JP-4 fuel vapor with that from Shale Oil derived JP-4 fuels since shale oils have been reported to be more potent carcinogens than petroleum oils when painted on mouse skin.

Mice and rats were exposed to JP-4 concentrations of 5000 mg/m^3 and 1000 mg/m^3 by the inhalation route in Thomas Dome inhalation chambers for 1 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.

Following the exposure period, 10% of the rodents from each group were sacrificed while the remaining rodents were held for postexposure observation for 1 additional year.

The experimental protocol for the 1-year inhalation exposure of rats and mice to Petroleum JP-4 vapor can be found in a previous annual report (MacEwen and Vernot, 1980). Body weight data as well as results of clinical tests through the 1-year postexposure period are detailed in a later annual report (MacEwen and Vernot, 1983).

Histopathologic evaluation of the tissues harvested at all sacrifice periods is presently taking place. It is anticipated that an evaluation of these data will be available for the next annual report.

NINETY-DAY CONTINUOUS INHALATION EXPOSURE TO PETROLEUM JP-4 JET FUEL

Petroleum derived JP-4 was selected as part of a comparative series of studies on the chronic effects of inhaled hydrocarbon fuels. Following the exposure portion of this study all dogs and one third of the rodents were necropsied to assess chronic toxicity effects in primary tissues. The remaining rodents were held for an additional 19 months at which time half the remaining rodents were killed. The final sacrifice of rodents took place at 21 months postexposure (2 years on study). Each exposure and control group of animals consisted of 75 male and 75 female

Fischer 344 rats, 150 female C57BL/6 mice, and 6 purebred beagle dogs equally divided by sex.

Information on the experimental protocol, methodology of inhalation exposure, and clinical data obtained during the 90-day exposure phase was given in a previous annual report (MacEwen and Vernot, 1980). Clinical observations during the postexposure observation period and histopathologic evaluation of the exposed animals necropsied at the end of the 90-day exposure phase were presented in a later annual report (MacEwen and Vernot, 1984).

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

NINETY-DAY CONTINUOUS INHALATION EXPOSURE OF RATS AND MICE TO SHALE JP-4 JET FUEL

The Air Force has embarked on a test and evaluation program to ensure the compatibility of shale derived turbine fuels with aircraft systems when introduced operationally at Air Force bases in late 1983. Shale derived JP-4 is included in this program.

Since the first test fuel supplied by Geokinetics Corporation and the fuel to be used later in the operational validation program will be produced from different shale crudes and at two different refineries, it was decided to perform acute and subchronic toxicity studies with both fuels. Studies performed with the Geokinetics JP-4 test fuel was to provide the data base for environmental and health assessments at the first operational base and allow comparison of source variability on bioassay results. Longer term oncogenic studies will be conducted on the operational validation fuel to assess the chronic toxicity and the carcinogenic properties of a fuel derived and processed differently from existing aircraft hydrocarbon fuels.

Previous studies of hydrocarbon fuels have shown histopathologic changes in the kidneys of male rats at exposure termination (MacEwen and Vernot, 1978, 1981). Due to the similarity of the fuels, it was highly likely that renal damage would result in male rats exposed to Shale JP-4. In order to further evaluate this renal toxicity, routine urinalysis and serial sacrifices of male rats for tissue examination were included in this study. Sampling in this manner would allow for microscopic evaluation of kidney tissue alterations prior to the onset of chronic nephropathy common to older rats.

This study was designed to determine the toxic effects of a 90-day continuous exposure of rats and mice to Shale derived JP-4 vapor for comparison with the previous 90-day Petroleum JP-4 study (MacEwen and Vernot, 1980). The conditions were selected to conform with the other fuel studies conducted in the THRU laboratory.

The JP-4 Shale fuel used was a complex mixture of aliphatic and aromatic hydrocarbon compounds including various additives. Minor revisions from Petroleum JP-4 military specifications were made to the procurement specification requirements. These are:

Aromatic content (min) - 9% (by volume)
Nitrogen (max) - 20 ppm by weight

Mice and rats were exposed to 500 mg/m^3 and 1000 mg/m^3 Shale JP-4 vapor on a continuous basis for 90 days. For these purposes, two Thomas Dome chambers were utilized. Sham exposed controls were maintained in a separate but identical chamber. Each chamber housed 95 male and 75 female Fischer 344 rats and 100 male and 100 female C57BL/6 mice.

The exposures of rats and mice began in December 1983 and terminated in March 1984. Following completion of the 90-day exposure, 15 rats and 25 mice of each sex were killed and tissue collected for examination. Additional samplings were conducted (10 male rats per group) at 2 weeks, 2 months, and 9 months post-exposure. All remaining animals will be necropsied during the 24th month of the study (December 1985).

At each of the interim sacrifice periods, blood and urine samples were also collected from the rats for examination. Rat organ weights were measured at exposure termination and at 9 months postexposure. Body weights of the rats and mice were routinely measured during the course of the exposure and are also being followed postexposure.

The last annual report (MacEwen and Vernot, 1984) details the experimental protocol for the 90-day inhalation exposure of rats and mice to Shale derived JP-4 fuel and gives results of exposure effects on urine, blood, and organ weights of animals killed at exposure termination and at 2 weeks postexposure.

Results

Mean body weights for the rat groups obtained on a biweekly schedule through 90 days of exposure and monthly thereafter are

shown in Tables 26 and 27. The Shale JP-4 exposed male rats show a dose-related depression in weight which began at the first exposure weighing and continues to date. Weight depression in the Shale JP-4 exposed female rats did not become significant until the end of the 90-day exposure and the difference was not dose-related.

TABLE 26. BODY WEIGHTS[a] OF MALE FISCHER 344 RATS EXPOSED TO SHALE JP-4 FOR 90 DAYS

Weighing Period	Control	500 mg/m^3	1000 mg/m^3
Exposure			
0 Days	211 ± 1.0	210 ± 1.0	210 ± 2.0
2 Weeks	248 ± 1.0	242 ± 1.0[b]	238 ± 1.0[b]
4 Weeks	271 ± 2.0	266 ± 1.0[b]	256 ± 1.0[b]
6 Weeks	282 ± 2.0	274 ± 2.0[b]	271 ± 2.0[b]
8 Weeks	298 ± 2.0	292 ± 2.0[b]	288 ± 2.0[b]
10 Weeks	308 ± 2.0	306 ± 2.0[c]	302 ± 2.0[b]
12 Weeks	317 ± 2.0	316 ± 2.0	307 ± 2.0[b]
Postexposure			
2 Weeks	329 ± 2.0	324 ± 2.0[b]	320 ± 2.0[b]
1 Month	350 ± 2.0	347 ± 2.0[b]	341 ± 2.0[b]
2 Months	372 ± 3.0	366 ± 3.0[b]	363 ± 2.0[b]
3 Months	392 ± 3.0	387 ± 3.0[b]	383 ± 3.0[b]
4 Months	410 ± 3.0	404 ± 3.0[b]	402 ± 3.0[b]
5 Months	418 ± 3.0	411 ± 3.0[b]	405 ± 4.0[b]
6 Months	426 ± 3.0	422 ± 3.0[b]	416 ± 3.0[b]
7 Months	445 ± 3.0	438 ± 3.0[b]	433 ± 3.0[b]
8 Months	448 ± 3.0	439 ± 3.0[b]	432 ± 3.0[b]
9 Months	454 ± 4.0	446 ± 4.0[b]	440 ± 3.0[b]
10 Months	455 ± 4.0	444 ± 3.0[b]	439 ± 3.0[b]
11 Months	455 ± 4.0	445 ± 4.0[b]	436 ± 4.0[b]

[a] Mean (grams) ± SE.
[b] Different from control, $p < 0.01$.
[c] Different from control, $p < 0.05$.

TABLE 27. BODY WEIGHTS[a] OF FEMALE FISCHER 344 RATS EXPOSED TO SHALE JP-4 FOR 90 DAYS

Weighing Period	Control	500 mg/m³	1000 mg/m³
Exposure			
0 Days	137 ± 1.0	133 ± 1.0[b]	136 ± 1.0
2 Weeks	156 ± 1.0	155 ± 1.0	152 ± 1.0[b]
4 Weeks	162 ± 1.0	162 ± 1.0	162 ± 1.0
6 Weeks	167 ± 1.0	163 ± 1.0[b]	165 ± 1.0
8 Weeks	174 ± 1.0	172 ± 1.0	171 ± 1.0[b]
10 Weeks	177 ± 1.0	176 ± 1.0	174 ± 1.0
12 Weeks	177 ± 1.0	177 ± 1.0	175 ± 1.0
Postexposure			
2 Weeks	190 ± 1.0	185 ± 1.0[b]	186 ± 1.0[b]
1 Month	198 ± 1.0	191 ± 1.0[b]	190 ± 1.0[b]
2 Months	205 ± 1.0	197 ± 1.0[b]	199 ± 1.0[b]
3 Months	211 ± 1.0	204 ± 1.0[b]	206 ± 1.0[b]
4 Months	217 ± 1.0	210 ± 1.0[b]	211 ± 1.0[b]
5 Months	221 ± 2.0	212 ± 1.0[b]	215 ± 1.0[b]
6 Months	224 ± 2.0	217 ± 1.0[b]	220 ± 2.0[b]
7 Months	236 ± 2.0	228 ± 2.0[b]	231 ± 2.0[b]
8 Months	237 ± 2.0	229 ± 2.0[b]	231 ± 2.0[b]
9 Months	246 ± 3.0	237 ± 2.0[b]	240 ± 2.0[b]
10 Months	249 ± 3.0	238 ± 2.0[b]	244 ± 3.0[b]
11 Months	247 ± 3.0	238 ± 2.0[b]	243 ± 3.0[b]

[a] Mean (grams) ± SE.
[b] Different from control, $p < 0.01$.

Blood values from male rats sacrificed at 2 months postexposure and from male and female rats sacrificed at 9 months postexposure are summarized in Tables 28, 29, and 30, respectively. Blood values determined in the male rats sacrificed at 2 months postexposure all are within normal limits for Fischer 344 male rats.

The blood values from both male and female rats appear to be within normal parameters. Although statistically significant differences are noted, the variations are within normal biological limits.

TABLE 28. MALE FISCHER 344 RAT BLOOD PARAMETERS[a] 2 MONTHS AFTER 90-DAY EXPOSURE TO SHALE DERIVED JP-4 VAPORS

	Control	500 mg/m^3	1000 mg/m^3
WBC (x10^3 cells/mm^3)	5.9 ± 0.3	5.9 ± 0.3	5.5 ± 0.2
RBC (x10^6 cells/mm^3)	8.40 ± 0.21	8.33 ± 0.19	8.20 ± 0.20
HGB (gm/dl)	15.4 ± 0.4	15.2 ± 0.3	15.0 ± 0.3
HCT (%)	42.1 ± 1.0	41.6 ± 1.0	40.7 ± 1.0
MCV (μm^3)	50.2 ± 0.2	50.5 ± 0.2	49.7 ± 0.1
MCH (pg)	18.3 ± 0.1	18.2 ± 0.1	18.3 ± 0.1
MCHC (gm/dl)	36.6 ± 0.2	36.5 ± 0.3	36.8 ± 0.3
BUN (mg/dl)	18.7 ± 1.2	20.0 ± 0.3	18.5 ± 0.6
Creatinine (mg/dl)	0.6 ± 0.03	0.6 ± 0.03	0.5 ± 0.03

[a] Mean ± SE, N = 10.

TABLE 29. MALE FISCHER 344 RAT BLOOD PARAMETERS[a] 9 MONTHS AFTER EXPOSURE TO SHALE DERIVED JP-4 VAPORS FOR 90 DAYS

	Control[b]	500 mg/m^3	1000 mg/m^3
WBC (x10^3 cells/mm^3)	7.4 ± 0.5	7.2 ± 0.3	7.8 ± 0.4
RBC (x10^6 cells/mm^3)	9.17 ± 0.14	9.21 ± 0.2	9.14 ± 0.1
HGB (g/dl)	16.0 ± 0.2	16.1 ± 0.4	16.2 ± 0.3
HCT (%)	44.9 ± 0.7	45.4 ± 1.0	45.6 ± 0.9
MCV (μm^3)	48.9 ± 0.3	49.3 ± 0.2	49.9 ± 0.4[d]
MCH (pg)	17.4 ± 0.2	17.5 ± 0.1	17.7 ± 0.1
MCHC (g/dl)	35.6 ± 0.3	35.5 ± 0.2	35.4 ± 0.2
Glucose (mg/dl)	196 ± 17	224 ± 26	254 ± 23
Tot. Pro. (g/dl)	7.2 ± 0.1	7.0 ± 0.1	7.1 ± 0.1
Albumin (g/dl)	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
Globulin (g/dl)	6.2 ± 0.1	6.0 ± 0.1	6.1 ± 0.1
A/G Ratio	0.157 ± 0.001	0.160 ± 0.002	0.157 ± 0.004
BUN (mg/dl)	20.1 ± 0.8	17.6 ± 0.5	17.5 ± 0.5[c]
Creatinine (mg/dl)	0.70 ± 0.04	0.74 ± 0.05	0.70 ± 0.02
Calcium (mg/dl)	11.2 ± 0.2	10.9 ± 0.1	11.3 ± 0.2
SGOT (IU/L)	150 ± 14	115 ± 5[d]	112 ± 8[d]
SGPT (IU/L)	84 ± 4	68 ± 4[c]	65 ± 5[d]
Alk. Phos. (IU/L)	114 ± 7	98 ± 5	92 ± 5[d]
Bilirubin (mg/dl)	0.12 ± 0.01	0.10 ± 0.0	0.11 ± 0.01

[a] Mean ± SE, N = 10.
[b] N = 9 in control group.
[c] Different from control, $p < 0.05$.
[d] Different from control, $p < 0.01$.

TABLE 30. FEMALE FISCHER 344 RAT BLOOD PARAMETERS[a] 9 MONTHS AFTER EXPOSURE TO SHALE DERIVED JP-4 VAPORS FOR 90 DAYS

	Control[b]	500 mg/m^3	1000 mg/m^3
WBC (x10^3 cells/mm^3)	4.1 ± 0.3	4.2 ± 0.2	4.2 ± 0.3
RBC (x10^6 cells/mm^3)	7.34 ± 0.29	8.09 ± 0.13[b]	7.98 ± 0.14
HGB (g/dl)	15.7 ± 0.3	16.1 ± 0.2	16.0 ± 0.2
HCT (%)	39.3 ± 1.6	43.6 ± 0.7	42.9 ± 0.8
MCV (μm^3)	53.5 ± 0.2	53.9 ± 0.1	53.8 ± 0.1
MCH (pg)	21.7 ± 1.0	19.9 ± 0.1	20.0 ± 0.2
MCHC (g/dl)	40.5 ± 1.8	36.9 ± 0.3	37.3 ± 0.3
Glucose (mg/dl)	232 ± 17	209 ± 14	248 ± 20
Tot. Pro. (g/dl)	8.39 ± 0.06	8.14 ± 0.11	7.99 ± 0.11[b]
Albumin (g/dl)	1.18 ± 0.01	1.15 ± 0.02	1.11 ± 0.02[b]
Globulin (g/dl)	7.20 ± 0.06	6.98 ± 0.09	6.88 ± 0.12
A/G Ratio	0.164 ± 0.001	0.165 ± 0.002	0.161 ± 0.002
BUN (mg/dl)	21.7 ± 1.0	19.9 ± 0.7	22.7 ± 1.0
Creatinine (mg/dl)	0.76 ± 0.03	0.74 ± 0.02	0.65 ± 0.02[b]
Calcium (mg/dl)	11.8 ± 0.1	11.5 ± 0.1	11.1 ± 0.2[c]
SGOT (IU/L)	87 ± 3.0	87 ± 5.0	80 ± 3.0
SGPT (IU/L)	55 ± 3.0	54 ± 2.0	55 ± 2.0
Alk. Phos. (IU/L)	93 ± 7.0	86 ± 5.0	84 ± 5.0
Bilirubin (mg/dl)	0.17 ± 0.02	0.18 ± 0.02	0.17 ± 0.02

[a] Mean ± SE (N).
[b] Different from control, $p < 0.05$.
[c] N = 8.

A summary of the organ weights of male and female rats sacrificed at 9 months postexposure is given in Tables 31 and 32, respectively. No significant differences were noted in any of the measured parameters in either sex of rats. Significant differences in the male kidney and liver weights previously seen at the sacrifice immediately following the 30-day exposure are no longer evident.

Values from urine samples collected from the male rats at the 2 and 9-month postexposure sacrifices were statistically analyzed for differences in osmolality and pH. A significant decrease in osmolality was seen in both test groups at each examination period when compared to the control values (Table 33). This is consistent with effects seen at exposure termination and at the 2-week interim sacrifice periods. No consistent pH differences were noted among the groups as shown in Table 34.

TABLE 31. BODY AND ORGAN WEIGHTS OF MALE FISCHER 344 RATS[a] EXPOSED TO SHALE JP-4 VAPORS FOR 90 DAYS; 9 MONTHS POSTEXPOSURE

Treatment Group	Mean Weight in Grams				% of Body Weight		
	Body	Spleen	Liver	Kidney	Spleen	Liver	Kidney
Control	438	0.73	11.90	3.12	0.17	2.72	0.71
500 mg/m^3	434	0.78	12.04	3.17	0.18	2.77	0.73
1000 mg/m^3	424	0.77	11.87	3.18	0.18	2.81	0.75

[a] N = 10.

TABLE 32. BODY AND ORGAN WEIGHTS OF FEMALE FISCHER 344 RATS[a] EXPOSED TO SHALE JP-4 VAPORS FOR 90 DAYS; 9 MONTHS POSTEXPOSURE

Treatment Group	Mean Weight in Grams				% of Body Weight		
	Body	Spleen	Liver	Kidney	Spleen	Liver	Kidney
Control	235	0.51	6.20	1.80	0.22	2.65	0.77
500 mg/m^3	227	0.52	6.14	1.80	0.23	2.71	0.79
1000 mg/m^3	232	0.51	6.13	1.74	0.22	2.66	0.75

[a] N = 10.

TABLE 33. URINARY OSMOLALITY VALUES OF MALE FISCHER 344 RATS EXPOSED TO SHALE JP-4 VAPORS

Treatment mg/m^3	Osmolality[a]				
	0 Days	90 Days	2 Wks Post	2 Mos Post	9 Mos Post
Control	1497 ± 100	1197 ± 109	1944 ± 206	2030 ± 125	1395 ± 129
500	1877 ± 174	882 ± 93[b]	1129 ± 91[c]	1238 ± 91[c]	961 ± 81[c]
1000	1645 ± 208	785 ± 69[c]	1126 ± 59[c]	1249 ± 77[c]	935 ± 110[c]

[a] Mean ± SE (expressed as millismols/liter), N = 10.
[b] Different from control, $p < 0.05$.
[c] Different from control, $p < 0.01$.

TABLE 34. URINARY pH VALUES OF MALE FISCHER 344 RATS EXPOSED TO SHALE JP-4 VAPORS

Treatment mg/m^3	pH Value[a]				
	0 Days	90 Days	2 Wks Post	2 Mos Post	9 Mos Post
Control	7.30 ± 0.15	6.75 ± 0.13	6.55 ± 0.05	8.30 ± 0.81	6.8 ± 0.10
500	7.35 ± 0.15	6.95 ± 0.16	6.70 ± 0.11	8.10 ± 0.15	7.2 ± 0.10[c]
1000	7.60 ± 0.15	7.05 ± 0.16	6.85 ± 0.08[b]	8.45 ± 0.50	6.9 ± 0.20

[a] Mean ± SE, N = 10.
[b] Different from control, $p < 0.05$.
[c] Different from control, $p < 0.01$.

This study is scheduled for termination in December 1985. Subsequent annual reports will contain additional experimental data as they become available.

PULMONARY MECHANICS, DYNAMICS, AND GAS EXCHANGE IN MALE RATS WITH EMPHYSEMA, FIBROSIS OR PNEUMONIA COMPARED WITH RATS EXPOSED TO SHALE JP-4 FUEL

As part of the testing designed to evaluate the effects of 90-day continuous exposure to Shale derived JP-4 jet fuel, pulmonary function parameters were evaluated in male rats immediately after 87 days continuous exposure to Shale JP-4. Papain and bleomycin were instilled into the lungs of male rats to induce emphysema and fibrosis, respectively. These groups, along with one found to have viral pneumonia on receipt, were also tested to provide positive controls for the Shale JP-4 group and to evaluate the sensitivity and pattern of response of the pulmonary function tests in characterizing these diseases.

METHODS

Plethysmograph System

All pulmonary function measurements were made with a semi-automated, constant pressure plethysmograph (Figure 2) which was constructed from acrylic plastic (25.4 cm long, 6.3 cm ID) and contained a removable hemicylindrical acrylic shelf upon which the intubated animals were placed. A system of straight-through, 3/8" diameter, computer controlled (Hewlett Packard 9845B) solenoid valves (Valves 1-7 in Figure 2, VACOA, Bohemia, New York)

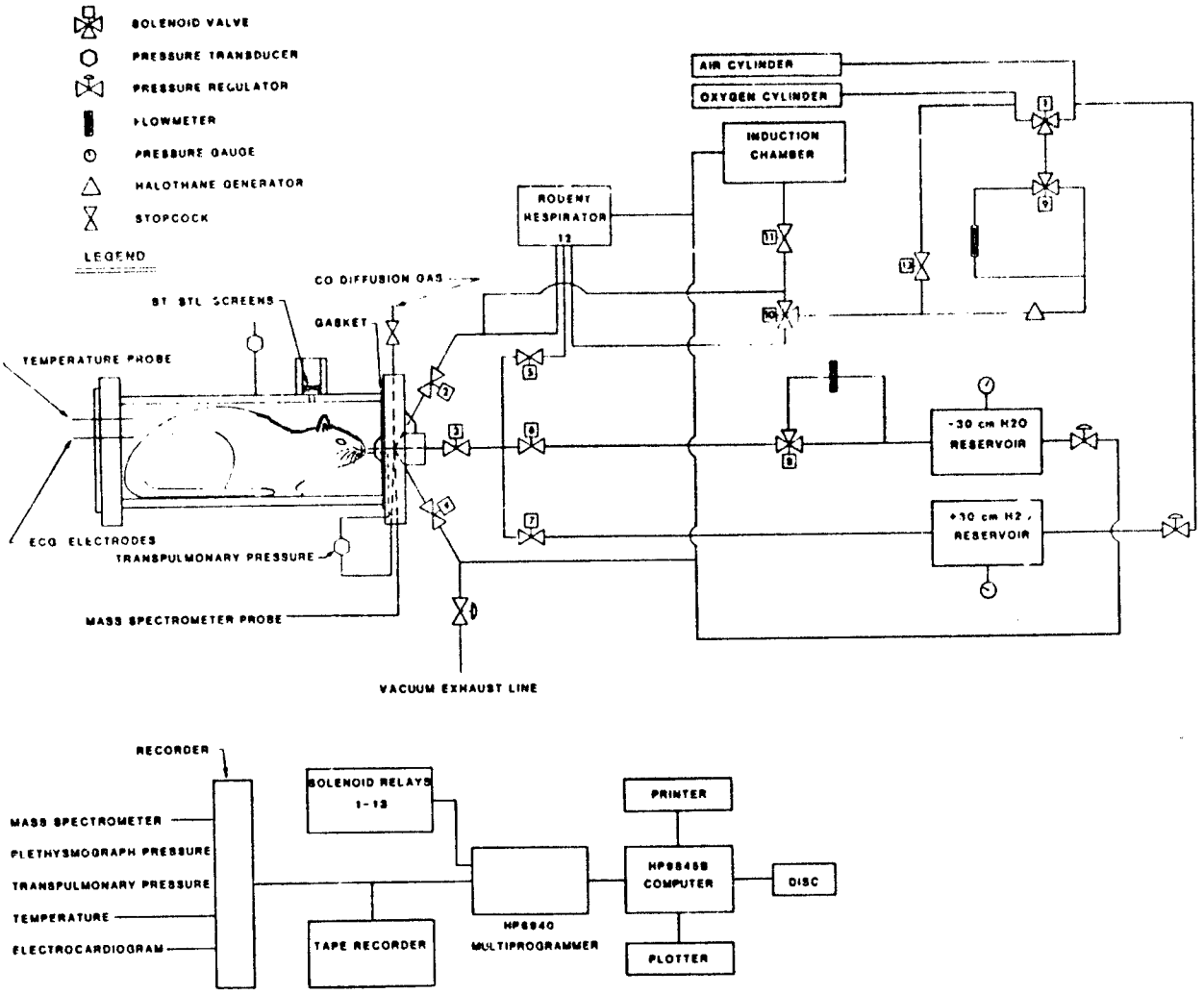


Figure 2. Computerized, semiautomated plethysmograph system for measuring pulmonary mechanics, dynamics, gas distribution, and gas exchange in rats.

and bidirectional valves (Valves 8-10; Skinner, New Britain, Connecticut) connected to the tracheal tube manifold allowed switching of the tube to: (1) a flow-by system containing a 4% Halothane-96% oxygen mixture for continued anesthesia; (2) a respirator (Harvard 680, Mills, Massachusetts) for hyperventilation; (3) positive or negative pressure reservoirs for forced maneuvers; (4) a port for carbon monoxide ($C^{18}O$) diffusion gas mixture insertion and withdrawal and a mass spectrometer probe (Balzers QMG511, Hudson, New Hampshire). The 20 L pressure reservoirs were regulated (Fairchild, Winston-Salem, North Carolina) at +30 and -30 cm H_2O, respectively. Flow rates were calculated by measuring the pressure drop (Validyne MP45 $\pm$ 2.25 cm H_2O)

across 8 layers of 400 mesh stainless steel screen (1.7 cm in diameter). Volume was obtained by computer integration. The frequency response of the plethysmograph was evaluated (Jackson and Vinegar, 1979) and the construction altered (Sinnett et al., 1981) until the gain of the plethysmograph was flat to 50 Hz.

Animal Preparation

Fischer 344 male rats (Charles River), 175-225 g, were used. Quality control examinations during the quarantine period including bacteriology, gross and histopathology, showed the animals were in good health. The animals had food (Purina Formulab #5008) and softened water (<17 ppm calcium carbonate) ad libitum. After anesthetization for 5 minutes in the Halothane-oxygen induction chamber, the rat was intubated (Nicholson and Kinkead, 1982) using a modified 14 gauge intravenous catheter (Mauderly, 1975). The rat was then placed on the hemicylindrical shelf and the intubation tube attached to the manifold. A shortened infant feeding tube (5 French) was then inserted to measure esophageal pressure (Century Technology, CP-01-DP, Inglewood, California) as an estimate of interpleural pressure. Transpulmonary pressure (PTP) was measured with the reference side of the esophageal pressure transducer connected to the airway with a second water filled catheter. The esophageal catheter was positioned within the esophagus to maximize the PTP signal. The depth of anesthesia was maintained by adjusting the Halothane concentration to produce a spontaneous breathing rate of 60 ± 5 B/M. The entire pulmonary function testing procedure took 30 minutes to perform.

Pulmonary Resistance (RL) and Compliance (Cdyn)

To produce a standard lung volume reference prior to each measurement, the respiration rate was stabilized at 60 ± 5 B/M and then the rat was hyperventilated (2 breaths: Vt = 6 mL, f = 80 B/M). After a 5 second pause, flow and PTP were sampled at 100 Hz for 10 seconds. Cdyn was calculated (Amdur and Mead, 1958) as the difference in flow integrated volume divided by the concomitant change in PTP at zero flow. RL was calculated as the difference in PTP divided by the concomitant difference in flow at points of equal volume. The RL, Cdyn, and tidal volume (VT) values were averaged over all breaths in the 10 second data acquisition period.

Diffusion Capacity (DLCO) and Lung Volumes

After again stabilizing the respiration rate at 60 ± 5 B/M, the rat was hyperventilated for 30 seconds (Vt = 6 mL, f = 80 B/M) to produce apnea and standardize the lung volume. During the apneic period, the lungs were inflated to PTP = 25 cm H_2O using a syringe attached to the tracheal tube manifold and containing 0.5% $C^{18}O$, 0.5% He, balance air. The lungs were held inflated for 6 seconds and then deflated to residual volume (RV) defined as the lung volume at PTP = -25 cm H_2O. PTP, flow, He, and $C^{18}O$ concentrations were measured through the entire maneuver using the mass spectrometer. DLCO (Takezawa et al., 1980 and Graham et al., 1981), total lung capacity (TLC), apneic expiratory reserve volume (AERV) and residual volume (RV) were calculated.

Partial Forced Vital Capacity

After stabilizing and hyperventilating to produce apnea, a partial followed immediately by a full forced vital capacity maneuver was performed (Bouhuys, 1977). The rat was sequenced through: (1) slow inspiration (3 mL/sec) to PTP = 10 cm H_2O, (2) fast expiration to PTP = -25 cm H_2O, (3) slow inspiration to PTP = 25 cm H_2O, and (4) fast expiration to PTP = -25 cm H_2O. These forced vital capacities (FVC; both partial and full) were added to the previously determined RV and the following parameters were calculated: peak expiratory flow rate (PEFR), maximum expiratory flow rates at 80% TLC (MEFR), difference in flow rates between the full and partial manuevers at 80% TLC and forced expired volume in 0.1 seconds (FEV.1).

Quasistatic Compliance and Closing Volume

After switching to a 4% halothane - 96% air mixture, stabilizing and hyperventilating to produce apnea, the flow-by system was rapidly reflushed with the halothane-oxygen mixture. A slow inspiration was then made to PTP = 25 cm H_2O followed by a slow expiration (1 mL/sec) to PTP = -25 cm H_2O. PTP, flow, and N_2 concentrations were sampled during the expiration maneuver. The slope of the transpulmonary pressure-volume curve between 0 and 7 cm H_2O was calculated. In addition, from the % N_2-volume curve, the slope of phase III was calculated by regression and the closing volume was measured as the volume at the inflection point of phase IV (Likens and Mauderly, 1982).

Emphysema, Fibrosis, and Pneumonia

To generate emphysema or fibrosis, the rats were anesthetized with 4% Halothane, intubated and one dose of 30 IU/100 g of papain (Sigma) in 0.9% saline or 0.7 IU/100 g bleomycin (Sigma) in 0.9% saline were intratracheally instilled, respectively. Control animals were dosed with 0.1 mL/100 g 0.9% saline. All animals were studied 2 weeks after dosing. The pneumonia study was performed in a group of rats found infected with rat coronavirus.

Histopathology

In order to provide rapid fixation and to maintain the dimensions and configurations of the lung tissue at approximately total lung capacity, the lungs taken for micropathologic examination were fixed with formalin for 4 hours by airway perfusion at a constant pressure of 20 cm of water. A complete horizontal section was embedded in paraffin blocks for slide preparation. The level of emphysema was quantitated by calculating the mean linear intercept (Weibel, 1963).

Data Analysis

Pulmonary function measurements were analyzed by multivariate analysis of variance for repeated measures (Dixon, 1983). Significant effects were defined as F values with $p < 0.05$.

RESULTS

Table 35 presents the results of the battery of pulmonary function tests obtained from rats intratracheally instilled with papain or bleomycin to produce emphysema or fibrosis. The bleomycin treated rats show significantly reduced lung volumes and flow rates consistent with a pattern of restrictive lung disease expected in pulmonary fibrosis. The papain treated rats show a different pattern with significant changes in lung volumes, compliance, flow rates, gas distribution, and CO diffusion consistent with the obstructive lung disease expected in emphysema.

Table 36 presents the results from a group of rats examined during the development of this battery of tests. Quality control examination of the lungs revealed mild, patchy pneumonitis typical of viral pneumonia. Serology in these animals was positive for rat coronavirus/sialodacryoadenitis.

TABLE 35. PULMONARY FUNCTION ($\bar{x}$ ± SE) IN RATS 2 WEEKS AFTER INTRATRACHEAL INSTILLATION OF 30 IU/100 G PAPAIN OR 0.7 IU/100 G BLEOMYCIN DISSOLVED IN 0.1 ML/100 G 0.9% SALINE

Variable	Control	Papain	Bleomycin
Weight	294.2 ± 5.6	282.1 ± 4.1	286.2 ± 2.1
Rdyn	0.34 ± 0.02	0.34 ± 0.04	0.30 ± 0.02
Cdyn	0.17 ± 0.01	0.19 ± 0.02	0.15 ± 0.01
Cqs	0.35 ± 0.02	0.44 ± 0.04[a]	0.34 ± 0.02
Vt	1.5 ± 0.05	1.4 ± 0.05	1.3 ± 0.05[b]
FVC	6.4 ± 0.23	5.8 ± 0.34	5.7 ± 0.21[a]
PEFR	75.7 ± 3.1	78.1 ± 3.1	77.2 ± 2.6
FEV.1/FVC	32.8 ± 1.3	34.6 ± 1.7	37.4 ± 1.6[a]
DFR 80%	12.4 ± 1.6	5.6 ± 1.7[b]	12.0 ± 1.6
CV	1.0 ± 0.09	1.3 ± 0.07[a]	0.95 ± 0.07
CC	2.81 ± 0.16	3.90 ± 0.21[b]	2.97 ± 0.18
Phase III	1.04 ± 0.05	0.87 ± 0.08	0.91 ± 0.11
DLCO	0.12 ± 0.01	0 10 ± 0.01[a]	0.10 ± 0.01[a]
DLCO/VA	0.017 ± 0.001	0.013 ± 0.001[b]	0.016 ± 0.001
TLC	9.8 ± 0.32	10.5 ± 0.55	9.1 ± 0.38
RV	1.8 ± 0.12	2.6 ± 0.21[b]	2.0 ± 0.15
AERV	0.69 ± 0.06	0.88 ± 0.05[b]	0.57 ± 0.06
MLI	58.8 ± 0.9	86.2 ± 3.6[b]	68.3 ± 1.0[b]
N	19	9	20

[a] Different from control, $p < 0.05$.
[b] Different from control, $p < 0.01$.

Where: weight (g); Rdyn: dynamic resistance at f = 60 B/M (cm H_2O/mL S); Cdyn: dynamic compliance at f = 60 B/M (mL/cm H_2O); Cqs: quasistatic compliance (mL/cm H_2O); Vt: tidal volume at f = 60 B/M; FVC: forced vital capacity; PEFR: peak expiratory flow rate (mL/s); FEV.1/FVC: forced expired volume in 0.1 second/forced vital capacity (%); DFR 80%: difference between partial and full flowrates at 80% TLC (mL/s); CV: closing volume (mL); CC: closing capacity (mL); Phase III: slope (% N_2/mL); DLCO: CO diffusion (mL CO/min mm Hg); VA: alveolar volume TLC: total lung capacity (mL); RV: residual volume (mL); AERV: expiratory reserve volume at apnea (mL); and MLI: mean linear intercept.

TABLE 36. PULMONARY FUNCTION ($\bar{x} \pm SE$) IN RATS INFECTED WITH RAT CORONAVIRUS (SEE TABLE 35 FOR PARAMETER DEFINITIONS)

Variable	Control	Infected
Weight	334 ± 14	311 ± 22[b]
Rdyn	0.24 ± 0.13	0.24 ± 0.07
Cdyn	0.50 ± 0.12	0.40 ± 0.11[b]
Cqs	1.01 ± 0.26	0.93 ± 0.25
Vt	1.66 ± 0.17	1.42 ± 0.26[b]
TLC	15.2 ± 0.8	13.7 ± 2.6[a]
RV	2.35 ± 0.78	2.49 ± 1.20
AERV	0.77 ± 0.56	0.68 ± 0.92
N	10	10

[a] Different from control, $p < 0.05$.
[b] Different from control, $p < 0.01$.

The data in Table 36 are consistent with a pattern of restrictive lung disease shown in viral pneumonia.

Table 37 presents the pulmonary function test results after 87 days of exposure to 1000 mg/m^3 Shale JP-4. Multivariate analysis found no significant difference due to the Shale JP-4 exposure.

TABLE 37. PULMONARY FUNCTION ($\bar{x} \pm SE$) AFTER A 90-DAY CONTINUOUS EXPOSURE TO 1000 MG/M^3 SHALE JP-4 (SEE TABLE 35 FOR PARAMETER DEFINITIONS)

Variable	Control	Exposed
Weight	351 ± 6	350 ± 6
Rdyn	0.24 ± 0.03	0.34 ± 0.05
Cdyn	0.36 ± 0.06	0.32 ± 0.03
Cqs	0.75 ± 0.02	0.75 ± 0.07
Vt	1.3 ± 0.07	1.1 ± 0.08
FVC	8.7 ± 0.51	9.7 ± 0.56
PEFR	66.5 ± 4.5	55.6 ± 5.1
FEV.1/FVC	25.9 ± 1.8	21.0 ± 2.1
DFR 80%	11.6 ± 6.6	6.8 ± 2.5
CV	1.1 ± 0.1	1.7 ± 0.3
CC	3.1 ± 0.3	3.7 ± 0.3
Phase III	0.55 ± 0.07	0.45 ± 0.07
DLCO	0.07 ± 0.01	0.07 ± 0.01
DLCO/VA	0.009 ± 0.001	0.009 ± 0.001
TLC	10.5 ± 0.5	10.5 ± 0.4
RV	1.9 ± 0.3	2.0 ± 0.2
AERV	0.56 ± 0.1	0.37 ± 0.1
N	13	11

DISCUSSION

Plethysmograph System

The plethysmograph system developed and depicted in Figure 2 represents the product of extensive efforts to provide an accurate, precise, and repeatable testing capability of pulmonary mechanics, dynamics, gas distribution, and gas exchange in rats. This development has included innovations developed here but has also benefited by inclusion of techniques published by others.

Use of halothane as the anesthetic agent (Mauderly, 1975) permitted control and standardization of the anesthesia level by regulating the halothane concentration to produce a breathing frequency of 60 ± 5 B/M. Such adjustable control is not possible using injections of pentobarbital for example. In addition, the rats recover from halothane anesthesia in a matter of minutes as opposed to hours for pentobarbital.

The use of modified arterial catheters as intratracheal tubes and insertion using a lighted-speculum (Nicholson and Kinkead, 1982) provided an intubation technique which was quick and repeatable. It sealed the trachea sufficiently to allow measurement of test parameters during forced maneuvers.

Construction of a pressure-wave generation device (Jackson and Vinegar, 1979) for computerized analyses of the frequency response of the plethysmograph system, permitted alterations in connectors, fittings, and the size and number of pneumotach screens to maximize the frequency response of the plethysmograph. Procurement of straight-through solenoid valves reduced the inertial loss found in more common solenoid valves and improved the response during forced maneuvers. The essentially flat response out to 50 Hz is sufficient to measure the forced maneuver flow rates which have been shown to have components up to 30 Hz (Harkema et al., 1982).

The battery of tests developed allowed assessment of pulmonary mechanics (lung volumes, quasistatic pressure-volume curve), pulmonary dynamics (resistance, compliance, partial and full forced maneuvers), gas distribution (closing volume), and gas transfer (CO diffusion). All of these tests are currently used for clinical evaluation of lung function in man and should therefore be useful in extrapolating from animal to man. Computerization of these tests allowed for test performance with quick and precise calculation of test parameters which otherwise would be too time consuming.

Shale JP-4

Aspiration of hydrocarbon substances (e.g. kerosene), leads to development of chemical pneumonitis and bronchopneumonia and, in some cases, emphysema (Scharf et al., 1981). Previous pathologic results in animals showed exposure to Shale JP-5 produced nasal inflammation and RJ-4 and RJ-5 produce pulmonary irritation (Haun, 1975). Epidemiologic results suggested jet fuel exposure induced dyspnea or feelings of "suffocation, pain upon inhalation, slight cough, or ache in the chest" (Knave et al., 1976 and Davies, 1964). All these data supported the hypothesis that Shale JP-4 could produce pulmonary dysfunction. The results of this study, however, do not show any effect of the continuous 90-day exposure to 1000 mg/m^3 Shale JP-4 on measured pulmonary function parameters.

EVALUATION OF STRAIN SUSCEPTIBILITY TO CHRONIC NEPHRITIS IN RATS EXPOSED TO SHALE DERIVED JP-4 JET FUEL

Previous subchronic inhalation studies with hydrocarbon vapors, including petroleum and shale derived jet fuels, have shown a pattern of toxic nephropathy in male rats (Carpenter et al., 1975a, 1975b, 1975c, 1975d; Gaworski et al., 1979a, 1979b, 1984; Phillips, 1982; and Bruner and Pitts, 1982). The lesions were described as greatly accentuated hyaline droplets in proximal tubular epithelium and dilated, cystic tubules near the corticomedullary junction which were plugged with necrotic cellular debris. The characteristic lesion of hydrocarbon nephropathy has not been reported in female rats or in either sex of other animal species exposed to hydrocarbon vapors.

This lesion has been described in male rats of various strains, including Sprague-Dawley, Wistar, and Fischer 344. In addition to the nephropathy, Phillips (1982) reports a significant increase in urine volume and decrease in osmolality in Fischer 344 rats following exposure to Stoddard Solvent. Bruner and Pitts (1982) postulate that one mechanism which may contribute to the nephropathy is α_{2u} globulin, a low molecular weight protein produced by the liver of male rats at puberty and readily filtered by the kidney. This protein has not been found in the livers of normal female rats. Bruner believes that this protein could be the major constituent of hyaline droplets, and factors resulting in its excessive accumulation in proximal tubular cells could contribute to the pathogenesis of hydrocarbon nephropathy.

This study was designed to determine the relative susceptibility to chronic nephropathy of 4 rat strains during and following inhalation exposure to 1000 mg/m^3 Shale derived JP-4 jet fuel. Urine metabolite analysis and protein measurement are made at selected times. These will be analyzed for correlation with the degree of chronic nephritis found in each rat species. The exposure conditions were selected to conform with previous fuel studies conducted in the Toxic Hazards Research Unit laboratory.

The Shale derived JP-4 sample used in this study is from the same source as that used in the Shale JP-4 90-day study previously described and was subjected to the same quality control measures.

Four strains of male rats were exposed to 1000 mg/m^3 Shale JP-4 vapor on a continuous basis for 90 days. For these purposes, two Thomas Dome inhalation chambers were utilized. One chamber contained a 1000 mg/m^3 JP-4 fuel concentration while sham exposed controls were housed in an identical chamber. Each chamber housed 60 each of the following male rat strains: Fischer 344, Wistar, Sprague-Dawley, and Osborne Mendel.

The exposures began in December 1983 and terminated in March 1984. Interim sacrifices of 10 rats per strain occurred at 45 and 90 days of exposure and at 6 and 12 months postexposure. All remaining rats will be necropsied during the 24th month of the study (December 1985).

Organ weights were measured and blood and urine samples taken after 45 and 90 days of exposure in animals scheduled for interim sacrifice. Organ weights were measured at the 6 and 12-month postexposure sacrifices. Body weights were routinely measured during the course of the exposure and are also being followed postexposure.

The previous annual report (MacEwen and Vernot, 1984) details the experimental protocol for the 90-day inhalation exposure of the 4 rat strains and gives the results of the blood, urine, and organ weight measurements of rats killed after 45 and 90 days exposure.

Results

Mean body weights for the rat strains obtained on a biweekly schedule through 90 days of exposure and monthly thereafter are shown in Table 38. The Shale JP-4 exposed Sprague-Dawley and Wistar rats began to show a statistically significant ($p < 0.01$)

TABLE 38. BODY WEIGHTS[a] OF 4 STRAINS OF MALE RATS EXPOSED TO SHALE JP-4 FOR 90 DAYS

Weighing Period		Fischer 344	Sprague-Dawley	Wistar	Osborne Mendel
0 Days	C	179.3 ± 1.3	332.6 ± 2.4	324.5 ± 2.0	279.1 ± 2.6
	T	183.9 ± 1.3	332.2 ± 2.2	323.1 ± 2.2	285.6 ± 1.4[b]
3 Weeks	C	246.5 ± 1.6	428.1 ± 3.5	429.0 ± 3.9	370.4 ± 2.5
	T	244.3 ± 1.4	410.0 ± 3.3[b]	415.8 ± 3.6[b]	368.8 ± 3.4
5 Weeks	C	265.8 ± 1.8	450.3 ± 5.3	467.8 ± 4.7	402.1 ± 2.9
	T	258.4 ± 1.6[b]	456.4 ± 4.2[b]	463.8 ± 4.5	403.2 ± 3.2
7 Weeks	C	277.5 ± 2.2	485.0 ± 5.3	495.7 ± 6.1	423.5 ± 3.8
	T	270.6 ± 1.5[b]	473.3 ± 4.9[b]	484.0 ± 5.2[b]	419.1 ± 3.8
9 Weeks	C	288.3 ± 2.3	512.4 ± 5.9	517.5 ± 6.5	440.9 ± 4.0
	T	285.3 ± 2.0	493.5 ± 5.2[b]	507.9 ± 5.5[b]	428.8 ± 4.2[b]
11 Weeks	C	297.4 ± 2.4	530.6 ± 6.6	533.9 ± 7.7	449.2 ± 4.3
	T	298.0 ± 2.3	516.9 ± 5.2[b]	517.9 ± 7.3[b]	454.8 ± 5.2
13 Weeks	C	307.9 ± 2.5	556.0 ± 6.6	562.7 ± 7.6	461.1 ± 4.7
	T	304.9 ± 2.6	538.8 ± 5.1[b]	547.7 ± 6.9[b]	466.2 ± 5.1
Postexposure					
1 Month	C	325.5 ± 2.9	577.6 ± 7.0	563.9 ± 9.3	460.3 ± 5.1
	T	322.3 ± 2.7	559.7 ± 7.1[b]	554.6 ± 6.4[b]	469.4 ± 4.5[b]
2 Months	C	350.7 ± 3.4	618.5 ± 7.7	608.8 ± 9.7	484.0 ± 5.5
	T	347.3 ± 3.0	600.5 ± 7.2[b]	590.5 ± 7.2[b]	491.3 ± 4.8[b]
3 Months	C	376.3 ± 3.4	639.0 ± 8.4	624.9 ± 10.8	495.4 ± 5.6
	T	369.7 ± 3.0[b]	622.1 ± 7.9[b]	614.6 ± 8.4[b]	505.7 ± 5.0[b]
4 Months	C	393.9 ± 3.6	667.5 ± 8.9	642.9 ± 12.4	512.2 ± 5.8
	T	384.9 ± 3.3[b]	646.8 ± 9.0[b]	635.8 ± 8.6[b]	521.0 ± 5.0[b]
5 Months	C	410.5 ± 3.7	687.2 ± 9.6	664.5 ± 13.4	526.7 ± 5.8
	T	401.4 ± 3.2[b]	662.7 ± 10.0[b]	658.2 ± 9.6	531.5 ± 5.3
6 Months	C	423.1 ± 3.9	705.5 ± 10.3	681.6 ± 13.5	539.8 ± 6.2
	T	416.0 ± 3.6[b]	685.5 ± 11.1[b]	678.4 ± 10.1	544.6 ± 5.5
7 Months	C	437.7 ± 4.6	716.5 ± 11.7	697.9 ± 18.0	543.0 ± 7.7
	T	426.2 ± 4.2[b]	699.6 ± 14.0[b]	683.4 ± 12.9[b]	551.9 ± 9.4[b]
8 Months	C	448.9 ± 4.7	732.1 ± 13.1	708.4 ± 17.9	563.6 ± 7.2
	T	437.4 ± 4.5[b]	714.0 ± 14.2[b]	694.9 ± 12.8[b]	572.1 ± 6.5[b]
9 Months	C	455.7 ± 5.0	743.8 ± 15.2	712.8 ± 18.1	564.8 ± 7.8
	T	444.3 ± 4.6[b]	714.9 ± 14.7[b]	701.9 ± 13.2[b]	573.1 ± 6.9[b]
10 Months	C	458.3 ± 5.2	760.0 ± 16.2	723.3 ± 18.0	549.9 ± 9.3
	T	440.7 ± 4.3[b]	709.4 ± 15.4[b]	700.8 ± 13.6[b]	565.8 ± 6.2[b]
11 Months	C	458.7 ± 5.0	776.1 ± 15.5	732.8 ± 18.2	557.1 ± 9.6
	T	456.3 ± 4.1[b]	724.3 ± 17.8[b]	708.3 ± 14.4[b]	571.9 ± 5.8[b]

[a] Mean, (grams) ± SE.
[b] Different from control, $p < 0.01$.
[c] C = control group, T = test group.

depression in body weight during the exposure period which has continued through the postexposure period. The fuel-exposed Fischer 344 rats have shown a depressed body weight since the third month postexposure while the exposed Osborne Mendel rats have outgained their respective controls during the postexposure period.

A summary of the organ weights of the rats sacrificed at 6 and 12 months postexposure are shown in Tables 39 and 40, respectively. At 6 months postexposure a statistically significant increase in the kidney weight to body weight ratio was seen in the Fischer 344 rats. At 12 months postexposure none of the 4 strains showed a statistically significant kidney weight effect. In previous sacrifices (45 and) days exposure) all 4 strains showed statistically significant increases in mean kidney weights as well as the ratios of kidney weight to the mean body weight.

TABLE 39. BODY AND ORGAN WEIGHTS OF 4 RAT STRAINS 6 MONTHS AFTER BEING EXPOSED TO SHALE DERIVED JP-4 VAPORS FOR 90 DAYS

Rat Strain	Treatment Group	Mean Weight in Grams				% of Body Weight		
		Body	Testis	Liver	Kidney	Testis	Liver	Kidney
F 344	Control	411	3.22	13.22	2.76	0.78	3.22	0.67
F 344	1000 mg/m^3	406	3.33	12.57	2.90	0.82	3.10	0.72[a]
S-D	Control	692	3.71	23.18	4.77	0.54	3.34	0.69
S-D	1000 mg/m^3	676	3.96	21.00	4.89	0.59	3.12	0.73
Wistar	Control	681	3.93	22.61	4.44	0.58	3.34	0.66
Wistar	1000 mg/m^3	700	3.83	21.47	4.47	0.55	3.07	0.64
O M	Control	538	4.39	18.96	4.41	0.82	3.51	0.82
O M	1000 ng/m^3	532	4.34	18.81	4.55	0.82	3.55	0.86

[a] Different from control, $p < 0.05$.

TABLE 40. BODY AND ORGAN WEIGHTS OF 4 RAT STRAINS 12 MONTHS AFTER BEING EXPOSED TO SHALE DERIVED JP-4 VAPORS FOR 90 DAYS

Rat Strain	Treatment Group	Mean Weight in Grams				% of Body Weight		
		Body	Testis	Liver	Kidney	Testis	Liver	Kidney
F 344	Control	440	3.66	13.40	3.12	0.83	3.05	0.71
F 344	1000 mg/m^3	430	3.92[a]	13.24	3.22	0.91	3.08	0.75
S-D	Control	791	3.64	23.81	4.92	0.47	3.01	0.62
S-D	1000 mg/m^3	709[a]	3.48	23.64	5.40	0.50	3.41	0.78
Wistar	Control	769	3.86	22.37	4.72	0.51	2.92	0.62
Wistar	1000 mg/m^3	660[a]	3.42[a]	22.37	5.29	0.52	3.42	0.84
O M	Control	547	4.20	20.15	4.33	0.77	3.67	0.79
O M	1000 mg/m^3	581[b]	4.50[a]	22.66	5.06	0.78	3.90	0.87

[a] Different from control, $p < 0.01$.
[b] Different from control, $p < 0.05$.

The study is continuing and further data will be presented in future annual reports of THRU activities.

THE EXPERIMENTAL DETERMINATION OF SAFE ATMOSPHERIC CONCENTRATIONS OF RJ-5

A 6-month chronic inhalation toxicity exposure to 0.15 mg/L 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 mice held 1-year postexposure following the 6-month exposure to 0.15 mg/L 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 oncology studies, Fischer 344 and C57BL/6, respectively.

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 previous annual reports (MacEwen and Vernot, 1980, 1981).

Animal body weights and results of various clinical determinations are detailed in a more recent annual report (MacEwen and Vernot, 1983). Tissues harvested at the conclusion of the study have been processed and are currently being evaluated by an independent pathology group. The results of the histologic examinations should soon be available for evaluation and interpretation and we expect to publish a technical report on this subject in the near future.

EVALUATION OF 90-DAY INHALATION TOXICITY OF PETROLEUM AND OIL SHALE JP-5 JET FUEL

These studies began in 1977 and various phases have been presented in previous annual reports. This report presents a complete summary of the relevant biological changes discussed in

the earlier reports, in addition to the results of the recently completed evaluation of the tissues obtained during the Shale JP-5 study.

Petroleum distillates have been used as large scale sources of energy for over 100 years, and since the advent of the internal combustion engine, vast quantities of distillate fractions have been introduced into man's working environment. The development of jet engines as almost universal power plants for commercial and military aircraft has led to the use of a number of petroleum distillate fuels with special properties. These are less volatile than the gasoline fractions used in conventional internal combustion engines.

Despite long industrial and environmental experience with petroleum distillates, little investigative work was done on the toxicological characteristics of these fuels until Drinker et al. (1943) exposed groups of human volunteers to known concentrations of gasoline vapor. They found that for concentrations up to 0.03% (1060 mg/m^3) the major complaint was eye irritation. When the concentration reached 0.26% (9150 mg/m^3) symptoms appeared of mild exhilaration and muscular incoordination characteristic of moderate ethanol ingestion. At a concentration of 1.1% or 38,800 mg/m^3, the subjects were described as appearing intoxicated, most within 5 minutes.

As a result of these studies and industrial experience, the ACGIH assigned a TLV of 500 ppm or 1760 mg/m^3 to gasoline. Then in 1963, Elkins et al. pointed out that the relative concentration of benzene in air after evaporation of gasoline, either totally or partially, would be greater than its volume or weight concentration in the liquid phase, leading to the possibility that the Threshold Limit Value (TLV) of benzene at that time, 25 ppm, might be exceeded in a mixed fuel concentration below the gasoline TLV. In response, the ACGIH in 1967 changed its approach in favor of determining the TLV on the basis of the content of benzene, other aromatics, and additives in gasoline or petroleum distillates.

In 1973, the THRU undertook an 8-month study of JP-4 jet fuel under a 6 hour/day, 5 day/week exposure regimen (MacEwen and Vernot, 1974). In this experiment, the aim was to generate concentrations of JP-4 which contained 25 and 12.5 ppm of benzene and, as a positive control, 25 ppm benzene alone which was the current TLV. Preliminary experiments indicated the required concentrations of JP-4 were 5 and 2.5 mg/L. As different containers of JP-4 were used, the benzene content changed slightly,

and the concentrations of JP-4 vapor were changed to keep benzene concentrations constant.

Activity depression was noted during the initial 3 weeks of the study in dogs and monkeys exposed to benzene or JP-4 vapors. A statistically significant increase was noted in RBC fragility in female dogs between the 10th and 27th weeks of exposure to 5 mg/L. The increase was not seen in dogs exposed to the lower concentration of JP-4 or benzene. At sacrifice, immediately postexposure, the liver, spleen, and kidney weights of rats exposed to 5 mg/L JP-4 were significantly higher than controls, and there was a higher incidence of chronic murine bronchitis in the rats exposed to either concentration. The only exposure related pathologic lesions at 1-year postexposure were increased hemosiderin deposits in the spleens of rats exposed to both concentrations of JP-4 and benzene alone.

The jet fuel of interest in the present study is designated JP-5. JP-5 is less volatile than JP-4 and also contains less benzene. The Naval Medical Research Institute Toxicology Detachment (NMRI/TD) requested that the Toxic Hazards Research Unit conduct comparative toxicity tests with JP-5 derived from both conventional petroleum and oil shale. Oil shale represents one of the largest underdeveloped sources of fossil energy in the United States. It is estimated that the Green River formation in Colorado, Utah, and Wyoming contains 600 billion barrels of recoverable oil, an amount that exceeds the known world liquid petroleum supply (Weaver and Gibson, 1979). The military is participating in an interagency effort to produce and refine large quantities of crude oil shale into military specification fuels for subsequent evaluation.

As part of the overall evaluation of the oil shale fuels, it is desirable to assess the toxicity associated with typical use exposure. Data of this type allow for a comparison of the hazards of the oil shale and petroleum fuels and are valuable in establishing proper workplace procedures and controls. Since inhalation will be a prime route of exposure for personnel working with JP-5, inhalation exposures were conducted to compare the effects at expected worker exposure levels. A 90-day, continuous inhalation exposure period was chosen to simulate conditions where Naval personnel may be exposed during a cruise situation. While this type of exposure is less traditional than a 6 hour/day, 5 day/week regimen, it does create a maximum exposure situation and increases the probability of observing exposure related effects.

Because of the unavailability of the Shale JP-5 at the onset of this study it was impossible to conduct simultaneous tests. The Shale JP-5 sample did not become available for testing until 2 years after initiation of the Petroleum JP-5 exposures. Despite this lengthy time period between the studies, care was taken to insure that the experiments were conducted in as similar a manner as possible.

METHODS

Test Material

JP-5 is a high flashpoint, kerosene-type aviation turbine fuel described in military specification MIL-T-5624K, (1 April 1976), and in Table 41. Petroleum JP-5 was obtained from the stock supply at Wright-Patterson Air Force Base. Shale JP-5 was refined by SOHIO from hydrotreated Paraho crude. The fuels were supplied to the THRU by NMRI/TD in clean 55-gallon drums.

TABLE 41. PHYSICAL-CHEMICAL SPECIFICATIONS (MIL SPECS) FOR JP-5 JET FUEL

Distillation Temperature (°C)	
Initial Boiling Point	--
10% Recovery	205°
End Point, Maximum Temperature	290°
Aromatics, Vol. %, Maximum	25
Olefins, Vol. %, Maximum	5
Sulfur, Total Weight, % Maximum	0.4
Sulfur, Mercaptan, Weight %	0.001
Hydrogen Content, Weight %	13.5
Freezing Point, °C, Maximum	-46°
Density, g/mL at 15°C, Minimum	0.788
Maximum	0.845
Flashpoint, °C, Minimum	60°

JP-5 Generation and Monitoring

The petroleum and oil shale studies were both conducted in the same manner. The basic design for the JP-5 generation system was adapted from the previous study on JP-4. Since JP-5 jet aircraft fuel is a multicomponent material with a wide boiling range, it was necessary to operate the animal exposure chambers from a single master generation system to assure similar exposure.

Two identically operated solvent evaporator towers were used to generate fuel vapor for the assigned chamber concentrations which were then controlled by dilution in the chamber air supply system.

A Beckman Model 400 hydrocarbon analyzer was used for mass analysis. Chamber concentrations were analyzed using a single analyzer by dilution of the higher JP-5 concentration chamber sample to a similar concentration as the low concentration using input chamber air for diluent and as the source of baseline air.

In the Petroleum JP-5 study a Varian 1200 gas chromatograph (GC) equipped with a FID detector and a Spectra Physics Model I computing integrator was used for quality control analysis of each drum of fuel prior to use, analysis of spent fuel to monitor generation system operation, and chamber atmosphere fingerprint analysis. The GC was operated isothermally with the oven set at 40°C. Routine chromatographs were limited to peaks eluted in the first 20 minutes. The Shale JP-5 study employed a Varian 3700 GC equipped with a FID detector and a Spectra Physics Model I computing integrator.

Animals

Young, adult purebred beagle dogs were selected from a colony maintained by the Air Force at Wright-Patterson Air Force Base. Fischer 344 rats (9-11 weeks old) were purchased from Charles River Breeding Laboratories (Wilmington, Massachusetts). C57BL/6 mice (9-11 weeks old) were purchased from Jackson Laboratories (Bar Harbor, Maine). Test animals were gang-caged by species in stainless steel, wire-mesh cages during exposure. Animals had access to food (Purina, St. Louis, Missouri) and water ad libitum. All cage areas were cleaned daily during which time food remaining in the feeders was discarded and replaced with a fresh supply.

Exposure Conditions

Groups of dogs, rats, and mice (Table 42) were exposed via inhalation to Petroleum or Shale JP-5 vapor continuously for a period of 90 days. Exposures were conducted on a 24-hour basis and personnel servicing the chambers during the exposure were provided with respiratory protection and disposable protective clothing. Exposures were conducted in 25 m^3 Thomas Dome inhalation chambers. Control groups were maintained in Bioclean®

TABLE 42. ANIMAL GROUPS EXPOSED TO EACH JP-5 CONCENTRATION (CONTROL, 150 MG/M^3, 750 MG/M^3)

Contaminant	Animal	No. of Males	No. of Females
Petroleum JP-5	Beagle dogs	3	3
	Fischer 344 rats	75	75
	C57BL/6 mice	--	111[a]
Shale JP-5	Beagle dogs	3	3
	Fischer 344 rats	75	75
	C57BL/6 mice	--	150

[a] Reduced number of mice due to reinitiation of exposure.

laminar air flow rooms in a separate facility. Because of space limitations, male mice were not included in the exposure.

Upon termination of the 90-day exposure period, all of the dogs and 1/3 of the rodents were killed for detection of pathologic lesions caused by exposure. The remaining rodents were held for long-term postexposure observation. An interim sacrifice was conducted at 19 months postexposure, with a final sacrifice during the 24th month of the study.

All animals were carefully observed throughout the exposure and postexposure periods for signs of altered physical condition. Rats and dogs were weighed individually at biweekly intervals during exposure, and rats were weighed monthly during the post-exposure period. Mice were weighed monthly throughout the study, and the group mean weights were monitored. All animals that died or were killed were necropsied and approximately 38 tissues were collected for histopathologic examination. The liver, spleen, and kidneys of individual rats were weighed during necropsy at exposure termination and 19 months postexposure. Liver, spleen, and kidney weights of dogs in the Shale JP-5 study were also measured. Dog red blood cell osmotic fragility tests were conducted at exposure termination. Blood samples were drawn from fasted dogs biweekly and from fasted rats at exposure termination and interim necropsy for hematology and clinical chemistry tests.

Data Analysis

Body weights, blood test results, and organ weights were analyzed by an independent t-test, and a Fisher exact test was

used to analyze the incidence of histopathologic lesions (Zar, 1974).

RESULTS

Analytical

Initially, animal exposures to JP-5 vapors were intended to be based on air concentrations that would result in benzene concentrations equal to 10 ppm and 1 ppm. In preliminary tests of Petroleum JP-5 vapor generation in the exposure chambers, it was determined that even though military specifications for refining of JP-5 fuel permitted up to 25% aromatic hydrocarbon content, the measured benzene content was very low. The highest benzene concentrations we were able to achieve in the exposure chamber ranged from 0.5 ppm to 0.7 ppm. These levels were reached at a petroleum JP-5 vapor concentration of 1500 mg/m^3, which also was the highest stable concentration attainable.

Shortly after initiation, unexpected deaths occurred in the exposed mice exposed to 1500 mg/m^3 Petroleum JP-5. Seventy-five of the group of 150 mice were dead by the end of 6 exposure days. Dogs were lethargic and appeared to be sleeping more than normal. Oily deposits were noticeable on the fur of both species and, in addition, oil deposits were seen on the chamber windows. Examination of the daily records from the hydrocarbon analyzer showed no significant deviation from the desired concentration of 1500 mg/m^3 JP-5. This evidence suggested that generation of this concentration of Petroleum JP-5 was producing an aerosol which was lethal to mice after a few days exposure.

The rate of introduction of JP-5 into the 1500 mg/m^3 chamber was subsequently lowered to give a chamber concentration of 750 mg/m^3. This resulted in an exposure atmosphere that had no visible aerosol and the particle counts were only slightly higher than room air, as measured by a Royco particle counter.

Based on the evidence that Petroleum JP-5 concentrations of 1500 mg/m^3 produced an aerosol exposure that was fatal to mice after several days, the initial study was terminated. New JP-5 subchronic exposure concentrations of 750 mg/m^3 and 150 mg/m^3 were selected. A new group of dogs was chosen from the original stock group for use in the Petroleum JP-5 exposure. The number of mice in each group had to be reduced to avoid delays from ordering new animals. Spare mice from the original lot were available and were incorporated into the pool for redistribution of the exposure groups. Each Petroleum JP-5 study group then

consisted of 111 mice instead of the original 150. At the time of the early termination, the rats had not yet entered the exposure chambers. They were therefore maintained until the Petroleum JP-5 study was restarted. Reduction of the concentration allowed the Petroleum JP-5 study to be conducted without further incident. Exposures were well controlled throughout both studies with daily mean values ranging within 5% of the target chamber concentrations.

Gas chromatographic analyses performed during the Petroleum JP-5 study were intended only to determine the benzene concentration. Use of this method showed a benzene concentration in the 150 mg/m^3 exposure chamber averaging about 0.1 ppm (range: 0.06 - 0.12 ppm). The 750 mg/m^3 chamber contained approximately 0.5 ppm (range: 0.33 - 0.57 ppm). The use of a single pass vapor generation system in this study resulted in the vaporization of about 8% of the total fuel mass. Because of the temperature limitations imposed on the generation towers there was a selective vaporization of the more volatile components of JP-5. The slightly different GC technique used in the Shale JP-5 study established that the majority of the constituents in the chamber were between C_{10} and C_{14}. This type of specific analysis was not conducted in the Petroleum JP-5 study.

Dogs

The growth of beagle dogs exposed to either Petroleum or Shale JP-5 was unaffected by 90 days of continuous exposure, and no deaths resulted from exposure to either fuel.

Dogs exposed to Petroleum JP-5 for 90 days had increased red blood cell osmotic fragility when compared to controls (Figure 3). Although there was a slight shift in the curve toward increased fragility for the dogs exposed to 750 mg/m^3 Shale JP-5, the difference from the control values was not significant at $p < 0.05$. Slightly decreased red blood cell counts, hematocrit, and hemoglobin levels were seen in dogs exposed to either Petroleum or Shale JP-5 at 750 mg/m^3 when compared to respective controls. Although this was a persistent trend, the differences were not always statistically significant at $p < 0.05$. All other hematalogic and clinical chemistry parameters measured were well within normal limits and failed to show any consistent exposure related effect.

Increased liver weight was noted in dogs exposed to 750 mg/m^3 Shale JP-5. The increase was not evident in the dogs

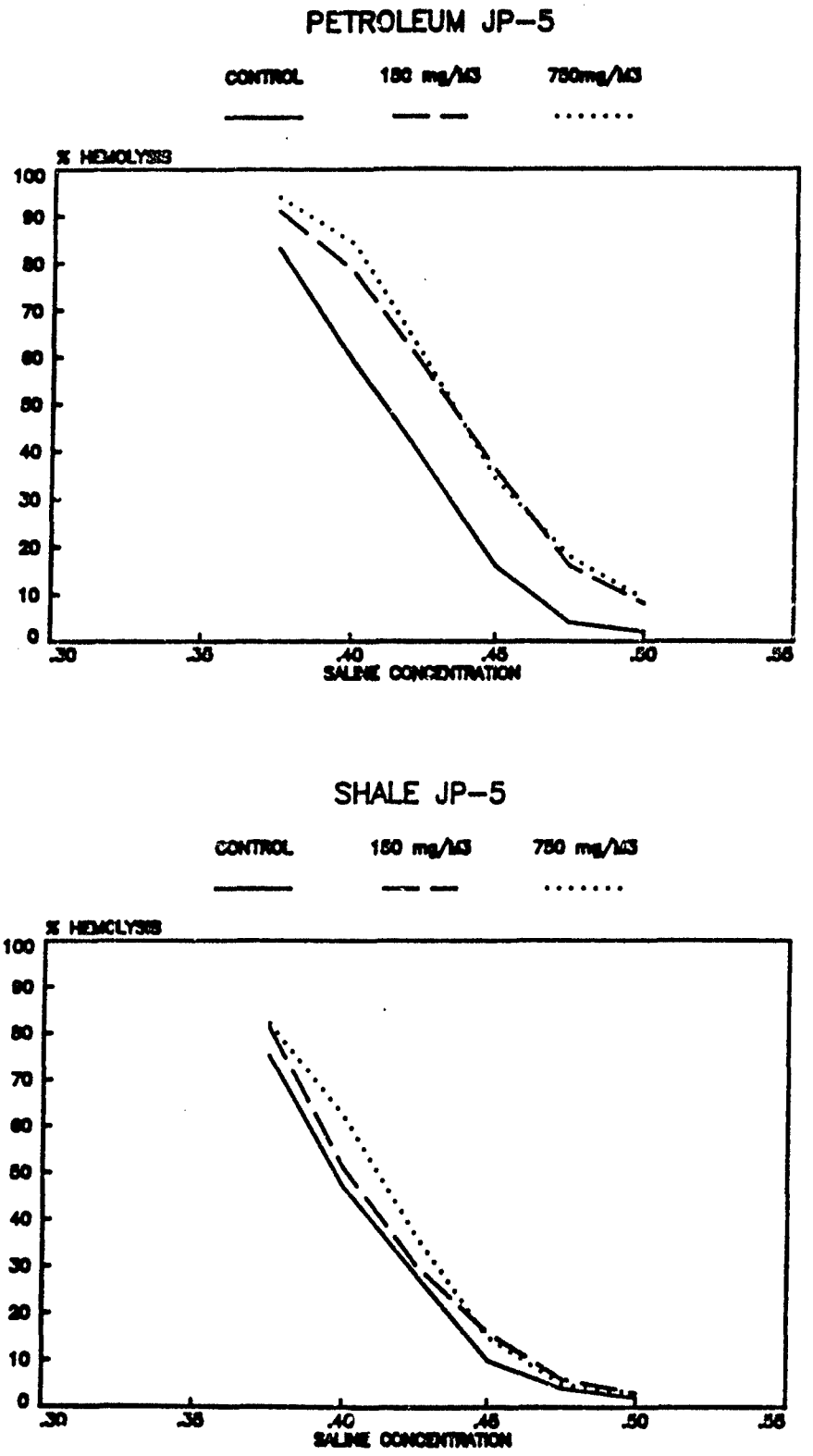


Figure 3. Effect of JP-5 vapor exposure on dog red blood cell osmotic fragility.

exposed to 150 mg/m^3 Shale JP-5. Organ weights were not measured in dogs exposed to Petroleum JP-5.

The majority of the lesions noted in the tissues of dogs exposed to either Petroleum or Shale JP-5 were not exposure related, occurring with equal distribution among all groups. The only exception was diffuse cloudy swelling of hepatocytes of dogs exposed to Petroleum JP-5. This occurred in all dogs exposed to 750 mg/m^3, 2 of the 6 dogs exposed to 150 mg/m^3, and none of the controls. Affected hepatocytes were moderately swollen, pale, and had a "foamy" cytoplasm. Transmission electron microscopic examination of a small number of these livers indicated this to be excessive glycogen accumulation.

Mice

Exposure of mice to Petroleum JP-5 produced a slightly shorter mean survival time when compared to their control group. This effect was not seen in Shale JP-5 exposed mice. However, the control group in the petroleum study survived approximately 2.5 months longer than the control group in the shale study, and the survival time of the 750 mg/m^3 Petroleum JP-5 exposure group was not inconsistent with any of the other groups.

Mouse body weights were unaffected by exposure to either Petroleum or Shale JP-5.

Hepatocellular fatty change and vacuolization were the only remarkable findings in mouse tissues examined at the completion of the 90-day exposure period. Although most of the mice exposed to either Petroleum or Shale JP-5 displayed fatty livers after 90 days of exposure, the incidence was not dose related.

The major histopathologic changes noted in mice held for postexposure observation are shown in Table 43. The list has been abbreviated by excluding common lesions that appeared with low incidence. Chronic dermal inflammation and ulceration were common observations in all groups. However, ulceration did occur with a greater frequency in the mice exposed to 750 mg/m^3 JP-5. The appearance of granulocytic hyperplasia of the bone marrow was also common in all exposure groups and often corresponded with the presence of ulcerative dermatitis.

Lesions noted in the respiratory system of control and exposed mice included cytoplasmic hyaline change in the nose and

TABLE 43. HISTOPATHOLOGIC LESIONS[a] IN MICE HELD FOR POSTEXPOSURE OBSERVATION AFTER 90 DAYS OF CONTINUOUS INHALATION EXPOSURE TO JP-5

Tissue	Petroleum JP-5 Concentration (mg/m³) 0	Petroleum JP-5 Concentration (mg/m³) 150	Petroleum JP-5 Concentration (mg/m³) 750	Shale JP-5 Concentration (mg/m³) 0	Shale JP-5 Concentration (mg/m³) 150	Shale JP-5 Concentration (mg/m³) 750
Skin						
Ulcer	1/68 (1)	7/65 (11)[b]	13/66 (20)[c]	11/90 (12)	23/96 (24)	24/93 (26)[b]
Inflammation	18/68 (26)	15/65 (23)	8/66 (12)	3/90 (3)	1/96 (1)	0/93 (0)
Bone Marrow						
Granulocytic hyperplasia	26/65 (40)	26/61 (43)	23/65 (35)	35/86 (41)	26/93 (28)	38/94 (40)
Fibrosis	1/65 (2)	1/61 (2)	1/65 (2)	0/86 (0)	4/93 (4)	16/94 (17)[c]
Respiratory						
Nose - hyaline degeneration	19/68 (28)	8/66 (12)[b]	5/67 (7)[c]	65/93 (70)	53/97 (55)	62/96 (65)
Lung -						
inflammation	2/70 (3)	5/67 (7)	3/68 (4)	28/93 (30)	38/97 (39)	31/96 (32)
crystals	26/70 (37)	11/67 (16)	17/68 (25)	23/93 (25)	8/97 (8)	1/96 (1)
alveolar adenoma	5/70 (7)	1/67 (1)	1/68 (1)	1/93 (1)	2/97 (2)	2/96 (2)
alveolar carcinoma	0/70 (0)	1/67 (1)	0/68 (0)	2/93 (2)	0/97 (0)	1/96 (1)
Liver						
Clear cell focus	0/69 (0)	0/69 (0)	0/67 (0)	2/93 (2)	12/97 (12)[b]	4/95 (4)
Fatty Change	41/69 (59)	28/69 (41)[b]	23/67 (34)[c]	57/93 (61)	56/97 (58)	66/95 (69)
Necrosis	2/69 (3)	2/69 (3)	0/67 (0)	8/93 (9)	5/97 (5)	5/95 (5)
Carcinoma	1/69 (1)	0/69 (0)	0/67 (0)	2/93 (2)	0/97 (0)	0/95 (0)
Adenoma	3/69 (4)	1/69 (1)	0/67 (0)	0/93 (0)	1/97 (1)	6/95 (6)[b]
Urinary						
Kidney - membranous glomerulonephritis	7/66 (11)	6/69 (9)	9/70 (13)	19/93 (20)	14/97 (14)	19/96 (20)
hyaline degeneration	0/66 (0)	5/69 (7)	1/70 (1)	6/93 (6)	0/97 (0)[b]	1/96 (1)
hydronephrosis	2/66 (3)	3/69 (4)	0/70 (0)	4/93 (4)	3/97 (3)	7/96 (7)
Reproductive & Endocrine						
Ovaries - cyst	11/63 (17)	13/57 (23)	7/57 (12)	17/94 (18)	17/93 (18)	13/96 (14)
Uterus - endometrial cyst	8/66 (12)	8/68 (12)	19/67 (28)[b]	48/91 (53)	58/97 (30)	31/93 (33)[b]
Pituitary - adenoma	28/54 (52)	24/45 (53)	21/51 (41)	49/82 (60)	40/85 (47)	38/76 (5)
- carcinoma	0/54 (0)	1/45 (2)	0/51 (0)	15/82 (18)	4/85 (5)[c]	2/76 (3)[c]
Thyroid -						
adenoma	1/68 (1)	5/31 (8)	3/64 (5)	9/91 (10)	11/96 (11)	6/90 (7)
carcinoma	0/68 (0)	1/61 (2)	0/64 (0)	1/91 (1)	0/96 (0)	0/90 (0)
papillary hyperplasia	38/68 (56)	27/61 (44)	34/64 (53)	71/91 (78)	62/96 (65)	70/90 (78)
Parathyroid - adenoma	0/33 (0)	0/25 (0)	0/16 (0)	0/52 (0)	1/69 (1)	0/39 (0)
- hyperplasia	0/33 (0)	0/25 (0)	0/16 (0)	1/52 (2)	0/69 (0)	0/39 (0)
Adrenal - carcinoma	0/67 (0)	0/64 (0)	0/67 (0)	1/95 (1)	0/96 (0)	0/96 (0)
- pheochomocytoma	0/67 (0)	0/64 (0)	0/67 (0)	1/95 (1)	0/96 (0)	0/96 (0)
Lymphoreticular						
Malignant lymphomas	8/70 (11)	14/69 (20)	9/71 (13)	40/91 (44)	30/94 (32)	25/95 (26)[b]
Digestive						
Stomach-ulcer	0/68 (0)	0/67 (0)	0/66 (0)	0/90 (0)	1/97 (1)	3/95 (3)
Gallbladder						
Hyaline degeneration	7/53 (13)	12/56 (21)	8/52 (15)	14/89 (57)	2/93 (2)[c]	24/86 (28)
Spleen						
Hematopoiesis	29/69 (42)	28/67 (39)	29/64 (45)	38/91 (42)	37/94 (39)	54/95 (57)[b]
Salivary Gland						
Perivascular cuffing	6/65 (9)	0/63 (0)[b]	9/64 (14)	37/84 (44)	37/94 (39)	53/90 (59)

[a] Number observed/Number examined (%).
[b] Different from control, $p < 0.05$.
[c] Different from control, $p < 0.01$.

inflammatory processes in the lung, including the combined diagnoses of acute and chronic inflammation, perivascular cuffing, lymphocytic infiltrates, interstitial inflammation, granulomatous inflammation, and alveolar macrophages. These lesions were more frequently noted in the group of mice used in the Shale JP-5 study. Most importantly, primary lung tumors were not increased in mice exposed to either Petroleum or Shale JP-5 compared to respective controls.

Six hepatocellular adenomas were observed in the female mice exposed to 750 mg/m^3 Shale JP-5 while no primary liver cell tumors were observed in controls. Liver adenomas were not seen in mice exposed to Petroleum JP-5 at 750 mg/m^3, while 3 of the 71 respective control mice developed liver adenomas. Hepatocellular fatty change was commonly diagnosed in all groups of mice examined postexposure.

Lesions noted in the urinary system were generally unremarkable. All groups demonstrated a moderate incidence of membranous glomerulonephritis. Frequency of this change was slightly greater in the Shale JP-5 study. This was diagnosed when the glomerular capillary tufts appeared markedly thickened with homogenous, eosinophilic deposits. In some cases these deposits may have been amyloid fibrils while in other animals, immune complex deposition associated with murine oncornavirus infection was probably responsible. Tumors of the kidney were not seen in any of the JP-5 exposed mice.

Ovarian and uterine cystic changes were present with high frequency and variable distribution among all groups. Pituitary and thyroid follicular adenomas were also common in all groups. Adrenal tumors were noted only in 2 control mice in the Shale JP-5 study.

The other lesions noted with some frequency in mice included gallbladder hyaline degeneration, splenic hematopoiesis, malignant lymphomas, and salivary gland perivascular cuffing. These are generally regarded as typical of aging, and the occasional increased incidence in the exposed groups compared to controls is considered to be incidental.

Rats

Exposure to either Petroleum or Shale JP-5 did not adversely affect survival of rats. The mean survival time for all groups in each study was approximately 22 months.

Depressed weight gains were noted in male rats exposed to either Petroleum or Shale JP-5. The weights of male rats exposed to 750 mg/m^3 Petroleum JP-5 vapor were significantly ($p < 0.01$) less than unexposed control male rats through the exposure and postexposure phases of the study. Male rats exposed to 150 mg/m^3 Petroleum JP-5 vapor also weighed significantly ($p < 0.01$) less than unexposed control rats. However, this difference continued only through the 16th month of the study at which time the weights of this exposure group returned to the level of the unexposed control rats. Both groups of Shale JP-5 rats weighed significantly ($p < 0.05$) less than controls throughout the exposure and postexposure periods, with the weights generally following a dose response pattern. Exposure to Petroleum JP-5 had no effect on female rat growth. Shale JP-5 at 750 mg/m^3 produced slight but statistically significant ($p < 0.05$) reduced growth through exposure and postexposure periods.

A slight but statistically significant increase in relative kidney weight was noted at both the exposure termination and postexposure examinations in male rats exposed to either Petroleum or Shale JP-5 at 750 mg/m^3. This effect was not dose related as the kidneys of male rats exposed to JP-5 at the lower 150 mg/m^3 concentration generally weighed less than the respective control group rather than greater. At 19 months postexposure the spleens of 3 control male rats from the Petroleum JP-5 study were unusually large (42, 39, and 17 g). Other changes noted in JP-5 exposed male rat organ weights were sporadic and were considered incidental. Female rat organ weights were unremarkable.

Male rat hematology, BUN, and creatinine values were measured during the study. Although all of the values determined at exposure termination were within normal variation for this species, there were some trends that were consistent with those mentioned previously for dog blood examinations. Slight but statistically significant ($p < 0.01$) reductions in red blood cell counts, hematocrit, and hemoglobin levels were noted in JP-5 exposed male rats examined at exposure termination. BUN levels were also increased at that time in male rats exposed at the 750 mg/m^3 JP-5 level. Examination at 19 months did not indicate consistent reductions in erythrocyte parameters. The 3 male control rats from the Petroleum JP-5 study that had abnormally large spleens had WBC counts in excess of 48,000 cell/m^3. In these rats 80-90% of the WBC's were leukemic mononuclear cells. Blood values from these rats were excluded from statistical comparison. WBC abnormalities of this type were not found in Petroleum JP-5 exposed male rats nor in male rats from the Shale JP-5 study. Although BUN levels for the 750 mg/m^3 JP-5 exposure

groups were greater than controls at 19 months postexposure, they were not statistically significant. Other blood parameters measured in male and female rats during the study were within normal species variation, and any differences between control and test groups were considered to be unrelated to exposure.

Exposure of rats to Shale JP-5 resulted in mild hepatocellular vacuolization (fatty change). This effect was not dose-related in female rats and was absent in rats exposed to Petroleum JP-5. Mild nasal inflammatory changes occurred in Shale JP-5 exposed rats. The incidence was not dose-related. Petroleum JP-5 exposed rats were free of this lesion.

The most notable and obviously dose-dependent lesions found in rats were restricted to the kidneys of males. Male rats examined immediately following exposure to either Petroleum or Shale JP-5 exhibited moderate to marked cytoplasmic hyaline droplets in proximal tubular epithelium followed by necrosis and exfoliation of tubular cells. Hyaline droplets were regarded as microscopically visible aggregates of protein. Their presence in the tubular epithelium suggested that cells were unable to efficiently transport resorbed protein from glomerular filtrate to the capillary blood. Subsequently, tubular segments near the corticomedullary junction were plugged and dilated with casts of necrotic cell debris. Affected segments corresponded with the juncture of the pars recta of the proximal tubule and the descending limb of Henle's loop. Incidence of necrosis in all exposed groups was at or near 100%. However, a clear dose response was seen in severity with minimal renal changes at 150 mg/m^3, while lesions found at 750 mg/m^3 were characterized as moderate. Glomeruli were morphologically unremarkable by both electron and light microscopic analysis.

Results of microscopic examination of tissues collected from male rats maintained for postexposure observation are shown in Table 44. Only the lesions occurring with some frequency are shown. As at exposure termination, the most striking lesions associated with JP-5 exposure were found in the kidneys of male rats. These changes included focal diffuse papillary hyperplasia of the pelvic urothelium over the surface of the renal papillus, moderate to severe deposits of mineralized debris in medullary tubules (probably at the loop of Henle), and tubular degeneration. Hyperplastic areas along the pelvic urothelium were thought to result from mechanical irritation by mineralized debris shed from the tubules into the pelvis during exposure, combined with panrenal hypertrophy associated with chronic progressive nephropathy (CPN). In turn the mineralized tubular deposits probably represent calcium-impregnated debris resulting from

TABLE 44. HISTOPATHOLOGIC LESIONS[a] IN MALE RATS HELD FOR POSTEXPOSURE OBSERVATION AFTER 90 DAYS OF CONTINUOUS INHALATION EXPOSURE TO JP-5

Tissue	Petroleum JP-5 Concentration (mg/m^3) 0	150	750	Shale JP-5 Concentration (mg/m^3) 0	150	750
Skin						
Mammary gland -						
hyperplasia/dilatation	4/18 (14)	7/19 (24)	6/32 (19)	6/34 (18)	12/34 (35)	12/37 (32)
adenocarcinoma	0/28 (0)	0/29 (0)	0/32 (0)	0/34 (0)	1/34 (3)	0/37 (0)
fibroadenoma	1/28 (4)	2/29 (7)	1/32 (3)	0/34 (0)	0/34 (0)	2/37 (5)
Cardiovascular						
Myocardial fibrosis	6/49 (12)	2/50 (4)	4/49 (8)	23/50 (46)	15/49 (31)	21/50 (42)
Pulmonary artery mineralization	29/50 (58)	25/50 (50)	30/50 (60)	24/50 (48)	25/50 (50)	27/50 (54)
Respiratory						
Nose - inflammation	4/49 (8)	4/49 (8)	8/49 (16)	12/50 (24)	8/50 (16)	6/48 (13)
Lung -						
alveolar adenoma	0/50 (0)	0/50 (0)	0/50 (0)	0/50 (0)	1/50 (2)	0/50 (0)
alveolar carcinoma	0/50 (0)	1/50 (2)	2/50 (4)	1/50 (2)	0/50 (0)	0/50 (0)
Liver						
Focal cell change	23/50 (46)	29/50 (58)	32/49 (65)	38/50 (76)	38/50 (76)	41/50 (82)
Neoplastic nodule	1/50 (2)	1/50 (2)	0/49 (0)	2/50 (4)	0/50 (0)	4/50 (8)
Carcinoma	0/50 (0)	0/50 (0)	0/49 (0)	0/50 (0)	1/50 (2)	0/50 (0)
Adenoma	1/50 (2)	2/50 (4)	0/49 (0)	0/50 (0)	0/50 (0)	0/50 (0)
Urinary						
Kidney -						
tubular degeneration	42/50 (84)	46/48 (96)	48/50 (96)	34/50 (68)	44/49 (90)[b]	48/49 (98)[c]
papillary hyperplasia	0/50 (0)	2/48 (4)	23/50 (46)[c]	0/50 (0)	8/49 (16)[c]	31/49 (63)[c]
mineralization	0/50 (0)	29/48 (60)[c]	37/50 (74)[c]	0/50 (0)	49/49 (100)[c]	49/49 (100)[c]
cysts	0/50 (0)	0/48 (0)	1/50 (2)	0/50 (0)	3/49 (6)	3/49 (6)
Reproductive & Endocrine						
Pituitary -						
adenoma	7/48 (15)	18/49 (36)[b]	13/47 (28)	5/46 (11)	9/47 (19)	13/46 (28)
carcinoma	0/48 (0)	0/49 (0)	1/47 (2)	0/46 (0)	1/47 (2)	3/46 (4)
Thyroid -						
follicular cell tumors	2/45 (4)	0/44 (0)	0/44 (0)	0/49 (0)	2/49 (4)	1/50 (2)
c cell tumors	5/45 (11)	4/44 (9)	7/44 (16)	2/50 (4)	13/49 (26)[c]	7/50 (14)
hyperplasia	1/45 (2)	1/44 (2)	10/44 (23)[c]	5/50 (10)	3/49 (6)	3/50 (6)
Parathyroid -						
adenoma	1/35 (3)	2/35 (6)	0/33 (0)	1/38 (3)	2/42 (5)	1/42 (2)
Testes -						
interstitial cell tumor	46/49 (94)	44/46 (96)	40/50 (80)	48/49 (99)	48/50 (96)	43/47 (91)
Adrenal -						
cell change	18/50 (36)	13/50 (26)	15/50 (30)	10/50 (20)	12/50 (24)	19/47 (40)
carcinoma	0/50 (0)	0/50 (0)	0/50 (0)	1/50 (2)	0/50 (0)	0/47 (0)
adenoma	1/50 (2)	0/50 (0)	0/50 (0)	0/50 (0)	0/50 (0)	0/47 (0)
pheochromocytoma	4/50 (8)	4/50 (8)	8/50 (16)	1/50 (2)	0/50 (0)	8/47 (17)[b]
Lymphoreticular						
Thymus -						
thymoma	0/17 (0)	0/16 (0)	0/18 (0)	0/39 (0)	0/41 (0)	2/39 (5)
leukemia	14/50 (28)	5/50 (10)[b]	4/50 (8)[b]	12/50 (24)	7/50 (14)	11/50 (22)

[a] Number observed/Number examined (%).
[b] Different from control, $p < 0.05$.
[c] Different from control, $p < 0.01$.

tubular necrosis occurring during the exposure phase of the experiment. Tubular degenerative changes were entirely compatible with CPN which is common in aged Fischer 344 rats. The severity of CPN, however, did appear to be slightly greater in the JP-5 exposed male rats. No kidney tumors were found in JP-5 exposed male rats.

Select tumors and tissue changes were recorded with increased frequency in several endocrine organs in exposed male rats. Although a slight increase in the incidence of pituitary tumors was found, these were not considered to be highly significant. Pituitary tumors are extremely common in aged Fischer 344 rats. Tumors of the thyroid parafollicular cells may be related to prolonged $Ca:PO_4$ imbalances secondary to severe nephropathy. Other endocrine lesions recorded in the studies were considered to be common findings in aged rats, and their apparent increase in some exposed groups is probably incidental. Neither Petroleum nor Shale JP-5 produced any increase in liver or lung tumors.

Results of the microscopic examination of the tissues collected from female rats at the end of 19 months postexposure observation are shown in Table 45. As at exposure termination sacrifice, the female rats failed to display any indication of JP-5 induced renal damage.

Unique to the female rats was the occurrence of bone hyperosteosis (osteosclerosis). This lesion was not recorded in any male rat and its etiology is believed to be related to increased levels of estrogen in aged females. Estrogen competes with parathyroid hormone for osteoclastic cell receptors and prevents parathyroid hormone from initiating bone resorption. Although this lesion occurred more frequently in JP-5 exposed females, there was no dose response relationship. Lesions noted in other organ systems of female rats were similar in nature to those previously discussed for male rats.

DISCUSSION

Male rat nephrotoxicity was a consistent effect produced by 90 days of continuous inhalation of Petroleum or Shale JP-5. Renal toxicity was apparent in virtually all exposed male rats immediately upon exposure termination, even at the lowest level of exposure tested, 150 mg/m^3. The renal tubular necrosis observed in male rats was considered to be an end product of severe hyaline degeneration. Hyaline droplets are normal components of the protein resorption process of the proximal convoluted tubule. Droplets are frequently accentuated in male rats where tubular cells must resorb and degrade increased levels of a highly filterable protein unique to male rats, an α_{2u} globulin (Alden et al., 1983). This globulin is produced in the liver of male rats only at puberty, and its low molecular weight facilitates rapid glomerular filtration. The biological fate of α_{2u} globulin is unknown. The inability of the kidney tubular cell to efficiently degrade resorbed α_{2u} globulin may be central to

TABLE 45. HISTOPATHOLOGIC LESIONS[a] IN FEMALE RATS HELD FOR POSTEXPOSURE OBSERVATION AFTER 90 DAYS OF CONTINUOUS INHALATION EXPOSURE TO JP-5

Tissue	Petroleum JP-5 Concentration (mg/m^3) 0	150	750	Shale JP-5 Concentration (mg/m^3) 0	150	750
Skin						
Mammary gland -						
hyperplasia/dilatation	3/36 (8)	16/43 (37)[b]	15/44 (3)[b]	11/46 (24)	23/45 (5)[b]	17/48 (35)
adenocarcinoma	0/36 (0)	2/43 (5)	3/44 (7)	1/46 (2)	1/45 (2)	0/48 (0)
cystadenocarcinoma	0/36 (0)	2/43 (5)	3/44 (7)	0/46 (0)	0/45 (0)	1/48 (2)
fibroadenoma	3/36 (8)	4/43 (9)	8/44 (18)	2/46 (4)	4/45 (9)	4/48 (8)
Musculoskeletal						
Bone -						
hyperostosis	3/43 (7)	10/45 (22)	9/47 (19)	7/47 (15)	20/49 (41)	18/50 (36)
Respiratory						
Nose - inflammation	2/44 (5)	0/46 (0)	4/44 (9)	0/49 (0)	3/50 (6)	1/48 (2)
Lung -						
adenoma	0/44 (0)	0/45 (0)	0/47 (0)	0/50 (0)	1/50 (2)	0/50 (0)
carcinoma	0/44 (0)	1/45 (2)	0/47 (0)	1/50 (2)	0/50 (0)	0/50 (0)
Liver						
Focal cell change	17/44 (37)	21/46 (48)	22/47 (50)	25/50 (50)	30/50 (60)	32/50 (64)
Neoplastic nodule	0/44 (0)	1/46 (2)	0/47 (0)	1/50 (2)	2/50 (4)	2/50 (4)
Urinary						
Kidney -						
tubular degeneration	5/44 (11)	5/44 (11)	4/47 (9)	4/49 (8)	1/50 (2)	0/50 (0)
mineralization	0/44 (0)	0/44 (0)	1/47 (2)	0/49 (0)	0/50 (0)	1/50 (2)
cysts	0/44 (0)	0/44 (0)	0/47 (0)	0/49 (0)	1/50 (2)	1/50 (2)
Reproductive & Endocrine						
Pituitary -						
adenoma	16/42 (38)	22/46 (48)	22/44 (50)	10/45 (22)	23/47 (49)[b]	11/45 (24)
carcinoma	0/42 (0)	1/46 (2)	0/44 (0)	1/45 (2)	0/47 (0)	1/45 (2)
Thyroid -						
c cell tumors	3/37 (8)	3/40 (8)	6/44 (14)	5/49 (10)	7/49 (14)	4/49 (8)
hyperplasia	2/37 (5)	0/40 (0)	12/44 (27)[c]	6/49 (12)	7/49 (14)	2/49 (12)
Parathyroid -						
adenoma	0/25 (0)	0/32 (0)	0/35 (0)	0/36 (0)	2/41 (5)	0/40 (0)
Adrenal -						
cell change	9/44 (20)	5/45 (11)	9/47 (19)	18/49 (37)	13/50 (26)	19/50 (38)
carcinoma	0/44 (0)	0/45 (0)	0/47 (0)	1/49 (2)	0/50 (0)	0/50 (0)
adenoma	0/44 (0)	0/45 (0)	0/47 (0)	0/49 (0)	1/50 (2)	1/50 (2)
pheochromocytoma	3/44 (7)	1/45 (2)	1/47 (2)	0/49 (0)	1/50 (2)	3/50 (6)
Uterus -						
carcinoma	1/44 (2)	1/44 (2)	0/44 (0)	2/47 (4)	5/50 (10)	5/48 (10)
stromal polyps	11/44 (25)	14/44 (32)	8/44 (18)	4/47 (9)	5/50 (10)	7/48 (15)
Lymphoreticular						
Spleen -						
hemosiderosis	10/42 (24)	11/45 (24)	3/46 (7)[c]	10/50 (20)	29/49 (59)[b]	3/50 (6)[c]
leukemia	6/44 (14)	4/46 (9)	6/47 (13)	8/50 (16)	7/50 (14)	7/50 (14)

[a] Number observed/Number examined (%).
[b] Different from control, $p < 0.01$.
[c] Different from control, $p < 0.05$.

the pathogenesis of JP-5 induced nephrosis in male rats. Changes occurring in the kidneys subsequent to the 90-day exposure included increased deposits of mineralized debris in medullary tubules, papillary hyperplasia of the pelvis, and more severe chronic progressive nephropathy. Consistent with the pathologic changes observed in male rats exposed to 750 mg/m^3 JP-5 were

increased kidney weight and serum creatinine and BUN levels. Body weights of male rats exposed to 750 mg/m^3 JP-5 were also consistently less than unexposed rats. Although structural changes were also observable at the 150 mg/m^3 level, kidney weights and blood kidney function indicators were generally not increased.

A similar type of male rat nephropathy has been observed after gavage with Petroleum or Shale JP-5 (Parker et al., 1981). Inhalation of hydrocarbon solvents has also been reported to produce tubular changes in male rats (Carpenter et al., 1975a, 1975b; Gaworski et al., 1984; Phillips and Egan, 1984a, 1984b). In the present study of JP-5 the renal toxicity was limited to male rats and did not occur in female rats or female C57BL/6 mice. Easley et al. (1982) has reported renal toxicity consisting of cortical degeneration, possibly due to ischemia related dehydration, in C3Hf/Bd mice treated dermally with Petroleum or Shale JP-5, JP-8, or Diesel Fuel Marine.

The vacuolization in the hepatocytes of mice and rats inhaling JP-5 for 90 days was probably an indication of fatty metamorphosis associated with excess lipid accumulation. Parker et al. (1981) found vacuolization in livers of rats gavaged with Petroleum or Shale JP-5 and also observed elevated liver function indices. In the present study, examination of rat serum chemistry parameters at exposure termination failed to suggest abnormal liver function. At 19 months postexposure, clinical liver function indices were normal. Additionally, at that time liver vacuolization was not a significant finding in rats and was noted with equal distribution in all mouse groups. These results suggest that the type and extent of liver damage produced by inhalation of JP-5 vapor at concentrations up to 750 mg/m^3 was mild and reversible.

Exposure to either Petroleum or Shale JP-5 produced an apparent slight increase in endocrine tumors in rats, particularly tumors of the pituitary gland. However, these tumors are generally regarded as common aging neoplasms in Fischer 344 rats, and, in the absence of any strong dose response relationship, this increase is considered incidental. Most important was the absence of any definite indication of increased tumor formation in any of the major organs of rats including the lungs, liver, and kidneys.

In conclusion, this comparative examination of the toxic effects of 90 days of continuous inhalation of JP-5 derived from Petroleum or Shale sources established no substantial difference between fuels. The major effect of exposure to JP-5 from either

source was the production of histologic changes in male rat kidneys suggesting renal tubular nephropathy. The results of the study are consistent with other published reports of hydrocarbon fuel and solvent toxicity.

EVALUATION OF THE CHRONIC TOXICITY OF JP-7 JET FUEL

As part of the program to evaluate the toxicity of hydrocarbon fuels used by the Air Force, the THRU conducted an inhalation exposure study using the jet fuel JP-7. JP-7 is a complex mixture of aliphatic and aromatic hydrocarbons which closely resembles the U. S. Navy jet fuel, JP-5. The study conducted was an industrial type involving exposure of Fischer 344 rats and C57BL/6 mice to JP-7 vapors for 6 hours/day, 5 days/week. The exposure phase of the study ran from April 1981 to April 1982. A small sample of the animals was taken for evaluation of chronic toxicity response during exposures. The remaining animals were held for 1-year postexposure observation.

Two chambers were utilized for the exposures; one contained a concentration of 150 mg/m^3 and the other contained a concentration of 750 mg/m^3. Animal groups consisted of 100 male and 100 female Fischer 344 rats and C57BL/6 mice. An additional group with the same numbers of animals was housed in another Thomas Dome chamber to serve as sham operated controls. All animals had food and water ad libitum during nonexposure hours. Food was removed during the exposure period.

Following the 1-year exposure period, 12 animals of each species and sex from all groups were killed for tissue collection and examination. The remaining rodents were held for 1-year of postexposure observation. At the conclusion of this period (April, 1983), all remaining rodents were killed for tissue collection and examination.

Exposure to JP-7 produced a slight, but statistically significant ($p < 0.05$), decrease in body weight gain in male rats exposed to 750 mg/m^3. No other adverse weight effects were seen in any of the other groups. Examination of rat hematology and clinical chemistry parameters generally failed to suggest any exposure related effect. In addition, liver, kidney, and spleen weights of rats exposed to JP-7 were comparable to controls.

Tissues collected from mice and rats in this study were sent to Brooks Air Force Base, San Antonio, Texas for processing and evaluation.

THE EXPERIMENTAL DETERMINATION OF THE CHRONIC TOXICITY OF JP-TS JET FUEL

The U. S. Air Force requested a long-term inhalation study to determine the chronic toxicity of the high altitude jet fuel designated JP-TS. This jet fuel is similar in composition to the jet fuel JP-4 previously investigated by the Toxic Hazards Research Unit. Current information from two studies of the toxicity of JP-4 are included in this report.

The present study was designed to determine the chronic effects, including oncogenic, of long-term inhalation exposure of rats and mice to JP-TS jet fuel vapor. The same exposure regimen was followed as in previous experiments to investigate chronic toxicity. The results of this experiment will be used for comparison with studies done previously on fuels of a similar chemical nature.

Mice and rats were exposed for 1 year to 200 and 1000 mg/m^3 JP-TS vapor by the inhalation route in Thomas Dome chambers using an industrial work week schedule of 6 hours/day, 5 days/week with holidays and weekends excluded to simulate a human exposure regimen. Each exposure group consisted of 100 male and 100 female Fischer 344 rats and 100 male and 100 female C57BL/6 mice. A sham treated control group was housed in a Thomas Dome chamber.

Following the exposure period, 12 animals from each group were killed, 2 for electron microscopic and 10 for light microscopic examination. The remaining rodents were held 1 year for postexposure observation. All animals have now been necropsied and tissues are undergoing histopathologic examination.

Previous annual reports (MacEwen and Vernot, 1982, 1983) detail the experimental protocol for the 1-year inhalation exposure of rats and mice to JP-TS fuel and give results of effects on blood parameters and organ weights of male and female rats killed at exposure termination. Mean body weights and mortality effects through 24 months is also included. Histopathologic evaluation of tissues is being conducted by the Veterinary Pathology Group at Brooks Air Force Base.

A 90-DAY CONTINUOUS INHALATION EXPOSURE TO JP-8

As part of a series of 90-day inhalation tests of various hydrocarbon fuels used by the military, the THRU conducted a study of JP-8. The test was designed to conform with previously

conducted 90-day tests. In addition, more frequent sacrifices with emphasis on kidney examination were incorporated into the study protocol. A detailed discussion of the experimental procedures and tests performed during the study along with the JP-8 generation and analysis methods was presented in an earlier annual report (MacEwen and Vernot, 1983).

Exposure of rats and mice to JP-8 vapors began in late 1982 and terminated in early 1983. Two exposure concentrations were tested, 500 mg/m^3 and 1000 mg/m^3. Sham exposed controls were maintained in Thomas Dome inhalation chambers. Experimental groups consisted of 95 male and 75 female Fischer 344 rats and 100 male and 100 female C57BL/6 mice. Following completion of the exposure 15 rats and 25 mice of each sex were killed and tissues were collected for microscopic examination. Additional sacrifices were conducted at 2 weeks, 2 months, and 9 months postexposure. Results of these examinations have been presented in previous annual reports (MacEwen and Vernot, 1983, 1984).

Since the last annual report, the scheduled postexposure observation period concluded after 2 years on study. This represented 21 months postexposure. All remaining rats and mice were sacrificed in late December 1984 for tissue collection and examination, rat organ weights were measured, blood samples were taken for hematology and clinical chemistry measurements, and urinalyses were performed.

Results

Final mortality results are shown in Table 46.

TABLE 46. MORTALITY[a] IN ANIMALS EXPOSED TO JP-8 JET FUEL

	Control	500 mg/m^3	1000 mg/m^3
C57BL/6 Mice, Male	37/100	47/100[b]	47/100[b]
C57BL/6 Mice, Female	37/100	42/100	45/100
Fischer 344 Rats, Male	22/95	19/95	18/95
Fischer 344 Rats, Female	9/75	11/75	19/75

[a] Values represent number of natural or moribund deaths/original number of animals.
[b] Different from control, $p < 0.05$.

The Mantel-Cox test was used to examine the survival data for rats and mice. Both groups of male mice exposed to JP-8 had significantly higher mortality rates compared to controls. However, no dose response relationship was indicated. Examination of the gross pathology records indicated that the vast majority of these mice were sacrificed moribund with necrotic dermatitis. Mortality rates of female mice, male rats and female rats were comparable to controls. Because of the high mortality rate in the male mouse group it was necessary to sacrifice the remaining mice at 20 months postexposure rather than at 21 months as originally planned. This early sacrifice allowed for the collection of adequate numbers of fresh tissue. Rats were allowed to complete the full 21 months of postexposure holding.

The body weights of male rats are shown in Figure 4. Both groups of male rats exposed to JP-8 demonstrated reduced weight gain when compared to controls. Differences were significant at $p < 0.01$ throughout the exposure and postexposure phases of the study, but there was no clear dose response relationship. During the first year of the study male rats exposed to 1000 mg/m^3 had

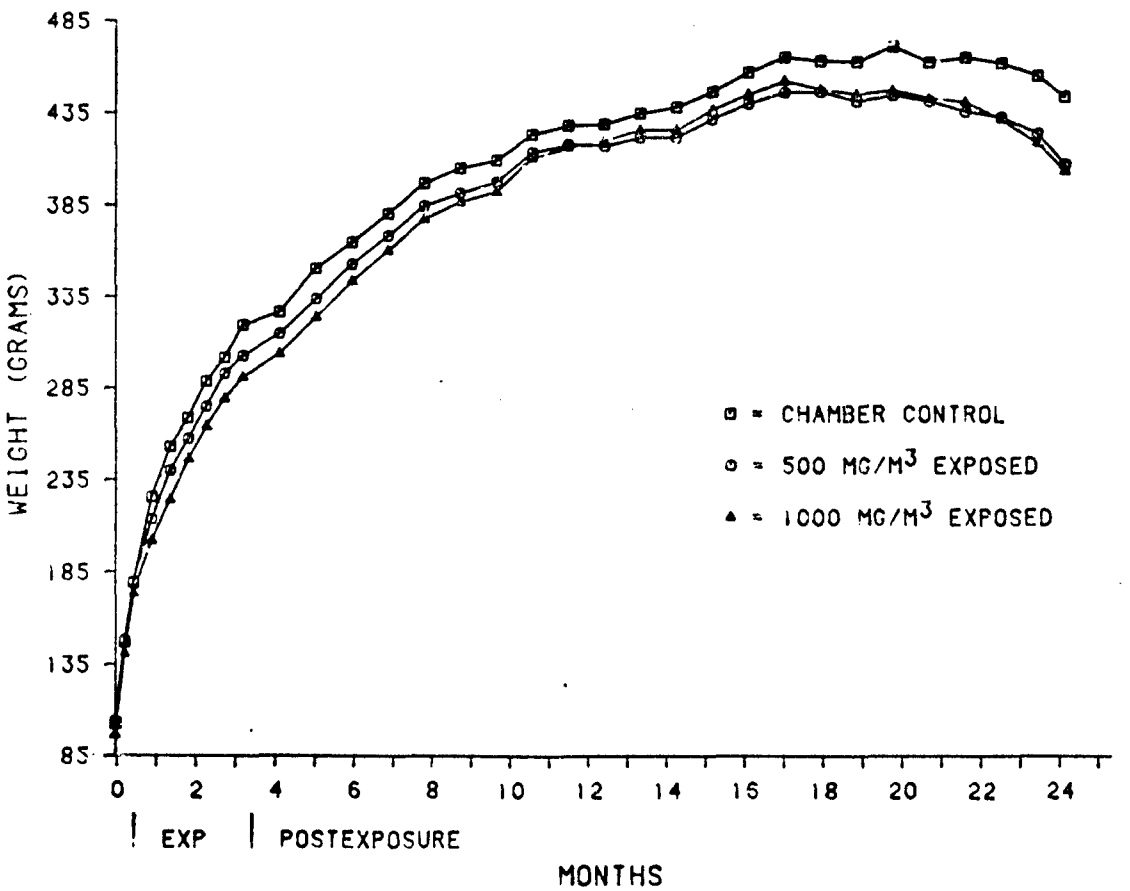


Figure 4. Effect of JP-8 on male rat body weight gain.

the lowest body weights, but during the subsequent year the 500 mg/m^3 male rat exposure group showed lower body weights. Female rat body weights (Figure 5) were generally unaffected by exposure to JP-8. Exposure to 1000 mg/m^3 did result in weight differences ($p < 0.05$) near exposure termination; however, this was a transient effect with no further indication of weight differences noted in subsequent measurements.

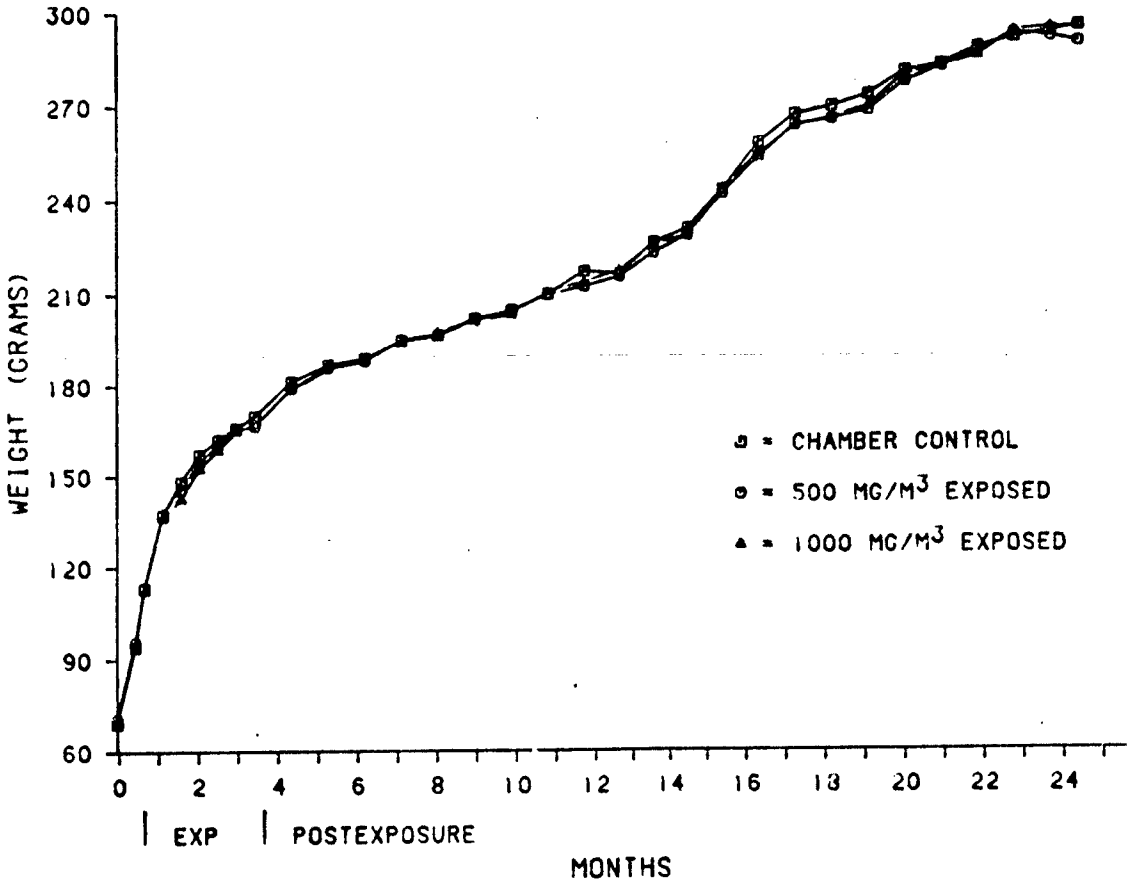


Figure 5. Effect of JP-8 on female rat body weight gain.

Rat organ weights measured at study termination are shown in Table 47. Previously, at exposure termination, increased kidney and liver weights had been noted in male rats exposed to JP-8. At 21 months postexposure there was a slight, but significant, dose related increase in relative liver and kidney weights in exposed male rats compared to controls. Absolute liver and kidney weights failed to demonstrate this dose response relationship. No significant organ weight effects were seen in female

TABLE 47. ORGAN WEIGHTS[a] OF RATS 21 MONTHS AFTER 90-DAY CONTINUOUS EXPOSURE TO JP-8

Concentration (mg/m^3)	Male Rats						
	Weight (g)				% of Body		
	Body	Liver	Kidney	Spleen	Liver	Kidney	Spleen
Control	440 ± 9	13.88 ± 0.52	3.24 ± 0.15	1.92 ± 0.41	3.16 ± 0.11	0.74 ± 0.04	0.45 ± 0.10
500	370 ± 10[b]	11.89 ± 0.62[b]	3.10 ± 0.16	1.16 ± 0.15[b]	3.25 ± 0.22[b]	0.85 ± 0.06[b]	0.31 ± 0.04
1000	376 ± 9[b]	13.53 ± 0.69	3.64 ± 0.23[b]	1.64 ± 0.53	3.61 ± 0.19[b]	0.98 ± 0.09[b]	0.44 ± 0.15
Concentration (mg/m^3)	Female Rats						
	Weight (g)				% of Body		
	Body	Liver	Kidney	Spleen	Liver	Kidney	Spleen
Control	278 ± 4	7.69 ± 0.20	1.94 ± 0.03	0.56 ± 0.04	2.77 ± 0.04	0.70 ± 0.01	0.20 ± 0.02
500	269 ± 5	7.06 ± 0.21	1.89 ± 0.04	0.59 ± 0.03	2.63 ± 0.05	0.70 ± 0.01	0.22 ± 0.01
1000	277 ± 7	7.37 ± 0.25	1.89 ± 0.04	0.78 ± 0.22	2.67 ± 0.09	0.69 ± 0.02	0.28 ± 0.08

[a] Mean ± SE, N = 9 or 10 samples/group.
[b] Different from control, $p < 0.01$.

rats examined at 21 months postexposure. Increased liver weight had been noted in both groups of female rats examined at exposure termination.

The results of the blood examinations conducted at the study termination sacrifice are shown in Tables 48 and 49 for male and female rats, respectively. Slight reductions in red blood cell counts, hematocrit, and hemoglobin levels were noted in male rats exposed to 1000 mg/m^3 JP-8. These findings were consistent with the reductions in these erythrocyte parameters previously noted in male rat blood examined at exposure termination, 2 weeks post-exposure and 9 months postexposure. Increased serum BUN was also noted in male rats exposed to 1000 mg/m^3 JP-8. This effect had also been seen in the earlier examinations. Female rat blood failed to indicate any effects that could be considered exposure related.

Male rat urine osmolality and pH values are shown in Table 50. For comparison, results of previous examinations are also shown. Decreased osmolality was noted in male rats exposed to 1000 mg/m^3 JP-8 immediately upon exposure termination and at subsequent postexposure examination periods. Examination of urine pH values failed to suggest any consistent exposure related effect.

Although the complete examination of all tissues collected from the animals in this study has not been completed, there have been limited examinations of respiratory tissues collected from rats at exposure termination. There was a high incidence of pulmonary inflammatory lesions noted in control groups indicating that the rats were experiencing a subclinical pneumonia at the

TABLE 48. MALE RAT BLOOD PARAMETERS[a] 21 MONTHS AFTER 90-DAY CONTINUOUS EXPOSURE TO JP-8

	Control	500 mg/m^3	1000 mg/m^3
WBC (x10^3 cells/mm^3)	7.2 ± 1.0	6.7 ± 0.6	6.7 ± 0.7
RBC (x10^6 cells/mm^3)	9.11 ± 0.43	8.39 ± 0.37[b]	8.09 ± 0.27[c]
HGB (g/dl)	17.1 ± 0.8	15.8 ± 0.7	15.6 ± 0.4[b]
HCT (%)	47.5 ± 1.9	44.2 ± 1.8	43.7 ± 1.3[b]
MCV (μm^3)	52.3 ± 0.4	52.8 ± 0.5	54.1 ± 0.3
MCH (pg)	18.8 ± 0.3	18.9 ± 0.2	19.4 ± 0.2
MCHC (g/dl)	36.0 ± 0.5	35.7 ± 0.3	35.8 ± 0.3
Glucose (mg/dl)	179 ± 14	221 ± 28[b]	202 ± 8
Tot. Pro. (g/dl)	6.59 ± 0.10	6.57 ± 0.12	6.61 ± 0.12
Albumin (g/dl)	0.85 ± 0.02	0.82 ± 0.03	0.79 ± 0.03
Globulin (g/dl)	5.74 ± 0.09	5.75 ± 0.09	5.81 ± 0.10
A/G Ratio	0.15 ± 0.01	0.14 ± 0.01	0.14 ± 0.01
BUN (mg/dl)	19.0 ± 0.5	22.0 ± 2.1	23.8 ± 1.5[c]
Creatinine (mg/dl)	0.66 ± 0.02	0.81 ± 0.09[c]	0.77 ± 0.04
Calcium (mg/dl)	10.7 ± 0.2	11.1 ± 0.2	11.2 ± 0.2[b]
SGOT (IU/L)	86 ± 3	118 ± 11[c]	92 ± 6
SGPT (IU/L)	45 ± 4	49 ± 7	44 ± 3
Alk. Phos. (IU/L)	73 ± 8	67 ± 5	58 ± 4
Bilirubin (mg/dl)	0.23 ± 0.03	0.34 ± 0.12[c]	0.29 ± 0.03

[a] Mean ± SE, N = 7-10 samples/group.
[b] Different from control, $p < 0.05$.
[c] Different from control, $p < 0.01$.

time of sacrifice. The etiologic agent for the pneumonia was most likely a virus, but no distinct morphologic features were present which would incriminate a singular viral disease. Because viral agents or environmental conditions causing pneumonia in rodents may subsequently influence tumorigenesis in both positive and negative fashions, it was decided to submit serological samples to an independent laboratory for analysis.

Samples were collected from 20 control, 13 low dose, and 8 high dose rats sacrificed at 9 months postexposure. Results of the tests for rat coronavirus (RCV) and sialodacryoadenitis (SDA) using ELISA methodology were positive in the vast majority of cases. A positive response for this test is > 0.17 absorbance units. Most of the rats tested demonstrated values in excess of 1.00 absorbance units, indicating that at some time during their life these rats had become infected with these viral agents.

TABLE 49. FEMALE RAT BLOOD PARAMETERS[a] 21 MONTHS AFTER 90-DAY CONTINUOUS EXPOSURE TO JP-8

	Control	500 mg/m^3	1000 mg/m^3
WBC (x10^3 cells/mm^3)	4.8 ± 0.2	4.8 ± 0.4	4.5 ± 0.2
RBC (x10^6 cells/mm^3)	7.40 ± 0.13	7.47 ± 0.09	7.56 ± 0.20
HGB (g/dl)	15.0 ± 0.3	15.1 ± 0.1	15.1 ± 0.3
HCT (%)	40.2 ± 0.8	40.7 ± 0.5	40.9 ± 1.1
MCV (μm^3)	54.3 ± 0.2	54.4 ± 0.3	54.2 ± 0.2
MCH (pg)	20.3 ± 0.1	20.2 ± 0.1	20.1 ± 0.2
MCHC (g/dl)	37.4 ± 0.1	37.1 ± 0.3	37.0 ± 0.4
Glucose (mg/dl)	240 ± 29	176 ± 10[b]	178 ± 8[b]
Tot. Pro. (g/dl)	7.95 ± 0.2	7.45 ± 0.07[c]	7.49 ± 0.12
Albumin (g/dl)	1.03 ± 0.02	1.00 ± 0.01	1.02 ± 0.03
Globulin (g/dl)	6.93 ± 0.20	6.45 ± 0.06[b]	6.47 ± 0.11[c]
A/G Ratio	0.15 ± 0.02	0.15 ± 0.01	0.16 ± 0.01
BUN (mg/dl)	16.7 ± 1.0	14.6 ± 0.3	15.2 ± 0.5
Creatinine (mg/dl)	0.78 ± 0.02	0.64 ± 0.03[b]	0.63 ± 0.02[b]
Calcium (mg/dl)	13.4 ± 2.0	10.9 ± 0.3	11.1 ± 0.2
SGOT (IU/L)	134 ± 9	104 ± 6[c]	130 ± 13
SGPT (IU/L)	70 ± 12	53 ± 3[c]	52 ± 4[c]
Alk. Phos. (IU/L)	88 ± 17	73 ± 7	66 ± 6
Bilirubin (mg/dl)	0.23 ± 0.02	0.17 ± 0.02	0.19 ± 0.02

[a] Mean ± SE, N = 8 to 10 samples/group.
[b] Different from control, $p < 0.01$.
[c] Different from control, $p < 0.05$.

TABLE 50. URINE OSMOLALITY AND pH VALUES[a] OF RATS 21 MONTHS AFTER 90-DAY CONTINUOUS EXPOSURE TO JP-8

	Examination Period - Osmolality (mOsm/L)				
Concentration (mg/m^3)	Preexposure	Exposure Termination	2-Week Postexposure	9-Month Postexposure	21-Month Postexposure
Control	1450 ± 89	1005 ± 60	1496 ± 114	1078 ± 155	890 ± 118
500	1462 ± 83	925 ± 141	1169 ± 57[b]	765 ± 80	463 ± 47[b]
1000	1412 ± 113	794 ± 60[c]	1223 ± 43[c]	799 ± 84	517 ± 47[b]

	Examination Period - pH Value				
Concentration (mg/m^3)	Preexposure	Exposure Termination	2-Week Postexposure	9-Month Postexposure	21-Month Postexposure
Control	8.1 ± 0.1	7.7 ± 0.7	7.4 ± 0.2	7.9 ± 0.2	7.0 ± 0.1
500	7.7 ± 0.1	7.1 ± 0.1[b]	6.5 ± 0.0[b]	8.4 ± 0.1[c]	6.5 ± 0.0[c]
1000	7.8 ± 0.2	7.2 ± 0.1[c]	7.9 ± 0.3[c]	7.8 ± 0.2	6.8 ± 0.1

[a] Mean ± SE, N = 9 to 10 samples/group.
[b] Different from control, $p < 0.01$.
[c] Different from control, $p < 0.05$.

The effects noted in rats exposed to JP-8 appear to be similar to those observed in rats exposed to other hydrocarbon fuels. Although most of these changes are slight they include anemia, increased kidney and liver weight, and decreased urine concentrating ability. Tissue samples from the animals from this study are being sent to an independent pathological services group for examination. Results of these examinations will be presented in a future annual report.

THE EXPERIMENTAL DETERMINATION OF SAFE ATMOSPHERIC EXPOSURE CONCENTRATIONS OF JP-10 JET FUEL

JP-10 is used 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. It is also used as a missile propellant in air breathing turbojet engines. JP-10 is a synthetic saturated polycyclic hydrocarbon identified as tricyclo ($5.2.1.0^{2,6}$) decane. It is the exo-isomer of tetrahydrodicyclopentadiene. Gas chromatographic analysis of this fuel indicated that it has a 98% purity with the endo-isomer of tetrahydrodicyclopentadiene as the major impurity (about 2%). This information was presented in a detailed review of the chemistry and the use of JP-10 at the Thirteenth Conference on Environmental Toxicology (1982) by Inman.

The acute toxicity of JP-10 was reported by Kinkead et al. (1979). Oral LD_{50} values were unobtainable for male or female Fischer 344 rats and for Golden Syrian hamsters since the maximum usable volume dose of 20 mL/kg caused only partial mortality in either species. MacEwen and Vernot (1979) reported an LD_{50} for female C57BL/6 mice of 3.9 mL/kg. Deaths occurred within 48 hours of treatment with convulsions preceding death. The ip LD_{50} values for rodents were 1.2 mL/kg and 1.6 mL/kg for male and female Fischer 344 rats, respectively, and for female C57BL/6 mice, 1.1 mL/kg. Male Golden Syrian hamsters were reported to have an ip LD_{50} value of 1.4 mL/kg. JP-10 caused no irritation to eyes or skin of New Zealand White rabbits, but was found to produce mild dermal sensitization in Hartley strain guinea pigs. MacEwen and Vernot also reported that the 4-hour LC_{50} for inhaled JP-10 was 1221 ppm for male rats and 1194 ppm for female rats. The ALC_{50} for female mice was given as 930 ppm. No mortality was seen in hamsters exposed 6 hours to saturated vapor pressure concentrations. JP-10 produced no deaths in a group of New Zealand White rabbits following dermal applications of 20 mg/kg (MacEwen and Vernot, 1980).

Emergency exposure limit studies of inhaled JP-10 vapors were reported by Kinkead et al. (1979). After single short high level exposures of beagle dogs, rats, and mice they found slight CNS responses at very high concentrations. They recommended concentrations of 1000, 600, and 150 ppm for 10, 30, and 60 minutes duration for short-term exposure limits.

JP-10 was not embryotoxic for pregnant ICR mice treated with doses of this fuel up to 0.8 mg/kg during organogenesis (Lyng, 1981). In studies reported by Keller et al. (1983) JP-10 did not produce change in fetal weight or difference from controls of malformations or resorption of rat litters from dams treated with doses of JP-10 up to 1000 mg/kg on gestation days 6 through 15. Inhalation exposures of pregnant rats during this same period to a 600 ppm concentration for 6 hours/day caused some convulsions in the dams but no measureable changes in the fetuses or pups in resulting litters.

Short-term bioassays were described by Arthur D. Little Inc. (1982) in which only a marginal clastogenic effect was reported for the CHO/chromosome abberation assay. Negative or inconclusive responses were reported for the Ames Salmonella/mammalism microsomal mutagenicity assay, the CHO/HGPRT[2] gene mutation assay, the CHO/sister chromatid assay, and the BALB/C-3T3 neoplastic transformation assay.

The tissue distribution of JP-10 after intraperitoneal injection of radiolabeled fuel was reported by Inman et al. (1982) who also identified the major urinary metabolite as 5-hydroxy exo-tetrahydrodicyclopentadiene excreted as the glucuronide conjugate.

Because the use of JP-10 in operational missiles was being expanded, the numbers of fuel handlers exposed to this material were also increasing, and there was a need to develop data for hazard evaluation and to establish safe exposure limits.

Preliminary acute inhalation experiments had shown that mice were the most sensitive species to JP-10 when 6 animals exposed to 1000 ppm died 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 days' exposure. One mouse had a slight convulsion early on 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, a concentration of 100 ppm (556 mg/m^3) JP-10 was selected for chronic inhalation studies with animals to determine safe exposure limits.

The JP-10 used for these animal exposures was obtained by the Air Force from Suntech, Inc., Marcus Hook, Pennsylvania.

Purebred beagle dogs were selected from a baseline group on the basis of examination and general observation of good health and several preexposure clinical chemistry determinations. Fischer 344 rats and Golden Syrian hamsters were obtained from the Charles River Breeding Laboratories. C57BL/6 mice were purchased from the Jackson Laboratory and beagle dogs from Ridgelan Farms Inc. Two-hundred female mice, 100 male hamsters, 50 rats and 4 dogs of each sex were used in the exposure group and an equal number of animals of each sex and species served as untreated controls. The dogs and rats were housed in one exposure chamber with mice and hamsters in a separate companion chamber.

Animal exposures to JP-10 were conducted for 1 year, using an industrial work week schedule of 6 hours/day, 5 days/week, with holidays and weekends off to simulate a human exposure regimen. The Thomas Dome exposure chambers (Thomas, 1965) were operated with nominal airflows of 30 cfm at a slightly reduced pressure, 725 mm Hg, to avoid leakage of JP-10 vapor into the laboratory environment.

Details of the exposure methods, tests performed, and some results were presented in a previous annual report (MacEwen and Vernot, 1983). During the current report period, results of histopathologic examination of tissues from the exposed and control dogs were received.

Histopathologic findings in the 15 dogs that were sacrificed 5 years postexposure were considered to be common changes typically seen in aging dogs. Two exposed males had patchy, mild testicular atrophy characterized by loss of spermatogonia. Since evidence of long-term testicular damage was lacking, this lesion was probably not exposure related. Three tumors were seen in the dogs used in this study. One control male had an edematous polyp of the anus, while an adenoma was seen in the adrenal cortex of another male control. An adrenal pheochromocytoma was observed in an exposed female dog. All of these tumors are common in dogs.

Histologic changes noted in rats, mice, and hamsters were previously reported. The important findings in these species were an increased incidence of fatty livers in JP-10 exposed mice, and of more significance, renal tubular nephrosis together with a significant increase in benign and malignant renal cell tumors in male rats. The significance of renal carcinoma and increased renal nephropathy in the male rat is not clearly understood. Nephropathy, common to many hydrocarbon fuels, has not been seen in the female rat nor in mice, hamsters or dogs. To date there has been no conclusive evidence of renal neoplasia in man resulting from exposure to hydrocarbon fuels.

The results of this study provide evidence that 100 ppm JP-10 may not be a safe exposure level for man. Based on the information developed in this study, a time weighted average (TWA) interim exposure limit of 25 ppm JP-10 has been recommended (McNaughton, 1981; McNaughton et al., 1984).

SUBCHRONIC 90-DAY CONTINUOUS INHALATION EXPOSURE TO DIMETHYL METHYLPHOSPHONATE

Dimethyl methylphosphonate (DMMP) is used by the military as a nerve gas simulant in training exercises. Since this type of use may result in human exposure through inhalation of DMMP vapor or physical contact with skin and clothing, the Air Force requested that the THRU evaluate the toxic hazard associated with DMMP.

A 90-day continuous exposure regimen was developed to test DMMP. Mice and rats were exposed to concentrations of either 25 ppm or 250 ppm DMMP. A sham exposed control group was also maintained. Groups consisted of 85 male and 85 female Fischer 344 rats and 100 male and 100 female C57BL/6 mice. Following the exposure period 15 rats and 25 mice of each sex from each group were sacrificed for tissue collection and examination. An additional 10 rats and 10 mice of each sex and group were sacrificed at 3 and 12 months postexposure. All groups will be terminated for examination at the 24th month of the study.

A complete description of the DMMP generation and monitoring system was provided in the previous annual report (MacEwen and Vernot, 1984). Also included in that report were the results of the examinations of blood samples and organ weights conducted at exposure termination and 3 months postexposure. Since that report the 12 month postexposure sacrifice has been completed.

Results

Mortality in animals exposed continuously to DMMP for 90 days is shown in Table 51.

TABLE 51. MORTALITY[a] IN ANIMALS EXPOSED TO DMMP

	Control	25 ppm	250 ppm
C57BL/6 Mice, Male (17)[b]	15/55	17/54	28/55[c]
C57BL/6 Mice, Female (17)	26/55	31/54	43/61[c]
Fischer 344 Rats, Male (18)	7/50	10/47	26/48[c]
Fischer 344 Rats, Female (18)	12/47	11/48	16/45

[a] Denominator censored to exclude scheduled sacrifices, missing, and accidental deaths. Original N values = 100 mice/sex, 85 rats/sex.
[b] Postexposure month.
[c] Different from control, $p < 0.05$.

Analysis of the mortality data with a Mantel-Cox test indicated significant difference between the male and female mice and male rat exposure groups and respective controls.

Male rat body weights are shown in Figure 6. Male rats exposed to 250 ppm DMMP demonstrated reduced weight gains during the 90 day exposure period when compared to controls. Subsequently, the body weights returned to normal. However, during the later period of postexposure observation, the male rats exposed to 250 ppm DMMP have shown a considerable weight loss. Although both controls and 25 ppm DMMP exposed male rats are also showing a weight loss, it is not as severe as that seen in the high level exposure group. Female rats exposed to 250 ppm DMMP also gained less weight than control rats during the exposure (Figure 7). Subsequent postexposure weighings showed sustained weight gain in the 250 ppm exposure group, and at 3 months after removal from exposure no significant differences were noted between the body weights of this group and control female rats. Exposure to 25 ppm DMMP did not affect female rat body weight gain.

Blood was collected from the rats sacrificed at 12 months postexposure. The results of hematology and clinical chemistry measurements are shown in Tables 52 and 53 for males and female

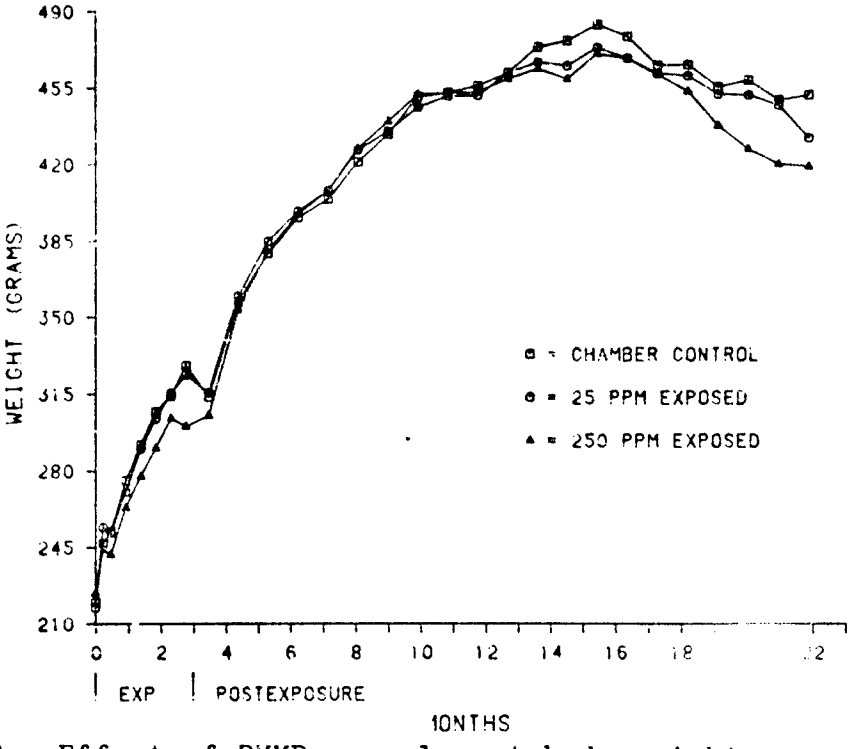


Figure 6. Effect of DMMP on male rat body weight.

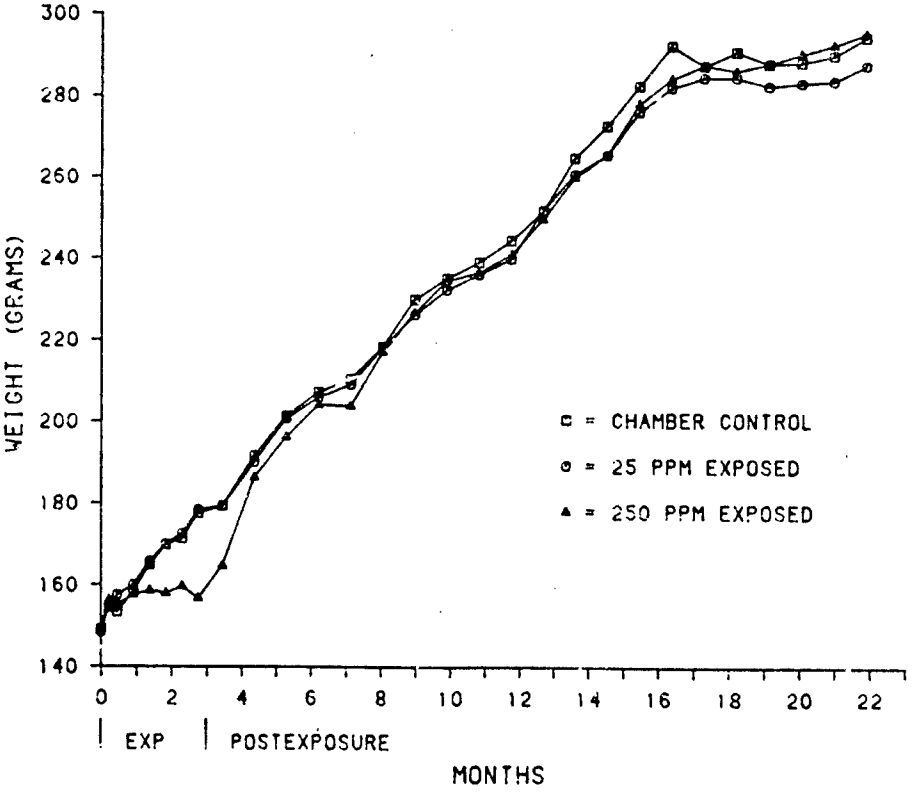


Figure 7. Effect of DMMP on female rat body weight.

TABLE 52. MALE RAT BLOOD PARAMETERS[a] 12 MONTHS AFTER EXPOSURE TO DMMP

	Control	25 ppm	250 ppm
WBC ($x10^3$ cells/mm^3)	6.2 ± 0.2	6.6 ± 0.4	6.6 ± 0.3
RBC ($x10^6$ cells/mm^3)	8.81 ± 0.13	8.20 ± 0.10[b]	8.12 ± 0.22[b]
HGB (g/dl)	15.6 ± 0.2	14.9 ± 0.2	14.8 ± 0.3[b]
HCT (%)	43.5 ± 0.8	40.0 ± 0.5[b]	40.8 ± 1.1[b]
MCV (μm^3)	49.4 ± 0.2	48.8 ± 0.2	50.3 ± 0.3
MCH (pg)	17.8 ± 0.2	18.2 ± 0.9	18.2 ± 0.2
MCHC (g/dl)	36.0 ± 0.4	37.3 ± 0.1[b]	36.3 ± 0.4
Glucose (mg/dl)	300 ± 33	209 ± 9	285 ± 44
Tot. Prc. (g/dl)	7.25 ± 0.04	7.15 ± 0.06	6.90 ± 0.07[c]
Albumin (g/dl)	0.91 ± 0.01	0.88 ± 0.02	0.82 ± 0.01[c]
Globulin (g/dl)	6.35 ± 0.04	6.27 ± 0.06	6.09 ± 0.07[b]
A/G Ratio	0.14 ± 0.002	0.14 ± 0.003	0.13 ± 0.002[b]
BUN (mg/dl)	15.7 ± 0.4	15.6 ± 0.9	18.4 ± 0.7[b]
Creatinine (mg/dl)	0.6 ± 0.02	0.5 ± 0.02	0.5 ± 0.02
Calcium (mg/dl)	11.6 ± 0.2	11.1 ± 0.1	11.2 ± 0.1
SGOT (IU/L)	97 ± 5	115 ± 7	92 ± 14
SGPT (IU/L)	80 ± 4	79 ± 5	78 ± 9
Alk. Phos. (IU/L)	95 ± 7	92 ± 6	93 ± 10
Bilirubin (mg/dl)	0.2 ± 0.01	0.2 ± 0.02	0.2 ± 0.02

[a] Mean ± SE, N = 10.
[b] Different from control, $p < 0.05$.
[c] Different from control, $p < 0.01$.

rats, respectively. Although there were some statistical differences between the test and control male rats, all values were within normal ranges. Male rats exposed to DMMP continued to show reduced red blood cell counts, hematocrit and hemoglobin levels. These reductions were slight, but they were consistent with the effects noted in male rats examined at exposure termination and 3 months postexposure. No abnormal values were noted in the female rats examined. At exposure termination blood samples obtained from female rats exposed to 250 ppm DMMP had shown reduced erythrocyte parameters.

Organ weights measured at the 12-month postexposure sacrifice are shown in Table 54. Organ weights relative to body weights are presented in Table 55. Reduced fasted body weights were seen in both groups of exposed male rats when compared to controls. This was not considered to be an exposure-related effect since there was no dose response and normal non-fasted body

TABLE 53. FEMALE RAT BLOOD PARAMETERS[a] 12 MONTHS AFTER EXPOSURE TO DMMP

	Control	25 ppm	250 ppm
WBC ($x10^3$ cells/mm^3)	4.9 ± 0.3	4.1 ± 0.4	3.9 ± 0.2
RBC ($x10^6$ cells/mm^3)	7.33 ± 0.43	7.51 ± 0.16	7.61 ± 0.92
HGB (g/dl)	15.4 ± 0.3	15.1 ± 0.2	15.2 ± 0.2
HCT (%)	39.2 ± 2.2	40.6 ± 0.8	41.2 ± 0.6
MCV (μm^3)	53.6 ± 0.3	54.1 ± 0.2	54.2 ± 0.4
MCH (pg)	22.2 ± 2.3	20.2 ± 0.3	19.9 ± 0.2
MCHC (g/dl)	41.2 ± 4.0	37.4 ± 0.7	36.8 ± 0.4
Glucose (mg/dl)	179 ± 12	194 ± 24	163 ± 5
Tot. Pro. (g/dl)	7.61 ± 0.11	7.49 ± 0.16	7.33 ± 0.09
Albumin (g/dl)	0.96 ± 0.02	0.98 ± 0.02	0.95 ± 0.01
Globulin (g/dl)	6.64 ± 0.10	6.53 ± 0.15	6.39 ± 0.09
A/G Ratio	0.15 ± 0.003	0.15 ± 0.003	0.15 ± 0.002
BUN (mg/dl)	17.5 ± 0.9	15.8 ± 0.6	16.4 ± 0.6
Creatinine (mg/dl)	0.5 ± 0.02	0.5 ± 0.03	0.4 ± 0.04
Calcium (mg/dl)	11.3 ± 0.1	11.0 ± 0.3	10.8 ± 0.1
SGOT (IU/L)	127 ± 17	93 ± 9	94 ± 6
SGPT (IU/L)	82 ± 16	60 ± 6	72 ± 6
Alk. Phos. (IU/L)	88 ± 7	72 ± 4	74 ± 4
Bilirubin (mg/dl)	0.2 ± 0.02	0.2 ± 0.02	0.2 ± 0.02

[a] Mean ± SE (N).

TABLE 54. ORGAN WEIGHTS[a] OF RATS 12 MONTHS AFTER 90-DAY CONTINUOUS EXPOSURE TO DMMP

Concentration (ppm)	Male Rats Weight (g) Body	Liver	Kidney	Spleen	Testes
Control	472 ± 8	12.70 ± 0.48	3.09 ± 0.08	0.75 ± 0.03	3.67 ± 0.09
25	436 ± 7[b]	12.64 ± 0.43	3.01 ± 0.06	0.76 ± 0.02	3.64 ± 0.07
250	442 ± 8[c]	13.19 ± 0.41	3.28 ± 0.08[c]	0.85 ± 0.03[c]	2.27 ± 0.23[b]

Concentration (ppm)	Female Rats Weight (g) Body	Liver	Kidney	Spleen
Control	254 ± 13	6.54 ± 0.23	1.82 ± 0.05	0.50 ± 0.01
25	257 ± 5	6.46 ± 0.15	1.78 ± 0.03	0.52 ± 0.02
250	255 ± 5	6.46 ± 0.15	1.82 ± 0.03	0.54 ± 0.02

[a] Mean ± SE, N = 9 or 10 samples/group.
[b] Different from control, $p < 0.01$.
[c] Different from control, $p < 0.05$.

TABLE 55. RELATIVE ORGAN WEIGHTS[a] OF RATS 12 MONTHS AFTER 90-DAY CONTINUOUS EXPOSURE TO DMMP

Concentration (ppm)	Male Rats			
	Liver	Kidney	Spleen	Testes
Control	2.69 ± 0.08	0.66 ± 0.01	0.16 ± 0.01	0.78 ± 0.02
25	2.90 ± 0.07	0.69 ± 0.01	0.17 ± 0.003	0.84 ± 0.03
250	2.99 ± 0.08[b]	0.74 ± 0.02[b]	0.19 ± 0.01[c]	0.52 ± 0.06[c]

Concentration (ppm)	Female Rats		
	Liver	Kidney	Spleen
Control	2.63 ± 0.18	0.74 ± 0.05	0.20 ± 0.01
25	2.52 ± 0.06	0.70 ± 0.01	0.20 ± 0.01
250	2.54 ± 0.05	0.71 ± 0.02	0.21 ± 0.01

[a] Mean ± SE (% of body weight), N = 9 or 10 samples/group.
[b] Different from control, $p < 0.01$.
[c] Different from control, $p < 0.05$.

weights for the same period are not greatly different. Consistent with the organ weight measurements taken at the 90-day exposure termination and 3 months postexposure were increased liver and kidney weights and reduced testicular weights in male rats exposed to 250 ppm. These changes were indicated in both absolute and relative organ weights. Changes in organ/body weight ratios of male rats at the 25 ppm level probably reflect the reduced body weight of the groups. No abnormal organ weight effects were seen in the female rats examined at 12 months postexposure. Increased liver and kidney weights had previously been noted in female rats exposed to 250 ppm DMMP when examined at the 90-day exposure termination sacrifice.

Discussion

Dunnick et al. (1984) reported reproductive effects in male rats receiving oral doses of DMMP. In that study no testicular weight loss was observed in rats treated with 63 doses of DMMP at concentrations up to 2000 mg/kg. Chapin et al. (1984) saw significantly reduced epididymis weight in male rats receiving daily oral doses of DMMP at 1750 mg/kg, 5 days/week for 12 weeks. However, no significant testicular weight loss was seen. These studies established male rat reproductive dysfunction as well as testicular tissue alteration.

The present study has demonstrated that inhalation exposure to 250 ppm DMMP for 13 weeks results in significant testicular weight reduction. Even after 1-year postexposure the testes of male rats exposed to 250 ppm DMMP continued to weigh substantially less than controls. Although microscopic examination of the tissues collected during the study has not yet begun, testicular tissue collected from male rats included in a dominant lethal study conducted by the Air Force as an adjunct to the 90-day study has shown degenerative change (unpublished data). These effects were limited to rats exposed to 250 ppm DMMP and coincided with a reduction in reproductive competency. Whether these effects also occurred in male mice exposed to DMMP vapor is unknown.

This study is continuing with terminal sacrifices of experimental animals scheduled for mid 1985. The results obtained at study termination and histologic evaluations will be presented in a future annual report.

A SUBCHRONIC INHALATION TOXICITY STUDY OF O-ETHYL-O'-(2-DIISOPROPYLAMINOETHYL)METHYLPHOSPHONITE (EDMP)

The THRU has been conducting a series of studies to characterize the toxic hazard associated with the Army chemical O-ethyl-O'-(2-diisopropylamincethyl)methylphosphonite (EDMP) also known by the Army designation, QL. Tests already completed by the THRU include single dose oral, intraperitoneal and dermal toxicity, irritation of skin and eye tissues, sensitization, and neurotoxicity (MacEwen and Vernot, 1984). Because of the possibility of EDMP inhalation exposure by personnel during manufacture, processing, or transportation, subchronic inhalation toxicity studies have also been conducted (McNamara et al., 1981).

As part of the THRU's investigation of the toxic effects of EDMP, a 13-week inhalation study was initiated. To establish the maximum tolerated concentration for the 13-week subchronic exposure, a 2-week inhalation study was also conducted. The results of these studies are being reviewed and will be presented in the next annual report.

THE DETERMINATION OF A 6-HOUR INHALATION LC_{50} OF O-ETHYL-O'-(DIISOPROPYLAMINOETHYL)METHYLPHOSPHONITE (EDMP) USING MALE AND FEMALE SPRAGUE-DAWLEY RATS

Because of the possibility of exposure of military and civilian personnel to EDMP during manufacturing, processing, or transportation, the Army has a strong interest in characterizing its acute toxicity. Previous experiments have been conducted to determine the effects of acute inhalation exposure to EDMP (Dimmick Jr. et al., 1979). Exposure to 12,300 mg/m^3 for 1 hour and 2,350 mg/m^3 for 6 hours failed to produce 50% mortality in rats or guinea pigs. Intravenous (iv) administration yielded an LD_{50} of 203.7 mg/kg in mice and 164.4 - 207.5 mg/kg in rabbits. The iv values would classify this material as toxic but not highly toxic. McNamara et al. (1981) performed 27-week subchronic inhalation studies to 5.0 and 22.4 mg/m^3. Experimental animals exposed were Sprague-Dawley/Wistar rats, ICR Swiss and "A" strain mice, and Hartley guinea pigs. Measurements were made of hematologic and clinical chemistry parameters, pulmonary resistance in guinea pigs, spontaneous activity in rats, and organ pathology.

Additionally specific tests were run to determine sensitization potential, dominant lethal mutation capability, teratogenicity, fetal toxicity, and effect on reproduction. In a multigeneration study, 3 generations of rats were examined after a 10-week exposure of the progenitors. The only positive finding was depression of red blood cell cholinesterase after 27 weeks of exposure to both concentrations in rats and in mice exposed to the higher level.

The unstable character of EDMP was demonstrated in the subchronic inhalation studies in which analyzed concentrations ranged from 13-41% of nominal.

Six-hour inhalation studies were performed in this laboratory using CDF®(Fischer 344)/CrlBR male and female rats and hybrid B6C3F1/CrlBR male and female mice (MacEwen and Vernot, 1984). The 6-hour LC_{50} values and 95% confidence limits for male and female rats are 2520 (2130-3280) and 209 (142-339) mg/m^3, respectively. The values for male and female mice are 3500 (3040-3480) and 2360 (2180-2570) mg/m^3, respectively. Female rats were found to be much more susceptible to the toxic effects of this compound than male rats or either sex of mice. This would indicate that the EDMP is more toxic than was found in the previous inhalation tests.

The unusual disparity in the toxic response of the female Fischer 344 rats was not noted in previous studies using Sprague-Dawley rats. To determine if the female Fischer 344 female rat reacts differently from females of other rat strains, the 6-hour inhalation exposures were repeated using male and female Sprague-Dawley rats.

The EDMP was supplied by the U. S. Army Chemical Systems Laboratory and was the same as was used in the previous studies.

Groups consisting of 10 male and 10 female Sprague-Dawley rats aged 9 to 11 weeks were used for the determination of the 6-hour LC_{50}. Control groups were maintained for comparative purposes.

The compound was aerosolized into a 1 m^3 Rochester chamber. A Solo-Sphere® and/or Collison Nebulizer were used for the generation of the aerosol. Andersen impactor samples were taken for particle size analysis.

Nominal concentrations were obtained by material balance calculation to correlate with methods for continuous chemical analysis by an infrared analyzer. A gas chromatographic analysis was run once/hour to determine relative concentration of EDMP and its decomposition products.

Ten rats were exposed at each concentration level, and the LC_{50} with its 95% confidence limits calculated using the probit method of Finney (1971). Deaths which occurred during the 14-day observation period were included in the final mortality tally.

The rats were observed frequently during the exposure and twice daily during the 14-day holding period. Visible signs of toxicity were recorded. Body weights of all animals were obtained prior to exposure and at 1, 2, 4, 7, and 14 days postexposure.

Any rat that died during exposure or during the 14-day observation period received gross examination. The surviving animals were observed for 14 days or until signs of reversible toxicity subsided at which time they were sacrificed for gross examination.

An additional group of 10 male and 10 female Sprague-Dawley and Fischer 344 rats were exposed for 6 hours to 500 mg/m^3 EDMP for determination of cholinesterase activity. The activity (Ellman, 1961) was measured in both serum and red cells.

The mortality data shown in Table 56 show the results of both male and female rat exposures. As was seen in the previous study using Fischer 344 rats, the toxicity of the compound appears to be biphasic in nature. Also, a sex-related toxicity difference was apparent in the Sprague-Dawley rat as it was in the Fischer 344 rat. The difference beteween LC_{50} of the male and female Fischer 344 rats was of a factor of 10 while the difference in the LC_{50} of male and female Sprague-Dawley rats is a factor of 4.

TABLE 56. MORTALITY AFTER 6-HOUR INHALATION EXPOSURES OF MALE AND FEMALE SPRAGUE-DAWLEY RATS TO EDMP

Concentration, mg/m^3	Mortality Ratio	
	Males	Females
3617	10/10	--
2955	8/10	--
2399	5/10	10/10
2026	1/10	8/10
1715	2/10	6/10
1534	0/10	3/10
1093	2/10	7/10
733	3/10	5/10
503	3/10	5/10
342	6/10	2/10
118	0/10	--
72	2/10	0/10

Female rat LC_{50} and 95% C.L. = 844 (535-1220) mg/m^3
Male rat LC_{50} and 95% C.L. = 2971 mg/m^3[a]

---

[a] Due to the wide spread in mortality effects, a confidence limit could not be calculated.

A group consisting of 10 male and 10 female Sprague-Dawley and 10 male and 10 female Fischer 344 rats were exposed for 6 hours to a mean concentration of 512 mg/m^3 EDMP. One female Sprague-Dawley, 1 male Fischer 344 and all 10 female Fischer 344 rats died following exposure.

Forty-eight hours after exposure, blood was sampled from 5 rats from each of the surviving groups for determination of cholinesterase activity. The activity was measured in both plasma and red cells with the results shown in Table 57. Cholinesterase was severely inhibited at 48 hours.

TABLE 57. EFFECT OF 6-HOUR INHALATION EXPOSURE TO 512 MG/M^3 EDMP ON CHOLINESTERASE RATS (N=5)

Strain	Sex	Percent of Pretreatment Cholinesterase Activity[a]	
		Plasma	Erythrocyte
Sprague-Dawley	M	28.7 ± 2.5	6.8 ± 1.6
Sprague-Dawley	F	36.2 ± 4.8	6.3 ± 1.7
Fischer 344	M	35.1 ± 2.3	5.9 ± 0.4

[a] Mean ± SE; 48 hours postexposure.

This study and the previous 6-hour LC_{50} inhalation study on Fischer 344 rats had indicated the possibility of a biphasic concentration effect on mortality. Repeat 6-hour exposures were done using only Fischer 344 male rats at preselected concentrations to determine if the biphasic effect was reproducible. Groups of 10 male Fischer 344 rats each were exposed to nominal concentrations of 2400, 2000, 1600, 1200, 800, or 400 mg/m^3. A summary of the results is shown in Table 58.

TABLE 58. EFFECT OF 6-HOUR INHALATION EXPOSURES TO EDMP ON MALE FISCHER 344 RATS (N=10)

Nominal Conc., mg/m^3	Analyzed Conc., mg/m^3	Mortality Ratio
2400	2460	10/10
2000	1937	10/10
1600	1618	10/10
1200	1175	10/10
800	800	10/10
400	397	10/10

All exposures resulted in 100% mortality. Analysis of this sample of EDMP has shown that the proportions of O-(diisopropylaminoethyl)methyl phosphinate and O-ethyl, O'-diisopropylaminoethyl)methyl phosphonate are much higher than what was found in the sample used for the earlier LC_{50} tests. The latest exposures have shown that the biphasic nature of previous mortality responses was probably a reflection of the imprecision of the LC_{50} determinations caused by differences in toxicity of EDMP aerosols of different composition. The results also imply that the LC_{50} obtained for female rats (209, 95% confidence limits = 142-339)

is a better measure of the acute toxicity of EDMP to rats than the previous value obtained for male rats (2520, 95% confidence limits = 2130-3280).

THE ACUTE TOXICITY TESTING OF FOUR COMPOUNDS ASSOCIATED WITH MANUFACTURE OF EDMP

Because of the possibility of exposure of military and civilian personnel to EDMP manufacturing byproducts, the Army has a strong interest in characterizing the acute toxicity of some of the precursors, intermediates, and byproducts associated with EDMP production. Three of the materials proposed for testing are also decomposition products of EDMP. The unstable character of EDMP has been previously demonstrated, and the breakdown products are thought to directly affect the acute toxicity attributed to EDMP.

This study, currently in progress, is designed to characterize the toxic effects of acute exposure of rats, mice, and rabbits to 4 compounds associated with EDMP. The compounds are being tested in the same manner as was used for the acute toxicity testing of EDMP. The data obtained from these experiments should permit objective assignment of sound occupational exposure limits for personnel working with these compounds. The compounds selected for testing are listed below with U. S. Army designations in parentheses:

1. Diisopropylaminoethanol (KB)
2. O,O'-Diethylmethylphosphonite (TR)
3. Bis (diisopropylaminoethyl)methylphosphonite (LT)
4. Triethylphosphite (TEP)

Compounds 1 and 3 were provided by the U. S. Army while compound 4 was purchased by UCI. The second compound (O,O'-diethylmethylphosphonite) has not been received from the Army to date.

Methods

Oral Toxicity

All compounds were dissolved or suspended in corn oil and the suspensions kept in a turbulent state while in use with a magnetic stirring platform.

Glass syringes with oral dosing needles were used to administer the compounds to the rats and mice that had been fasted for

at least 16 hours prior to administration of the oral dose. The dose volume for the test animals was 0.01 mL/gm body weight, and the animals were weighed individually at the time of dosing. Originally, male and female Sprague-Dawley rats and male and female CD-1 mice were used. However, following the peroral testing of diisopropylaminoethanol, the rat strain was changed to Fischer 344. Geometrically spaced doses were administered to determine the LD_{50} in ten animals of each sex at each level. The LD_{50} values with 95% confidence limits were calculated using the probit method of Finney (1971).

Skin Irritation

A patch-test method was utilized to determine the degree of primary skin irritation on the intact skin of 6 albino rabbits. The rabbits were clipped of all possible hair on the back and flanks 24 hours prior to exposure to allow for recovery from any abrasion. Undiluted test materials were applied in the amount of 0.5 mL to the designated patch area and covered by surgical gauze, held in place with strips of surgical adhesive tape. The entire area was covered with polyethylene plastic wrap and secured with more surgical adhesive tape. The patch remained in place on the rabbits for 4 hours, after which, the wrap and patch were carefully removed, and the test area evaluated for irritation using the Draize (1959) table as a reference standard. Readings were also made at 72 hours after treatment. A primary index rating was calculated using the following formula:

$$\text{Primary Index Score} = \frac{\text{Total Score}}{\text{No. Rabbits} \times \text{No. Sites} \times \text{No. Observations}}$$

The primary index score was interpreted using the NIOSH Skin Test Ratings (Campbell et al., 1975).

Eye Irritation

One tenth milliliter of the test compound was applied to one eye of each of 9 albino rabbits. The opposite eye was untreated and served as a control. The eyes were examined with fluorescein stain prior to use to ensure absence of lesions or injury and a topical anesthetic (Alcaine; Proparacaine HCl 0.5%) was instilled in the eyes, treated and control, of all rabbits approximately 2 minutes prior to application of the test substance. The treated eye of 6 rabbits remained unwashed while the other 3 rabbits had

the treated eye flushed for one minute with lukewarm water starting no sooner than 20-30 seconds after instillation. Examinations for gross signs of eye irritation were made 1, 2, 3, 4, and 7 days following application. If an injury occurred, the animals were scored three times a week until the lesion subsided or was deemed irreversible. The irritative effects were scored according to the method of Draize (1959).

Dermal Toxicity

Male and female albino New Zealand rabbits weighing between 2 and 3 kilograms were used. The back of the rabbits and the sides down to about halfway to the abdomen were clipped from the saddle area of the shoulders to the top of the rear leg area.

The animals were individually weighed prior to dosing to determine the proper dose volume which was applied undiluted to the back of the rabbit and divided as equally as possible between the two sides. The dose was kept in place by applying 8 ply gauze patches over the liquid on each side of the back. A patch of clear plastic wrap was then applied over the entire clipped back area and elastoplast tape was used to wrap the entire midsection of the rabbit. Specially designed rabbit restraining harnesses were fitted to each rabbit at the time of dosing and kept in place during the entire dosing period. These harnesses prevent excessive movement of the rabbits and prevent the rabbit from chewing on the taped area. The harnesses do, however, allow the rabbit access to food and water during the dosing period.

All doses were kept in contact with the rabbit's skin for 24 hours. The rabbits were observed for mortality or other signs of toxicity during the 14 days immediately following exposure. Any deaths that occurred in this period were included in the final tally.

The animals were observed frequently on the day of dosing and twice daily thereafter and all symptoms were recorded. Body weights were obtained at the time of dosing and on days 1, 2, 4, 7, 10, and 14 posttreatment.

Testing was initiated by dosing 5 animals of each sex with 2 mL of test material/kg of body weight. If there was no appreciable mortality at this level, then no additional dose levels were administered. If mortality was produced, then testing continued with 5 animals of each sex per dose level. LD_{50} calculations were made using the probit method of Finney (1971).

Inhalation

Groups consisting of 10 male and 10 female Fischer 344 rats and 10 male and 10 female CD-1 mice, all aged 9 to 11 weeks, were used for the determination of the 6-hour LC_{50}. Control groups were maintained for comparative purposes.

The compounds were aerosolized into a 1 m^3 Rochester chamber using A Solo-Sphere® Nebulizer for generation of the aerosol. Andersen impactor samples were taken for particle size analysis. Nominal concentrations were obtained by material balance calculation to correlate with methods for continuous chemical analysis. A gas chromatographic analysis was run once per hour to determine relative concentration of the test compound and its decomposition products.

Ten animals of each species were exposed at each concentration level, and the LC_{50} with its 95% confidence limits calculated using the probit method of Finney (1971). The animals were observed frequently during the exposure and twice daily during the 14-day holding period. Visible signs of toxicity were recorded and all animals were weighed prior to exposure as well as at 1, 2, 4, 7, and 14 days postexposure.

Any animal that died during exposure or the 14-day observation period received gross examination. The surviving animals were observed for 14 days or until signs of reversible toxicity subsided, at which time they were sacrificed and gross examination of lung, liver, kidney, heart, stomach, small and large intestine, spleen, and brain performed on each animal. If signs of neurotoxicity (ataxia, paralysis) were observed, sections of spinal cord and sciatic nerve were sampled for histologic examination.

RESULTS

Oral Toxicity

Diisopropylaminoethanol

Ten male and 10 female Sprague-Dawley rats and 10 male and 10 female CD-1 mice received oral doses of diisopropylaminoethanol in corn oil. Concentrations, mortality ratios, and LD_{50} values are shown in Tables 59 and 60 for mice and rats, respectively.

TABLE 59. ACUTE ORAL TOXICITY OF DIISOPROPYLAMINOETHANOL IN MALE AND FEMALE CD-1 MICE

Concentration, g/kg	Mortality Ratios	
	Males	Females
2.00	9/10	9/10
1.00	6/10	2/10
0.50	4/10	3/10
0.25	0/10	0/10
LD_{50} & (95% C.L.) g/kg =	0.77(0.52-1.14)	1.08(0.75-1.86)

TABLE 60. ACUTE ORAL TOXICITY OF DIISOPROPYLAMINOETHANOL IN MALE AND FEMALE SPRAGUE-DAWLEY RATS

Concentration, g/kg	Mortality Ratios	
	Males	Females
2.00	10/10	10/10
1.50	9/10	---
1.00	5/10	6/10
0.75	3/10	---
0.50	0/10	1/10
LD_{50} & (95% C.L.) g/kg =	0.96(0.81-1.14)	0.86(0.64-1.17)

Peroral doses of diisopropylaminoethanol produced tremors and convulsions in both rats and mice prior to death. Deaths normally occurred within 24 hours. Survivors had porphyrin exudate around eyes and noses for several days following the peroral dose. Normal weight gains were evident throughout the 14-day observation period in all surviving animals.

Triethylphosphite

Ten male and 10 female Fischer 344 rats and 10 male and 10 female CD-1 mice received oral doses of triethylphosphite in corn oil. Concentrations, mortality ratios, and LD_{50} values are shown in Tables 61 and 62 for mice and rats, respectively.

Peroral doses of triethylphosphite produced rapid breathing and tremors in the animals prior to death. Most deaths occurred within 24 hours of dosing. Surviving rats and mice showed normal weight gains during the subsequent 14-day observation period.

TABLE 61. ACUTE ORAL TOXICITY OF TRIETHYLPHOSPHITE IN MALE AND FEMALE CD-1 MICE

Concentration, g/kg	Mortality Ratios Males	Females
5.0	9/10	10/10
4.5	---	7/10
4.0	6/10	5/10
3.5	---	6/10
3.0	2/10	0/10
2.0	0/10	0/10
LD_{50} & (95% C.L.) g/kg =	3.7(3.2-4.3)	3.8(3.5-4.1)

TABLE 62. ACUTE ORAL TOXICITY OF TRIETHYLPHOSPHITE IN MALE AND FEMALE FISCHER 344 RATS

Concentration, g/kg	Mortality Ratios Males	Females
4.0	10/10	---
3.0	9/10	10/10
2.5	5/10	---
2.0	1/10	7/10
1.5	---	1/10
1.0	0/10	0/10
LD_{50} & (95% C.L.) g/kg =	2.5(2.2-2.7)	1.8(1.6-2.2)

Skin Irritation

Diisopropylaminoethanol

A summary of the skin irritation effects of diisopropylaminoethanol is shown in Table 63. This compound proved to be severe irritant and a corrosive (indicated by the necrosis in al 6 rabbits). The primary index score of 8.4 indicates that diisopropylaminoethanol is too irritating for human skin contact.

The effects of diisopropylaminoethanol contact was evident on the rabbits skin through 4 weeks following treatment. By 1-week posttreatment the contact areas showed signs of corrosion with sloughing of the dead tissue. By 8 weeks posttreatment the necrotic areas were completely healed.

TABLE 63. SUMMARY OF RABBIT SKIN IRRITATION EFFECTS AFTER 4-HOUR CONTACT WITH DIISOPROPYLAMINOETHANOL

Symptoms		
4 Hours	24 Hours	72 Hours
Well defined (5) to severe (1) erythema. Very slight (4) to slight (2) edema. No necrosis (6).	Well defined erythema (6). Very slight (2) to slight (3) edema. Slight necrosis (6).	Moderate to severe erythema (6). Slight edema (6). Moderate necrosis (6).

Primary Index Score = 8.4
Classification: severe irritant
( ) Number of rabbits showing symptom.

Triethylphosphite

A summary of the skin irritation effects of triethylphosphite is shown in Table 64. This primary index score of 1.7 would indicate that triethylphosphite is a mild irritant using the interpretation of the NIOSH skin test ratings.

TABLE 64. SUMMARY OF RABBIT SKIN IRRITATION EFFECTS AFTER 4-HOUR CONTACT WITH TRIETHYLPHOSPHITE

Symptoms		
4 Hours	24 Hours	72 Hours
Very slight erythema (4). Very slight edema (3).	Well defined erythema (6).	Well defined erythema (6).

Primary Index Score = 1.7
Classification: mild irritant
( ) Number of rabbits showing symptom.

The irritation effect of triethylphosphite was persistent with erythema being evident at the contact sites for 4 weeks posttreatment. The skin returned to normal appearance by 8 weeks posttreatment.

Eye Irritation

Diisopropylaminoethanol

Because diisopropylaminoethanol proved to be a severe irritant and a corrosive in the skin irritation test, it was deemed unnecessary to perform the eye irritation test with this compound.

Triethylphosphite

Examination of the eyes of the 9 rabbits following treatment with triethylphosphite revealed slightly constricted pupils in all treated eyes. By 24 hours the pupils of the treated eyes were still slightly constricted and showed sluggish reaction to light. Two of the treated eyes showed a diffuse corneal opacity when stained with fluoroscein. One of these had been washed following treatment while the other remained unwashed. No irritation was found in the iris or conjunctivae. By 48 hours all pupils appeared normal and responded to light. The rabbit with the unwashed eye still showed a diffuse corneal opacity which disappeared by 72 hours.

Dermal Toxicity

Diisopropylaminoethanol

Diisopropylaminoethanol, proved to be a corrosive in the skin irritation test. A dermal LD_{50} was not attempted as it was felt the corrosive properties of the compound are much more hazardous than its toxicity.

Triethylphosphite

Five male and 5 female rabbits were dosed with a triethylphosphite concentration of 2 mL/kg of body weight. Two of 5 female rabbits died within 24 hours following treatment while a single male rabbit died 12 days after treatment. Since 2 mL/kg is the upper dosing limit for dermal testing, no further testing is planned with triethylphosphite.

Inhalation Toxicity

Diisopropylaminoethanol

A summary of the 6-hour inhalation toxicity effects of diisopropylaminoethanol on male and female rats and mice is shown in Tables 65 and 66, respectively. No appreciable difference in toxic response was noted between male and female rats as was the case with EDMP. The LC_{50} of this compound is similar to the male 6-hour LC_{50} of EDMP which was 2520 mg/m^3. Whatever caused the increase in EDMP toxicity in female rats (6-hour LC_{50} of 209 mg/m^3) does not appear to be directly related to the amount of diisopropylaminoethanol present in the EDMP exposures.

TABLE 65. SUMMARY OF 6-HOUR INHALATION TOXICITY RESULTS OF DIISOPROPYLAMINOETHANOL ON FISCHER 344 RATS (N = 10)

Concentration, mg/m^3	Mortality Ratio	
	Male	Female
2592	10/10	10/10
2105	6/10	8/10
1836	2/10	2/10
1766	0/10	1/10

Male Rat LC_{50} (95% C.L.) = 2041 (1942-2223) mg/m^3
Female Rat LC_{50} (95% C.L.) = 1965 (1877-2108) mg/m^3

TABLE 66. ACUTE (6-HOUR) INHALATION TOXICITY OF DIISOPROPYLAMINOETHANOL IN CD-1 MICE (N = 10)

Concentration, mg/m^3	Mortality Ratio	
	Male	Female
2169	8/10	10/10
1989	5/10	9/10
1727	--	7/10
1676	0/10	10/10
1584	--	2/10
1568	0/10	0/10

Male Mouse LC_{50} (95% C.L.) = 2011 (1869-2119) mg/m^3
Female Mouse LC_{50} (95% C.L.) = 1661 mg/m^3 (95% C.L. unobtainable)

Diisopropylaminoethanol appears to be somewhat more toxic to female mice than male mice when comparing the relative LC_{50} values. The apparent increased toxicity is caused by the reversal in toxicity which occurred in a group of female mice exposed to 1676 mg/m^3. This reversal also precludes the calculation of confidence limits for the female mouse LC_{50}.

The acute toxicity testing of bis (diisopropylaminoethyl) methlyphosphonite has been started while O,O'-diethylmethylphosphonite has not yet been received for evaluation.

INVESTIGATION OF THE 1-HOUR EMERGENCY EXPOSURE LIMIT OF JP-5

The THRU has investigated the toxic hazards associated with a number of jet fuels used in military applications. JP-5 is a kerosene type fuel comprised of a mixture of aromatic, paraffinic and alicyclic hydrocarbons, and is less volatile than other jet fuels. It is used in Naval aircraft. Because JP-5 is carried and transferred in confined areas aboard ships, where continuous exposure by personnel might be a hazard, a 90-day continuous inhalation study was conducted by the THRU to evaluate potential toxic effects. Another possible inhalation exposure hazard is created during an accidental spill of JP-5. Concentrations generated in these situations may be extremely high for short periods of time.

In recognition of the hazards associated with accidental brief high level exposures, the Committee on Toxicology of the National Academy of Sciences - National Research Council has established Emergency Exposure Limit (EEL) guidelines. EEL's are concentrations of contaminants that can be tolerated without adverse effects. These concentrations may cause acute discomfort or other evidence of irritation or intoxication. Intoxication to the point of preventing self-rescue is unacceptable, despite the fact that the toxic effects are reversible.

In previous animal studies on JP-5 derived from Petroleum or Shale sources the most significant and consistent effect has been the development of renal tissue changes in male Fischer 344 rats (Gaworski et al., 1984, 1985). The microscopic changes consisted of hyaline droplet formation and renal tubular epithelial cell necrosis. Female Fischer 344 rats, female C57BL/6 mice, male beagle dogs, and female beagle dogs were free of the renal damage. Knave et al. (1976) reported that aircraft factory workers exposed to jet fuel vapors experienced acute effects including dizziness, headache, nausea, respiratory tract symptoms, heart

palpitations and a feeling of tightness in the chest. Similar symptoms were described by a pilot acutely exposed to JP-4 vapors (Davies, 1964).

The EEL for JP-5 is presently 5000 mg/m^3. This value was chosen on the basis of the toxicities of the individual components of the fuel rather than the fuel itself. The Committee on Toxicology of the National Academy of Sciences recently reviewed the EEL value for this fuel and considered a downward revision to 150 mg/m^3 based on the renal effects seen in male rats after 90 days continuous exposure. There was no information in the literature on the toxicity of a single exposure to JP-5 at a concentration of 5000 mg/m^3. The purpose of this study was to investigate the effect of an acute single inhalation exposure of JP-5 at high concentrations to determine if renal injury would result. The initial concentration tested was 5000 mg/m^3. Subsequently a 2500 mg/m^3 JP-5 exposure was also conducted. Because of the signs of renal toxicity in rats exposed continuously to JP-5 vapors, the kidneys of all animals were examined closely.

Methods

JP-5 vapors were generated by passing a known liquid flow through spiraled glass evaporation towers. A measured air flow through the evaporators carried the vapors into the exposure chamber. An airflow minimum of 20 CFM was maintained in the exposure chamber.

Chamber concentrations were measured with a hydrocarbon analyzer. Calibration was accomplished using Mylar bags containing known standard concentrations.

Rats and mice were exposed to vapor concentrations of 5000 mg/m^3 or 2500 mg/m^3 in a 2.5 m^3 Longley chamber. Groups consisting of 20 male Fischer 344 rats and 20 male C57BL/6 mice were exposed for 1 hour. Two separate control groups consisting of a similar number of animals were also maintained. Ten animals of each species from the control and exposed groups were sacrificed 24 hours following exposure while the remaining animals were observed for 28 days postexposure.

Rodents were housed in large wire mesh cages during exposure to allow for a greater freedom of movement and to provide more opportunity for visual observations. All animals were observed for signs of toxic stress during the exposure period. In particular, signs that could be interpreted as preventing self rescue were carefully evaluated. The rats were weighed immediately

prior to exposure and at 1, 2, 3, 7, 14, 21, and 28 days post-exposure. Mice were weighed immediately prior to exposure and at days 7, 14, and 28.

Blood samples were collected from rats (vena cava) at the time of sacrifice and complete blood counts were performed. Blood chemistry analyses were also made for SGOT, SGPT, BUN, creatinine, and alkaline phosphatase.

Whole body, liver, and kidney weights were measured on all rats sacrificed at 24 hours and 28 days postexposure. All animals received a complete gross examination at the time of necropsy. Liver and kidney tissue was collected from each animal for microscopic examination.

After completion of the 1-hour exposure period the 10 rats from each group selected for postexposure holding were transferred to the NMRI/TD laboratory for periodic urine collection and analysis. In addition, water consumption and urine volume were measured and a microscopic evaluation of the urine was conducted.

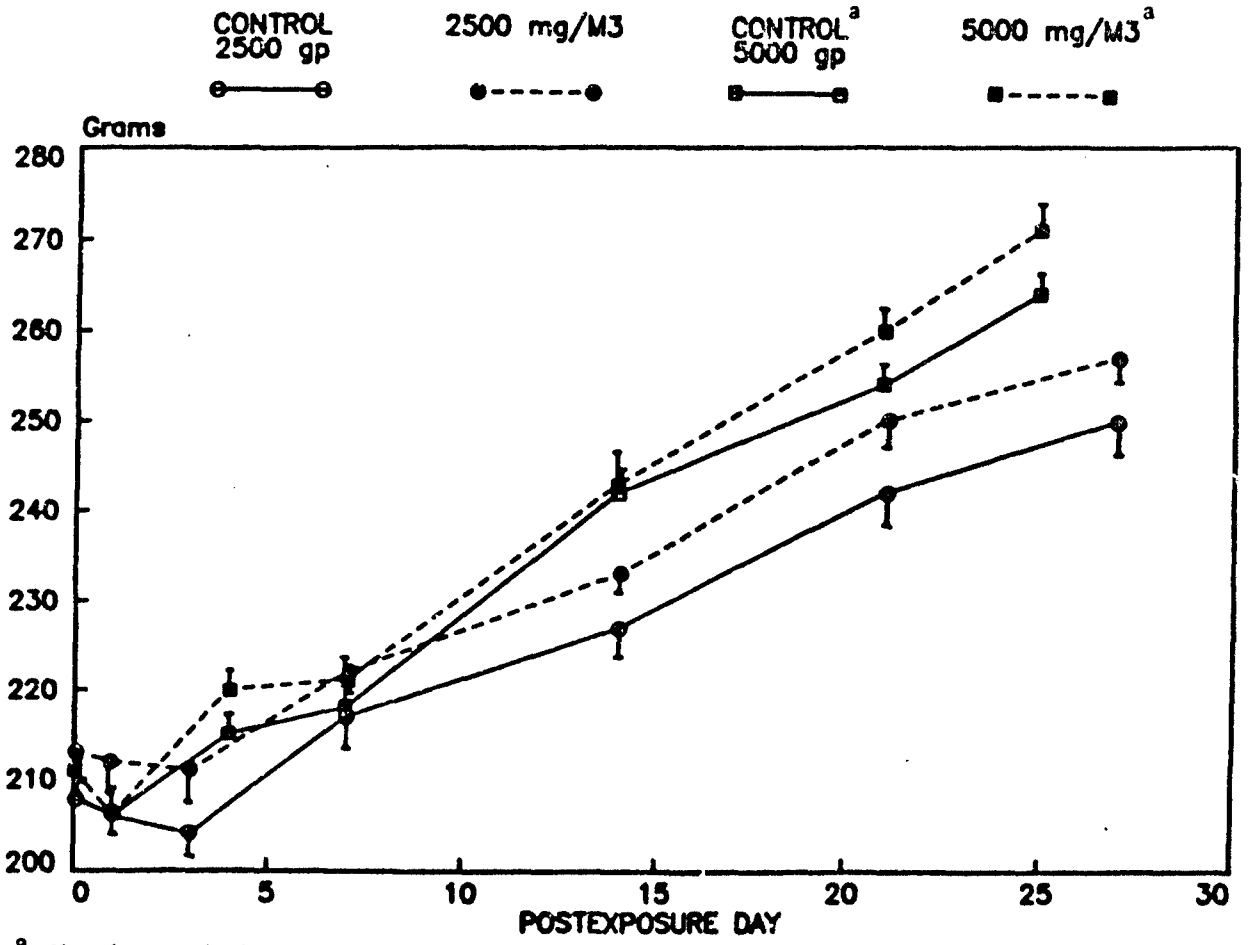
Results

Analyzed exposure concentrations were 2483 ± 63 mg/m^3 and 5085 ± 78 mg/m^3. Although the JP-5 contaminant was generated as a vapor, a very dense condensate aerosol developed in the chamber, particularly during the 5000 mg/m^3 exposure. Most of the animals had a coating of JP-5 on their fur at termination of the 5000 mg/m^3 exposure. The coating was not observed on animals exposed at the lower level.

Sensory eye irritation and CNS depression developed in rats and mice exposed to 5000 mg/m^3. The eye effects were indicated by mild lacrimation, eye closure, and pawing of the eyelids. Rats were lethargic and demonstrated delayed righting reflex for about 2 hours postexposure. One of the 20 mice exposed to 5000 mg/m^3 exhibited hind limb paralysis upon removal from exposure. None of the other 19 mice demonstrated any loss of mobility. At 24 hours postexposure all animals, with the exception of the mouse with hind limb paralysis, appeared normal. At that time the paralyzed mouse had regained partial use of the hind limbs and was able to move around the cage. At 1-week postexposure the mouse still had an unsteady gait but continued to show improvement until the scheduled sacrifice at 28 days postexposure. Eye irritation also occurred in animals exposed to 2500 mg/m^3 JP-5, but disappeared upon removal from exposure. No CNS effects were observed in rats or mice exposed to 2500 mg/m^3 JP-5.

Because of the effects noted in the single mouse exposed to 5000 mg/m^3, a second exposure was conducted using 40 male B6C3F1 mice. During this exposure the mice were inactive and showed signs of eye irritation. Most of the mice had wet coats due to the JP-5 condensate aerosol present in the chamber. Upon removal from the chamber the mice became active and started preening to remove JP-5 deposited on their coats. None of the 40 mice demonstrated any loss of mobility, either during the exposure or during the 28 days observation period.

No adverse effects were seen on the body weight gains of rats exposed to either level of JP-5 (Figure 8). Mouse body weight changes were so slight during the 28-day observation period that analysis of growth was difficult; however, it was noted that no weight loss occurred in the mouse groups exposed to JP-5 at either concentration.



[a] Nonfasted days 7, 14, 21, and 25.

Figure 8. Effect of JP-5 exposure on rat body weights. Values represent mean ± SE.

Serum BUN and alkaline phosphatase levels were increased at 24 hours postexposure in rats exposed to 5000 mg/m^3 (Table 67). These increases were mild and the values were well within normal biological variation for the species. No exposure related blood effects were seen in rats exposed to JP-5 at the 2500 mg/m^3 level.

TABLE 67. EFFECT OF 1 HOUR EXPOSURES TO JP-5 ON RAT BLOOD PARAMETERS

Parameter	(Day)	JP-5 Concentration mg/m^3			
		0[a]	2500	0[b]	5000
RBC (x10^6 cells/mm^3)	1	7.18 ± 0.61[c]	6.35 ± 0.62	7.91 ± 0.08	7.92 ± 0.13
	28	8.25 ± 0.12	8.17 ± 0.15	8.24 ± 0.26	8.39 ± 0.07
HCT (%)	1	40 ± 9	35 ± 3	45 ± 1	45 ± 1
	28	43 ± 1	42 ± 1	44 ± 1	43 ± 1
HGB (g/dl)	1	15.6 ± 0.2	15.3 ± 0.2	14.9 ± 0.2	15.2 ± 0.2
	28	15.6 ± 0.2	15.6 ± 0.3	15.4 ± 0.2	15.6 ± 0.1
WBC (x10^3 cells/mm^3)	1	7.9 ± 0.3	8.2 ± 0.4	5.2 ± 0.2	5.5 ± 0.2
	28	6.1 ± 0.2	7.1 ± 0.4	9.0 ± 0.6	8.9 ± 0.3
BUN (mg/dl)	1	20.2 ± 0.4	17.0 ± 0.4	17.3 ± 0.3	18.5 ± 0.4[d]
	28	17.9 ± 0.3	17.2 ± 0.2	14.0 ± 0.3	13.2 ± 0.4
Creat. (mg/dl)	1	0.43 ± 0.02	0.46 ± 0.02	0.42 ± 0.03	0.43 ± 0.02
	28	0.60 ± 0.00	0.60 ± 0.00	0.51 ± 0.03	0.54 ± 0.03
SGOT (IU/L)	1	84 ± 2	84 ± 7	89 ± 2	90 ± 3
	28	92 ± 2	89 ± 2	98 ± 3	93 ± 2
SGPT (IU/L)	1	46 ± 2	39 ± 4	32 ± 2	30 ± 2
	28	31 ± 1	32 ± 2	50 ± 2	43 ± 2[d]
Alk. Phos. (IU/L)	1	337 ± 15	312 ± 16	242 ± 12	283 ± 9[d]
	28	161 ± 7	158 ± 8	194 ± 11	190 ± 9

[a] Control group for the 2500 mg/m^3 study.
[b] Control group for the 5000 mg/m^3 study.
[c] Mean ± SE, N = 7 to 10 samples/group.
[d] Different from control, $p < 0.05$.

Exposure to JP-5 at either 2500 mg/m^3 or 5000 mg/m^3 did not affect liver or kidney weight (Table 68).

Rat urinalysis conducted by NMRI/TD revealed elevated glucose and LDH levels in JP-5 exposed rats during the first 24

TABLE 68. EFFECT OF 1 HOUR OF EXPOSURES TO JP-5 ON RAT ORGAN WEIGHT

Parameter	(Day)	JP-5 Concentration mg/m³ 0[a]	2500	0[b]	5000
Fasted Body Weight (g)	1	202 ± 3[c]	202 ± 3	189 ± 3	191 ± 3
	28	231 ± 2	237 ± 3	248 ± 3	250 ± 3
Kidney (g)	1	1.65 ± 0.03	1.70 ± 0.04	1.55 ± 0.03	1.62 ± 0.03
	28	1.75 ± 0.03	1.80 ± 0.04	1.87 ± 0.03	1.82 ± 0.04
Liver (g)	1	7.51 ± 0.15	7.34 ± 0.19	6.56 ± 0.14	6.68 ± 0.12
	28	6.72 ± 0.24	6.84 ± 0.14	8.36 ± 0.14	8.46 ± 0.21
Kidney % of Body	1	0.82 ± 0.01	0.84 ± 0.01	0.82 ± 0.01	0.85 ± 0.01
	28	0.76 ± 0.02	0.76 ± 0.01	0.76 ± 0.01	0.73 ± 0.01
Liver % of Body	1	3.72 ± 0.06	3.62 ± 0.05	3.47 ± 0.04	3.50 ± 0.04
	28	2.91 ± 0.12	2.90 ± 0.03	3.38 ± 0.04	3.38 ± 0.06

[a] Control group for the 2500 mg/m³ study.
[b] Control group for the 5000 mg/m³ study.
[c] Mean ± SE, N = 10 samples/group.

hours postexposure. Subsequent examinations indicated a return to normal levels in exposed rats. Urinary GOT was increased in rats exposed to 5000 mg/m³ for up to 4 days postexposure. This trend was also seen in rats exposed to 2500 mg/m³; however, the values were not significantly different from controls at $p < 0.05$. Rats exposed to 2500 mg/m³ consistently excreted more protein than controls. This effect was not seen in rats at the 5000 mg/m³ level. Exposure to JP-5 at either level produced sloughing of renal epithelial cells. At 1-week postexposure, cells remained in the urine of exposed rats, but on greatly reduced numbers. Even at 28 days postexposure renal epithelial cells were observed in exposed rat urine. No significant exposure related effect was observed in the urine concentrating ability of exposed rats. In addition, all other urine parameters measured failed to suggest any exposure related effects.

No significant gross lesions were observed in animals exposed to JP-5 at either 2500 mg/m³ or 5000 mg/m³. Mild, diffuse

hyaline droplet formation was noted in the proximal convoluted tubules of all rats examined 24 hours after exposure to 5000 mg/m^3 JP-5 (Table 69). This condition was similar to that occurring in the male rats exposed to JP-5 for 90 days, although it was far less severe. At 28 days postexposure 7 of 10 exposed rats displayed hyaline droplets. No liver tissue changes were observed in rats exposed to 5000 mg/m^3.

TABLE 69. EFFECT OF 1 HOUR EXPOSURES TO 5000 MG/M^3 JP-5 ON RAT LIVER AND KIDNEY

Concentration (mg/m^3)	Liver Lesions		Kidney Hyaline Droplets	
	24 hrs	28 days	24 hrs	28 days
0	0/10[a]	0/10	0/10	1/10
5000	0/10	0/10	10/10	7/10

[a] Number observed/Number examined.

Examination of mouse tissues collected from either study and rat tissues collected during the 2500 mg/m^3 study was not complete for this report.

Discussion

A single 1-hour exposure to 5000 mg/m^3 JP-5 produced renal tissue hyaline droplet formation in male rats similar to changes noted in animals exposed to JP-5 for longer time periods. This is considered to be one of the first indications of the process ultimately leading to necrosis of renal epithelial cells. Although significant numbers of renal cells were sloughed in the male rat urine postexposure, no microscopic evidence of cellular necrosis was observed. This suggests that the toxic renal insult was mild even at an extremely high dose. Examination of tissues from rats exposed to 2500 mg/m^3 may give additional evidence toward establishing a "no effect" level for this type of renal damage.

Urinary markers proved to be sensitive indicators of JP-5 induced renal injury, while organ weights, serum BUN, and serum creatinine were less reliable. Recovery of urinary markers generally occurred within 4 days postexposure, indicating that the

effects of exposure were reversible. It was notable that despite the indications of renal tissue changes and urinary enzyme changes, no detrimental effect was seen on urine concentrating ability.

The CNS depression noted in rats exposed to 5000 mg/m^3 for 1 hour suggests that this level may be too high to allow for self rescue. In addition, 1 of 20 mice exposed to 5000 mg/m^3 JP-5 displayed hind limb paralysis. It must be emphasized, however, that a repeat exposure of 40 mice to 5000 mg/m^3 failed to produce any indication of severe neurologic impairment. The CNS depression noted in rats and mice exposed to 5000 mg/m^3 is consistent with the disturbances noted in aircraft factory workers and a pilot exposed to jet fuel (Knave et al., 1976; Davies, 1964). Absence of CNS effects at a concentration of 2500 mg/m^3 suggests that this level may be more appropriate as the basis for an EEL concentration. However, the recommendation for an EEL must await completion of microscopic examination of tissues collected from all animals in the study.

ACUTE DELAYED NEUROTOXICITY STUDY ON CHLOROTRIFLUOROETHYLENE OLIGOMER

Chlorotrifluoroethylene (CTFE) oligomer has become a primary nonflammable hydraulic fluid candidate. As current and other candidate hydraulic fluids have been suspected neurotoxins, the neurotoxic potential of CTFE must be evaluated.

This study was designed to determine if delayed neurotoxic effects result from exposure of adult chickens to peroral doses of CTFE. A vehicle control as well as a triorthocresylphosphate (TOCP) positive control were tested concurrently with CTFE. Final determination of an injury effect was based on a comparison of the test chickens with the TOCP control chickens.

Leghorn hens (Gallus domesticus, Carey Nick:300-320 hybrid) 5 to 7 months of age and weighing between 1.10 and 1.95 kilograms were purchased from Carey Farms, La Rue, Ohio. The debeaked hens were group housed in 3' by 6' pens to allow for freedom of movement. Food and water were available ad libitum.

The CTFE was supplied by the U. S. Air Force. Included was an additive of 1.0% of a 50% effective anti-rust additive, neutral barium dinonylnaphthalene sulfonate. This is a currently used additive present in many rust inhibited fluids. A second additive is 0.05% of a company proprietary anti-wear additive. At the manufacturer's request, no further analysis was made.

Preliminary to the neurotoxicity testing, the acute oral toxicity of the hydraulic fluid was determined. Oral intubation was accomplis employing a syringe fitted with a 6" infant catheter. The nonfasted hens were weighed individually to determine the proper dose volume. None of 3 hens died after receiving a single dose of 5 mL/kg. To determine if hens could survive this regimen for 5 consecutive days, doses of 5 mL/kg were administered to 3 naive hens over a 5-day period. All survived the following 14 days.

The hydraulic fluid was administered to unfasted hens in an undiluted state. The positive control, TOCP, was diluted in corn oil to achieve a dose volume of 5 mL/kg. A negative control group received appropriate doses of corn oil. The method follows that of Siegel et al. (1965) recommended for testing of U. S. Navy materials. The following dosing regimen was followed:

CTFE - Groups of 4 hens treated with 4 doses. The highest nontoxic dose followed by lower doses decreased arithmetically to a no-effect level.

TOCP - Groups of 4 hens treated with each of the following doses: 60, 75, 90 mg/kg/day.

Corn Oil - Twelve hens given the same total volume of fluid dose as test animals.

Observations and grading by 3 observers began 7 days after the first dose, and continued 3 times a week (Monday, Wednesday, and Friday) until 30 days after the first dose. The following point score system was used:

Symptom Free	0 Points
Doubtful or Minor Symptoms	2 Points
Positive Paralytic Symptoms	8 Points
Advanced Paralytic Symptoms	12 Points
Death	16 Points

During observation and grading, the chickens were removed from their enclosure and placed on a rubber mat to provide sure footing. Symptoms of test hens noted during the observation period were compared with those seen in the TOCP treated hens.

All test and control hens were examined for gross pathology at death. Longitudinal and cross sections of the spinal cord (cervical, lumbar, and thoracic regions) and a section of the sciatic nerve were sampled from representative hens from each group for histopathology examination.

Results

Neurotoxic signs were observed in nearly all hens that received TOCP. The corn oil control group showed no signs of neurotoxicity. Two of 4 hens dosed at 5 mL/kg with CTFE died within 1 week, neither showing neurotoxic symptoms prior to death. Of the remaining CTFE hens, none showed any neurotoxic signs during the 30 day period.

Samples of the spinal cord and sciatic nerve were removed from representative hens of each group. Although the results of the histopathologic examination of the nerve tissue are not yet available, there were no signs or symptoms indicating that CTFE has neurotoxic potential.

THE INVESTIGATION OF THE ABSORPTION AND METABOLISM OF CHLOROTRIFLUOROETHYLENE OLIGOMER (MLO 83-322) IN MALE RATS AFTER ORAL AND INHALATION EXPOSURE

Chlorotrifluoroethylene (CTFE) oligomer is an inert nonflammable chlorofluorocarbon oil. It is noncorrosive, saturated, and hydrogen free. It also has high thermal stability, good lubricity, and high dielectric strength. Recent tests with CTFE indicate that it has a low degree of toxicity. There were no deaths among male and female rabbits exposed to 2 g CTFE/kg body weight (Gargus, 1983). Furthermore, there were no deaths among male and female rats exposed to atmospheres containing saturated vapor concentrations of CTFE for a 4-hour period (Coate, 1984). CTFE did not show neurotoxic potential in adult hens.

There is a possibility that free fluorides may be released during the in vivo metabolism of CTFE. The ingestion of fluorides has been shown to result in vomiting, abdominal pain, diarrhea, convulsions, etc. (Patty, 1981). Continuous exposure to fluorides also results in effects on bone and teeth. Therefore it was considered to be of interest to determine if CTFE exposure could result in metabolism to free fluorides. This study was designed to determine if CTFE is absorbed and, if so, whether it is metabolized.

Materials and Methods

The CTFE was supplied by the U. S. Air Force. One additive present is 1.0% of an anti-rust additive, neutral barium dinonylnaphthalene sulfonate. This compound is present in many rust

inhibitor fluids. The second additive is 0.05% of a proprietary anti-wear additive (composition unknown).

Oral - CTFE was administered dissolved in corn oil, using glass syringes equipped with ball tipped oral dosing needles. A dose volume of 1.0 mL/100 grams of body weight was used. Test animals were male Sprague-Dawley rats weighing 200-300 grams.

For the fluoride determinations a group of 10 male rats was orally administered CTFE at 5.0 mL/kg. Five animals were bled via the orbital sinus at 4 hours posttreatment and the remaining 5 animals were bled at 24 hours posttreatment. At 48 hours posttreatment the group bled at 4 hours was again bled. A fourth bleeding was conducted at 1-week posttreatment. All 10 rats were held in metabolism cages for the first 24 hours posttreatment for urine collection and analysis. Additional 24-hour urine samples were collected at days 8 and 16 posttreatment. The same basic procedure was repeated using a fresh group of rats for the CTFE determinations. A separate group of 10 rats was maintained and bled at the same intervals to serve as a control group for both the CTFE and fluoride determinations.

Inhalation - Concentrated vapors of CTFE were generated in a gas wash bottle equipped with a fritted disk. The bottle contained known amounts of the test material. Dried air was blown through the bottle at a known rate. The resulting air-vapor mixture was conducted to a 60-liter plastic chamber which contained 10 male Sprague-Dawley rats. The exposure period was 6 hours. Analytical concentrations were not measured; however, estimated concentrations were calculated by material balance. Blood and urine for CTFE and fluoride ion analysis were to be collected from these animals in the same manner as that used in the oral exposure portion of this study. A separate group of control animals was used.

CTFE Analysis

Blood and urine was analyzed for the presence of CTFE with gas chromatography using electron capture detection. CTFE in hexane served as a standard.

Fluoride Ion Analysis

Fluoride ion concentration in urine and plasma was measured using a fluoride ion specific electrode. The urine fluoride ion determinations were made using the method of Neefus et al. (1970)

which used a synthetic urine for standardization and a buffer (TISUB) to correct for pH and ionic strength. Plasma fluoride ion determinations were made using a method described by Singer and Ophaug (1979) which measures the ionic form directly, following dilution in a simple buffer system.

Results

Summaries of the urine and plasma fluoride concentrations in male rats following oral administration of CTFE are presented in Tables 70 and 71. These results indicate that CTFE was absorbed after oral dosing and conversion to free fluoride occurred. The fluoride levels were still elevated in plasma at 7 days postadministration and in urine at 16 days postadministration.

TABLE 70. PLASMA FLUORIDE CONCENTRATIONS[a] IN MALE RATS FOLLOWING ORAL ADMINISTRATION OF CTFE

Dose CTFE (mL/kg)	Fluoride Ion Conc. (µg/mL) Time Postadministration			
	4 hrs	24 hrs	48 hrs	7 Days
0	0.10 ± 0.004(5)	0.10 ± 0.006(5)	0.11 ± 0.004(5)	0.10 ± 0.001(10
5	0.39 ± 0.02(5)	0.39 ± 0.01(5)	0.23 ± 0.004(5)	0.20 ± 0.002(16

[a] Mean ± SE (N).

TABLE 71. URINARY FLUORIDE CONCENTRATIONS[a] IN MALE RATS FOLLOWING ORAL ADMINISTRATION OF CTFE

Dose CTFE (mL/kg)	Fluoride Ion Concentration (µg/24 hrs) Days Postadministration		
	1	8	16
0	22 ± 1 (10)	19 ± 1 (5)	20 ± 1 (5)
5	526 ± 14 (10)	146 ± 4 (5)	92 ± 5 (5)

[a] Mean ± SE (N).

Summaries of the CTFE concentrations in blood and urine following oral administrations are presented in Tables 72 and 73. CTFE was absorbed after oral administration. The blood CTFE concentrations peaked at 48 hours, followed by a decline at 1 and 2 weeks. Urine CTFE levels demonstrated the same general trends.

TABLE 72. BLOOD CTFE LEVELS[a] IN MALE RATS FOLLOWING ORAL ADMINISTRATION

Dose CTFE (mL/kg)	CTFE (mg/mL) Time Postadministration				
	4 hrs	24 hrs	48 hrs	1 Week	2 Weeks
5	29.4 ± 0.6(5)	28.0 ± 2.8(5)	45.8 ± 8.6(5)	3.7 ± 0.2(5)	1.5 ± 0.2(5)

[a] Mean ± SE (N).

TABLE 73. URINARY CTFE LEVELS[a] IN MALE RATS FOLLOWING ORAL ADMINISTRATION

Dose CTFE (mL/kg)	CTFE (µg/24 hours) Days Postadministration		
	1	7	14
5	2600 ± 100 (4)	24 ± 3 (5)	2 ± 0.4 (5)

[a] Mean ± SE (N).

During the first 24-hour period the urinary output was 2.6 mg CTFE and declined at 1 and 2 weeks postdosing.

Although there are not many data points for plasma fluoride concentration, the points can be fitted to a first order elimination process with a half-life of approximately 3 days. Plasma CTFE concentrations appear to follow similar elimination kinetics, again with an approximate 3 day half-life.

The urine and plasma fluoride concentrations of male rats following a 6-hour CTFE saturated vapor exposure are summarized in Table 74. All 10 male rats in this exposure group (CTFE ≈ 2.77 mg/L) died within 3 days after exposure. Due to mortality of the exposed rats only the first urine collection and the first 2 bleedings were conducted. There were increased fluoride concentrations in both urine and blood. These increases were much smaller following inhalation exposure than oral exposure. The cause of death among the exposed animals appeared to be pulmonary edema, possibly induced by an aerosol. To investigate the possibility of aerosol formation, additional inhalation exposures were conducted using various filters in the generation system and a particle counter to sample the chamber atmosphere. The results of these trials are summarized in Table 75. In these repeat trials, animal mortality did not exhibit a consistent correlation

TABLE 74. URINARY AND PLASMA FLUORIDE CONCENTRATION[a] OF MALE RATS FOLLOWING 6-HOUR SATURATED VAPOR EXPOSURE TO CTFE

Estimated CTFE Conc. (mg/L)	Plasma Conc. (mg/L) Time Postexposure		Urinary Conc. (mg/24 hour) at 24 Hours	Mortalit
	Immediate	24 Hours		
0	0.11 ± 0.002(5)	0.12 ± 0.005(5)	17.9 ± 0.5(5)	0/10
2.77	0.33 ± 0.037(5)	0.15 ± 0.007(5)	55.7 ± 1.8(5)	10/10

[a] Mean ± SE (N).

TABLE 75. SUMMARY OF CTFE SATURATED VAPOR EXPOSURES TO MALE SPRAGUE-DAWLEY RATS

Exposure	Exposure Duration (Hour)	Filter	Nominal Conc. (mg/L)	Mortality (No. Dead/No. Exposed)
1	6	None	2.78	10/10
2	4	Glass Wool	--	2/5
3	6	Balston	2.86	4/4
4	6	Balston	3.51	1/5
5	6	Balston	2.48[a]	0/5

[a] Material from Exposure 4 was reused for this exposure.

with the presence or absence of an aerosol, indicating that aerosol was not necessary for lethality.

Additional inhalation exposures are planned. CTFE and fluoride ion concentrations in blood and urine will also be monitored in rats following dermal exposure. The results of these exposures will be presented in a future annual report.

SENSITIZING POTENTIAL OF NAVY HYDRAULIC FLUID PLURASAFE MC200

The THRU has examined samples of several Navy hydraulic fluids to evaluate their irritation and sensitization potentials. One of those which was previously examined was an ethylene glycol base fluid designated WGF-200D, produced by Wyandotte Corporation, Parsippany, New Jersey. The hydraulic fluid WGF-200D caused no eye or skin irritation but did produce high reaction scores in the guinea pigs and was classified as a sensitizer.

The hydraulic fluid has been reformulated to meet MIL SPEC 22072B, and is now designated Plurasafe MC200.

Plurasafe MC200, produced by BASF Wyandotte Corporation is an ethylene glycol base fluid. The fluid was supplied by the Naval Medical Research Institute/Toxicology Detachment, Wright-Patterson Air Force Base, Dayton, Ohio and has an NMRI/TD designation of #4179.

Since the exact composition of Plurasafe MC200 is unknown, there was some concern that it too, may be a sensitizer. The purpose of this study was to determine if Plurasafe MC200 was capable of sensitizing guinea pigs.

Ten male albino guinea pigs, Hartley strain, 6 to 8 weeks of age, were used for sensitization testing. The fluid was first tested for primary irritation on 3 guinea pigs by application to the clipped flank. Observation was made at 24 hours for signs of irritation. No erythema or edema was produced from this application and consequently, the sensitization test was conducted using undiluted hydraulic fluid.

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. The hydraulic fluid, 0.1 mL at each application, was applied to this area on a 1/2 x 1/2" cotton gauze square, covered with plastic wrap and held in place with adhesive tape. The first insult patch was allowed to remain in place for 2 days, then removed, and a second application of 0.1 mL was made. Two days later, this patch was removed, a total of 0.2 mL of a 50% aqueous dilution of Freund's[1] adjuvant per animal was injected intradermally, using 3 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 this application, the patch was removed and a new patch of 0.1 mL of the material applied. The last patch was removed 2 days later, and the animals were allowed to rest for 2 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 irritated area.

[1] Bacto Adjuvant Complete, Freund, Difco Laboratories, Detroit, Michigan.

After the 2-week rest period, the flanks of the animals were clipped and challenged with the test solution. The challenge applications were not occluded. The skin responses at these sites were observed and recorded at 24 and 48 hours after application. Any animal showing measureable erythema and/or edema at the test solution challenge site was rated as a positive responder.

One of 10 guinea pigs responded to the challenge dose of 0.1 mL Plurasafe MC200 and had a mild erythematous reaction at 24 hours which increased in severity at 48 hours. The other 9 guinea pigs showed a negative response to the challenge dose of the hydraulic fluid.

One week later the responding guinea pig and 2 others which showed no response to the initial challenge were given an additional challenge dose of 0.1 mL of the Plurasafe MC200. The reaction of each of the 3 guinea pigs was similar to this first challenge reaction; however, the response of the sensitized animal was more severe, showing a moderate erythematous response at 24 hours which progressed to a strong response by 48 hours. The 2 nonsensitized guinea pigs showed no response to the additional challenge dose at either 24 or 48 hours.

The response of the one guinea pig is a definite sensitization response. This response of 1 guinea pig in 10 is not nearly as dramatic as was seen with the hydraulic fluid WGF-200D where 78 and 100% of the guinea pigs responded to the two samples tested (MacEwen and Vernot, 1980). However, it does indicate that Plurasafe MC200 could present a problem to individuals previously sensitized to similar hydraulic fluids or who are allergy prone.

EVALUATION OF THE ACUTE TOXICITY OF A CYCLOTRIPHOSPHAZENE BASED HYDRAULIC FLUID

INTRODUCTION

The Toxicology Detachment of the Naval Medical Research Institute (NMRI/TD) requested that the Toxic Hazards Research Unit (THRU) determine the acute toxicity of a cyclotriphosphazene based hydraulic fluid. The Navy is developing candidate hydraulic fluids with chemical structures of cyclotriphosphazene cyclic esters. The candidate fluid of current interest contains 0.1% of tolyltriazole as a copper corrosion inhibitor.

No toxicity data other than an oral LD_{50} greater than 5 g/kg were available on the cyclotriphosphazene ester; however, information on related compounds indicates a low order of toxicity. The additive, tolyltriazole, is considered the most toxic of the two compounds with an oral LD_{50} of 675 mg/kg (HRCR, 1972). Since tolyltriazole is present at only 0.1% it is doubtful that this amount would increase the toxicity of the hydraulic fluid significantly.

MATERIALS AND METHODS

Test Material

The hydraulic fluid is a cyclotriphosphazene cyclic ester containing 0.1% tolyltriazole. The fluid was supplied by NMRI/TD, Wright-Patterson Air Force Base, Ohio. Pertinent data on components:

Cyclotriphosphazene ester:

NMRI/TD No.	4341-1
Vapor Pressure, mm Hg	65°C: 0.49
	149°C: 12.0

Tolyltriazole: $C_7H_7N_3$

CAS No.	29385-43-1
Synonyms	Methylbenzotriazole
	1,2,3-Triazole (methylphenyl)

Animals

Fischer 344 rats (CDF[F344]/CrlBR) purchased from Charles River Breeding Laboratories were used in the acute oral toxicity test. New Zealand White rabbits obtained from Clerco Research Farms were used in the dermal and irritation studies, and Hartley Albino guinea pigs purchased from Murphy Breeding Labs were used for sensitization testing. All animals used in this study were male.

Oral Toxicity

The hydraulic fluid was suspended in corn oil. The suspension was kept in a turbulent state with a magnetic stirring platform while in use. Glass syringes with oral dosing needles were used to administer the fluid to the rats. Animals were fasted

for at least 16 hours prior to the administration of the oral dose. The dose volume for the rats was 0.01 mL/g body weight, with the rats being weighed individually at the time of dosing.

An initial dose of 5 g/kg was given to a group of 5 male rats. If no deaths occurred at this level no additional oral dosing was planned. If deaths occurred at 5 g/kg, additional dosing was projected to determine an LD_{50}. Geometrically spaced doses would be administered and the LD_{50} calculated by the moving average method of Weil (1952). Deaths that occurred during the 14 days immediately following the administration of the single dose would be included in the final mortality tally. Surviving rats were weighed at 1, 2, 4, 7, 10, and 14 days postexposure and killed on the 14th day postexposure. Symptoms were recorded on symptomatology forms.

Dermal Toxicity

Male albino New Zealand rabbits weighing between 2 and 3 kilograms were used. All rabbits were clipped as closely as possible with an Oster® clipper equipped with surgical blades and a vacuum attachment.

The animals were individually weighed prior to dosing to determine the proper dose volume. The proper volume of the liquid material was applied undiluted to the back of the rabbit and divided as equally as possible between the 2 sides. The dose was kept in place by applying 8-ply gauze patches over the liquid on each side of the back. A patch of clear plastic wrap was then applied over the clipped back area and elastoplast tape used to wrap the entire midsection of the rabbit. Specially designed rabbit restraining harnesses were fitted to each rabbit at the time of dosing and kept in place during the dosing period. These harnesses prevent excessive movement of the rabbits and prevent chewing on the taped area while allowing the rabbit access to food and water during the dosing period.

All doses were kept in contact with the rabbit skin for 24 hours. After 24 hours, the tape, plastic wrap, and gauze were removed and the harnesses taken off. The rabbits were maintained in individual cages postexposure and observed for mortality or other signs of toxicity during the 14 days immediately following exposure.

The animals were observed frequently on the day of dosing and twice daily thereafter. Symptoms were recorded on symptomatology forms. Body weights were obtained at the time of dosing and on days 1, 2, 4, 7, 10, and 14 posttreatment.

Testing was initiated by dosing 5 rabbits with 2 mL of test material/kg of body weight. If there was no mortality at this level no additional dose levels were administered. If mortality occurred, testing would continue with 5 animals/dose level. LD_{50} calculations would be made using the moving average method of Weil (1952).

Skin Irritation

All rabbits dosed at 2 mL/kg in the acute dermal toxicity study were examined for skin irritation following 24-hour contact with the hydraulic fluid. If no skin irritation was present, no further skin testing was done. However, if irritation was noted at the 2 mL/kg level, a standard skin irritation evaluation was planned.

Eye Irritation

One tenth mL of the compound was applied to 1 eye of each of 9 albino rabbits. The opposite eye was untreated and served as a control. The eyes of the test animals were examined with fluorescein stain prior to use to ensure absence of lesions or injury. A topical anesthetic (Alcaine; Proparacaine HCl 0.5%) was installed in the eyes of all rabbits, treated and control, approximately 2 minutes prior to application of the test substance. The treated eye of 6 rabbits remained unwashed while the remaining three rabbits received test material and then had the treated eye flushed for 1 minute with lukewarm water starting no sooner than 20-30 seconds after instillation. Examination for gross signs of eye irritation were made at 1, 2, 3, 4, and 7 days following application. In case of injury, the animals would be scored 3 times a week until the lesion subsided or was deemed irreversible. Scoring of irritative effects was made according to the method of Draize (1959) in which cornea, 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 weighted because of the essential role of these tissues in vision.

Skin Sensitization

Ten male, albino guinea pigs, Hartley strain, 6 to 8 weeks of age, were used. The hydraulic fluid was tested for primary irritation on 3 guinea pigs by application to the clipped flank. Observations were made at 24 hours for signs of irritation.

Prior to the start of the study, all test guinea pigs were clipped on the left flank to evaluate baseline irritation response. An application of 0.1 mL of the hydraulic fluid was applied to the clipped area. The application was occluded and responses were recorded at 24 and 48 hours.

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" cotton gauze square, covered with plastic wrap and held in place with adhesive tape. The first insult patch was allowed to remain in place for 2 days, then removed, and a second application of 0.1 mL was made. Two days later, this patch was removed and a total of 0.2 mL of a 50% aqueous dilution of Freund's adjuvant per animal was injected intradermally, using 2 or 3 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 this application, the patch was removed and a new patch of 0.1 mL of the material applied. The last patch was removed 2 days later, and the animals were allowed to rest for 2 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 irritated area.

After the 2-week rest period, the flanks of the animals were clipped and challenged with the test solution. The challenge applications were not occluded. The skin responses at these sites were recorded at 24 and 48 hours after application. Any animal showing measureable erythema and/or edema at the challenge site was rated as a positive responder.

In scoring the Maguire Test, the important statistic is frequency of the reaction. The following table is used to classify test materials as to sensitization potential.

Sensitization Rate (%)	Grade
10	I Weak
20-30	II Mild
40-60	III Moderate
70-80	IV Strong
90-100	V Extreme

RESULTS

Oral Toxicity

An oral dose of 5 g/kg given to 5 male rats resulted in no mortality after 14 days of observation. No signs of toxic stress were noted during this period and all rats showed normal body weight gains.

Dermal Toxicity

Five rabbits were given a dermal dose of 2 mL/kg which was kept in contact with the skin for 24 hours. No deaths occurred during the subsequent 14-day observation period. No signs of toxic stress were noted during the 14-day period and all rabbits showed normal weight gains.

Eye Irritation

Nine rabbits were tested for eye irritation. The treated eye of 6 of the rabbits remained unwashed following treatment while the remaining 3 rabbits received the test material and then had the eye flushed for 1 minute with lukewarm water. Examinations for gross eye irritation were made at 1, 2, 3, 4, and 7 days following application. No signs of eye irritation were found at any of the observation periods.

Skin Irritation

The rabbits from the dermal study received doses approximately 8 times the recommended dose for primary skin irritation testing, as well as being in contact with the hydraulic fluid for 6 times the recommended time period. These rabbits showed no signs of skin irritation when examined at 24, 48, and 72 hours

following the dermal dose. Since no skin irritation occurred following this more severe test, it was concluded that the hydraulic fluid poses no hazard as a skin irritant.

Skin Sensitization

The sensitization test was performed on male guinea pigs using a modified Maguire method. No sensitization response occurred in any of the guinea pigs following the challenge dose of the hydraulic fluid.

DISCUSSION

The oral and dermal toxicity tests were done using an upper limit test of 5 g/kg and 2 mL/kg, respectively. As no deaths occurred during the subsequent 14-day observation period it was unnecessary to do further testing. The hydraulic fluid is considered non-toxic by either the oral or the dermal route of administration.

Eye and skin irritation and skin sensitization tests proved to be negative. The results of the acute toxicity tests reported herein indicate that this cyclotriphosphazene based hydraulic fluid should not pose an acute hazard to workers during production or use.

ACUTE TOXICITY OF THIONYL CHLORIDE VAPOR FOR RATS

The Air Force will be replacing existing standby power batteries at Minuteman Missile sites with new lithium/thionyl chloride batteries. During use, storage, or deactivation of lithium batteries, thionyl chloride may be released into the environment.

Little information was available on the toxicity of thionyl chloride. Generally, thionyl chloride ($SOCl_2$) vapor is thought to decompose in moist air to form hydrogen chloride and sulfur dioxide. For this reason, the toxic effect of thionyl chloride is often assumed to be the additive effect of hydrogen chloride and sulfur dioxide. Patty (1963) mentions a study by Flury and Zernik (1931) in which a 20-minute exposure to 17.5 ppm thionyl chloride proved fatal to cats. However, there appears to be an error in the original publication in conversion of units which leads to uncertainty in the actual concentration tested.

Studies have been conducted in this laboratory on the acute toxicity of hydrogen chloride and sulfur dioxide. Darmer et al. (1972) reported a 5-minute and 30-minute male rat LC_{50} for hydrogen chloride vapor of 40898 and 4701 ppm, respectively. MacEwen and Vernot (1976) report a 1-hour rat LC_{50} of 3120 ppm for hydrogen chloride vapor. The 1-hour inhalation LC_{50} value of sulfur dioxide vapor for male rats has been reported by MacEwen and Vernot (1977) as 2520 ppm.

This study was designed to determine the male rat 1-hour LC_{50} of thionyl chloride when delivered to an exposure chamber in an atmosphere of low humidity which would result in minimum decomposition of the contaminant. It was also our purpose to determine whether the acute toxicity of thionyl chloride could be considered as the additive effect of its decomposition products.

METHODS AND MATERIALS

Test Agent

Thionyl chloride was purchased from Aldrich Chemical Company, Milwaukee, Wisconsin. Quality control analyses on $SOCl_2$ were conducted using a Beckman Acculab 4, infrared (IR) analyzer. IR scans were obtained for approximately 10 mg/mL concentrations of $SOCl_2$ in carbon tetrachloride which were read against a solvent blank.

Generation

Exposures were conducted in a 60-liter, Plexiglas®, exposure chamber. The chamber air supply consisted of high purity dry nitrogen (99.99% min., Matheson Gas Products) and Zero-Gas® dry oxygen (99.8% min., Matheson Gas Products) combined in a 4:1 flow ratio monitored with calibrated, Fisher & Porter flowmeters. High purity dry gases were used to minimize hydrolytic decomposition of thionyl chloride.

Liquid thionyl chloride was supplied by a syringe pump (Sage, Model #355) to a helical, glass evaporating tower where it was vaporized in a counterstream of nitrogen. Because of $SOCl_2$ volatility and the possibility of thermal decomposition, the evaporating tower was not heated.

Total Chloride Contaminant Analysis

The exposure chamber atmosphere was monitored for chloride ion concentration using a flow cell containing a chloride ion electrode (Orion, Model #94-17B) and a reference electrode (Orion, Model #90-02). Electrode response was read on a pH meter set on expanded millivolt scale and referenced to a strip-chart recorder. A timer-controlled, electromechanical valve switched from tower effluent to baseline absorbing solution from a tower by-pass.

Thionyl Chloride Analysis

The concentration of unhydrolyzed $SOCl_2$ in the chamber atmosphere was measured by IR analysis of carbon tetrachloride (CCl_4) solutions of the contaminant obtained by impinger sampling. Chamber air was drawn through a train of three impingers, each containing 20 mL of CCl_4, at a metered flow of 1.5 L/min. The resultant solutions were read on the Beckman Acculab 4 IR analyzer against a CCl_4 blank using liquid cells with sodium chloride windows and set at 0.1 mm path lengths. The instrument scanned each impinger sample from a wavelength of 2.5 μm to 16 μm. Absorbance at 8.3 μm was indicative of $SOCl_2$.

The instrument was calibrated by measuring the absolute transmittance at 8.3 μm of standard solutions of $SOCl_2$ dissolved in CCl_4. Transmittance was converted to absorbance units and plotted as a function of standard concentration in mg/mL.

Sampling for thionyl chloride analysis was done once during each exposure. The chamber was allowed to achieve a stable total chloride contaminant concentration as indicated by the chloride ion electrode analysis before impinger sampling was initiated. In most cases, impinger sampling continued for the duration of the exposure.

Animals

The animals used in these experiments were male CDF® (Fischer 344)/CrlBR rats, nine to eleven weeks of age, obtained from Charles River Breeding Labs, Wilmington, Massachusetts. Exposure groups consisted of 5 animals.

EXPERIMENTAL RESULTS

The concentrations of decomposed and undecomposed $SOCl_2$ determined during the inhalation exposures are given in Table 76. Although dry gases were used to generate the atmospheres, enough moisture was produced by the rats to produce relative humidities of around 50%. Under these conditions, SOCl was completely hydrolyzed, or almost so, in all experiments so that rats were exposed to SO_2 and HCl in a ratio of 1:2.

TABLE 76. CONCENTRATIONS OF DECOMPOSED AND UNDECOMPOSED THIONYL CHLORIDE IN INHALATION EXPOSURE CHAMBERS

Decomposed Conc., ppm	SD ppm	Undecomposed Conc., ppm	Chamber Temp., °C	% Rel. Humidit
661	± 68	11	22.6	47
503	± 46	0	25.0	39
413	± 24	0	23.5	46
360	±110[a]	0	27.0	58
302	± 44	0	25.0	50

[a] Large standard deviation due to syringe pump jamming during exposure.

Mortality in the 1-hour inhalation exposures is shown in Table 77. Low non-lethal concentrations were irritating to the eyes and respiratory system resulting in shallow breathing and, eventually, gasping. Deaths were directly attributed to severe lung irritation with resultant edema formation. Deaths usually occurred within 24 hours of exposure termination. No deaths occurred beyond 48 hours after exposure.

Survivors of thionyl chloride concentrations in which partial lethality occurred never regained original body weight, with the exception of the single survivor from the highest concentration exposure. Rats exposed at non-lethal concentrations regained their original body weight during the second week of postexposure observation.

TABLE 77. EFFECT OF 1-HOUR INHALATION EXPOSURES OF MALE FISCHER 344 RATS TO THIONYL CHLORIDE

Concentration, ppm[a]				Mortality	Number Dead At	
$SOCl_2$	SO_2	HCl	SO_2-HCl Total	Ratio	24 hrs	48 hrs
11	661	1322	1983	4/5	3	1
0	503	1006	1509	3/5	3	-
0	413	826	1239	2/5	2	-
0	360	720	1080	0/5	-	-
0	302	604	906	0/5	-	-

[a] $SOCl_2$ measured concentrations, others calculated.

LC_{50} of SO_2 + HCl mixture (95% confidence limits) = 1480 (1170-2110) ppm.

LC_{50} calculated as $SOCl_2$ (95% confidence limits) = 500 (420-660) ppm.

Pathology

Gross examination of the rats that died during or shortly following exposure showed that the respiratory tract was the primary target for the $SOCl_2$ damage. Mild to moderate multifocal congestion with multiple areas of moderate to severe ecchymotic hemorrhage were observed in the rats. There was also evidence of atelectasis and consolidation with some residual alveolar damage.

DISCUSSION

The acute effects of exposure to $SOCl_2$ were similar to those observed with exposure to other pulmonary irritants such as OF_2 (Davis, 1970), HF (DiPasquale and Davis, 1971), ClF_5 (Darmer, 1971), CF_3 (Dost et al., 1968), and HCl (Darmer et al., 1972). Deaths were attributed primarily to the irritative effects of the compound on the respiratory tract.

The exposures of male rats to $SOCl_2$ were carried out by generating the contaminant in very dry air to minimize decomposition. However, it was impossible to eliminate all water from the exposure chamber due to the moisture from the animals breath and excreta. Except for one exposure, the complete breakdown of $SOCl_2$ to SO_2 and HCl was not prevented.

Finney (1952) gives the following equation for calculation of mixture LC_{50} values:

$$\frac{1}{\text{Predicted } LC_{50} \text{ Mixture}} = \frac{p_a}{LC_{50} \text{ Component}_a} + \frac{p_b}{LC_{50} \text{ Component}_b}$$

Where: p_a = Proportion of component a, p_b = Proportion of component b and $p_a + p_b = 1.00$.

For the 1:2 mixture of SO_2 and HCl, this becomes:

$$\frac{1}{LC_{50} \text{ Mixture}} = \frac{0.33}{2520} + \frac{0.67}{3120}$$

Predicted LC_{50} mixture = 2890 ppm or 960 ppm as $SOCl_2$.

Since the measured LC_{50} of the mixture was 1480 ppm, or 500 ppm as $SOCl_2$, the acute toxicity of the mixture is greater than would be expected from simple additivity but not enough to place it in a higher toxicity class. Moreover, the major reason for measuring the acute toxicity of $SOCl_2$ was that Flury and Zernik (1931) stated that cats were killed during a 20-minute inhalation exposure to 17.5 ppm, a value which appears to be in error. Our data obtained with rats demonstrate that $SOCl_2$ is not extremely toxic.

SECTION III

FACILITIES

ANALYSIS OF γ-HYDROXYBUTYRIC ACID (GHBA) AND γ-BUTYROLACTONE (GBL) IN RAT URINE

Single dose IP administration or 2-week continuous inhalation of dimethyl methylphosphonate (DMMP) leads to urinary excretion of γ-butyrolactone (GBL) or γ-hydroxybutyric acid (GHBA) in rats (MacEwen and Vernct, 1984). A hypothesis was developed that the presence of either GBL or GHBA in the urine is due to the

inhibition of the major metabolic pathway of succinic semialdehyde dehydrogenase (SSA-DH). Succinic semialdehyde is an intermediate in the metabolism of the neurotransmitter γ-aminobutyric acid (GABA) (Figure 9).

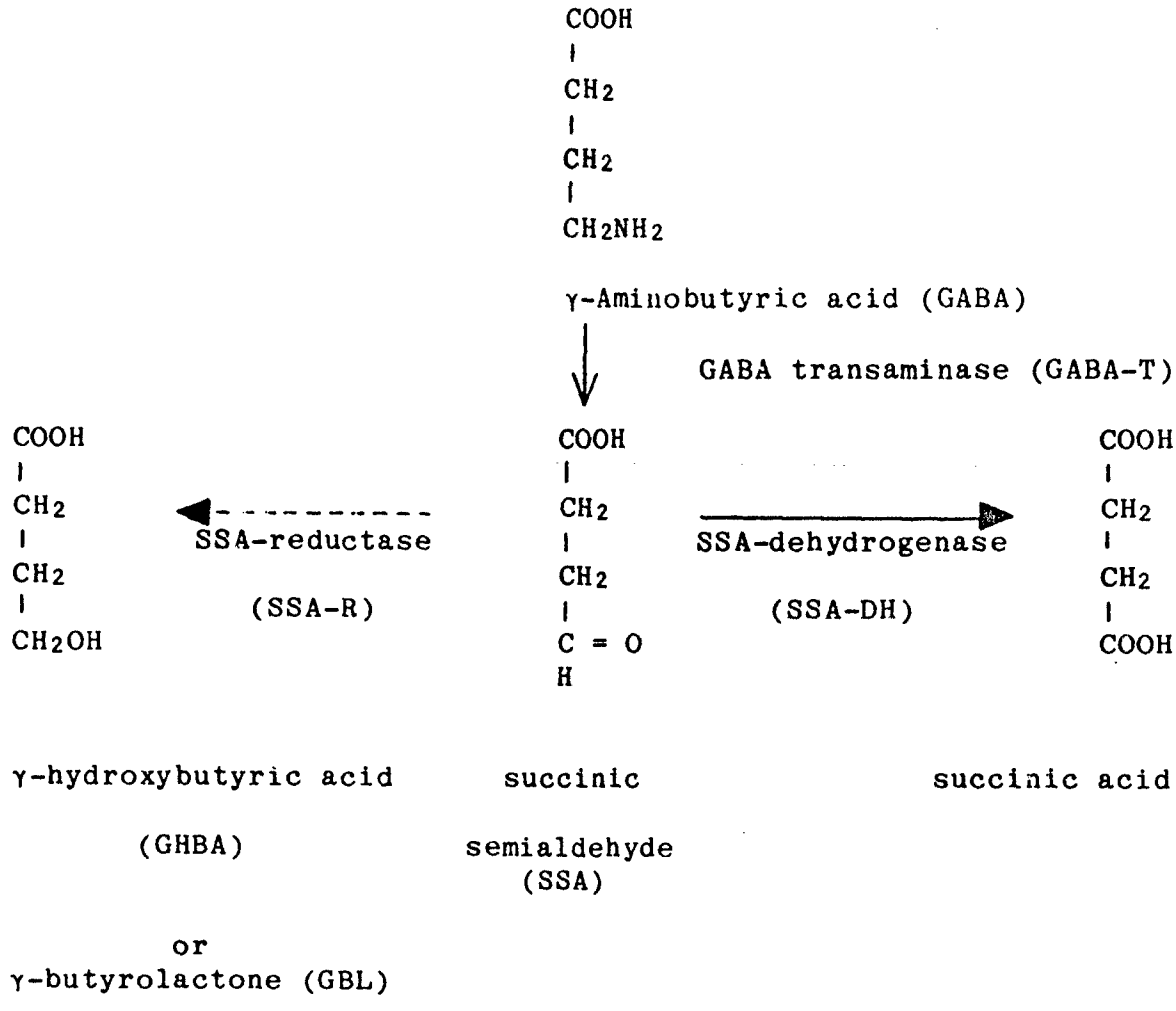


Figure 9. γ-Aminobutyric acid metabolic pathway.

Blockage of SSA-DH may lead to the increased utilization of the alternate metabolic route (SSA-R) thereby increasing the products GBL or GHBA.

In order to test this hypothesis, experiments were designed to measure urinary GHBA and GBL levels after administration of DMMP, GBL itself, and 2-propylpentanoic acid, a known inhibitor of SSA-DH. To carry out this objective, it was necessary to develop analytical methods which could distinguish between GHBA and GBL and determine each with accuracy and precision. Since it had previously been shown that GHBA was converted to GBL in a gas chromatograph, procedures were developed to differentiate between them by their extraction characteristics into chloroform. The method first measures GBL + GHBA by lactonization of the GHBA using strong acid and heat before extraction. In a separate sample of the same urine, GBL alone is determined by extraction without acid treatment.

Procedure for Analysis of GBL and GHBA in Rat Urine

Urine samples are analyzed for GBL and GHBA the day after collection, if possible, but are stored at -80°C when necessary. Two 1 mL samples are taken from the 24-hour collection of urine following treatment. They are placed in two 4 mL TR-Vials. The first sample is treated with acid and heat to convert the GHBA to GBL. The treatment steps are as follows:

1. Add 0.06 mL concentrated sulfuric acid to 1 mL urine.
2. Heat at 83°C for 15 minutes.
3. Cool to room temperature.

Both samples are then individually extracted to remove the GBL for analysis. The extraction steps in the 4 mL TR-Vial are as follows:

1. Extract 1 mL urine/2 mL chloroform with a 1 minute swirl.
2. Add 100 mg Alconox® and swirl to mix.
3. Separate off the chloroform extract.
4. Extract 1 mL urine/2 mL chloroform with a 1 minute swirl.
5. Remove chloroform layer and pool with the first extract.
6. Evaporate to 1 mL and inject 2 µL into gas chromatogram.

The extracted samples are analyzed by GC and recorded using a computing integrator under the following conditions:

GC:	Varian 3700 with FID
Sample Size:	2 µL
Column Flow:	3 mL/min
Splitter Flow:	50 mL/min
Column:	50 m x 0.32 mm Carbowax® CP57 WCOT
Column Temperature:	130°C
Injector Temperature:	210°C
Detector Temperature:	260°C
Integrator:	Hewlett-Packard 3388

The retention time is about 8 minutes, varying slightly from day to day. A standard of GBL in chloroform is run daily to determine retention time and to check detector sensitivity which also varies from day to day. GBL and GHBA excreted per day are calculated from the concentrations and the total urine volume. This procedure was applied to urine from rats given IP doses of 2 mL/kg DMMP and to control rats. It was shown that:

1. Control urine contains a negligible concentration of GBL and a reproducible low level of GHBA.
2. IP administration of DMMP results in significant levels of urinary GBL and increases in urinary GHBA.

The urine samples used to check the GC procedure were also analyzed using the HP 5993 GC/MS under chromatographic conditions listed below:

Column:	Chrompak-57 capillary, 50 m x 0.32 mm
Column Temperature:	130°C isothermal
Injector Temperature:	210°C
Carrier Flow:	Helium at 3 mL/min, 20 psi
Splitter Flow:	10 mL/min
Run Time:	12 min
Scan Delay:	5 min
Injection Volume:	5.0 µL

In order to maximize GC/MS sensitivity, GBL was measured by selected ion monitoring (SIM). The GBL peak assignment was made on the basis of retention time coincidence of mass fragments at 42, 56, and 86 amu which are evident in the mass spectrum of GBL shown in Figure 10.

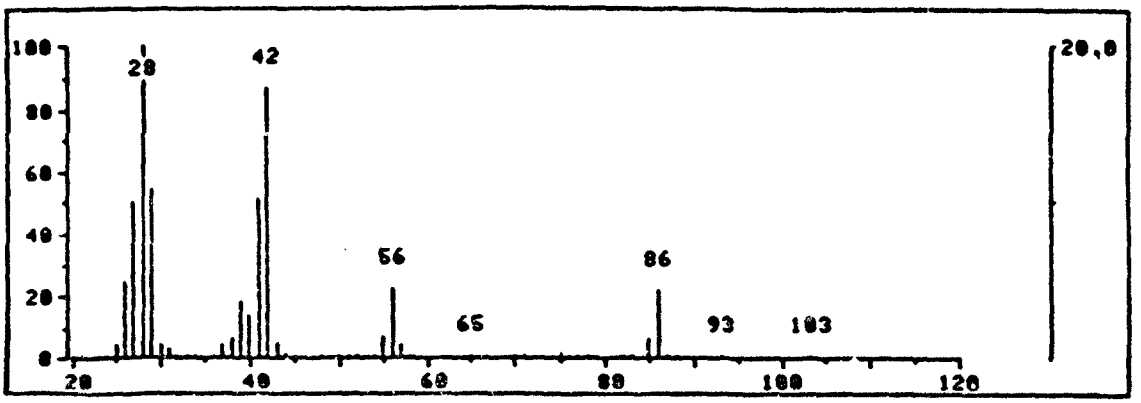


Figure 10. Library mass spectrum of γ-butyrolactone (GBL).

GBL concentration in the urine was quantitated using chromatographic peak area achieved by monitoring the ion at 42 amu. Because of fluctuations in detector sensitivity from run to run, GBL response had to be normalized to the response to an internal standard which, in this case, was γ-valerolactone (GVL). The peak area obtained by monitoring mass fragment 56 amu in the GVL spectrum, shown in Figure 11, served as the reference response. All urine samples analyzed for GC/MS quantitation contained GVL at a concentration of 17 µg/mL. This was accomplished by doping each 1 mL sample of urine extract with 4.25 µL of a 4000 µg/mL GVL solution in chloroform.

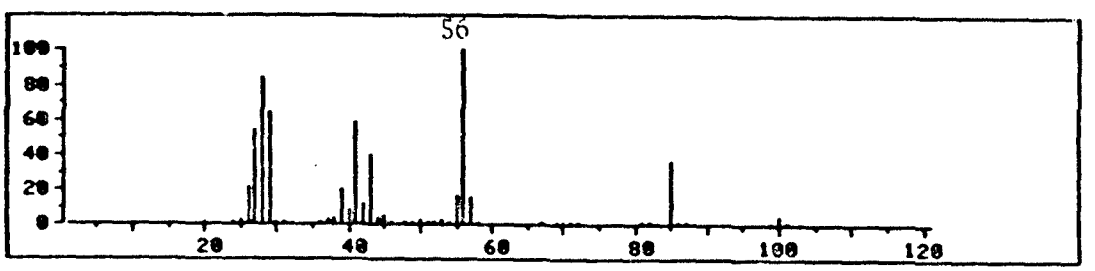


Figure 11. Library mass spectrum of γ-valerolactone (GVL).

A 17 µg/mL GVL solution served as diluent for all GBL standards. This solution was prepared by dissolving 4.0 µL (4.228 mg) of GVL in chloroform and diluting to 250 mL. A 25 µg/mL GBL standard was then prepared by dilution of 2.50 mg of GBL to 100 mL with the diluent. The remaining standards were prepared by further dilution of the 25 µg/mL standard.

For each 5.0 µL injection of standard, peak areas were calculated for response to GVL (56 amu) at RT = 7.2 minutes and to GBL (42 amu) at RT = 7.6 minutes. The GBL/GVL area ratio was calculated and plotted as function of GBL concentration.

This procedure confirmed the identity of the compound being chromatographed as GBL and also duplicated the quantitative and qualitative results of the GC/FID procedure.

CYCLOTRIPHOSPHAZENE QUALITY CONTROL

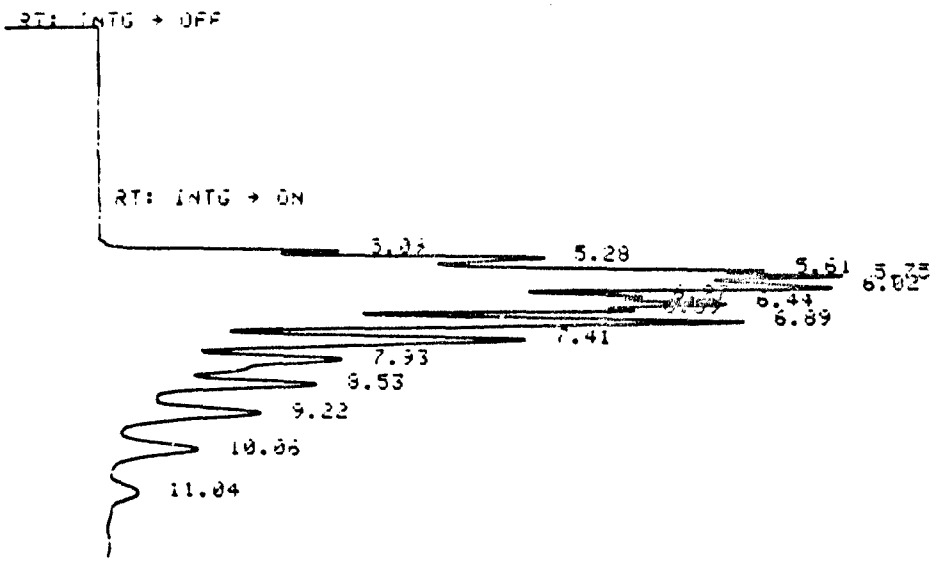
Quality control (QC) analysis was conducted on cyclotriphosphazene, hydraulic fluid (PN) as part of the oral, ocular, and dermal studies on this material. PN is composed of a mixture of heterosubstituted, cyclotriphosphazene cyclic esters, tolyltriazole (Cobratec® TT-100), and a copper-based corrosion inhibitor. The test agent has a vapor pressure of 12 mmHg at 149°C and undergoes thermal decomposition at 185°C. These properties negate the possibility of gas chromatographic analysis (GC) on the intact material. It was therefore determined that liquid chromatography (HPLC) would be the method of choice for QC analysis of PN.

A reverse phase separation of PN components was conducted with the Waters M-6000 Liquid Chromatograph using a C-18 column with a methanol/water mobile phase. Chromatographic bands were read by UV spectrophotometer (Kratos Spectroflow 773) at a wavelength of 259.2 nanometers. Figure 12 is a representative chromatogram obtained by this method. Chromatographic conditions are detailed in the figure. Results of eight replicate chromatograms are presented in Table 78. Reproducibility is good and is acceptable for purposes of QC characterization.

TABLE 78. CHROMATOGRAPHIC RESULTS OF CYCLOTRIPHOSPHAZENE QUALITY CONTROL ANALYSIS. MATRIX OF % AREA BY PEAK AND SAMPLE

RT	Sample #								Mean	
(min)	1	2	3	4	5	6	7	8	% Area	SD
5.09	2.42	2.40	2.43	2.39	2.25	2.13	2.14	2.19	2.29	0.12
5.28	6.21	6.45	6.37	6.32	6.44	6.49	6.14	6.38	6.35	0.12
5.61	7.66	8.14	8.06	7.91	8.27	8.43	7.30	8.16	7.99	0.36
5.75	7.79	7.83	7.80	7.82	8.21	8.49	9.53	8.44	8.24	0.60
6.02	13.05	13.87	13.68	13.41	13.80	13.94	12.61	13.59	13.49	0.46
6.27	5.40	5.53	5.67	5.56	5.89	6.19	5.53	5.82	5.70	0.26
6.44	7.59	8.16	7.85	7.76	7.81	7.79	7.41	7.75	7.77	0.21
6.59	5.65	5.91	5.89	5.80	5.99	6.11	5.45	5.91	5.84	0.21
6.89	12.86	12.73	12.70	12.78	13.06	12.98	15.12	13.50	13.22	0.81
7.41	9.04	9.20	9.14	9.15	9.20	9.32	8.68	9.12	9.11	0.19
7.93	6.53	6.44	6.41	6.51	6.37	6.39	5.97	6.32	6.37	0.18
8.53	4.98	4.40	4.44	4.69	4.49	4.26	6.27	4.82	4.79	0.64
9.22	4.91	4.41	4.45	4.73	4.30	4.17	3.62	4.24	4.35	0.39
10.06	3.52	2.73	2.85	3.23	2.64	2.41	2.36	2.59	2.79	0.40
11.04	1.96	1.25	1.52	1.69	1.28	0.91	1.01	1.09	1.34	0.36

Cyclotriphosphazene Quality Control Analysis
HPLC - Waters M-6000 System
Column: Spherisorb ODS, 50, (20 x 0.4) CM.
Mobile Phase: 90/10% Methanol/Water (Y/Y)
Elution Mode: Isocratic
Flow Rate = 0.7 C/min
Inject.: 1.5 µL of 1/20 Dilution of PN in Mob. Ph.



[illegible] 3388A MANUAL INJECTION @ 15:06 JAN 22, 1985
AREA %

RT	AREA	TYPE	WIDTH	HEIGHT	BASELINE	AREA %
0.00			BASELINE @ START RUN = 24.09			
0.00			THRESHOLD @ START RUN = 7			
0.00			PEAK WIDTH @ START RUN = 0.04			
0.00			RT: INTG → OFF			
4.00			RT: INTG → ON			
5.09	17326.70	BM	-----	2039.63	29.51	2.251
5.28	49531.50	MM	-----*	3900.50	29.51	6.436
5.61	63681.50	MM	-----	5825.18	29.51	8.274
5.75	63149.30	MM	-----	6497.60	29.51	8.205
6.02	106202.00	MM	*-----	6398.79	29.51	13.799
6.27	45324.90	MM	-----	4747.79	29.51	5.889
6.44	60122.10	MM	-----	5467.68	29.51	7.812
6.59	46118.20	MM	-----	4665.51	29.51	5.992
6.89	100544.00	MM	-----*	5616.65	29.51	13.064
7.41	70769.40	MM	-----*	3700.68	29.51	9.195
7.93	49037.30	MM	-----*	2674.64	29.51	6.372
8.53	34551.90	MM	-----	1856.61	29.51	4.489
9.22	33103.70	MM	-----	1378.79	29.51	4.301
10.06	20313.00	MM	-----	836.95	29.51	2.639
11.04	9855.79	MM	-----	317.55	29.51	1.281

TOTAL AREA = 769634.00
MULTIPLIER = 1

Figure 12. Sample chromatogram by HPLC for cyclotriphosphazene quality control analysis.

IMPROVEMENTS TO THOMAS DOME CHAMBER SYSTEMS

Chamber Flow Measurement System

Inlet and exhaust chamber airflow measurements are made using Meriam laminar flow elements installed in both the inlet and exhaust chamber flow lines. Flow element output of 4" H_2O differential pressure (dp) is nominally equivalent to 50 cfm airflow through the laminar unit. Measurement of the dp output was initially accomplished by an inclined manometer air filter gage. The maximum range of these units was 0-7" H_2O differential pressure. The units had an accuracy of ± 3% of full scale. The scale consisted of a combination inclined and vertical configuration. This resulted in a much lower accuracy of readings in the scale range required for chamber readings. Another negative factor in the original manometer gages was their maximum scale reading of 7" H_2O dp. Normal chamber operation requires an increase in chamber air flows after each day's exposure to remove the contaminated atmosphere and permit entrance by technicians for daily chamber and animal maintenance. During this period, the flows required for effective contaminant removal may exceed the manometer's maximum rating. This often resulted in the measuring fluid being forced into the sensing lines and into the laminar flow elements, requiring their removal, cleaning, and recalibration. A third drawback of the original measurement configuration was the procedure for calibration of the units by operating personnel. Incorrect control settings during calibration could cause a blow-out of the manometer fluid and/or affect the flow controlling transducer causing a disturbance to chamber flow. To correct the problems, the tubing connections from manometers to transducers were redesigned to permit calibration without disturbing the flow controller. Additionally, the previously used manometers were replaced with panel mounted well-type units. These manometers have a reading accuracy of ± 1% and a maximum scale reading of 8" H_2O dp, accommodating the maximum chamber flow reading expected during blow-off procedures. Finally, in the event that the manometer is subjected to an excessive pressure drop, the units are equipped with safety traps on both the inlet and outlet ports to eliminate the possibility of fluid loss. For ease of reading and calibration, the manometers were mounted on control panels located near the flow transducers and laminar flow elements.

Chamber and Room Painting

A complete renovation of the chambers and chamber areas was accomplished during this report period. The chambers were completely stripped of paint and recovered with several layers of epoxy paint. All equipment was removed for cleaning and renovation. Concurrently, a subcontract was let to provide cleaning and painting of the chamber rooms. These areas still retained the black painted walls required for photographic and light incidence-related studies. These requirements were no longer appropriate, so light colors were chosen. Painting of these rooms resulted in a vastly improved lighting capability in the areas plus a significantly improved appearance. At this time the basement areas of each chamber facility were also repainted.

Bridge Crane Safety Modifications

As a result of the painting of the chamber rooms, a problem was discovered with the bridge cranes used for lifting and lowering of the Thomas Dome Chamber caps. Painting of the crane mechanisms resulted in some overspray coating the trolley surfaces of the crane superstructure. This reduced the grounding capability of the trolley and trolley tracks. Inspection of the two 5-ton bridge cranes revealed the absence of a direct ground connection to the building superstructure. Some operating personnel received minor electrical shocks when operating the bridge crane. Routine use of the crane was suspended until a satisfactory solution could be implemented. Of the two bridge cranes in the facility, the crane in Chamber Room A was the simplest to modify. Power was supplied to the crane superstructure by a 3-wire-440V cord-reel system. The reel had provisions for a fourth wire which was utilized for a ground connection. A #10 wire was connected to the bridge structure, routed to the cord-reel connection and from the cord-reel to the building structure. Measurement of the crane-to-ground voltage potential after this modification resulted in potentials of less than 1 volt. This eliminated any possibility of personnel exposure to hazardous electrical potentials due to improper grounding. The bridge crane in Facility B required more extensive modifications. This crane was supplied power by a 3-wire power system, supplied to the crane by a rail-rider system of electrical connections. This system contained only three rails, 1 for each leg of the 3-phase input electrical system. To accommodate a grounding connection, a fourth rail was installed and connected on one end to the building structure and on the other end to the crane structure. This eliminated hazardous electrical potentials from this equipment.

MULTI-USER TERMINAL SYSTEM

The system has been modified and improved significantly over the past year. Elements added or upgraded include:

1. Network Components Acquisition
2. IBM AT Acquisition for Network Fileserver
3. Communications Capability
4. Software Programs
5. Peripheral Devices

The network configuration completion was delayed until a network was available which not only had the desired operating features, but also which might be expected to have stability and support in a continually changing market. With IBM's announcement of a local area network in August 1984, that product became the focal point of our design. Deliveries were delayed but all components have now been received. The delivered network components include 7 network adapter boards, 1 for each of the multi-user data terminals. The system also includes a network translation unit which provides conversion and control of the PC-to-network communications. File communication between data terminals of the system is currently accomplished via either hard-wired connections at 9600 baud/960 characters per second or 1200 baud/120 characters per second. These utilize commercially acquired communications packages such as Crosstalk™ or Ascom™. With the completion of the network system, file transfers may be accomplished at the rate of 2 Mbits/sec or 200,000 characters/second. This increases file transfer speeds by a factor of 200 over previous capability. A network operating protocol is being developed which will encompass file transfer protocols, security requirements, file and hard disk backup procedures and peripheral sharing assignments. The IBM network system also includes an electronic mail function. This provides sending and receiving of messages to each terminal on the network system.

It is now possible to access the NBI 4000 word processor from the IBM terminals. Considerable modification to the software was required to achieve compatibility between the IBM systems and the NBI Model #4000 systems. Communications have also been established between the local THRU office and the University of California campus at Irvine.

An additional terminal was acquired to handle the network file-serving function. The unit acquired was an IBM-AT. This unit has a more powerful microprocessor, increased fixed disk storage capacity and a faster operating speed. The unit's fixed

disk has a maximum storage capacity of 20 megabytes. This capacity will allow backup of the total capacity of the 10 megabyte fixed disk drives on each of the IBM-XT's in the network system. The IBM-AT operates at approximately 3 times the speed of the IBM-PC or IBM-XT. This unit has been installed in the Engineering Department. The faster speed will enhance the heavy requirements required of software programming involved in assembling, linking, and compiling computer languages. The unit was installed in place of an existing IBM-XT which was relocated to the Administration Department. The unit functioned without difficulty in operating the peripherals which had been connected to the IBM-XT, including a 35 megabyte fixed disk manufactured by Tallgrass. Full implementation of the IBM-AT into the IBM network system will allow backing up of data onto cartridge tapes from any terminal attached to the network or the downloading of software updates to any terminal from the central system. Procedures such as these should greatly simplify the administration of network programs and security.

Several software programs have been implemented on the various terminals and applications are being developed. Lotus 1-2-3™ has been established on the Technical Services terminal, the Administration terminal, the Chemistry/Toxicology terminal, and the Engineering terminal.

Symphony™ has also been implemented on these systems and is being heavily utilized on the Administration terminal in conjunction with Symphony™ applications also in use at the Irvine campus. Several data base applications are being evaluated and developed by the Technical Services Data personnel. These include evaluations of the feasibility of Lotus 1-2-3™ in database applications such as the Engineering Work Order Program. Further evaluation of different software packages is being conducted to arrive at a standardized software group to be installed on each terminal system. This software grouping will be implemented and routinely updated as new software versions become available. The package of standard software currently anticipated would include the following categories:

1. Word Processing
2. Spread Sheet
3. Data Base
4. Communications
5. Graphics

Other specialized software would be available on a per terminal basis. Allocating software in this manner should simplify maintaining control of software applications. Control techniques

should also prevent the presence of multiple software programs being used to accomplish the same requirements. This is particularly critical in the areas of experimental data analysis and reduction.

Additional peripheral units are being acquired for utilization with the Multi-User Terminal System. Several plotters are being acquired for graphical output from the system data terminals. Plotters will be located at the Statistics data terminal, the Quality Assurance data terminal and the Engineering data terminal. These plotters will provide graphical output from software programs such as Lotus 1-2-3™ spread sheet packages and graphics output packages such as Energraphics™. These units are capable, with the proper software, of providing camera-ready copy. Another peripheral being added to the system is a laser-printer. The laser printer will be a shared peripheral on the network system which will provide high-speed letter-quality printing for any terminal on the system. Location of the printer may be at any of the data terminals. Nominal speed of these types of laser printers is 8 pages/minute of letter-quality copy.

PARTICLE DEPOSITION AND CLEARANCE FROM THE LUNGS OF 4 STRAINS OF MALE RATS

Interspecies or strain differences in toxicity can often be useful in elucidating a specific toxic mechanism or pathway. Conversely, unknown species or strain differences can cause confusion in comparing data from several laboratories when different strains are used. In the case of inhaled particle deposition and clearance, variable results have been reported in studies using different rat strains. Ferin and Morehouse (1980) showed a 2-component alveolar clearance curve for Long-Evans rats but only one component for Fischer 344 rats. Chan et al. (1981) and Wolff et al. (1982), however, did find a 2-component curve for the Fischer 344 rats. In addition, Bianco et al. (1980) found only one alveolar clearance component for Sprague-Dawley rats, whereas Newton and Pfledderer (1985) found a 2-component curve. Some of the disparities may be attributed to particle size and composition or to measurement techniques but they also may result from true interstrain differences. The only interstrain study using the same particles and techniques (Ferin and Morehouse, 1980) did show a difference between the Fischer 344 and Long-Evans rats.

In order to better document interstrain differences, the pattern of deposition, recruitment of macrophages, and clearance of inhaled radiolabeled particles was measured in 4 strains of

rat commonly used in toxicologic investigations: Sprague-Dawle Fischer 344, Wistar and Long-Evans.

EXPERIMENTAL

Experimental Approach

The 4 strains of rats (33 rats/strain) were exposed via inhalation to radiolabeled microspheres. Immediately following particle deposition, 8 rats/strain were sacrificed with 50 mg/k pentobarbital i.p. and the radioactivity of their excised lungs determined to evaluate any differences in total deposition. In addition, 2 rats/strain were sacrificed for excised lung radioactivity measurements at 12, 24, 48, 72, and 96 hours postdepos tion to determine when gastrointestinal (GI) radioactivity no longer interfered with trachea-lung values measured externally the thorax (Newton and Pfledderer, 1985). Serial measurements thoracic radioactivity were made on an additional 15 rats/strai from 30 hours through 40 days postdeposition.

A second study was conducted similarly except the particle were deposited by intratracheal instillation which avoided the large bolus of nonpulmonary deposited particles passing through the GI tract. In addition, during this second study, 4 rats/strain were sacrificed at 2, 4, 6, 10, 14, and 17 days postdeposition and the number of alveolar macrophages was determined by pulmonary lavage.

Radiolabeled Microspheres

Polystyrene latex microspheres (PSL) labeled with a tightl bound ^{51}Cr isotope (28-day half life, 0.32 MeV gamma energy emi sion) were used (Applied Polymer Technology, Costa Mesa, California). In vitro leaching studies had indicated a leachin rate of ^{51}Cr from these particles of less than 0.1%/day (Hinric et al., 1978).

Microsphere Exposure System

In the first study, the microspheres were deposited by inh lation in an exposure system that consisted of an aerosol gener tor, exposure unit and containment enclosure. A Lovelace-type compressed-air nebulizer (ARIES, Davis, California) was operate at 40 psig to generate the aerosol. The reservoir contained a 0.1% suspension of ^{51}Cr labeled PSL particles with a total acti ity of about 5 mCi. The resultant aerosol was characterized

using a seven stage Mercer-type cascade impactor (ARIES, Davis, California) and had a mass median aerodynamic diameter of 1.6 μm and a geometric standard deviation of 1.4. After nebulization, the particles were mixed with a diluting air stream with a total flow of 17 L/m and heated to 70°C for drying. In order to neutralize the aerosol, the air stream then flowed through a 1 mCi 85 Kr deionizer (TSI, St. Paul, Minnesota). The aerosol was then introduced into a small animal, nose only exposure unit (INTOX, Albuquerque, New Mexico). The total activity within the chamber was approximately 250 μCi. The rats were held in 25.4 cm long, 6.3 cm diameter plastic tubes with an adjustable tailgate and were exposed for 20 minutes.

The aerosol was exhausted through absolute particle filters which entrapped the radiolabeled particles for subsequent disposal. The exposure unit was held slightly below ambient pressure to preclude loss of aerosol through the animal holding tubes.

The entire exposure unit was located within a secondary containment system with an airflow of 650 CFM and a downstream HEPA filter to entrap any radiolabeled particles which might escape.

Microsphere Instillation

In the second study, the radiolabeled microspheres were deposited by intratracheal instillation. The rats were anesthetized with a 4% Halothane-oxygen mixture for 5 minutes, intubated (Nicholson and Kinkead, 1982) and 0.3 mL of the diluted microsphere solution instilled to give the rats the same approximate thoracic load of particles as the rats exposed via inhalation.

Radioactivity Measurements

External thoracic radioactivity measurements were made by placing the rat in a holding tube within an annulus shaped NaI (Tl) detector 15 cm long and shielded by lead to favor detection of the radioactivity within the thoracic cavity. In the second study, a 7.5 cm long detector was used to further reduce measurement of emissions emanating from the lower GI tract. A multichannel analyzer (Model #30, Canberra, Meriden, Connecticut) integrated counts in the energy region of interest. All thoracic radioactivity measurements were made to obtain a minimum of 1000 counts. The holding tubes for the radioactivity measurements

were 25.4 cm long and 6.3 cm in diameter with an adjustable tailgate. The front end of the tube had a 1.3 cm diameter concentric hole into which the rats would voluntarily hold their noses. This standarized the position of the rat within the tube for the serial radioactivity measurements.

Lung Lavage

The rats were anesthetized with 50 mg/kg pentobarbital, intubated and then exsanguinated by cutting the abdominal aorta. The lungs were flushed in situ with 6 washes (23 mL/kg) of 0.9% saline. Each wash was injected by syringe, held for 1 minute with gentle external massage and then withdrawn. The washes were combined and spun down using a refrigerated centrifuge. The cell pellet was resuspended in 1 mL 0.9% saline and total white cell and differential count determinations made using a Papanicolaou's stain.

Experimental Animals

Sixty-day old male rats: Sprague-Dawley (250-313 g), Wistar (247-315 g), Long-Evans (252-326 g), and Fischer 344 (161-199 g) were used. Cultures from rats sacrificed for quality control determinations were free of Mycoplasma pulmonis. The animals had food and water ad libitum except during the aerosol exposure and radioactivity measurement periods. Food was Purina Formula #5008, and the water was softened and did not exceed 17 ppm hardness measured as calcium carbonate. For 12 days prior to the study, the animals were caged in a single layer in wire bottomed Rochester chambers in order to provide as dust-free a preexposure environment as possible. After the aerosol exposures, the animals were housed in wire bottomed cages in a laminar flow room.

Statistical Analysis

A 2-component exponential curve was fitted to the external thoracic radioactivity data for each rat using nonlinear regression analysis. All data were corrected for the physical decay of the ^{51}Cr. Comparison among the strains of the intercept for each component was made by a 1-way analysis of variance followed by Tukey's multiple comparison procedure. The Kruskal-Wallis method of analysis of variance followed by Dunn's nonparametric method of multiple comparisons were used for comparison of the half life data. An F-ratio or chi-square value with $p < 0.05$ was defined as statistically significant.

RESULTS

Figure 13 shows the alveolar clearance curves for the 4 ra strains in the inhalation study. The figure includes zero-hour excised lung data combined with external thoracic counts beginning at 96 hours postexposure when the external thoracic radioactivity data accurately reflected excised trachea-lung radioactivity. Because of the absorbtion of the gamma rays by the rat's body tissue surrounding the lungs, the external thoracic radioactivity measurements of the instilled particles were 91.1 ± 1 (SE) % of the excised lung values. Therefore, in Figure 13, excised lung data are adjusted by the above factor fo inclusion with the external thoracic measurements. All measurements were corrected for decay of the ^{51}Cr.

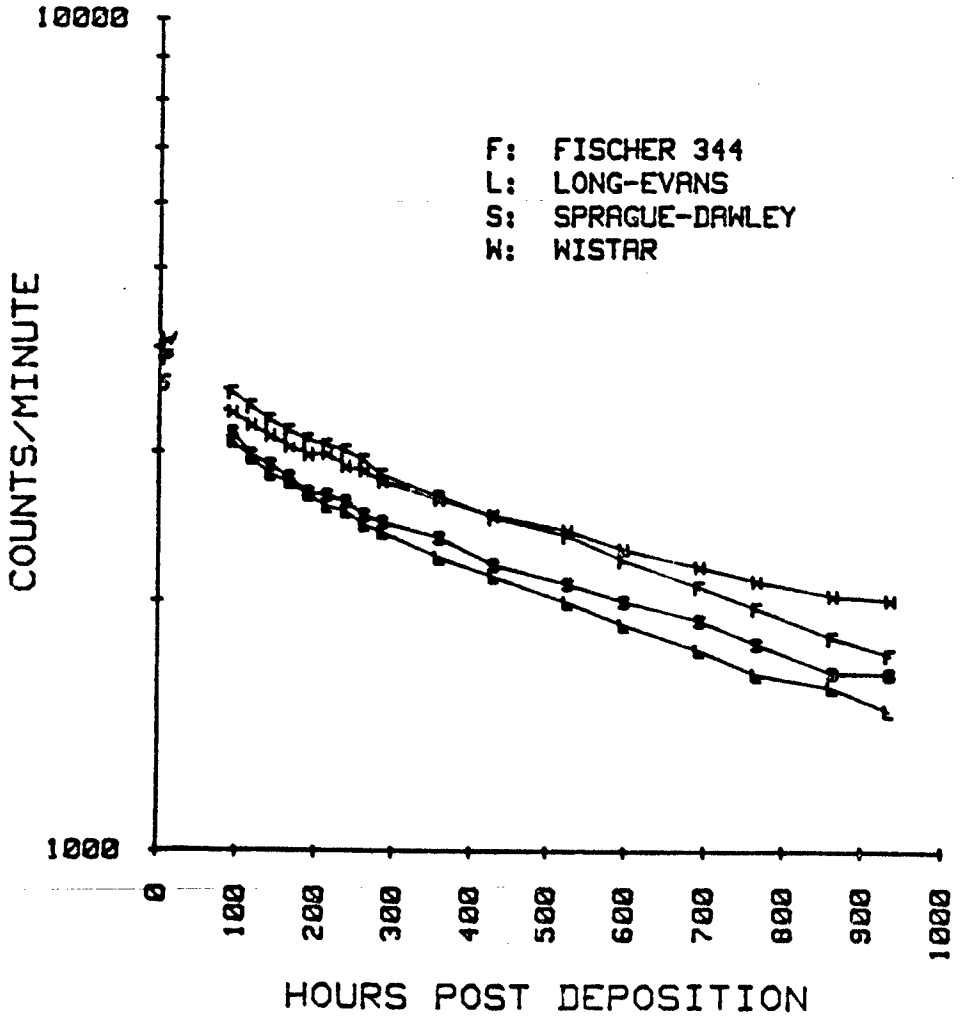


Figure 13. Clearance of ^{51}Cr labeled polystyrene latex microspheres (1.6 μm) deposited by inhalation from the lungs of 4 strains of male rats.

Figure 13 shows a 2-component alveolar clearance curve for 3 of the 4 strains (Sprague-Dawley, Long-Evans and Wistar) with a break point between the two components at about 8 days post-deposition. The Fischer 344 rats data did not show this first component statistically. The first three points did show a trend toward an early component but the data were insufficient for the nonlinear regression program to estimate this early component on an individual rat basis. Table 79 presents the calculated intercepts and half lives for the two components and shows significant differences among the strains. These differences occurred both in the intercepts or the amount being cleared in each component and in the half lives or rates of clearance in each component. There was no significant difference among the strains in the total amount of particles deposited.

TABLE 79. KINETIC CONSTANTS OF PULMONARY CLEARANCE OF RADIOLABELED MICROSPHERES DEPOSITED BY INHALATION

	Alveolar Component #1		Alveolar Component #2		
Rat Strain	Half Life[a]	Intercept[b]	Half Life[a]	Intercept[b]	N
Fischer 344	N/A	N/A	35.9 ± 0.7*	3628 ± 193*	16
Long-Evans	3.4 ± 0.6+	916 ± 147+	37.4 ± 2.9*+	2976 ± 255*	15
Sprague-Dawley	1.4 ± 0.2*	2027 ± 379*	39.9 ± 2.3*+	3116 ± 275*	12
Wistar	3.9 ± 1.0+	966 ± 366+	59.1 ± 9.2+	3254 ± 285*	9

[a] Values (days) with different symbols (+ or *) are significantly different by Dunn's nonparametric multiple comparison method.
[b] Values (counts/minute) with different symbols (+ or *) are significantly different by Duncan's multiple comparison method.

Previous results using a 7.5 cm detector (Newton and Pfledderer, 1985) showed that external thoracic radioactivity measurements accurately reflected excised trachea-lung measurements 30 hours postdeposition. The data reported here using a new 15 cm detector did not accurately reflect excised trachea-lung measurements until 96 hours postdeposition. Therefore, the instillation study was conducted using the original, smaller detector. Figure 14 shows the retention of lung radioactivity after the instillation. With the data now starting at 18 hours postdeposition instead of 96 hours, all four rat strains showed a two component alveolar retention curve over this time period.

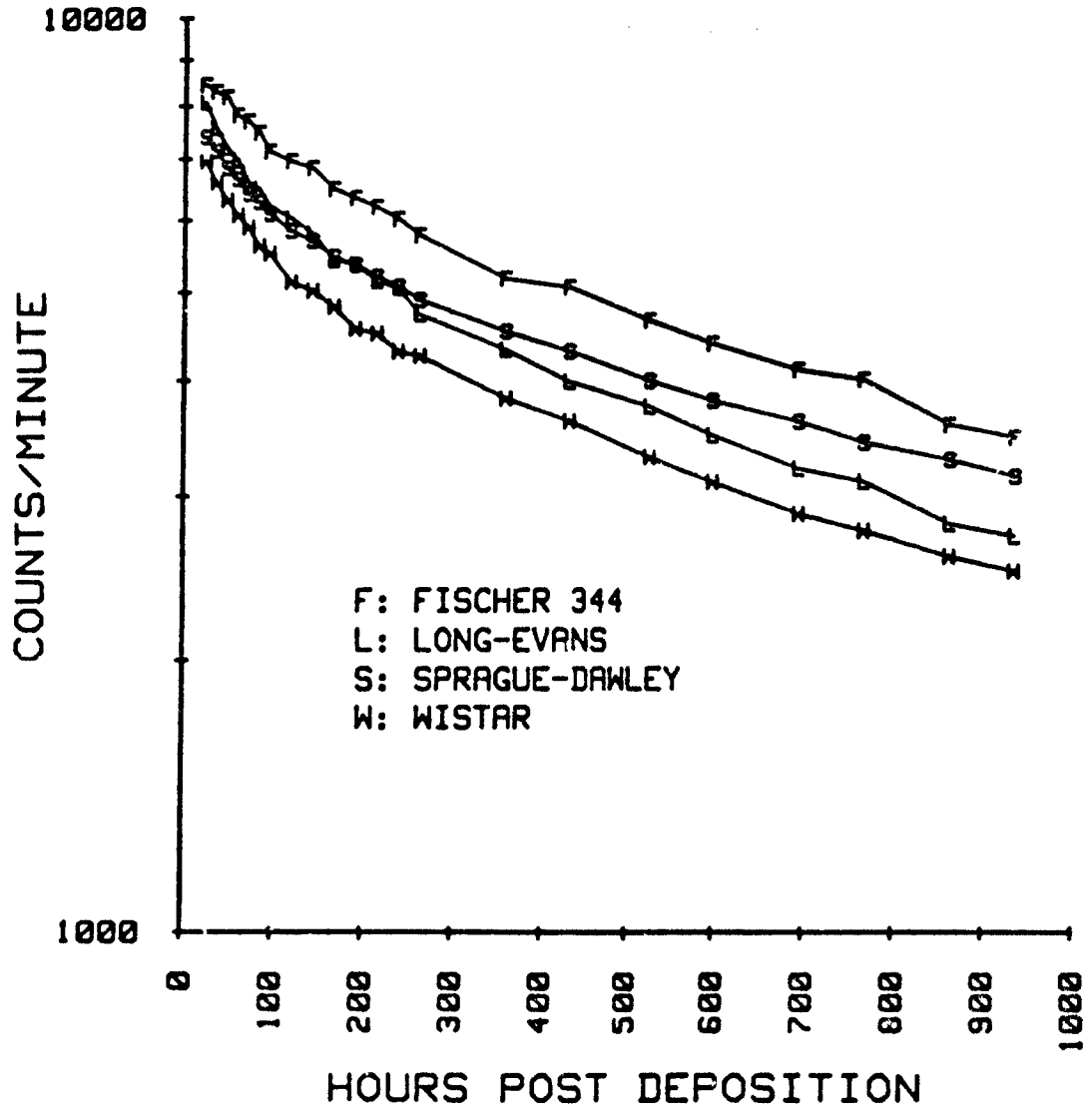


Figure 14. Clearance of ^{51}Cr labeled polystyrene latex microspheres (1.6 μm) deposited by intratracheal instillation from the lungs of 4 strains of male rats.

Table 80 presents the calculated intercepts and half lives for the two components and again significant differences among the strains are seen.

Figure 15 shows the recruitment of macrophages into the alveolar space which was induced by the instillation of the particles. Analysis of variance showed a significant rat strain effect in the number of particles recruited at 4 days post-instillation.

TABLE 80. KINETIC CONSTANTS OF PULMONARY CLEARANCE OF RADIOLABELED MICROSPHERES DEPOSITED BY INTRATRACHEAL INSTILLATION

	Alveolar Component #1		Alveolar Component #2		
Rat Strain	Half Life[a]	Intercept[b]	Half Life[a]	Intercept[b]	N
Fischer 344	3.9 ± 0.6*	2731 ± 254*	53.4 ± 10.5*	6370 ± 316*	11
Long-Evans	2.1 ± 0.2+	3187 ± 220+	35.0 ± 3.4*	5936 ± 230*	13
Sprague-Dawley	2.9 ± 0.6*	2580 ± 158*	49.9 ± 6.2*+	5617 ± 262*+	12
Wistar	2.7 ± 0.4+	3667 ± 773+	53.3 ± 17.3+	4881 ± 366+	9

[a] Values(days) with different symbols (+ or *) are significantly different by Dunn's nonparametric multiple comparison method.
[b] Values(counts/minute) with different symbols (+ or *) are significantly different by Duncan's multiple comparison method.

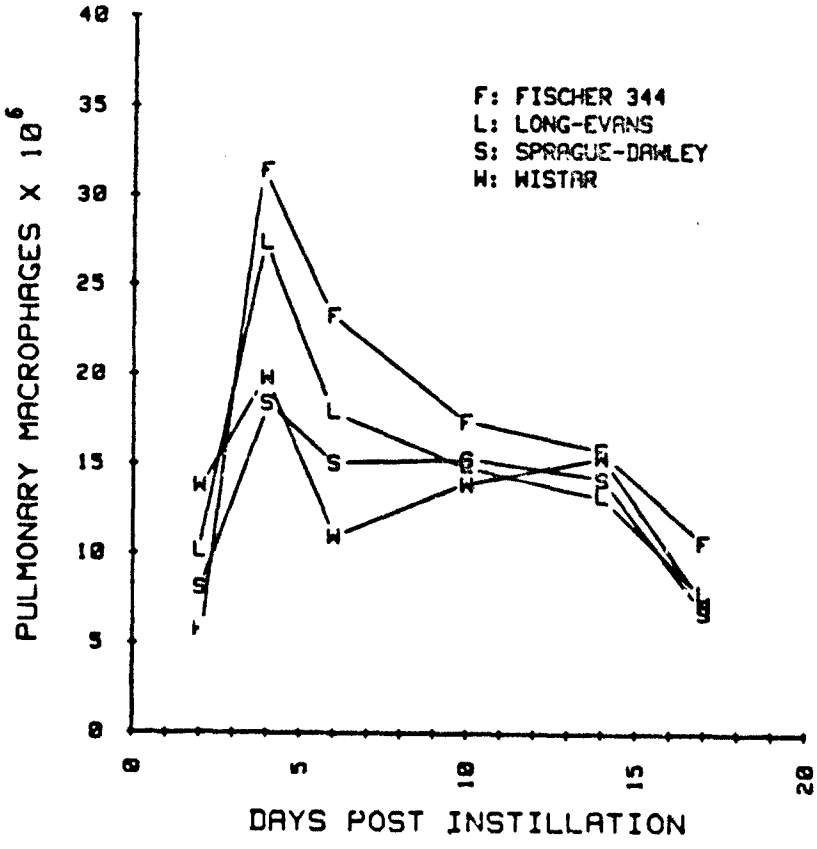


Figure 15. Recruitment in 4 rat strains of pulmonary macrophages induced by intratracheal instillation of ^{51}Cr labeled microspheres.

DISCUSSION

The rate of clearance of particles deposited within the lungs is intimately related to their site of deposition. Any alteration in the pattern of particle deposition can affect the subsequent clearance of these particles from the lungs. Therefore, it is essential to evaluate deposition in conjunction with any evaluation of clearance. Because the 4 strains were exposed in the same manner to the same particles, the only differences in deposition expected would be due to differences in ventilatory patterns or lung anatomy. The Fischer 344 rat was significantly smaller than the other 3 strains and therefore would be expected to have a slightly increased relative deposition (McMahon et al., 1977).

Conventionally, clearance of particles from the lungs during the first 24 hours postdeposition represents clearance of particles deposited on the tracheobronchial (T-B) tree, and clearance after 24 hours represents clearance of particles deposited in alveolar regions (Morrow et al., 1966). Therefore, the 2 components shown in Figures 13 and 14 and their corresponding half lives result from alveolar clearance. Ferin (1982) proposed that the first alveolar clearance component is mediated by macrophages. However, in this study alveolar clearance was inversely correlated with the maximum number of macrophages recruited. That is, the strain which cleared the most particles during these early clearance phases and therefore had the lowest alveolar phase 2 intercept, had fewer macrophages recruited.

The results of the present study do show statistical differences in clearance of these particles among the rat strains and quantitative differences from previous reports. However, comparison of the results with those in the literature (Lee et al., 1983; Chan et al., 1981; Ferin and Morehouse, 1980; Ferin, 1982; Bianco et al., 1980; Oberdoester et al., 1979; Snipes et al., 1983) indicates that the magnitude of the effect is similar to the intertest variabilty and is seen substantially during the first 24 hours postdeposition.

TECHNICIAN TRAINING PROGRAMS

Animal Technicians

All UCI animal technicians are currently certified by the American Association of Laboratory Animal Science (AALAS). One additional staff member passed the certification examination for Laboratory Animal Technologists and the current status of the animal technicians regarding certification is as follows:

7 - Laboratory Animal Technologist
3 - Laboratory Animal Technician
1 - Assistant Animal Technician

Most of the animal technician group are members of the local AALAS organization and attend various seminars and demonstrations dealing with animal husbandry.

The animal technicians successfully completed an AALAS sponsored correspondence course which was directed towards helping competent laboratory animal technicians become certified AALAS technologists. Every 2 months for a year a detailed study outline with study materials was received. After following the recommended study program, each technician completed an exam covering the material in that section. The study outlines and exams were not only graded, but written comments and corrections were developed for each technician. Six study packets were presented over a 1-year period.

Chamber Technicians

During the past year the Thomas Domes SOP's were reviewed and minor updates were made as necessary.

The monthly emergency training procedures program has been revised. Training procedures are conducted by the Senior Technician 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.

Date	Procedure
January	Care and operation of respirators
[a] February	Vacuum pump failure
April	Air supply fan failure
[a] May	Complete power failure
June	Building 429 alarm
July	Rescue of an incapacitated dome entrant
August	Fire in exposure laboratory area
[a] September	GLP procedures and toxicology SOP's
[a] October	Vacuum pump failure
[a] November	Air compressor failure
[a] December	Complete power failure

[a] Written examinations

A training program prepared by the American Institute of Biological Sciences concerning various types of bleeding procedures of rodents and rabbits was presented to all chamber technicans and animal technicians as well as interested Air Force and Navy personnel. This was a slide/cassette and study guide series. The most important aspect of this training program was the inclusion of practical demonstrations of bleeding techniques by professional veterinarians and experienced technicians. Personnel attending the training program were also allowed to carry out the techniques discussed during the demonstration session.

The chamber technician group presently has eight individuals certified in the AALAS program. The levels of AALAS certification are shown below:

Laboratory Animal Technologists - 2
Laboratory Animal Technicians - 4
Assistant Laboratory Animal Technicians - 2

AALAS Certification

Continuing efforts have been made to increase the involvement of all technicians in the certification program sponsored by the American Association of Laboratory Animal Science (AALAS). An examination of the requirements for AALAS Technologist certification revealed several UCI and Air Force personnel eligible. A training course is being presented to personnel considering applying for the AALAS technologist exam, which will be given in September 1985. Classes began in early May 1985 and will continue on a weekly basis until September 1985. Several personnel within the laboratory already certified as AALAS technologists will serve as instructors for this training course.

The training materials used for the course are as follows:

A. Syllabus for the Laboratory Animal Technologist.
B. AALAS Technologist Review Course.
C. AALAS Technologist Correspondence Course.
D. AAALAC slides (American Association for Accreditation of Laboratory Animal Care)

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