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TOXIC HAZARDS RESEARCH UNIT ANNUAL TECHNICAL REPORT: 1978

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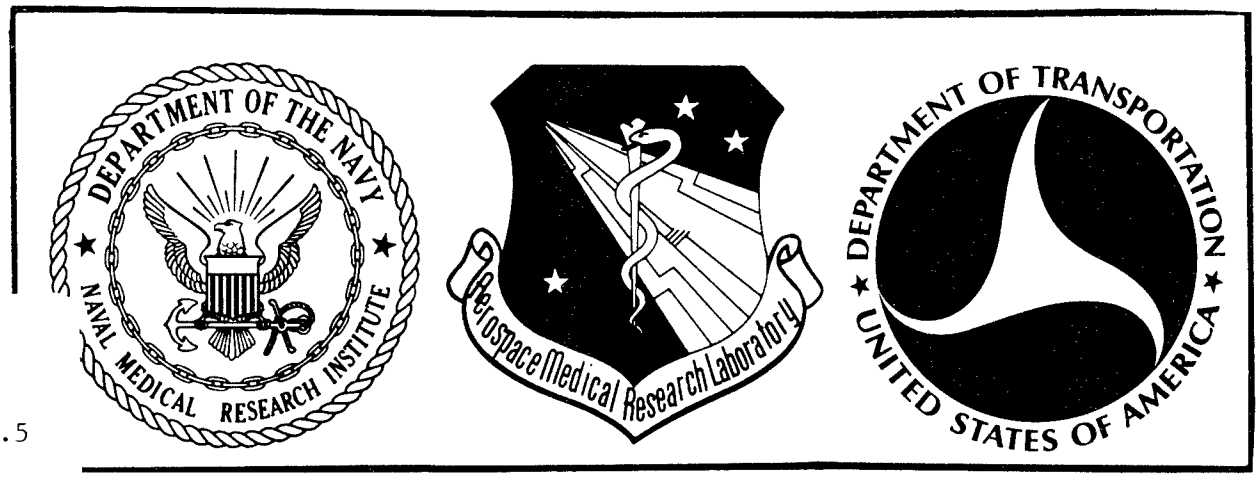

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TECHNICAL REVIEW AND APPROVAL

AMRL-TR-78-55

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 Information Office (OI) 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

Anthony A. Thomas

ANTHONY A. THOMAS, MD
Director
Toxic Hazards Division
Aerospace Medical Research Laboratory

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1,1-Dimethylhydrazine	JP-5	OMP-5
Hydrazine	Jet Fuels	SYFO
1,2-Dimethylhydrazine	DFM	Dimer Acid
Monomethylhydrazine	Diesel Fuel	Organometallic
Fluomine	OMP-4	Toxicity

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

The research programs of the Toxic Hazards Research Unit (THRU) for the period of June 1977 through May 1978 are reviewed in this report. Subchronic inhalation toxicity studies were conducted on decalin, JP-5 and DFM. Studies were carried out on the oncogenicity of inhaled hydrazine, 1,1-dimethylhydrazine and monomethylhydrazine. Acute oral, dermal and inhalation toxicity studies were conducted on a large variety of chemical agents used by the Air Force and Navy or transported in interstate commerce. Sensitization, eye and skin irritation studies were also made on these chemicals.

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Block 19.

Carcinogenesis
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Sensitization
Oral
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Irritation
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Acute
Chronic
bis(2,2-difluoroamino)-1,3-bis(dinitrofluoroethoxy)propane
1,4-dihydroxyanthraquinone
nonylphenol
1,2,3 benzotriole
Azelaic Acid
Phenothiazine
3-amino-1,2,4-triazole
N-benzyl-3,7-dioctylphenothiazine
Tris(β-chloroethyl)phosphate
Tricresylphosphate
Dioctylphenothiazine
2,6-ditert-butyl-dimethylamino-p-cresol

PREFACE

This is the fifteenth 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 F33615-76-C-5005. This document constitutes the third report under the current contract and describes the accomplishments of the THRU from June 1977 through May 1978.

The current contract for operation of the Laboratory was initiated in 1975 under Project 6302 "Occupational and Environmental Toxic Hazards in Air Force Operations," Task 01 "Toxicology of Propellants and Materials" Work Unit Number 63020115. K. C. Back, Ph.D., Chief of the Toxicology Branch was the technical contract monitor for the Aerospace Medical Research Laboratory.

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

TABLE OF CONTENTS

TABLE OF CONTENTS (CONT'D)

TABLE OF CONTENTS (CONT'D)

LIST OF FIGURES

LIST OF FIGURES (CONT'D)

LIST OF TABLES

LIST OF TABLES (CONT'D)

LIST OF TABLES (CONT'D)

SECTION I

INTRODUCTION

This document constitutes the 15th annual report of the Toxic Hazards Research Unit (THRU), 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 supported by Air Force pathologists, veterinarians, and medical technologists.

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

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

As part of its contract responsibilities, UCI/THRU presents an annual technical conference to disseminate new toxicological information to the U. S. Air Force, and other governmental and industrial scientists. This year's conference chaired by Ralph C. Wands, presented 22 technical papers covering a broad range of occupational and environmental toxicology problems. Three papers were presented by University of California faculty and staff members. The open forum discussion following each session resulted in significant contributions of additional technical information and scientific exchange. The conference, held 4 October through 6 October, 1977 drew 149 participants including speakers.

The papers presented at the conference were published as the Proceedings of the 8th Annual Conference on Environmental Toxicology, AMRL-TR-77-97, Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio.

Our next conference, currently in the development stage, will be held in April, 1979 at the Imperial House South Motel, Dayton, Ohio. The change in conference dates from fall to springtime was necessitated by the change in Federal fiscal years which occurred in 1977. This change makes travel in September or October difficult for many government workers who attend our conference.

SECTION II

RESEARCH PROGRAM

The research activity of the 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 are only summarized in this document. This year's research program was conducted on a broad range of chemical materials and includes inhalation studies of rocket and aircraft fuels. Acute oral and dermal toxicity studies on a large variety of materials were also conducted.

A Study of the Oncogenic Capacity of Inhaled Monomethylhydrazine

Evidence that hydrazines administered in drinking water led to carcinogenic activity in mice (Toth, 1972) and in hamsters (Toth and Shimizu, 1973) resulted in a series of chronic studies conducted to examine the inhalation hazard associated with monomethylhydrazine exposure.

The details of the experimental protocol and rationale were presented in the last annual report (MacEwen and Vernot, 1977). Since that report, the original groups of hamsters were replaced and they are currently being exposed. The exposure and postexposure observation schedule for each species is as follows:

Species	Start Exposures	Terminate Exposures	Terminate Observations
Dogs	8 March 1977	7 March 1978	7 March 1985
Mice	8 March 1977	7 March 1978	7 March 1979
Rats	14 April 1977	13 April 1978	13 October 1979
Hamsters	15 June 1977	14 June 1978	14 June 1979

Methods and Materials

Monomethylhydrazine for use in this experiment was prepared by Olin Corporation. The batch of MMH was purified as much as possible by bubbling nitrogen gas through it to drive off more

volatile contaminants. The batch of 5 liters volume was delivered in two packages and was then repackaged at THRU in 100 ml units under nitrogen to prevent oxidative changes. This repackaging was necessary to minimize oxidative degradation during use and also to reduce occupational health and safety hazards.

Liquid MMH for use as contaminant was analyzed for total MMH content by titration with an iodine solution. The impurities were separated by gas chromatography (GC) and identified by mass spectrometry (MS). MMH and the impurities were also quantitated by GC peak area calculation. The details of the methods used for MMH purity analysis may be found in the last annual report.

For clarity and better understanding of this report, the chamber assignments and concentrations are shown in Table 1.

TABLE 1. CHAMBER ASSIGNMENTS AND CONCENTRATIONS FOR INHALATION EXPOSURES TO MONOMETHYLHYDRAZINE

MMH Conc., ppm:	5.0	2.0	2.0	0.2	0.2	0.02	Unexposed
Chamber Number:	5	1	2	3	4	8	Controls
Species and Sex							
Rats Male	100	-	100	100	-	100	150
Female	100	-	100	100	-	100	150
Mice Female	-	400	-	-	400	400	400
Hamsters Male	200	200	0	0	200	0	200
Dogs Male	-	-	4	4	-	-	4
Female	-	-	4	4	-	-	4

Experimental Results

Currently the dogs, mice and rats are in the postexposure observation phase of the study while the hamsters are still being exposed. The postexposure animals are housed at the Vivarium with the rodents being maintained in the laminar airflow facilities. Mortality data for the rodents are shown in Table 2.

TABLE 2. MORTALITY IN RODENTS EXPOSED TO MMH

Species, Sex	Exposure Time, Weeks	Controls	0.02 ppm	0.2 ppm	2.0 ppm	5.0 ppm
Mice, ♂	52	44/400	63/400	51/400	39/400	-
Rats, ♂	52	6/150	1/100	3/100	10/100	10/100
Rats, ♀	52	4/150	2/100	4/100	7/100	20/100
Hamsters, ♂	43	13/200	-	16/200	34/200	49/200

The growth of male rats showed a dose response to MMH exposure throughout the entire study. This was particularly evident at the 5 and 2 ppm levels (Figure 1) where a large depression in mean body weight gain was demonstrated. The effects were much less dramatic at the 0.2 and 0.02 ppm levels but they were statistically different from the controls throughout the entire exposure period. The mean weights of the female rats (Figure 2) were more sporadic in nature; however, the two high level exposure groups remained significantly below the control group for the duration of the exposure.

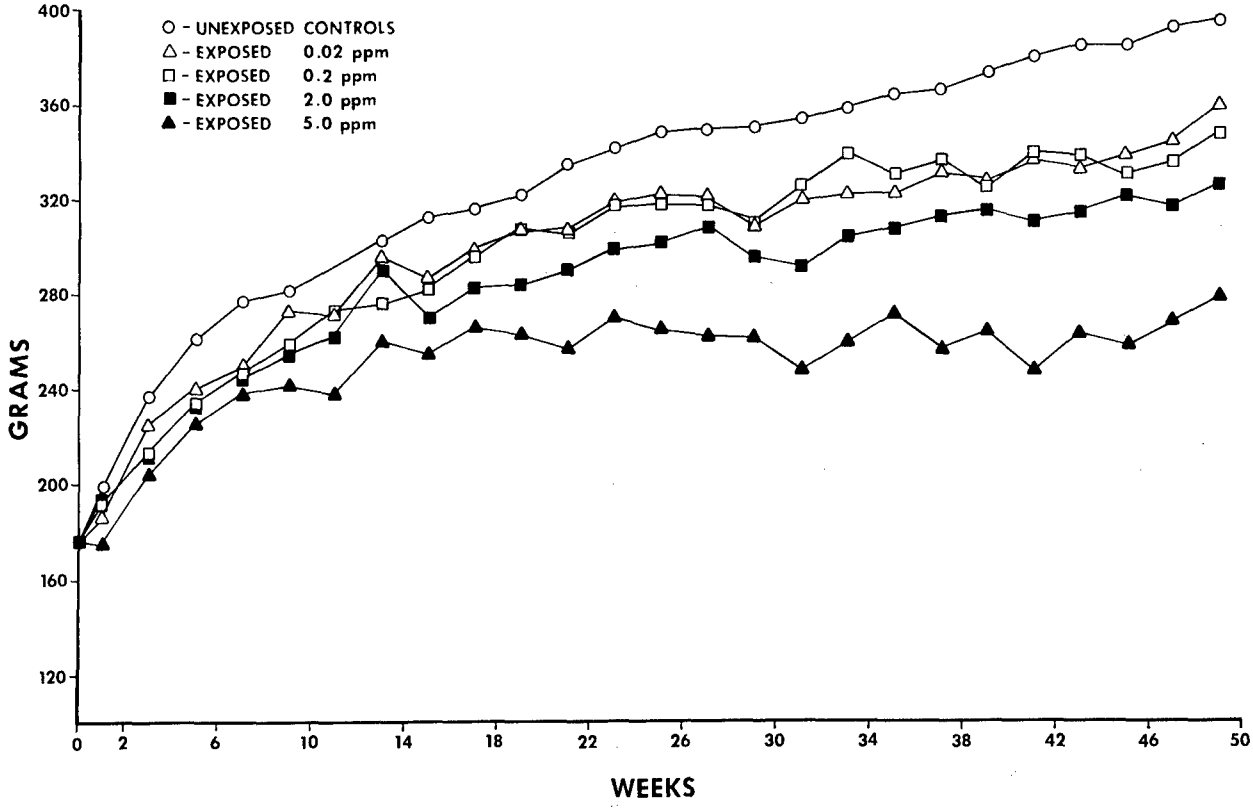


Figure 1. The effect of chronic inhalation exposure to MMH on the growth rate of male rats.

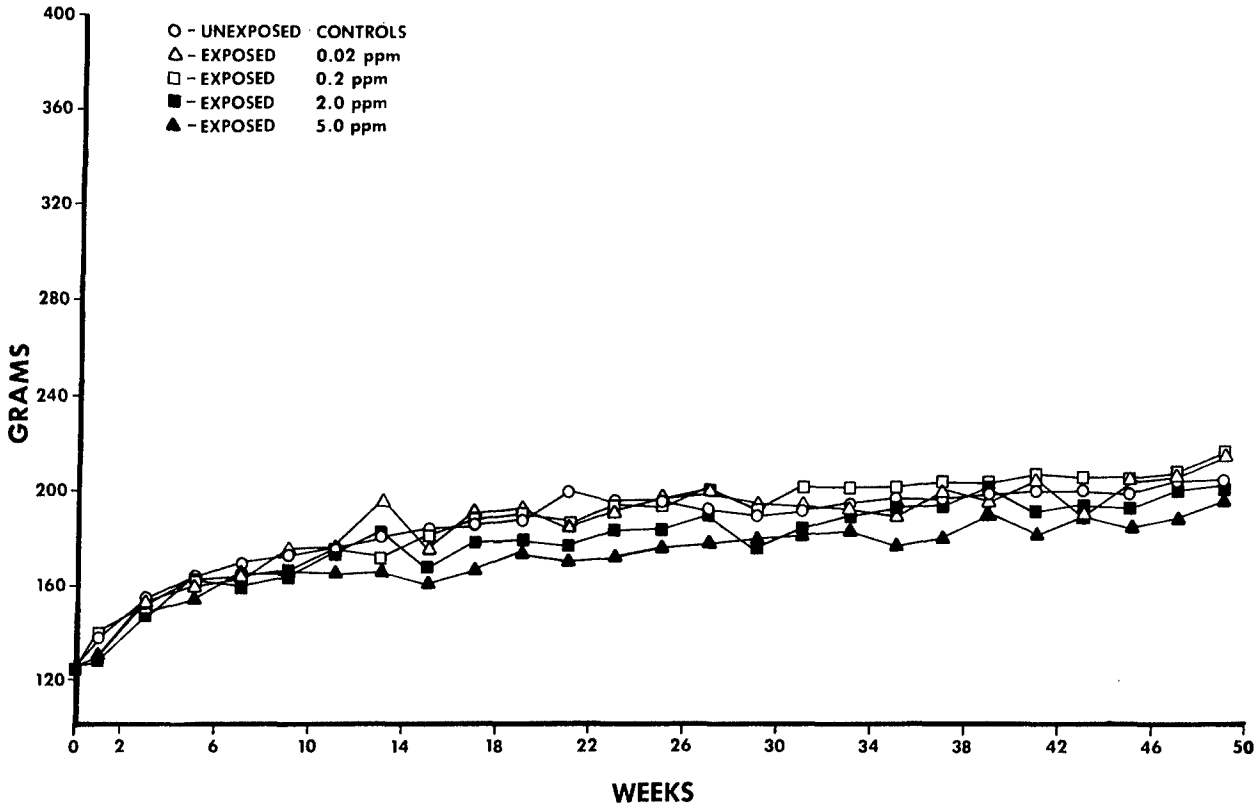


Figure 2. The effect of chronic inhalation exposure to MMH on growth of female rats.

The mean body weights of the 5 ppm exposure group of hamsters (Figure 3) showed a definite depression when compared to their respective 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. After the first week of exposure, the 5 ppm MMH exposure group was statistically different than the controls. Except for the 13th exposure week weighing the 2 ppm exposure group was also statistically smaller than controls in mean body weight and the 0.2 ppm MMH exposed hamster group was statistically lower than controls at 13 of the 20 weighing periods.

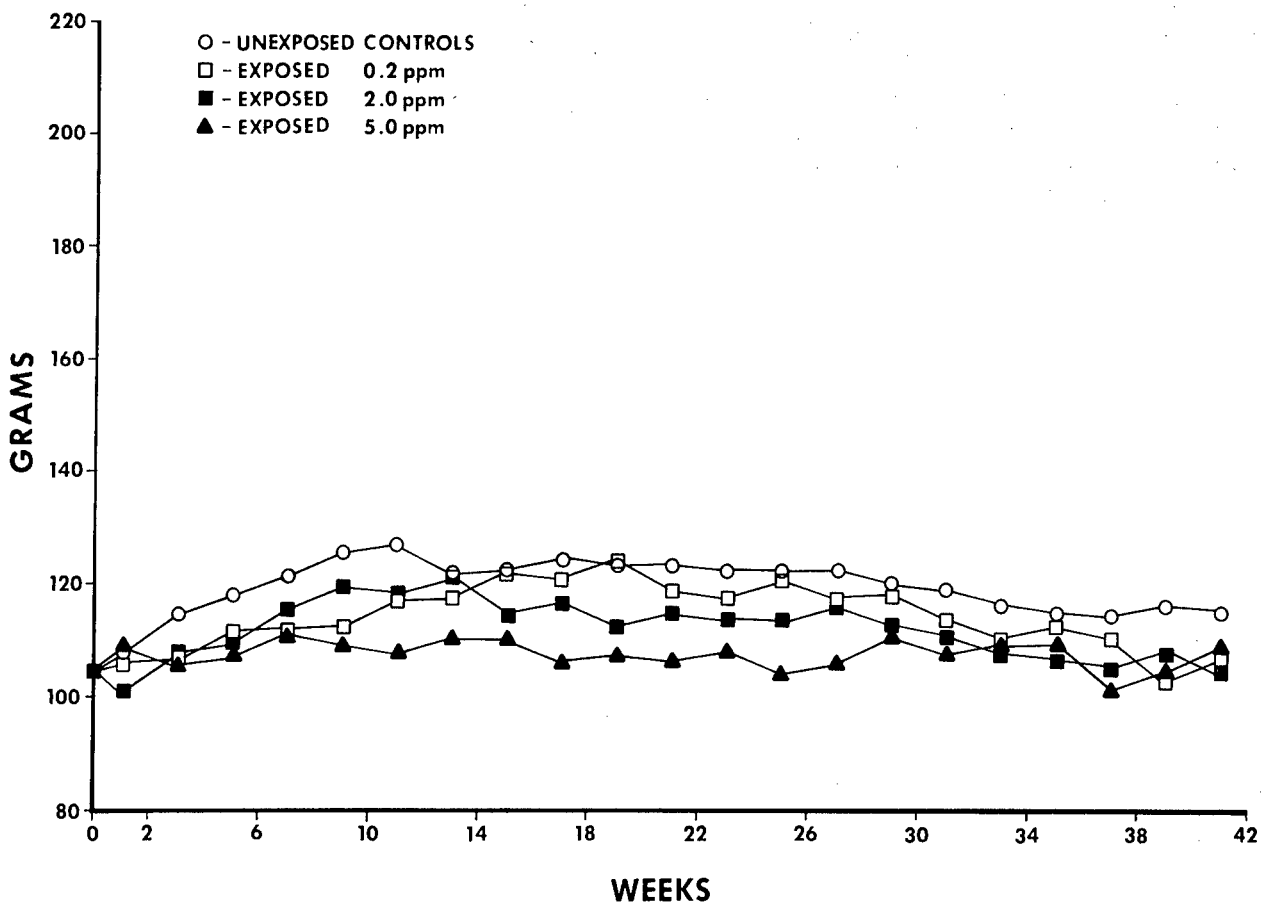


Figure 3. The effect of chronic inhalation exposure to MMH on the growth of male Golden Syrian hamsters.

Blood samples were drawn from dogs at biweekly intervals and determinations made for the following battery of clinical tests:

HCT
RBC
WBC
HGB
Alkaline Phosphatase
SGPT
Bilirubin
Glucose
Triglycerides
Iron
Sedimentation Rate

Methemoglobin determinations were made on each group of dogs at quarterly intervals during exposure (3, 6, 9 and 12 months).

The mean red blood cell, hemoglobin, hematocrit and SGPT values are shown graphically in Figures 4 through 7. The hemolytic effects seen in the exposed dog groups are similar to those reported in a previous long-term MMH inhalation study (MacEwen and Vernot, 1971). Statistical analyses of these hematology measurements revealed a significant difference between the test groups and the controls at almost every sampling point.

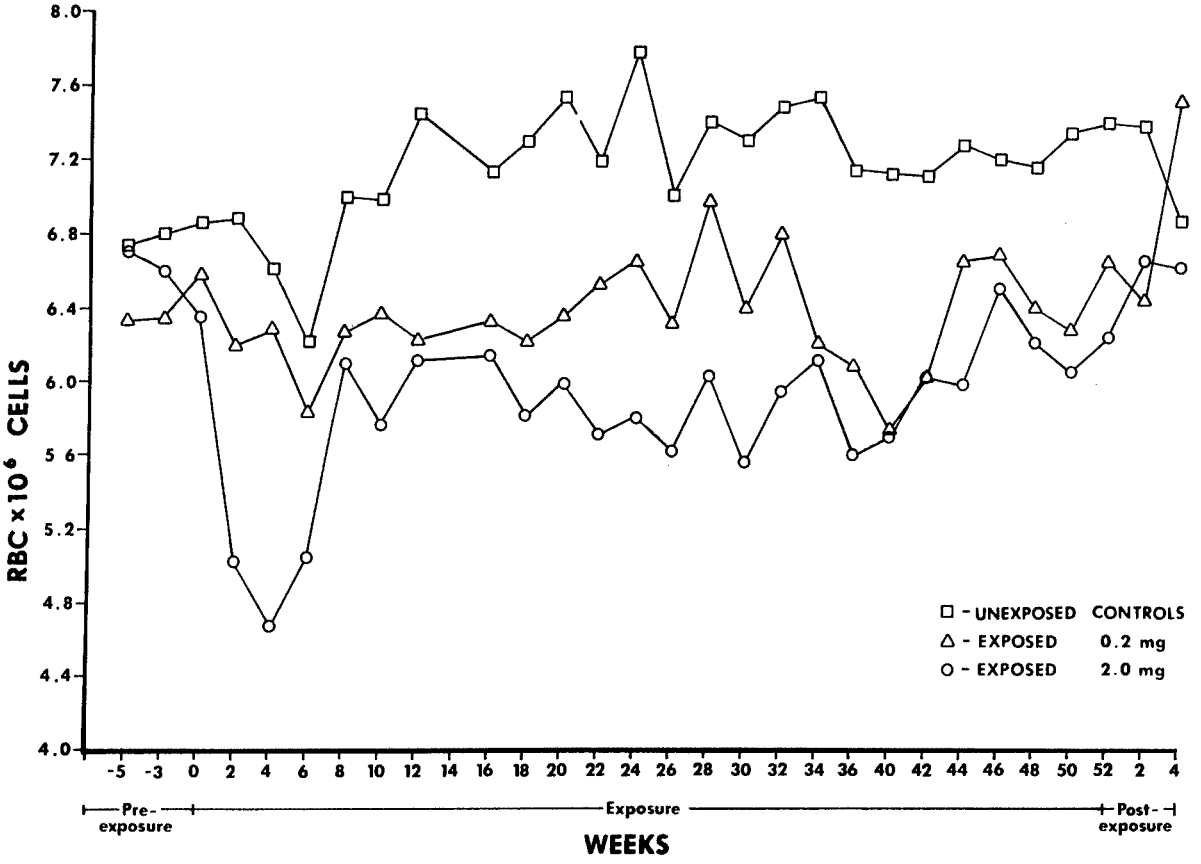


Figure 4. The effect of chronic inhalation exposure to MMH on beagle dog red blood cell counts.

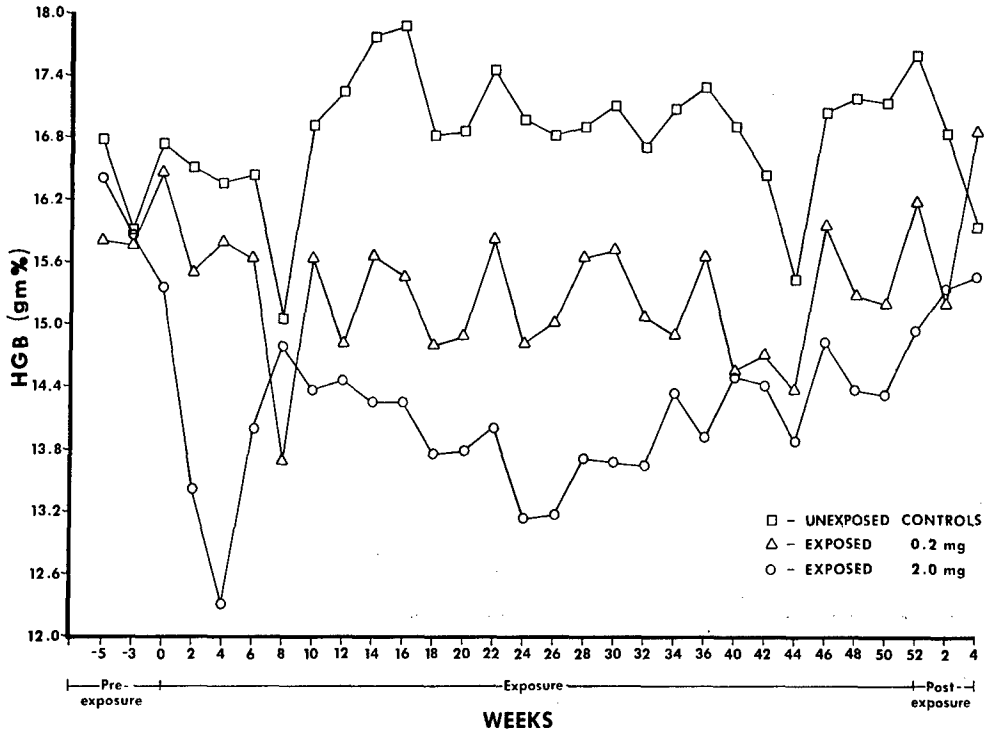


Figure 5. The effect of chronic inhalation exposure to MMH on beagle dog hemoglobin levels.

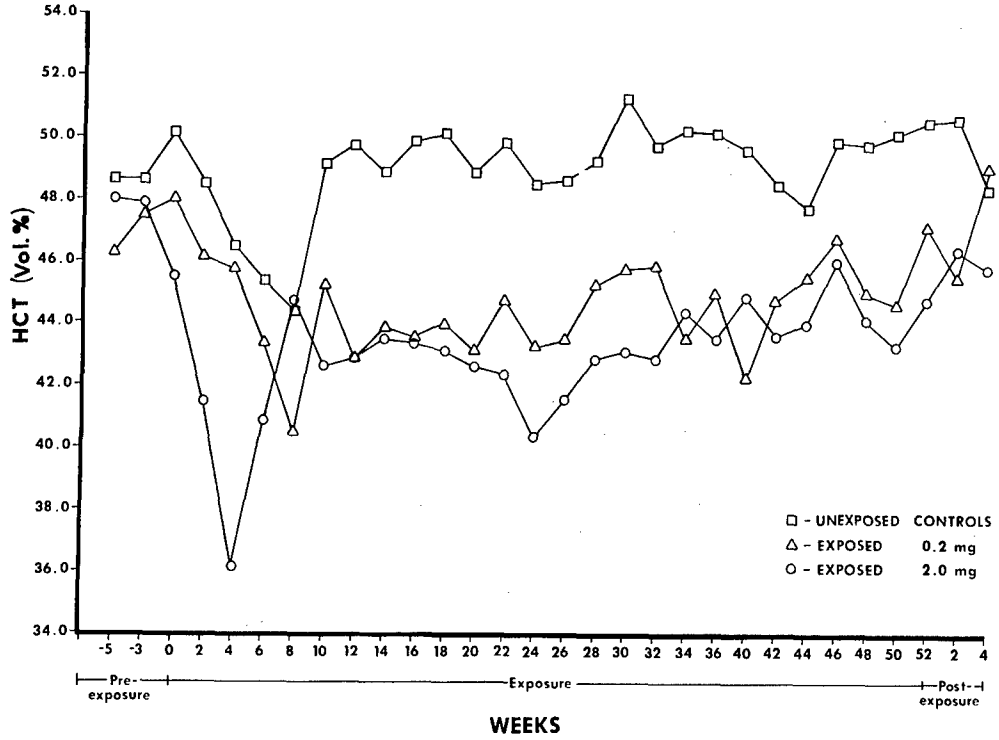


Figure 6. The effect of chronic inhalation exposure to MMH on beagle dog hematocrit levels.

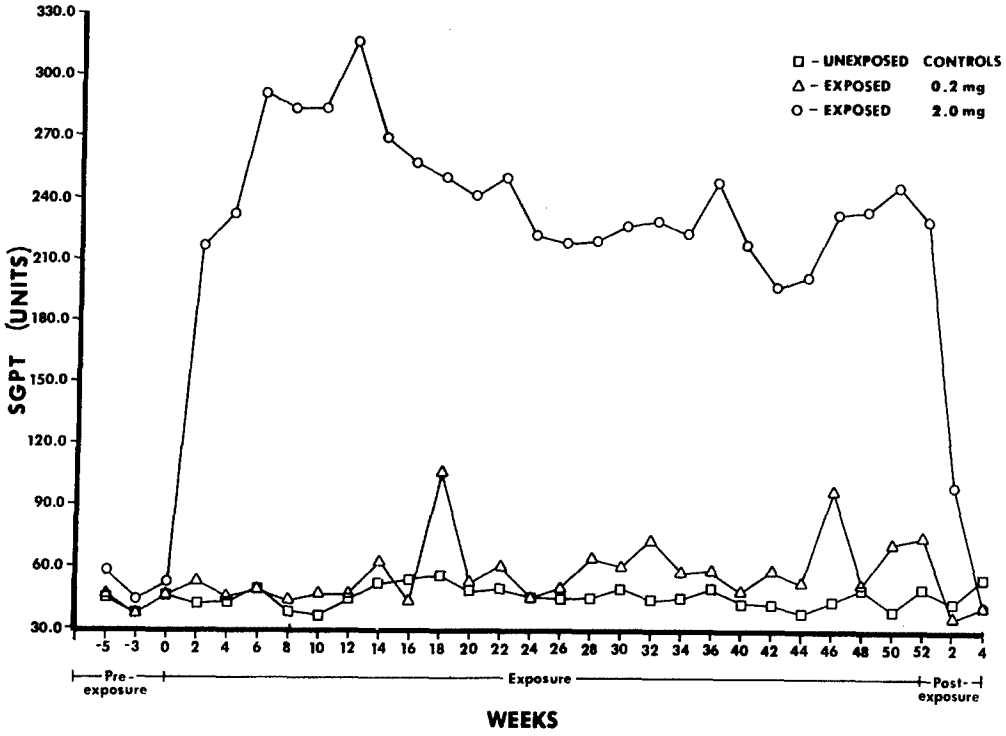


Figure 7. The effect of chronic inhalation exposure to MMH on SGPT values in beagle dogs.

The SGPT values increased significantly in the 2 ppm group at the first biweekly sampling and continued to increase for 12 weeks. This was followed by a slight decline and the values remained in the 200 to 250 unit range for the remainder of the exposure period. Twice during the study, at 18 and 46 weeks, one dog exposed to the 0.2 ppm MMH concentration level exhibited an extremely high SGPT value that returned to a normal level at the next sampling period. Concurrent with the high SGPT values and also indicative of liver stress, was an increase in the alkaline phosphatase and bilirubin values for the 2 ppm MMH exposed dogs.

A statistically significant difference was seen when the methemoglobin values determined for the 2 ppm exposed dogs were compared to the values of 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 percent. Dogs exposed to 0.2 ppm MMH were not different from the unexposed control dogs that had mean methemoglobin values ranging from 0.79 to 0.83%. Although the increase in methemoglobin in the 2 ppm MMH exposed dogs is a real treatment effect it is not believed to be clinically important.

An additional liver function test was performed on all dogs at exposure termination and at 2 and 4 weeks postexposure. Bromsulphalein (BSP) measured in the blood of the 2.0 ppm exposed dogs, 10 minutes after a 10 mg/kg injection, showed a significant retention at exposure termination (Table 3) but by the 2 week postexposure examination the BSP measurements had returned to unexposed control values.

After four weeks postexposure, all blood parameters in exposed dogs had returned to the ranges of unexposed control values. The return of SGPT values of the 2 ppm exposed dogs to normal levels by four weeks postexposure (Table 3) was surprising. This was a much more rapid recovery than seen in dogs exposed to 5 ppm UDMH that required more than 11 weeks to return to control levels as was described in the last annual report. The return of BSP and SGPT measurements in MMH exposed dogs to unexposed control levels indicated that the chronic liver injury was reversible.

TABLE 3. SGPT AND BSP RETENTION IN BEAGLE DOGS AFTER 12 MONTHS CHRONIC INHALATION EXPOSURE TO MMH

(N=8)

Sample Period	Mean SGPT Values (DuPont Int. Units/Liter) Controls	0.2 ppm	2.0 ppm
52 Weeks Exposed	50.0	75.0	228.3*
2 Weeks Postexposure	43.3	36.0	99.5*
4 Weeks Postexposure	55.0	41.5	41.8
	Percent BSP Retention		
52 Weeks Exposed	14.9	20.3	34.5*
2 Weeks Postexposure	22.9	14.3	23.5
4 Weeks Postexposure	15.1	16.2	23.9

*Statistically different from the control value at the 0.01 level of significance.

Although a clear cut dose response in rodent deaths was not demonstrated, rats and hamsters exposed to 5 ppm had a much greater mortality rate than unexposed controls and the groups exposed to lower MMH concentrations. Mice were not exposed to that concentration because of high mortality previously reported (MacEwen and Haun, 1971).

The concentrations of MMH were maintained in each individual chamber in a satisfactory manner throughout the exposure phase of the study. The active concentrations were held within 10% of the nominal air concentrations selected for evaluation. Monthly mean MMH exposure levels are shown in Table 4. The mean exposure concentration for the entire study is also shown.

TABLE 4. MONTHLY MEAN CONCENTRATIONS OF MEASURED MMH EXPOSURE IN CHAMBERS

(ppm)

Chamber No.	1	2	3	4	5	8
1977						
March	1.98	1.98	.21	.20	5.01	.022
April	1.92	1.89	.19	.21	4.95	.019
May	2.02	1.97	.21	.20	4.95	.022
June	2.01	1.97	.19	.20	4.96	.020
July	2.09	2.09	.20	.20	4.96	.019
August	2.03	1.97	.19	.19	4.96	.019
September	2.11	1.98	.19	.19	5.05	.019
October	2.06	2.04	.21	.20	4.99	.020
November	2.09	2.03	.22	.20	5.08	.020
December	2.00	2.05	.20	.21	5.10	.020
1978						
January	2.07	2.00	.21	.22	5.07	.021
February	2.08	2.02	.20	.19	5.04	.021
March	2.08	2.02	.19	.21	5.03	.021
12-Month Mean Chamber Concentration	2.04	1.99	.20	.20	5.01	.020

The animals of each species surviving the chronic inhalation exposure regime to monomethylhydrazine will be held for postexposure observation and will be necropsied at death for histologic examination of approximately 33 tissues for oncogenic changes. When the mortality in any one group of each species reaches 90% the remaining animals will be terminated and histopathologic examinations conducted. The study is continuing.

A Study of the Oncogenic Potential of Inhaled Hydrazine after Chronic Low Level Exposure

Previous annual reports (MacEwen and Vernot, 1975, 1976 and 1977) provide the rationale, purpose and details of the experimental protocol for the conduct of chronic inhalation studies to determine the oncogenic potential of hydrazine in dogs, rats, mice and hamsters. The latter two reports contain experimental data including mortality, body weight measurements, and clinical chemistry results for dogs tested during the 12 months of hydrazine exposure and through many months of the postexposure phase of the study.

The animal exposures were started during the summer and winter of 1975 and completed one year later. Not all experimental groups were in phase with respect to the starting dates of the generation of hydrazine concentration in the exposure chambers. The reasons for this were explained in previous annual reports and will not be repeated here.

Experimental Results

At the present time, except for the dogs, all animals are dead. They either died or were sacrificed due to moribund condition during the postexposure phase of the study or were sacrificed at selected time intervals culminating with the necropsy of survivors of the 1 ppm exposed mouse group and their controls in March of 1978.

Uncertainty in regard to the survival rate of hydrazine exposed rats and their controls prompted an interim sacrifice. Twenty rats of each sex from each exposure group and 30 of each sex from the control group were necropsied at 12 months postexposure (24 months in the study). For the same reason, 40 mice from the 0.05 ppm and 0.25 ppm hydrazine exposed groups and 40 of their control group were sacrificed at 15 months postexposure (27 months in the study). Remaining groups of rats, mice and hamsters were maintained until 90% mortality was reached per species at which time concluding sacrifices were made. The mouse group exposed to 1 ppm and their controls were treated separately in this regard since they were on a different time schedule from the other mice used in the study.

As of 14 July 1978, all dogs used in this study will be 24 months postexposure (36 months on test). The dogs were transferred from Brooks AFB to the Vivarium facility of the AMRL Veterinary Division on 4 April 1978 where they continue to receive quarterly comprehensive physical examinations and periodic clinical chemistry determinations.

Mentioned in a previous annual report (MacEwen and Vernot, 1977) was the observation of rectal bleeding in one dog in the 0.25 ppm exposure group at 4 months postexposure. A biopsy was taken from a 3 x 2 cm growth on the ventral surface of the rectum.

Histologic examination revealed a low grade adenocarcinoma. Subsequent examination resulted in a change in diagnosis to rectal polyps. All dogs are currently in good health and clinical chemistry determinations made on these animals fall within normal levels.

The body weights of male and female rats are shown in Figures 8 and 9, respectively, for the entire study. In general, growth was reduced in all hydrazine exposed rats during exposure but the effect is most significant in the rats exposed to the 5 ppm concentration. The differences between exposed and control animals were maintained at a relatively constant level during the first 12 months postexposure but became less significant during months 25 to 30 of the exposure as the weight decline of the aging animals was observed.

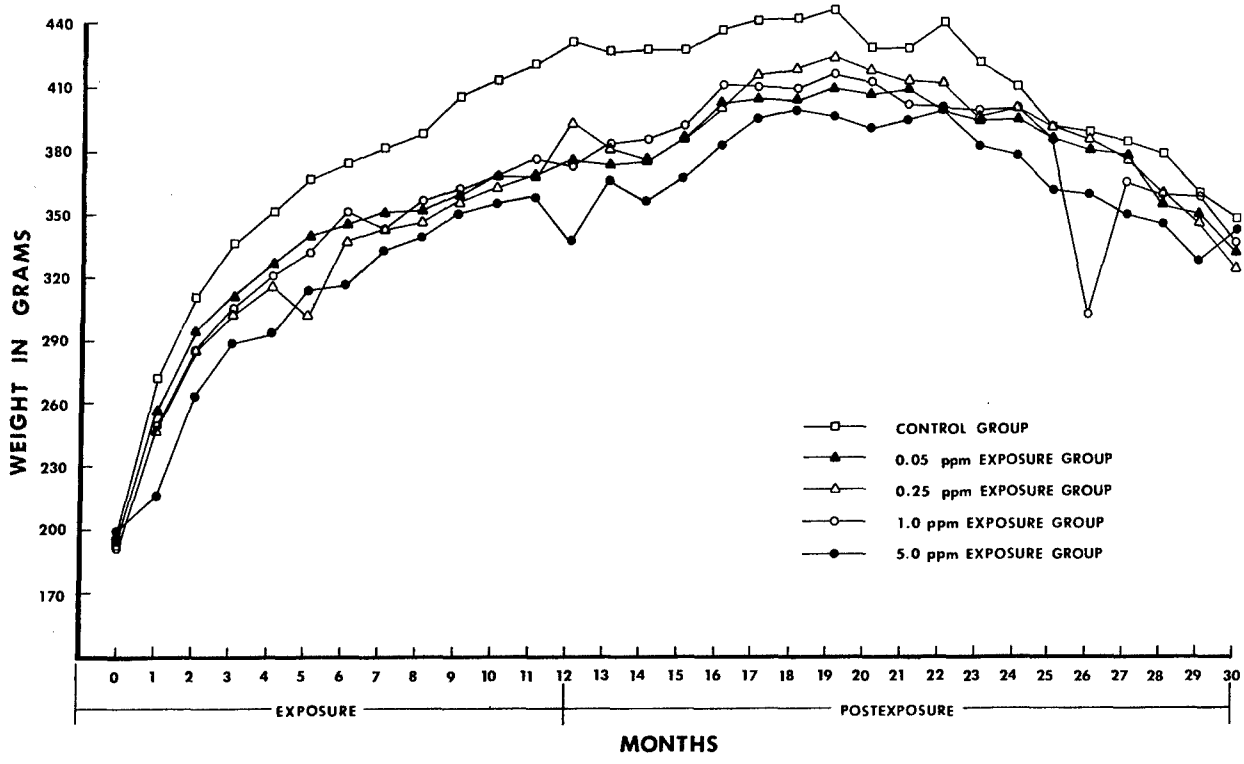


Figure 8. The effect of chronic inhalation exposure to hydrazine on the growth of male Fischer 344 rats.

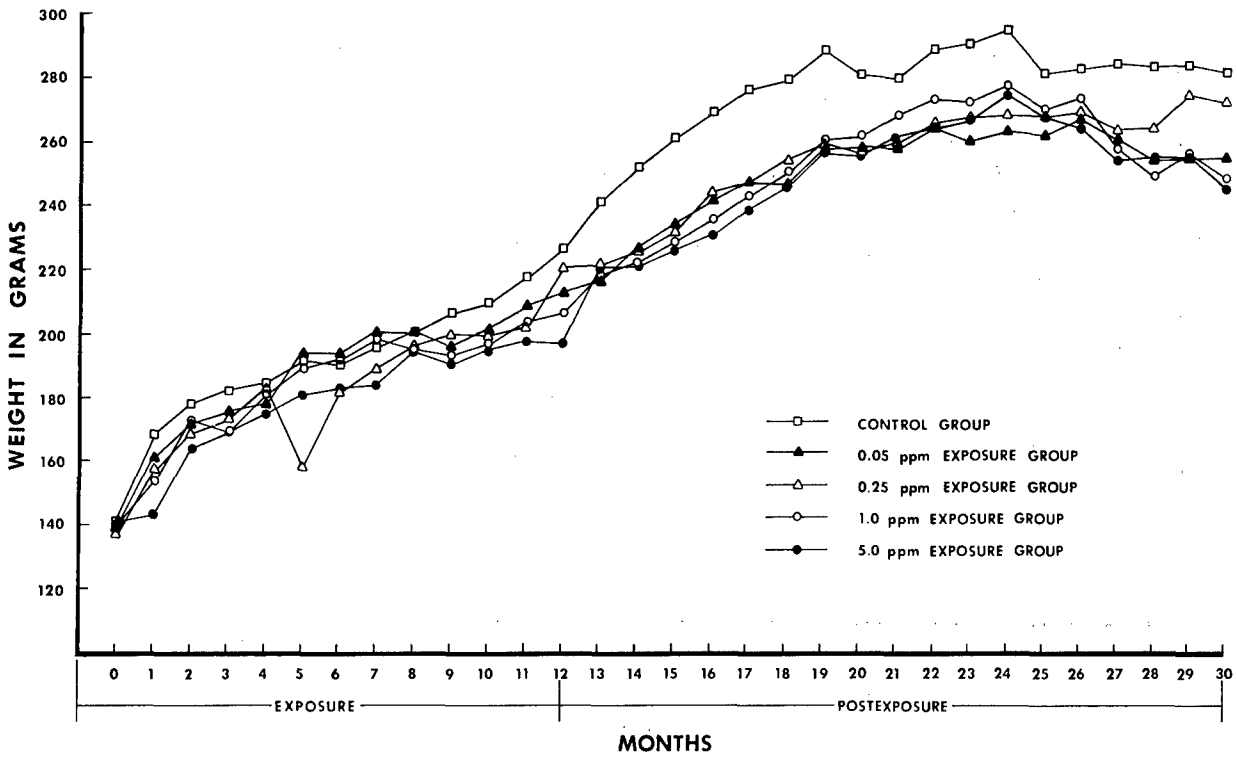


Figure 9. The effect of chronic inhalation exposure to hydrazine on growth of female Fischer 344 rats.

Hamster body weights shown in Figure 10 illustrate a cyclic phenomenon for which we have no explanation. This experience was common to all exposed groups as well as the unexposed control hamsters and was relatively severe in all groups. In the final months of observation of the hamsters only the 5 ppm hydrazine exposed group continued to show a significant weight difference from controls.

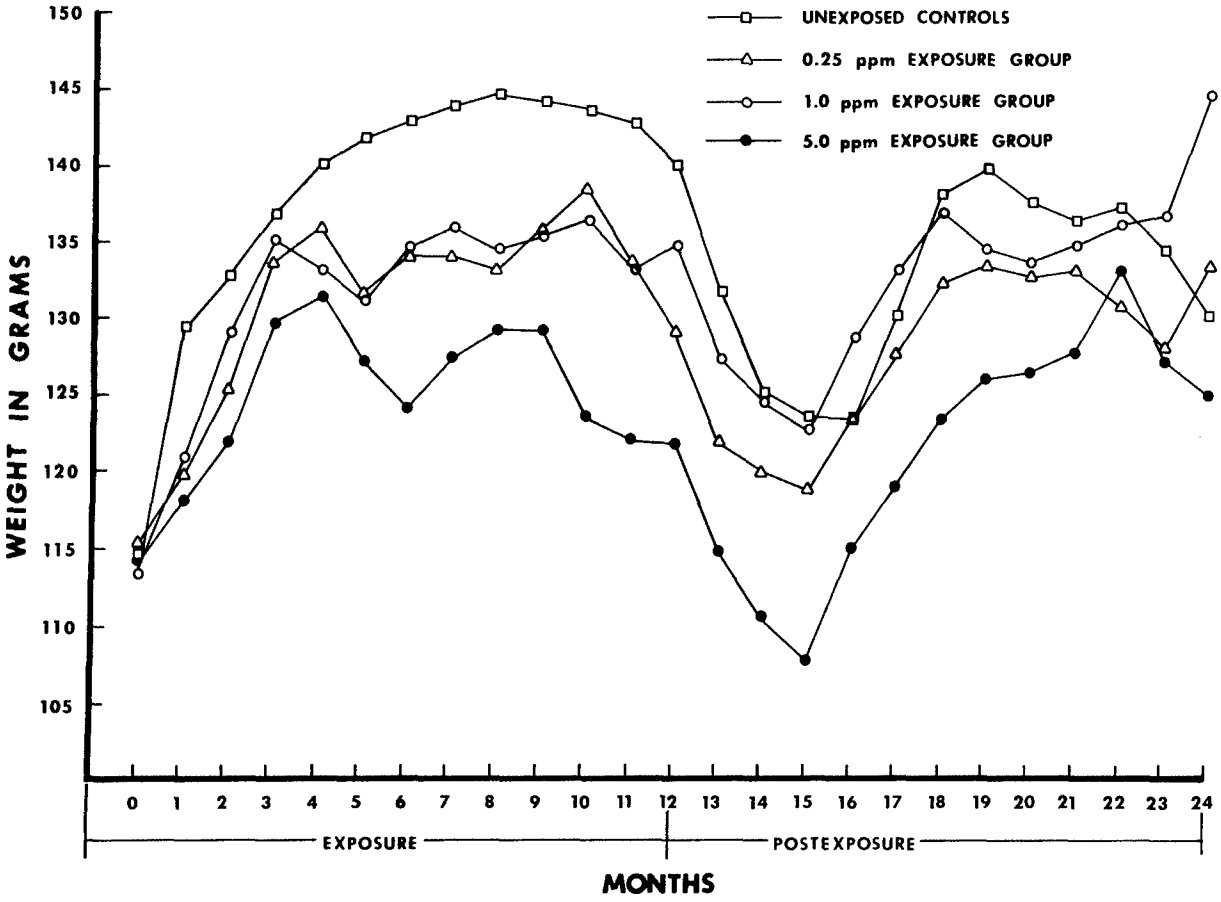


Figure 10. The effect of chronic inhalation exposure to hydrazine on the growth of male Golden Syrian hamsters.

Mean body weights of mice are shown in Table 5 along with their survival tally. Body weights of exposed mice were unaffected by hydrazine exposure when compared with their control groups.

Histopathology information is not available at this time, although all dead animals have received preliminary gross examination to determine cause of death. Paraffin embedded tissues from mice and rats have been sent to Huntingdon Research Center, England for final processing and definitive examination while hamster tissues were sent to the USAF School of Aerospace Medicine, Veterinary Sciences Division, Brooks Air Force Base, Texas. The study is continuing and no conclusion or comment on the oncogenic potential of hydrazine can be made at this time.

TABLE 5. MEAN BODY WEIGHTS AND SURVIVAL TALLY FOR MICE EXPOSED TO HYDRAZINE FOR ONE YEAR

Exposure Time Months	Controls for 0.05, 0.25 ppm		0.05 ppm		0.25 ppm		Controls for 1.0 ppm		1.0 ppm	
	No.	Weight,g	No.	Weight,g	No.	Weight,g	No.	Weight,g	No.	Weight,g
Pre	400	16.2	400	18.0	400	16.4	400	17.0	400	16.9
1	397	20.0	396	21.5	399	20.5	400	19.3	395	20.2
2	397	21.6	396	22.2	395	22.0	399	20.7	394	22.0
3	397	22.3	396	23.2	394	22.6	399	21.1	393	22.7
4	397	22.5	296	23.6	393	23.4	399	22.0	391	23.4
5	397	22.5	396	24.1	390	24.0	398	22.3	391	24.1
6	397	23.1	395	24.2	384	24.5	397	22.5	390	24.5
7	397	23.6	393	24.2	384	25.1	396	23.6	382	25.4
8	397	24.0	392	25.3	384	25.5	394	25.6	380	26.0
9	394	23.8	391	25.3	383	26.5	393	25.3	379	26.1
10	392	24.1	390	25.6	383	25.9	391	24.9	376	25.9
11	390	24.6	379	26.9	377	27.0	388	25.2	373	27.2
12	390	25.1	376	27.0	373	27.6	Not Weighed		371	26.8
Months Post										
1	387	26.5	372	26.4	373	26.5	385	25.2	368	26.0
2	382	27.5	369	26.9	370	27.5	375	25.5	350	26.6
3	377	27.4	364	27.0	367	27.2	372	26.6	349	27.4
4	374	27.2	344	26.6	364	26.8	367	26.4	345	26.8
5	369	27.0	335	26.2	358	27.0	358	27.5	342	27.8
6	350	27.6	319	26.0	351	26.1	337	27.6	320	27.3
7	344	28.7	312	26.7	342	27.1	313	28.1	309	27.7
8	337	28.4	295	27.0	330	27.2	293	29.1	309	27.7
9	324	29.1	285	27.8	310	27.7	284	29.5	270	28.3
10	298	28.5	249	27.4	272	27.7	264	30.0	248	28.3
11	272	29.0	233	27.3	252	27.7	242	27.6	226	27.3
12	249	28.9	200	28.0	232	27.8	218	28.7	201	27.5
13	227	28.8	186	27.8	210	27.7	184	27.3	173	26.7
14	210	29.4	152	28.1	179	27.5	153	27.1	133	26.3
15	102	26.2	78	27.0	67	26.1	116	26.1	83	25.1
16	100	27.0	51	27.5	55	27.3	83	27.3	55	25.9

1,2-Dimethylhydrazine Dihydrochloride as a Positive Tumorigenic Control for Experimental Animal Species used in Hydrazine Oncogenic Studies

1,2-Dimethylhydrazine dihydrochloride (SDMH dihydrochloride) has been shown to be a tumorigenic agent to rodents when given by oral or subcutaneous administration. Toth and Wilson (1971) gave SDMH dihydrochloride in the drinking water of mice for their lifetimes. Ninety-two percent of male and 98% of females developed angiosarcomas, mainly localized in the muscle, liver and pararenal tissues after median latent periods of 42 and 45 weeks. In addition, 24% of males and 44% of the females had lung adenomas after similar latent periods. Oral doses to rats (Druckrey et al., 1967; Druckrey, 1970) produced adenocarcinomas of the colon and rectum.

Hawks et al. (1971/1972) report subcutaneous injections of SDMH dihydrochloride to mice at doses of 15 mg/kg per week for a total of 22 weeks caused development of tumors in the descending colon and rectum in 52 of 58 surviving mice. Weekly subcutaneous injections of 20 mg/kg for 2 to 24 weeks in 34 CF_1 mice were reported by Thurnherr et al. (1973) to have produced multiple carcinomas of the colon in 90% of the animals. The earliest tumor was found in a mouse that died at 135 days.

Oncogenic studies conducted in this laboratory on the hydrazine compounds utilized specific strains of various rodent species. These were female C57Bl/6 mice, male and female Fischer 344 rats and male Golden Syrian hamsters.

This study was designed to determine the tumorigenic potential of the above mentioned animals when treated with a known oncogenic agent and to serve as a positive control for the current monomethylhydrazine study. The objective is to be better able to evaluate the tumorigenic effects found in these specific animal strains after long-term inhalation studies with the hydrazine compounds.

Materials and Methods

The SDMH dihydrochloride used in this study was procured from Curtin Matheson, Incorporated. A total of 100 grams was received in 5 gram units. The compound was prepared for injection by making a solution in distilled water. A fresh solution was prepared weekly, prior to the dosing regimen. Prior to injection, the pH of the solution was adjusted to 6.5 with sodium hydroxide.

Groups of 25 male and 25 female rats and 50 female mice received weekly subcutaneous injections of 20 mg/kg SDMH dihydrochloride. Groups of 50 each of male hamsters received dose levels of 10 and 5 mg/kg of the test compound. Similar groups of control animals were given weekly injections of distilled water. All species were scheduled to receive a maximum of 52 injections after which the survivors were to be held for one year of observation or until cumulative mortality exceeded 90%.

The rodents used in this study are listed below:

Species, Sex	Strain	Source
Rats, ♂ and ♀	Fischer 344	Chas. River Breeding Labs.
Mice, ♀	C57Bl/6	Jackson Laboratory
Hamsters, ♂	Golden Syrian	Chas. River Breeding Labs.

The animals were fed ad libitum and had a complete cage change twice per week. Assignment of the animals to groups was done using the THRU Computer Program RANDUM which utilizes the FORTRAN library subroutine RANF(X). The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," ILAR, National Research Council.

All animals were observed daily for general appearance and health status. Rats, hamsters and mice were weighed weekly, just prior to dosing; however, these weights were not recorded. A record of animal weights was kept utilizing the same weighing schedule as in the MMH dome study, i.e., biweekly for rats and hamsters and group means monthly for mice.

All animals that died or were sacrificed were necropsied. When cannibalism or autolysis precluded the examination, a completed record containing this information was filed. The necropsy was defined as external examination, including body orifices, and examination and fixation of all of the following tissues for histopathologic examination:

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

Experimental Results

Subsequent to beginning the repeated subcutaneous injections, a rangefinding LD_{50} was done for each species. Groups of the three species of animals were injected with various concentrations of the 1,2 SDMH dihydrochloride. The dosing concentrations were calculated on the weight of the SDMH alone. The pH of the solutions was not buffered or neutralized prior to injection. The pH of the solutions ranged between 0.48 and 1.0.

The concentration and mortality ratios for each species are shown below:

Mice, Female, C57Bl/6

Dose Level (mg/kg)	Mortality Ratio (N=10)
25	10/10
20	9/10
15	1/20
10	0/10

LD_{50} (95% C.L.) = 17.2 (15.6-19.1) mg/kg

Hamsters, Male, Golden Syrian

Dose Level (mg/kg)	Mortality Ratio (N=10)
30	10/10
25	5/10
20	4/10
15	0/10

LD_{50} (95% C.L.) = 22.5 (22.0-25.2) mg/kg

Rats, Male, Fischer 344

Dose Level (mg/kg)	Mortality Ratio (N=5)
100	5/5
80	4/5
60	3/5
40	1/5

LD_{50} (95% C.L.) = 54.8 (31.7-71.3) mg/kg

Rats, Female, Fischer 344

Dose Level (mg/kg)	Mortality Ratio (N=5)
80	5/5
60	3/5
40	0/5

LD_{50} (95% C.L.) = 56.6 (41.9-76.4) mg/kg.

The LD_{50} and 95% confidence limits for each species based on total dose of the compound are shown below:

Mice:	38.2 (34.6 - 42.4) mg/kg
Hamsters:	50.0 (44.4 - 56.0) mg/kg
Rats, male:	121.8 (70.4 - 158.4) mg/kg
Rats, female:	125.8 (93.1 - 169.8) mg/kg

The hamsters did not differ significantly from the mice in their response to the toxic effects of this compound. The rats, however, were more resistant than the other two species but no difference was noted between the sexes.

The original protocol for the repeated subcutaneous injections specified doses of 20 mg/kg calculated on the weight of SDMH alone. This dose proved to be too toxic, resulting in death of mice and debilitation of the other species. New groups of each species were then started using doses of 20 mg/kg SDMH dihydrochloride.

This dose produced a chronic toxicity response in the hamsters after 10 to 12 weekly injections. The hamsters began to lose weight and die in large numbers after the 12th injection until only two of the original 50 remained after 15 injections. Histologic examination of these animals showed diffuse hepatic toxic insult with changes as noted: hyalin droplet formation; vacuolation of cytoplasm; swollen hepatocytes and nuclei; nuclear membrane invagination; biliary hyperplasia; bizarre hepatocyte morphology; dilated sinusoids; early hepatocyte necrosis; bile laking and dissociation of hepatic cords. No tumors were found in any of these hamsters. Since the 20 mg/kg dose level proved to be toxic to the hamsters, additional groups of 50 each were started at dose levels of 10 and 5 mg/kg.

Deaths began to occur after 18 injections to male rats. The rate of mortality (Figure 11) increased following the 24th injection week. The first mouse and female rat death occurred after 24 injections. The mortality slope of the female rat group showed a more gradual increase than was seen with the male rats. The mouse mortality curve started very similar to the female rats but showed a sudden rise after 38 weeks. The cumulative mortality of female rats and mice had reached 90% after 44 weeks and therefore they were terminated along with their sham injected controls during May 1978.

Of 25 male rats examined grossly, 13 had lesions associated with the gastrointestinal tract. The most common of these were intussusceptions of the colon. Eight of 13 female rats showed similar lesions upon gross examination.

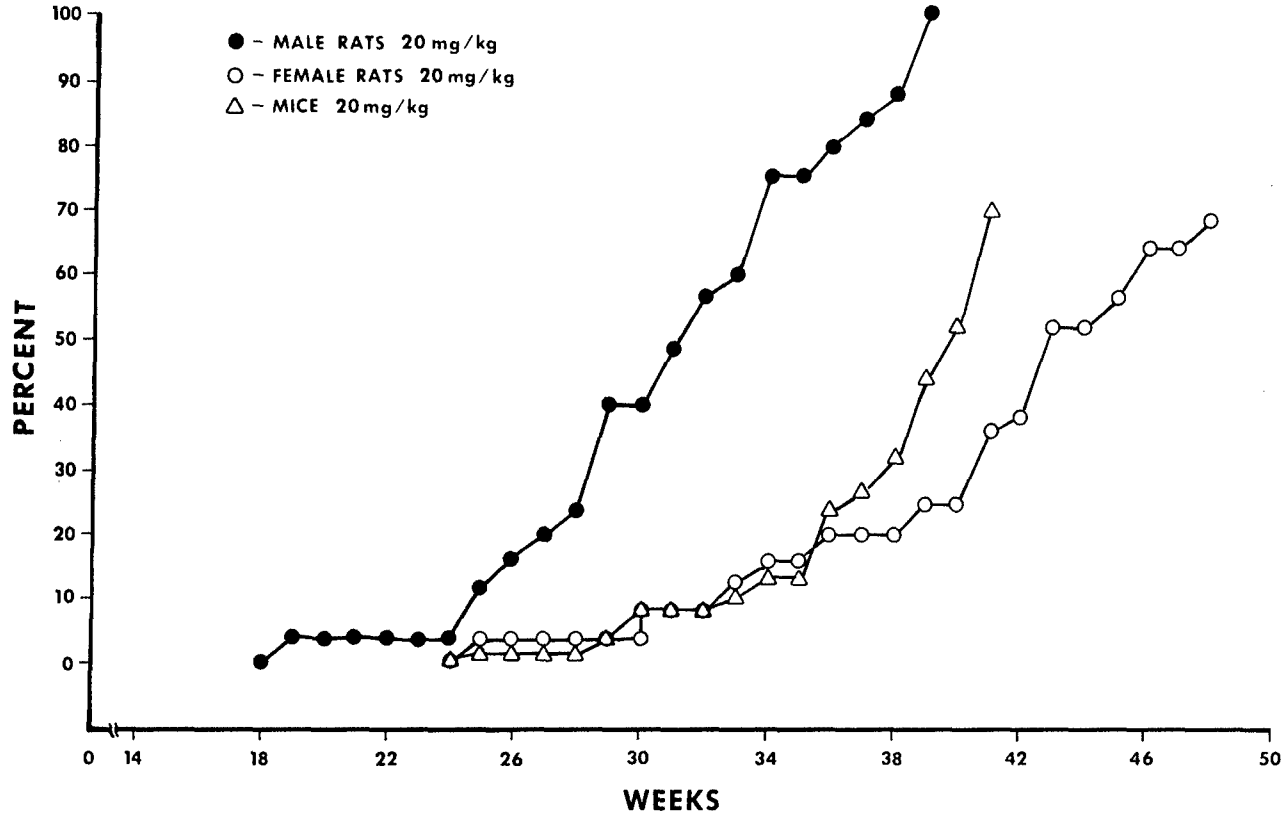


Figure 11. Cumulative mortality in SDMH injected animals.

Gross pathologic examination of the mice has shown many tumors in the gastrointestinal area but intussusceptions of the colon were not as common.

The results of histopathologic examination of rats to date are minimal, however, carcinomas appear to be quite common. Results from five male rats included two mucinous carcinomas of the colon; a duodenum carcinoma; three Zymbal's gland sebaceous adenocarcinomas and a ceruminous carcinoma of the ear.

The hamsters continue on study and will be discussed further in ensuing reports.

A 6-Month Chronic Inhalation Exposure of Animals to UDMH to Determine Its Oncogenic Capacity

Preliminary evidence that hydrazine (N_2H_4) was carcinogenic at concentrations near or at the industrial TLV (MacEwen and Vernot, 1974) led to concern for the oncogenic potential of unsymmetrical dimethylhydrazine (UDMH), another important military chemical which had been reported to be tumorigenic in animals by Roe et al. (1967), Toth (1972, 1973), and Druckrey et al. (1967). This concern resulted in a series of chronic inhalation toxicity experiments conducted to examine the hazard associated with UDMH exposure.

Materials and Methods

A careful review of carcinogenic testing methods was conducted to define what animal species and numbers should be used. Carcinogenic screening procedures generally utilized 100 animals of each species to be tested. To achieve our goal, it was decided that groups of 400 mice, 200 rats and hamsters, and 8 dogs would be exposed to 3 air concentrations of UDMH, hydrazine and MMH at or near the TLV value of 0.5 ppm. Exposures were conducted on a daily industrial exposure basis for 6 months followed by a prolonged observation period for potential cancer induction in each species. The numbers of animals used were based on the maximum numbers for each species that can be exposed in the THRU chambers and were selected to permit a statistically valid number of animals of each species to reach the required age for tumor induction with natural and toxicologic attrition. One large animal species (the dog) was selected because it is the most sensitive animal to other chronic effects of the hydrazines (hemolytic and CNS) and is also the most suitable species for monitoring hematologic and biochemical status during the experimental period. The long term postexposure holding of the dogs with periodic testing of hepatic function will also provide the data base for determining the capability of UDMH to produce delayed liver damage.

Animals used in this study, in the numbers mentioned previously, consisted of female C57 black/6 mice obtained from Jackson Laboratories, male CDF (Fischer 344 derived) albino rats from Charles River, male Engle Golden Syrian hamsters, and beagle dogs, 4 male and 4 female per group. A separate set of control animals was provided for the 0.05 ppm test since it was not started at the same time as 5 ppm and 0.5 ppm experiments. Two chambers were used for each UDMH air concentration. Each pair of chambers contained as few species as possible to minimize risk of cross infection. Dogs and rats were housed in one chamber and mice and hamsters in the companion chamber. All control animals were maintained in animal holding facilities.

The exposure chambers were operated with nominal airflows of 35 cfm at a slightly reduced pressure of 725 mm Hg to prevent leakage of UDMH into the laboratory. Exposures were conducted on a 6 hour/day, 5 day/week schedule. No exposures were made on weekends and holidays.

The chamber concentrations of UDMH were generated and continuously monitored with apparatus and instrumentation essentially the same as used for chronic hydrazine and MMH studies in the past. Details may be found in previous technical reports (Haun, 1970; MacEwen and Haun, 1971). Minor modifications in the air sampling system to increase the frequency of observations were described by MacEwen and Vernot (1975).

All test animals were observed hourly during exposure and nonexposure periods for signs of UDMH intoxication and mortality. Gross and histopathologic examinations were made on all dead animals. Rats, hamsters and dogs were weighed individually at biweekly intervals during exposure and monthly during the postexposure period. Mice were weighed in groups and group mean weights followed on a monthly basis throughout the experimental period. Blood samples were drawn from dogs at biweekly intervals and clinical determinations made for the following battery of tests:

RBC	Sodium	Albumin
WBC	Potassium	Globulin
HCT	Calcium	SGPT
HGB	Glucose	Alkaline Phosphatase
Differential Cell Count	Total Protein	

Blood measurements not included in regular biweekly schedule during the exposure phase of the study but made at the conclusion of the 5 ppm and 0.5 ppm experiments were:

Blood urea nitrogen	SGOT
Chloride	Prothrombin time
Cholesterol	Cephalin flocculation
Creatinine	Bromsulphalein

Of these, tests giving abnormal values were scheduled to be repeated postexposure at regular intervals until recovery. To examine for possible hemolytic effects in rodents, blood samples for hematocrit and red blood cell counts were taken from 5 rats and 5 hamsters from each group at the conclusion of the 5 ppm and 0.5 ppm exposures. Blood was withdrawn using a nondestructive suborbital sampling technique.

Experimental Results

Significant exposure effects of 0.5 and 5 ppm UDMH were limited to slight to moderate but transitory hepatotoxicity in dogs exposed to the 5 ppm concentration. Dogs exposed to 5 ppm UDMH on a 6 hour/day, 5 day/week schedule developed significantly elevated serum glutamic pyruvic transaminase (SGPT) levels by the fourth week of exposure. At 6 weeks the mean SGPT value for the exposed dogs was 3 times the control level. Throughout the remaining 20 weeks of exposure, SGPT values for the exposed dogs (Table 6) were stable at levels 3-4 times those of the control group. A trend to recovery, approximately 50% reduction, was seen in measurements made 2 weeks postexposure. Subsequent values at 4, 8 and 11 weeks postexposure showed no further reductions. However, when the dogs were sampled again (at Brooks AFB where they were being maintained) at 27 and 47 weeks postexposure, SGPT values were completely normal when compared with control animal values.

TABLE 6. EFFECT OF 6-MONTH INHALATION EXPOSURE TO 5 PPM UDMH ON SERUM GLUTAMIC PYRUVIC TRANSAMINASE LEVELS IN DOGS

[Group Mean Values (N=8)]

Weeks of Exposure	Control Group	Exposed Group
2	26[1]	32
4	27	79*
6	27	102*
8	25	118*
10	26	118*
12	31	116*
14	--	---
16	22	88*
18	23	107*
20	23	99*
22	20	97*
24	22	100*
26	25	86*
Weeks Postexposure		
2	22	37*
4	23	42*
8	22	36*
11	23	35*
27**	33	30
47**	40	37

[1]International Units
*Significant at the 0.01 level
**Measurements made at Brooks AFB.

Liver function tests were performed on dogs at exposure termination and at 4, 8, 11 and 38 weeks postexposure. Bromsulphalein (BSP) measured in the blood of the 5 ppm exposed dogs 10 minutes following a 10 mg/kg injection showed significant retention at exposure termination, 4 and 8 weeks postexposure. Although the mean BSP retention values for the exposed dogs at 4 weeks postexposure indicated no trend to recovery, an examination of individual values revealed 10-25% reduction in values for 6 of 8 dogs. As seen in Table 7, recovery occurred at 11 weeks postexposure. BSP measurements made at Brooks Air Force Base 38 weeks postexposure show no abnormal values for the exposed dogs. Their values for control and exposed dogs are noticeably less than ours, and probably represent differences in the BSP test method.

TABLE 7. MEAN BROMSULPHALEIN RETENTION VALUES* IN CONTROL AND 5 PPM UDMH EXPOSED DOGS

Time	Control	5 ppm
Exposure Termination		
26 Weeks	18.1	30.3**
Weeks Postexposure		
4 Weeks	20.7	29.5**
8 Weeks	12.8	30.0**
11 Weeks	18.0	21.8
38[1] Weeks	11.4	12.3

*Percent retention.
**Significantly higher than controls at the 0.05 level.
[1]Measurements made at Brooks Air Force Base.

Examination of hematocrit and RBC determinations made immediately postexposure on rats and hamsters showed no abnormalities. Likewise, results of hematocrit, hemoglobin, RBC and reticulocyte measurements on dogs showed no effects of exposure to UDMH.

Mean body weights measured biweekly during exposure and monthly postexposure to 5.0 and 0.5 ppm UDMH are shown for rats in Figure 12 and for hamsters in Figure 13. The rats exposed to UDMH grew at a slower rate than their controls but the rate increased after removal from the exposure at 6 months. Their growth thereafter exhibited a normal weight pattern for Fischer 344 rats when compared with other groups used in our laboratory. The weight loss seen in unexposed control rats was associated with a large number of deaths. Approximately 40 control rats died from an epizootic respiratory infection which occurred in an animal holding facility 3 months after the exposure phase of the study. Fortunately all other experimental animals were maintained in a different location.

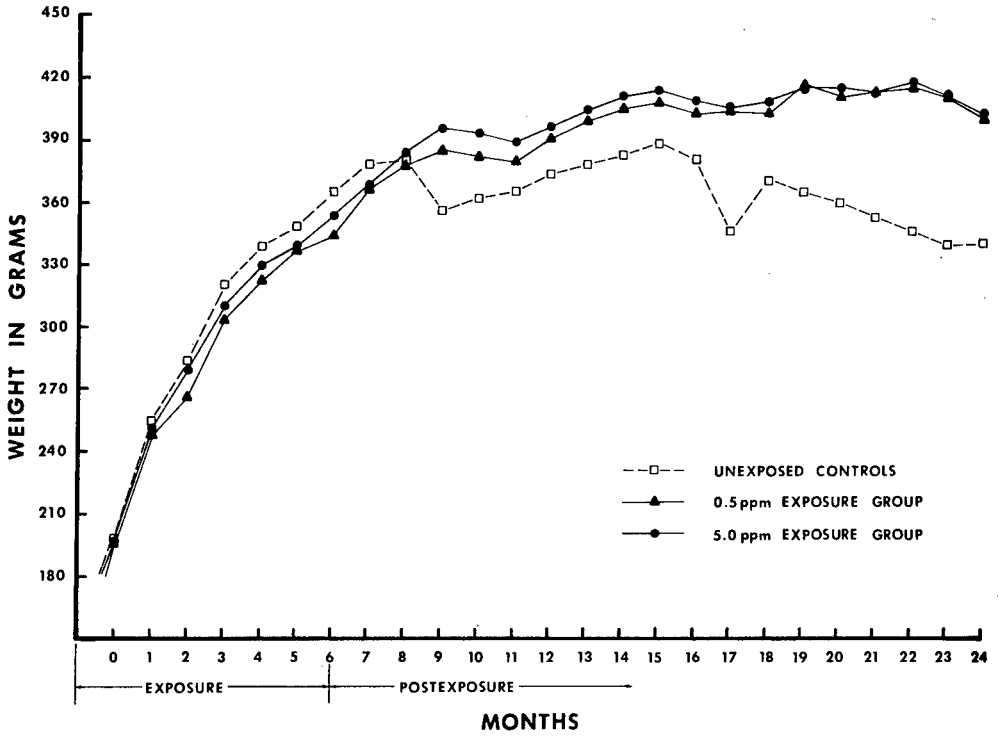


Figure 12. The effect of chronic exposure to inhaled UDMH on the growth of male rats.

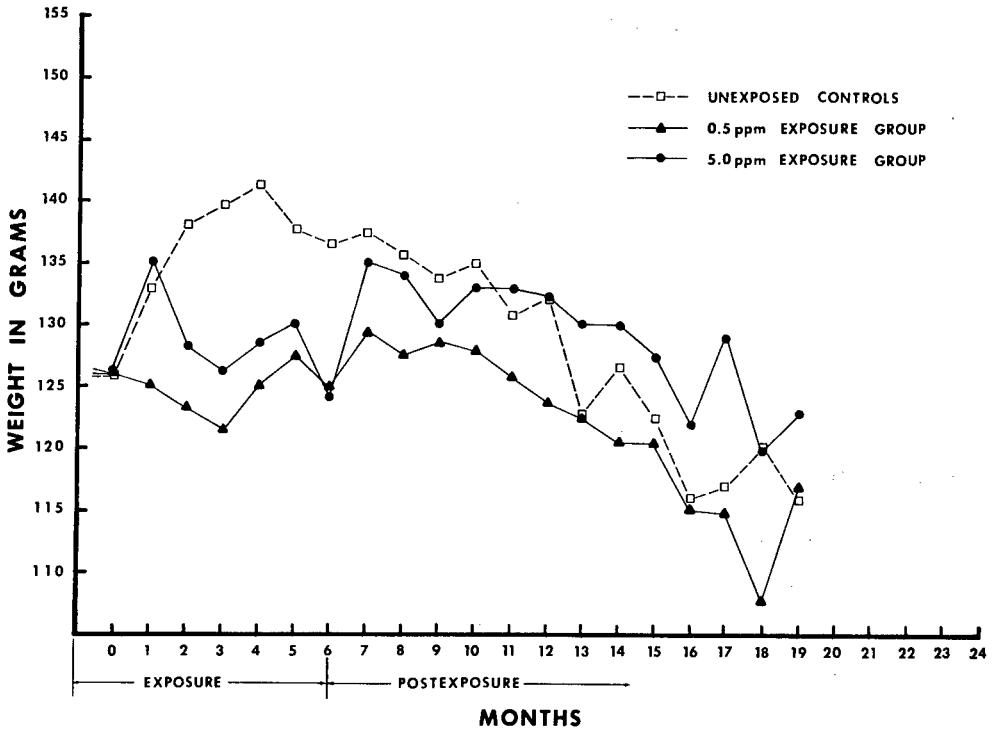


Figure 13. The effect of chronic exposure to inhaled UDMH on the growth of male hamsters.

TABLE 8. TUMOR INCIDENCE IN CONTROL AND UDMH EXPOSED C57BL/6 MICE

Tumor Type	Set 1 Unexposed Controls	Set 1 5 ppm Exposed	Set 1 0.5 ppm Exposed	Set 2 Unexposed Controls	Set 2 0.05 ppm Exposed
Lung					
Alveolar/Bronchiolar Adenomas	6/320	11/337	7/331	3/340	4/355
Alveolar/Bronchiolar Carcinomas	0/320	0/337	3/331	1/340	1/355
Liver					
Hepatocellular Carcinoma	2/332	4/342	7/344	2/349	1/363
Pituitary					
Carcinoma	2/255	1/251	1/257	3/270	3/320
Adenoma	1/255	3/251	3/257	13/270	10/320
Chromophobe Adenoma	29/255	24/286	2/257	48/270	34/320
Thyroid					
Follicular Cell Adenomas	25/258	13/286	20/278	20/293	17/311
Follicular Cell Carcinomas	2/258	5/286	8/278	0/293	1/311
Uterus					
Leimyosarcoma	0/304	3/312	3/311	1/328	3/348
Ovary					
Tubular Adenoma	3/279	1/308	4/287	2/324	4/336
Circulatory System					
Hemangioma	6/346	5/360	2/368	1/355	2/374
Hemangiosarcoma	3/346	19/360**	3/368	1/355	8/374*
Hematopoietic System					
Malignant Lymphomas	87/346	112/360*	98/368	94/355	102/374
Kupffer Cell Sarcoma	0/346	8/360*	0/368	1/355	4/374
Plasma Cell Tumor	1/346	2/360	1/368	1/355	4/374

*Significant at the 0.05 level as determined using Fischer's Exact Test.
**Significant at the 0.01 level as determined using Fischer's Exact Test.

TABLE 9. TUMOR INCIDENCE IN CONTROL AND UDMH EXPOSED FISCHER 344 RATS

Tumor Type	Set 1 Unexposed Controls	Set 1 5 ppm Exposed	Set 1 0.5 ppm Exposed	Set 2 Unexposed Controls	Set 2 0.05 ppm Exposed
Lung					
Bronchiolar Adenoma	1/196	8/191*	2/182	5/189	0/192
Squamous Cell Carcinoma	0/196	4/191	0/182	0/189	0/192
Liver					
Hepatocellular Carcinoma	0/193	6/188*	2/189	0/197	0/193
Pancreas					
Islet Cell Adenoma	3/132	8/158	12/169*	0/170	3/174
Pituitary					
Chromophobe Adenoma	39/166	89/174**	72/169**	69/171	76/182
Kidney					
Carcinoma	0/197	1/190	0/196	0/195	0/196
Adrenal					
Adenoma	0/189	1/188	1/177	0/194	1/188
Brain					
Astrocytoma	0/182	1/178	1/189	0/199	2/182
Glioblastoma	0/182	1/178	0/189	0/199	0/182
Malignant Neural Neoplasm	0/182	0/178	1/189	0/199	0/182
Pinealcytoma	0/182	1/178	0/189	0/199	0/182
Hematopoietic System					
Malignant Lymphoma and Leukemia	17/199	19/196	20/193	51/200	39/197
Skin & Miscellaneous Fibrous Histiocytomas	1/199	9/196*	6/193	1/200	0/197

*Significant at the 0.05 level as determined using Fischer's Exact Test.
**Significant at the 0.01 level as determined using Fischer's Exact Test.

The impact of UDMH exposure on hamsters was dramatic as seen in Figure 13. Growth was arrested during the 6 months of exposure and was erratic during the remainder of the study.

The growth of mice and beagle dogs was unaffected by UDMH exposure and the surviving dogs continue to be in good health. The dogs were returned to Wright-Patterson Air Force Base from Brooks Air Force Base in April 1978 for further postexposure observation.

Analysis of histopathology data revealed no differences in types of lesions seen in UDMH exposed and control mice. There were, however, significant differences in tumor incidence of several types between the unexposed controls and those mice exposed to 5 ppm UDMH as seen in Table 8. While most tumor types were similar in incidence, hemangiosarcomas, malignant lymphomas and Kupffer cell sarcomas occurred in excess in this UDMH exposure group. In particular Kupffer cell sarcomas were only seen in 1 of 701 control mice examined while 8 of 360 mice exposed to 5 ppm and 4 of 374 mice exposed to 0.05 ppm had this unusual tumor form.

Hemangiosarcoma incidence was excessive in both 5 ppm and 0.05 ppm exposed groups but was not dose related. The 0.5 ppm UDMH group had no excess tumors and the 0.05 ppm group was compared with its own set of controls which had a lower spontaneous incidence of hemangiosarcomas.

Fischer 344 rats were similar to UDMH exposed mice in that the same pathologic lesions were seen in both exposed and control animals. They differ as shown in Table 9 in that no excess tumors were found in the lowest exposure group and there was no specific dose response seen in the 5 ppm and 0.5 ppm UDMH exposure groups. The UDMH exposed rats in this study had an increased incidence of bronchiolar adenomas, hepatocellular sarcomas, pancreatic islet cell adenoma, pituitary chromophobe adenomas and miscellaneous fibromas and fibrous histiocytomas.

A single male dog exposed to 5 ppm UDMH died in the 19th month of the study, 65 weeks postexposure, after a rapid and unusual weight loss but with no apparent loss of appetite. At necropsy a large amount of food was found in the intestinal tract. The principal findings on gross examination were a large volume of serosanguineous fluid in the thoracic cavity and a very large white multilobulated neoplastic mass which appeared to encapsulate the heart, portions of the lung and to invade these tissues as well as the costal pleura. The right popliteal lymph node was also greatly enlarged. Histologic examination of the neoplastic tissue growth was performed and a diagnosis made of reticulum cell sarcoma of multicentric origin. The sarcoma was metastatic to the lung, pleura and vascular adventitia of thoracic vessels. The right popliteal lymph node also contained an area of reticulum cell sarcoma. No significant lesions were seen in any other tissue examined.

The variance in numbers of tissues examined for rats and mice in each group from the numbers placed in the study at onset was due to losses from autolysis, occasional cannibalism, escape from exposure cages and in the case of thyroid or pituitary glands, inability to find any tissue in some animals.

The histopathology data for hamsters exposed to UDMH and their controls are not yet complete but on the basis of the 3 species examined thus far it is clear that the impure UDMH used in these oncogenic studies is weakly tumorigenic when inhaled at the 5 ppm concentration.

We believe that the small amount of N-nitrosodimethylamine present in this UDMH as a 0.12% impurity contributed significantly to the oncogenic changes observed in the 5 ppm exposure group. We were able to demonstrate that this small amount of impurity of a very potent carcinogen was responsible for the hepatotoxicity observed in the dogs exposed to the highest UDMH concentration and as was reported in the 1976 annual report (MacEwen and Vernot), increased liver cell changes on orally dosed mice.

Daily exposures of C57Bl/6 mice to purified UDMH at a 5 ppm concentration were initiated in June of this year to determine if excess tumors can be produced without the N-nitrosodimethylamine contamination.

Chronic Effects of Low Level Inhalation Exposures to Fluomine Particulates

The compound fluomine [cobalt - bis(3-fluorosalicylaldehyde)-ethylenediimine], when activated, is capable of selectively absorbing oxygen from the air and upon heating will release pure molecular oxygen. This oxygen-scavenging property renders it useful as a possible component in life support systems for high altitude aircraft flights.

Materials and Methods

Preliminary studies of two-week duration as well as a 6-month chronic inhalation exposure were detailed in the previous annual report (MacEwen and Vernot, 1977). Included in that report were data obtained during the 6-month exposure period to 0.1 and 0.5 mg/m^3 aerosol. The animal groups for each exposure concentration and controls consisted of 100 male rats, 140 female mice, 24 guinea pigs and 8 dogs. The exposures were conducted 6 hours each day, five days per week. This report covers data accumulated since that time.

The fluomine particulates, produced by a Wright Dust Feeder®, were generated into a 200 liter mixing chamber prior to being drawn into the exposure domes by negative pressure. Regulation of the dust feeder gear ratios and/or the air passing through the mixing chamber controlled the concentration as well as the particle size entering the chambers.

Analysis of fluomine concentration was accomplished by taking hourly filter samples for colorimetric analysis. The fluomine samples were dissolved in 1N HNO_3 and absorbance at 365 nm measured using a GCA McPherson spectrophotometer. Checks were made by counting the particles in the 1.4 - 3.0 µm range using the Royco® analyzer. Since the greater part of the mass of the fluomine was included in this size range, fluctuations in chamber concentration could be easily detected by changes in the channel output representing this range.

Experimental Results

The mean concentration of fluomine aerosol in the planned 0.5 mg/m^3 exposure study was 0.51 mg/m^3 during the 117 days of exposure with a range of 0.37 to 0.79 mg/m^3. In the planned 0.1 mg/m^3 study the mean concentration for the entire study was 0.10 mg/m^3 with excursions of 0.06 to 0.17 mg/m^3.

All dogs and 10% of the rats and mice were sacrificed at the conclusion of the 6-month exposure portion of the study. Gross examination of these animals failed to reveal any exposure-related lesions. The only significant change in the organs examined by light microscopy was a higher incidence of diffuse, mild congestion with intraalveolar edema and the degree of severity of hydropic degeneration in the livers of the 0.5 mg/m^3 group of dogs. No differences were found in the organs of the mice or the rats when compared to their respective control groups. The significant findings are shown in the following table:

TABLE 10. SIGNIFICANT HISTOPATHOLOGY FINDINGS IN DOGS EXPOSED TO FLUOMINE AEROSOL (N=8)

Organ	Concentration, mg/m^3 0.5	0.1	0.0
Lung:			
Congestion, diffuse, mild	4	0	0
Intraalveolar edema	4	0	0
Liver:			
Vacuolation, hydropic, minimal	0	0	7
Vacuolation, hydropic, mild	2	4	0
Vacuolation, hydropic, moderate	3	0	0
Vacuolation, hydropic, severe	3	1	0

Fluomine exposed rats showed a statistically significant depression in mean body weight gain (Figure 14) throughout the study. Although the test rat groups differed from the control groups, they rarely differed from each other and a dose-effect relationship was not established. Following transfer to laminar flow animal rooms, the rat groups showed a slight increase in mean body weight gain. Although all groups showed this increase, the test groups remained below the control group.

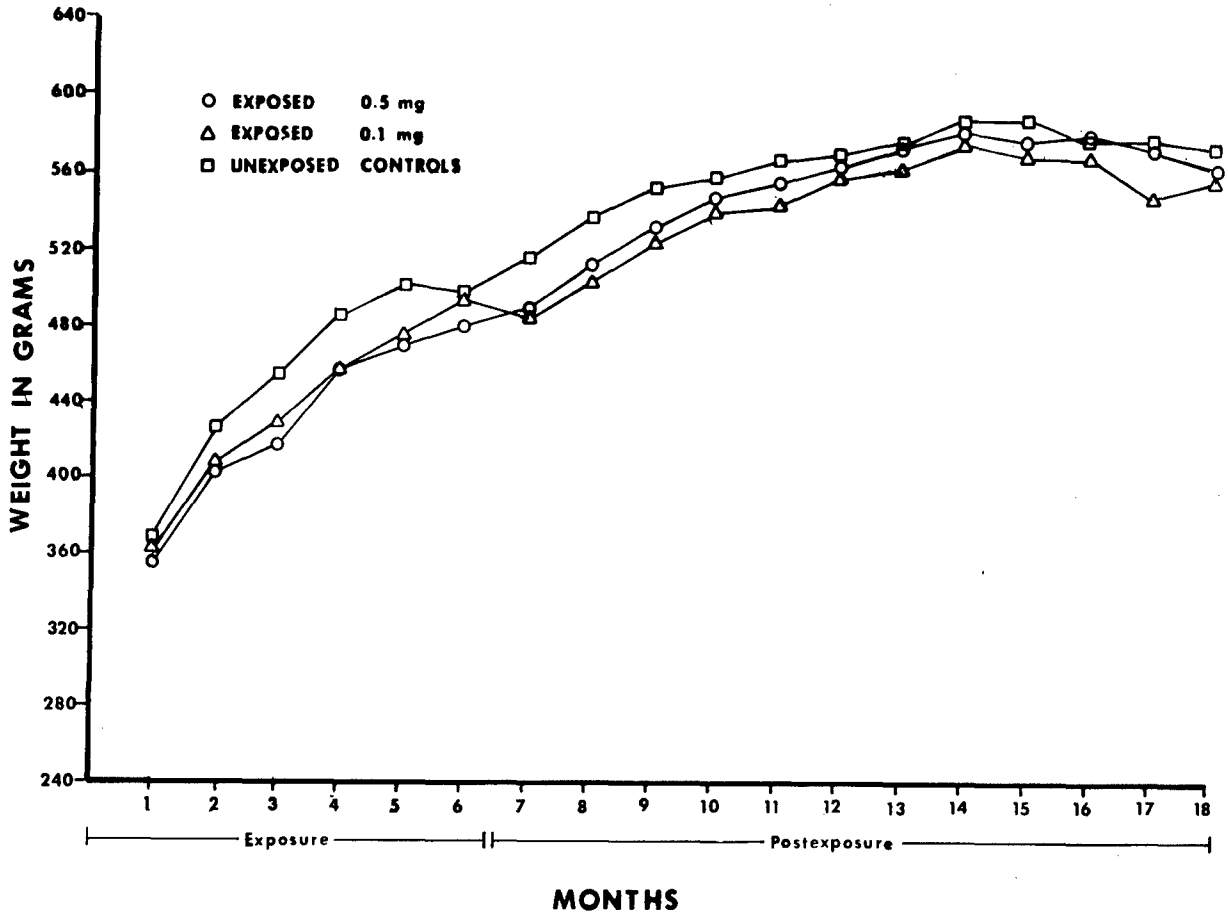


Figure 14. The effect of fluomine aerosol inhalation on growth of male rats.

The guinea pig mean body weights did not differ significantly from the control group during the exposure phase except for short periods of successive weighings as shown in Figure 15. The control group showed a drop in mean weights for a period of four months but then recovered to previous levels. The mean body weight differences noted in the guinea pig groups were transient in nature and not consistent throughout the study. A dose-response relationship was not seen.

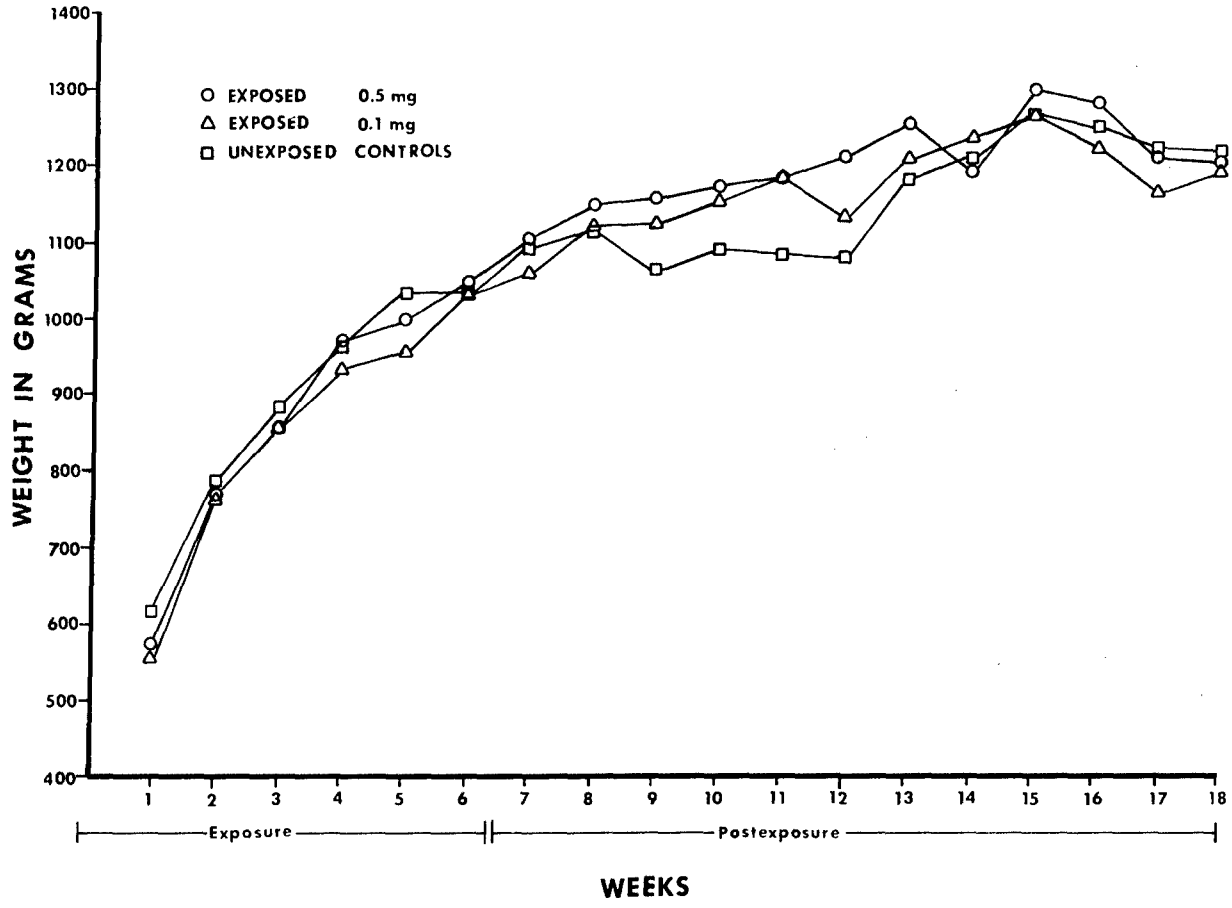


Figure 15. The effect of fluomine aerosol inhalation on growth of guinea pigs.

At the present time, these animals have completed the 12-month postexposure period. Initially, the protocol for this experiment scheduled that all surviving animals be sacrificed at the end of the observation period. However, only 16% of the rats and 23% of the mice had died by the 18th month and surviving animals appear to be in good health. Because holding animals for a longer period might reveal some long-term toxic effects of fluomine aerosol exposure, it was decided to sacrifice 50% of the remaining rats and mice at that time and hold the remainder for an additional six months at which time a sacrifice will again be considered. As mortality in the guinea pig group has been much higher, about 70%, all of the animals were sacrificed. The numbers of animals sacrificed in this study to date are shown in Table 11.

TABLE 11. POSTEXPOSURE SACRIFICE OF FLUOMINE AEROSOL EXPOSED ANIMALS

Fluomine Conc., mg/m^3	Animal Species & No. Sacrificed	Age at Sacrifice (weeks)	Number of Survivors
0.5	9 Guinea Pigs	93	0
	37 Rats	91	37
	50 Mice	91	52
0.1	5 Guinea Pigs	93	0
	35 Rats	91	34
	45 Mice	91	44
0.0	8 Guinea Pigs	93	0
	34 Rats	91	34
	46 Mice	91	46

Gross pathology examinations of the animals sacrificed at 18 months failed to reveal dose-related lesions in any of the animal species. Histopathologic results will not be available for several months and will be discussed in the next annual report.

The histopathologic information which will be available in the near future should provide us with the data necessary for predicting a safe exposure level for humans. Until that is available a safe level predicted on the dog histopathology data appears to be 0.1 mg/m^3.

The Effects of Subchronic Exposure of Rodents to Inhaled Decalin Vapors

Decalin (decahydronaphthalene), a commonly used alicyclic hydrocarbon solvent, does not have an established Threshold Limit Value (TLV) and available experimental data are insufficient for establishing a limit. The acute oral LD_{50} for rats is reported (Smyth et al., 1951) to be 4170 mg/kg of body weight indicating a low order of toxicity by this route although it is reported to be capable of producing dermatitis. Cardeni (1942) reported that repeated daily 8-hour inhalation exposures to three guinea pigs at 319 ppm of decalin resulted in one death at one day, a second death at 21 days, and the third animal died on the 23rd. On gross and microscopic examination of the tissues of these animals the principal positive pathologic findings were lung congestion, kidney and liver injury. Gage (1970) described the exposure of eight rats to 200 ppm decalin for 20 days on a 6 hour/day schedule with no toxic signs and grossly normal visceral organs at necropsy. A 4-hour single exposure of rats to 1000 ppm caused death in three of eight exposed rats. The deaths were preceded by tremors and convulsions.

These data were not sufficient for estimating safe interim exposure limits pending chronic toxicity studies since no controls were evaluated and histologic examinations were not conducted. Therefore, this study was designed with the purpose of gathering additional subchronic toxicity data on decalin and evaluating the need for chronic inhalation toxicity studies.

Acute Studies

Prior to initiation of the subchronic portion of these studies, some preliminary acute inhalation exposures were performed on male rats (Sprague-Dawley), female mice (CF-1) and male guinea pigs (Hartley-derived). The rats were obtained from Harlan Industries, Incorporated, the mice from Charles River Breeding Laboratories, Incorporated, and the guinea pigs from Sweetwater Farms, Incorporated. Prior to exposing the animals to known decalin vapor concentrations, rats and mice were exposed to essentially saturated vapors for various time periods.

Exposure of groups of five rats to saturated vapors for 4, 2 and 1-hour periods resulted in the death of 5, 5, and 2 rats, respectively. The rats were hyperactive early in the exposure but after approximately 40 minutes exhibited tonic convulsions, tremors, and prostration. Rats that survived two hours or more of exposure were paralyzed in the posterior half of the body and this paralysis persisted for several days. None of the paralyzed rats survived the 14-day postexposure observation period.

In these preliminary experiments, 5 of 8 mice died after 4 hours exposure to nominally saturated vapors while none of 10 died after 1 hour. As seen in rats, the primary signs of acute toxicity were tonic convulsions and tremors. However, none of the survivors showed the paralytic effect seen in rats.

Following the preliminary saturated vapor exposures, rats and mice were exposed to measured concentrations of decalin vapors to determine the 4-hour LC_{50}. The exposure concentrations and mortality results are shown in Table 12.

Rats were asymptomatic throughout the 4-hour exposure to 375 ppm decalin vapors and showed no apparent delayed effects during the subsequent 14-day observation period. Five guinea pigs were exposed to this same concentration of decalin to determine its suitability for chronic exposure testing, and they were also asymptomatic.

Gross pathology examination of the rats that died showed mild to severe congestion of the lungs with occasional areas of atelectasis. Reticulation of the liver and pale coloration of most organs was a common finding at all concentration levels.

TABLE 12. FOUR-HOUR INHALATION TOXICITY OF DECALIN TO MALE SPRAGUE-DAWLEY RATS AND FEMALE CF-1 MICE

Rats		Mice	
Conc., ppm	Mortality	Conc., ppm	Mortality
980	5/5	1085*	5/10
820	4/5	993	1/10
785	2/5		
625	2/5		
375	0/5		

LC_{50} (95% C.L.) = 710 ppm (619-816)

*Essentially saturated vapor (theoretical concentration at saturation = 1150 ppm).

Subchronic Studies

Three groups of 100 male Sprague-Dawley rats (Harlan Industries), 100 female CF-1 mice (Charles River Breeding Laboratories) and 25 male Hartley-derived guinea pigs (Sweetwater Farms) were exposed to decalin in inhalation exposure chambers at concentrations of 50 or 250 ppm, or served as controls in conventional housing for 30 days. The selection of 250 ppm as the high level exposure concentration for this study was based on its dosage equivalency to the 375 ppm 4-hour decalin exposure tests conducted in the acute studies that had not caused any ill effects in rats and guinea pigs.

The animal exposures were conducted for 6 hours per day, on a 5 day per week basis with continuous monitoring of the decalin concentrations. The rats and guinea pigs were weighed initially, at 15 days, and at termination. Organ weights (lungs, heart, liver and kidneys) were determined on 20 rats from each experimental group and organ to body weight ratios were calculated. These animals were killed by barbiturate overdose followed by exsanguination. Fasting body weights were used for this calculation. Terminal body weights for purposes of determining overall weight change were obtained on the previous day in the nonfasting state.

The remaining animals were sacrificed by chloroform inhalation. Representative tissues from these animals were preserved in formalin. Histologic evaluation was generally confined to trachea, lungs, liver, kidneys, and urinary bladder. In addition, any lesions noted during the gross necropsy were similarly examined. Guinea pigs exposed to decalin had statistically significant lower mean body weights at each interval as compared to the unexposed controls. The exposed groups of rats weighed more than controls after 15 days exposure but weighed significantly less than unexposed controls at termination of the experiment.

The body weight data for each group are presented in Table 13.

TABLE 13. MEAN BODY WEIGHTS OF MALE GUINEA PIGS AND MALE SPRAGUE-DAWLEY RATS EXPOSED TO DECALIN FOR 30 DAYS

Species	Conc., ppm	Mean Body Weight, g		
		Preexposure	Exposed 15 Days	Exposed 30 Days
Guinea Pig[a]	Control	600	726	807
	50	608	681[b]	757[c]
	250	598	662[c]	751[c]
Rat[d]	Control	211	288	351
	50	211	298[c]	330[c]
	250	212	300[c]	337[c]

[a] N=25
[b] Significantly different from control ($p < 0.05$)
[c] Significantly different from control ($p < 0.01$)
[d] N=100

Data obtained from rats sacrificed for organ weight measurement are shown in Table 14. Lung, heart and liver weights for the two test groups were significantly lower ($p < 0.01$) than the control group but there were no significant differences in organ-to-body weight ratios. There were no significant differences in the kidney weights; however, the ratios were significantly higher ($P < 0.01$) for the test groups when compared with the control group.

TABLE 14. MEAN TERMINAL BODY WEIGHTS, ORGAN WEIGHTS, AND ORGAN-TO-BODY WEIGHT RATIOS OF MALE SPRAGUE-DAWLEY RATS EXPOSED TO DECALIN FOR 30 DAYS[a]

	Unexposed Control	50 ppm Exposed	250 ppm Exposed
Body Wt.(g)	355	297	305
Lung Wt.(g)	1.82	1.65[b]	1.66[b]
Ratio (%)	0.543	0.555	0.546
Heart Wt.(g)	1.10	1.01[c]	1.01[c]
Ratio (%)	0.327	0.339	0.331
Liver Wt.(g)	10.07	8.86[b]	9.58[c]
Ratio (%)	3.00	2.98	3.15
Kidney Wt.(g)	2.20	2.18	2.23
Ratio (%)	0.659	0.733[b]	0.773[b]

[a] N=20
[b] Significantly different from control ($p < 0.01$).
[c] Significantly different from control ($p < 0.05$).

At necropsy no gross lesions were seen which could be attributed to exposure to decalin.

On microscopic examination, hyalin droplet formation characterized by multifocal to diffusely distributed mononuclear cells containing large, deeply eosinophilic globules was noted within the tracheal epithelium of the test and control rats. The incidence and severity of this finding was significantly greater in the test groups than in the control group. The difference in incidence of these findings between the two test groups was minimal; however, the severity of the changes was greater in the 250 ppm decalin exposure group.

Electron microscopic examination of the trachea, bronchus and alveolar tissues of rats exposed to decalin gave evidence that respiratory irritation was produced. This irritation effect was manifested in rats exposed to 50 ppm decalin by loss of ciliated cells and proliferation of cells interpreted to be goblet cells in tracheobronchial epithelium. Rats exposed to 250 ppm had these same changes, plus the presence of exudate on tracheobronchial epithelium and prominence of cells interpreted to be type 2 alveolar pneumocytes.

Hydropic change was observed in the hepatocyte cytoplasm of all rat groups and there appeared to be a definite dose relationship in the incidence and severity of these changes. The controls showed a 24% incidence of minimal change without any more severe changes. By contrast, the 50 and 250 ppm exposure groups, while showing approximately the same number of minimal changes - 25 to 31% - showed a dose-related response in the severity of changes. The 50 ppm group exhibited, on a scale of 0 to 4+, 15% 2+ and 8% 3+, whereas the 250 ppm group showed 21% 2+ and 29% 3+. We believe that these changes were the result of a reversible accumulation of water as the result of biochemical injury.

Hyalin droplet formation within the proximal tubular epithelial cytoplasm was observed in both exposed and control groups of rats. There was, however, a definite dose-response in both incidence and severity of change. On a scale of 0 to 4+, the unexposed rats showed a minimal change (1+) in 46% of the animals with the remainder showing no changes. Rats exposed to the 50 ppm decalin concentration showed changes of 1+ in 23%, 2+ in 39%, and 3+ in 25%. No grades of 4+ were reported. Rats exposed at the 250 ppm level showed changes of 1+ in 1%, 2+ in 33%, 3+ in 41%, and 4+ in 25%.

The primary change noted in the urinary bladder of rats consisted of a fine cytoplasmic vacuolization of the superficial epithelial cells which imparted a "foamy" appearance to them. Since this change was observed in 8% of the controls, 17% of the 50 ppm, and 32% of the 250 ppm rats, it would appear to be dose related. The significance of this finding is not known.

Histologic findings in the mice generally did not appear to be related to exposure to decalin with the exception of hepatocytic vacuolization of the cytoplasm in 7% of the controls, 5% of the 50 ppm exposed mice, and 52% of the 250 ppm decalin exposed mice. Again electron microscopy examination revealed evidence of respiratory irritation. The evaluation of tracheal sections was hampered by the loss of prepared control sections; however, there was loss of cilia in bronchial epithelium and the presence of exudate in both bronchi and alveoli in exposed animals. Cells believed to be Type 2 pneumocytes were seen in the alveolar areas of mice exposed to either 50 or 250 ppm decalin.

The only histopathologic finding in guinea pigs apparently due to decalin exposure was alveolar irritation. Mild to severe, focal to multifocal, chronic, active pneumonia consisting of thickened alveolar septae with exudation of mononuclear cells, neutrophils and septal macrophages into the alveolar spaces occurred in 4% of the controls, 36% of the 50 ppm group, and 20% of the 250 ppm group. While these lesions are not dose related, they may represent alveolar irritation due to decalin exposure.

No deaths occurred in groups of rats, mice and guinea pigs exposed for 30 days to 50 ppm and 250 ppm decalin on a 6 hour per day, 5 day per week schedule and there were no overt signs of toxicity during exposure. The animals appeared normal throughout the study. Both exposed groups of rats and guinea pigs gained less weight than their controls as a result of decalin exposure but the differences were not dose related. Histopathologic changes attributed to decalin exposure were seen in the trachea, liver, kidney and urinary bladder of exposed rats. Incidence and severity of these changes appeared to be dose related except for the hyalin droplet formation in the trachea. In mice, the only decalin exposure related changes were seen in the liver, and histopathologic findings in exposed guinea pigs were limited to alveolar irritation.

The incidence of micropathologic lesions noted in exposed animals indicate that the concentrations of decalin used were sufficient to produce tissue irritation characteristic of hydrocarbon toxicity and actual degenerative changes in rats. The environmental concentrations of decalin used in this series of animal exposures produced significant toxic changes in rodents after 22 days of repeated 6-hour industrial type exposures conducted over a 30 day period. These results also indicate that the 25 ppm decalin level suggested by Gerarde (1967) may be unsatisfactory for repeated daily exposure. Chronic inhalation exposure studies are recommended for evaluation of safe exposure levels. Chronic exposure studies should include tests of liver and kidney function since histologic changes of these tissues were seen in rats and liver changes were seen in decalin exposed mice.

Evaluation of Toxic Effects of 90-Day Continuous Exposure to Conventional JP-5 Jet Fuel

Petroleum distillates have been used as large scale sources of energy for over one hundred 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 decidely drunk, 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 possiblity that the Threshold Limit Value (TLV) of benzene at that time, 25 ppm, might be exceeded in a gasoline concentration that did not exceed its 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. 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 concentrations of JP-4 were 5 and 2.5 mg/liter. 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/liter. 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/liter JP-4 were significantly higher than controls, and there was a higher incidence of chronic murine bronchitis in the rats exposed to either concentration.

Twenty of each species of rodents in this study were held for postexposure observation and survivors sacrificed one year post-exposure. The only exposure related pathologic lesions were increased hemosiderin deposits in the spleens of rats exposed to both concentrations of JP-4 and benzene alone.

The jet fuel of interest to the U. S. Navy is JP-5 which differs from JP-4 primarily in being less volatile. The Navy has interest in determining chronic effects of exposure to JP-5 and in comparing these effects to those of JP-4. Because the Navy jet fuels are carried and transferred in enclosed areas aboard ship where crewmen might be continually exposed over the length of a cruise, a 90-day continuous exposure was selected for use.

Materials and Methods

This study was designed to determine the toxic effects, including oncogenesis, of 90 day continuous exposure of test animals to JP-5. Conditions were chosen for the experiment to simulate exposure conditions peculiar to the Navy and to permit comparison with previous exposures to JP-4.

Rats, mice and dogs were exposed to JP-5 by the inhalation route continuously in chambers for 90 days. The higher exposure concentration was intended to be that which produced a benzene concentration of 10 ppm, and the lower a 1 ppm benzene exposure.

One third of the rodents and all dogs were sacrificed immediately on termination of exposure for detection of any pathologic lesions caused by exposure. At 19 months postexposure one-half of the remaining rodents will be sacrificed. They should have attained almost a normal lifetime age at that time without having experienced a large number of deaths. This sacrifice should provide statistically satisfactory samples of animal tissues which will not be compromised by cannibalism or postmortem degeneration. The rest of the rodents will be held until mortality in any group of each species reaches 90% of the original number, at which time all representatives of that species will be sacrificed for gross and histopathologic examination.

Purebred beagle dogs used in this study were provided by the Air Force. Fischer 344 rats and C57Bl/6 mice were the rodent strains selected for use in the study. All animals were fed ad libitum and cleaned daily.

The experimental animals were randomized from the main group after quality control procedures and quarantine had been completed. Assignment of the animals from each species to each group was made by use of the THRU Computer Program RANDUM which utilizes the FORTRAN library subroutine RANF(X).

Each exposure chamber contained 3 each male and female dogs, 75 male and 75 female rats, and 150 female mice. Another group with the same numbers of animals was held at the Air Force Veterinary Services Division (Vivarium) as controls.

All animals were observed hourly during exposure and will be observed daily thereafter until the mortality rate becomes significant enough to warrant more frequent examination.

Rats and dogs were weighed individually at biweekly intervals during exposure and rats monthly during the postexposure period. Mice were weighed in groups and group mean weights followed on a monthly basis throughout the experimental period. Blood samples were drawn from dogs at biweekly intervals and clinical determinations made for the series of tests shown in Table 15.

TABLE 15. CLINICAL HEMATOLOGY AND CHEMISTRY TESTS PERFORMED ON DOGS AND RATS EXPOSED TO JP-5 VAPORS

Hematology	Chemistry
Hematocrit	Sodium
Hemoglobin	Potassium
Total RBC	Calcium
Total WBC	Albumin/Globulin
Differentials	Total Protein
Mean Corpuscular Volume (MCV)	Glucose
Mean Corpuscular Hemoglobin (MCH)	Alkaline Phosphatase
Mean Corpuscular Hemoglobin Concentration (MCHC)	SGPT
RBC Fragility	Bilirubin
	Creatinine
	BUN

Most of the above tests are included to detect the onset of any blood dyscrasias since these have been the major reported effects of exposure to benzene. Other tests could reveal toxicity to specific organs such as liver or kidney. All animals that died or were sacrificed in these studies were necropsied. The list of tissues taken for histopathologic examination is given in Table 16.

TABLE 16. TISSUES SAMPLED FROM ANIMALS EXPOSED TO JP-5 VAPORS

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

JP-5 Generation and Monitoring

The basic design for the JP-5 generation system was adapted from a previous study on JP-4. Since petroleum based jet aircraft fuels are multicomponent with a wide boiling range, it was necessary to operate both animal exposure chambers from a single master generation system (Figure 16) to assure similar exposure. To reduce potential fire hazard, an overheat alarm with a fuel shut off capability was incorporated into the generation apparatus.

The fuel was metered in the top of two electrically heated evaporator towers. Air entered the tower at the side near the bottom while spent fuel was drained from the bottom. The hot fuel vapor/air mix was split in the approximate volume ratio of the chamber concentration and entered the respective chamber air streams.

In general, the mass of hydrocarbon vaporized per unit time was dependent on the effective heat input while the vapor component concentration was affected by the ratio of components present in the fuel and the fuel flow rate. The system as operated had a fuel flow rate limit of about 15 ml/tower per minute. Fuel available for the study was another limitation on fuel usage.

The vapor concentrations for this study were finally determined empirically on the basis of fuel type and exposure system operational characteristics. The upper limit was determined by very rapid increases in aerosol concentration when saturation vapor pressures of high boiling components were exceeded. Exposure chamber air flow rate and/or heat input were used for control of mass concentration and aerosol generation once basic chamber operational parameters were determined.

Since it may be necessary to duplicate this system closely to validly compare various fuels, a detailed description of the generation and analysis equipment along with diagrams is presented here.

Two identically operated solvent evaporator towers were required to generate sufficient fuel vapor for the assigned chamber concentrations. The central zone of the glass tower is a cylinder 13 inches long by 1-3/4 inch O.D. It has a 13-turn spiral 9 inches long impressed in the wall to hold a heating coil and lengthen the evaporation path. The top reduced to a "T" with a 1 inch O.D. right arm for vapor exhaust and a 1/4 inch O.D. connector for input fuel. The bottom reduced to a 1 inch O.D. glass connector.

A double "T" of stainless steel tubing and pipe fittings allowed input air, spent fuel exit and temperature monitoring probe on the waste fuel. A valve downstream controlled air/spent fuel flow to reduce vapor loss from the bottom of the tower.

The primary source of heat is a 1/4 inch O.D. close coiled Nichrome wire (B&S-20 ga., 1.1 Ω/in., Wooge Manufacturing Co., Chicago, Illinois). The close coil length used on each solvent evaporator tower was approximately 72 inches in length. It is rated 400 watts with 115V on 28 inch coil length.

Additional heat was added to the system by wrapping the metal fittings at the bottom of the tower with a heat tape. Both tower and base heat sources were controlled by voltage regulation using variable voltage transformers.

Rather large volumes (100-150 gallons) of fuel were necessarily present in the area adjoining the chambers for prolonged periods of time. Because the system had to operate unattended except for hourly operational checks continuously for over 100 days for each study, an overheat alarm/shutdown system shown in Figure 17 was incorporated. Four temperature probes, each capable of system shutdown were placed at the most sensitive problem areas (fuel vapor/air mix leaving each tower and fuel draining from bottom of each tower). The temperature at each probe position was recorded hourly.

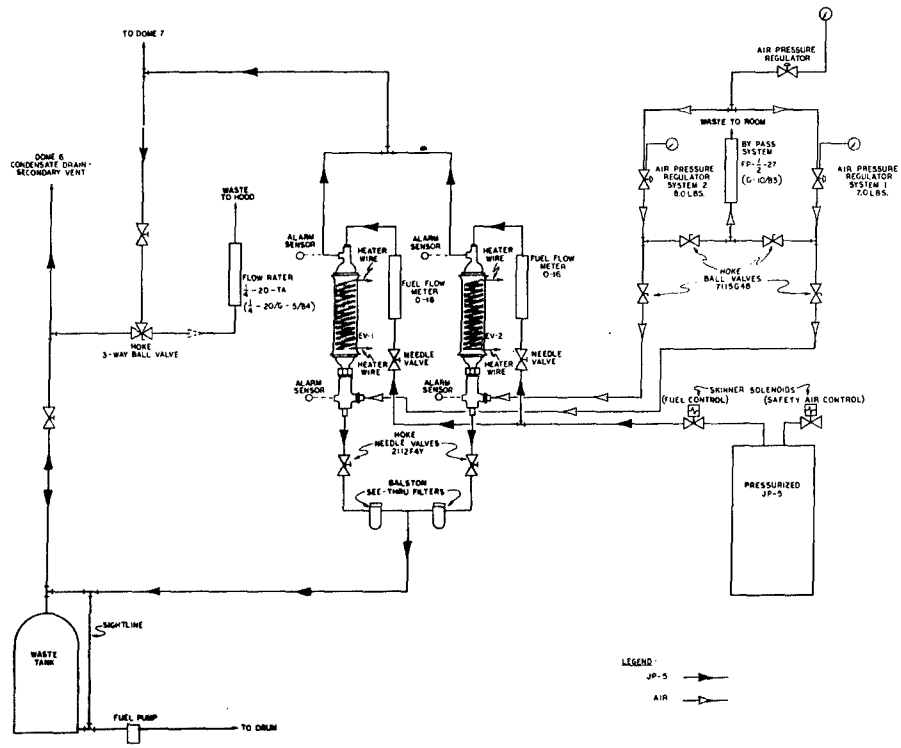


Figure 16. JP-5 contaminant introduction system.

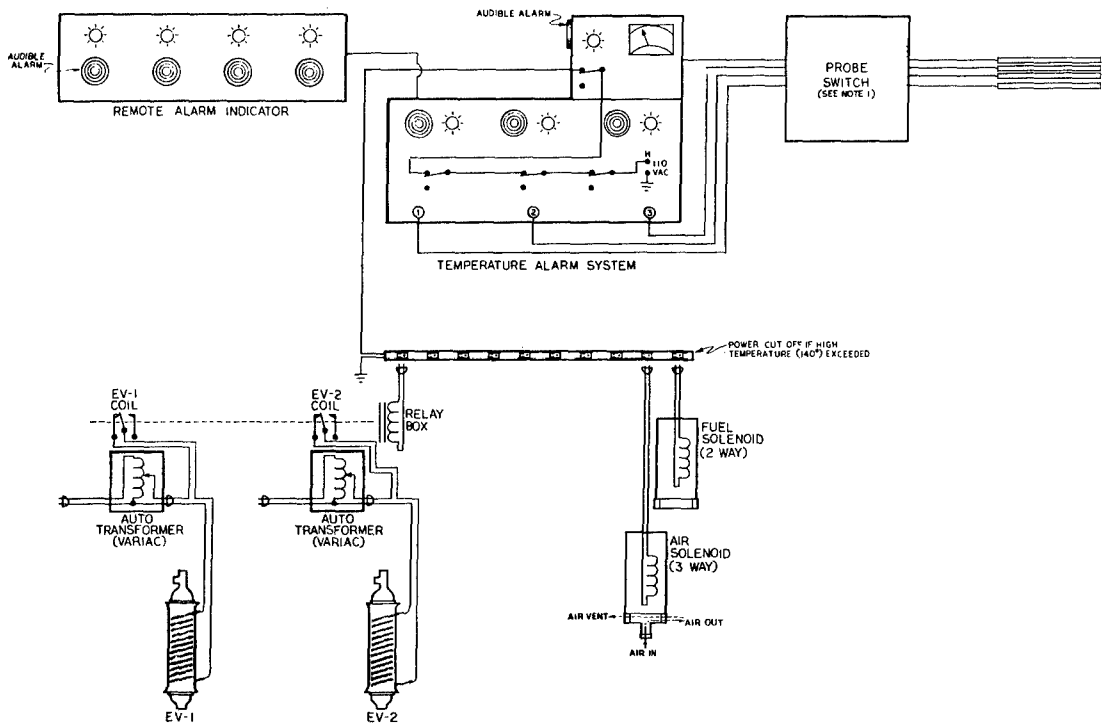


Figure 17. JP-5 contaminant introduction alarm system.

On shutdown, all heater power was turned off, fuel flow stopped and fuel pressure relieved and the fuel drums vented through the generation hood exhaust. In case of power failure, the whole system would shut down in the same manner. The temperature limit used was 120 F at the fuel vapor/air mix leaving the tower. Both upper and lower sensor system were set to trip off at 140 F.

A Beckman Model 400 Hydrocarbon Analyzer was used for mass analysis. Both chambers 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 (see Figure 18).

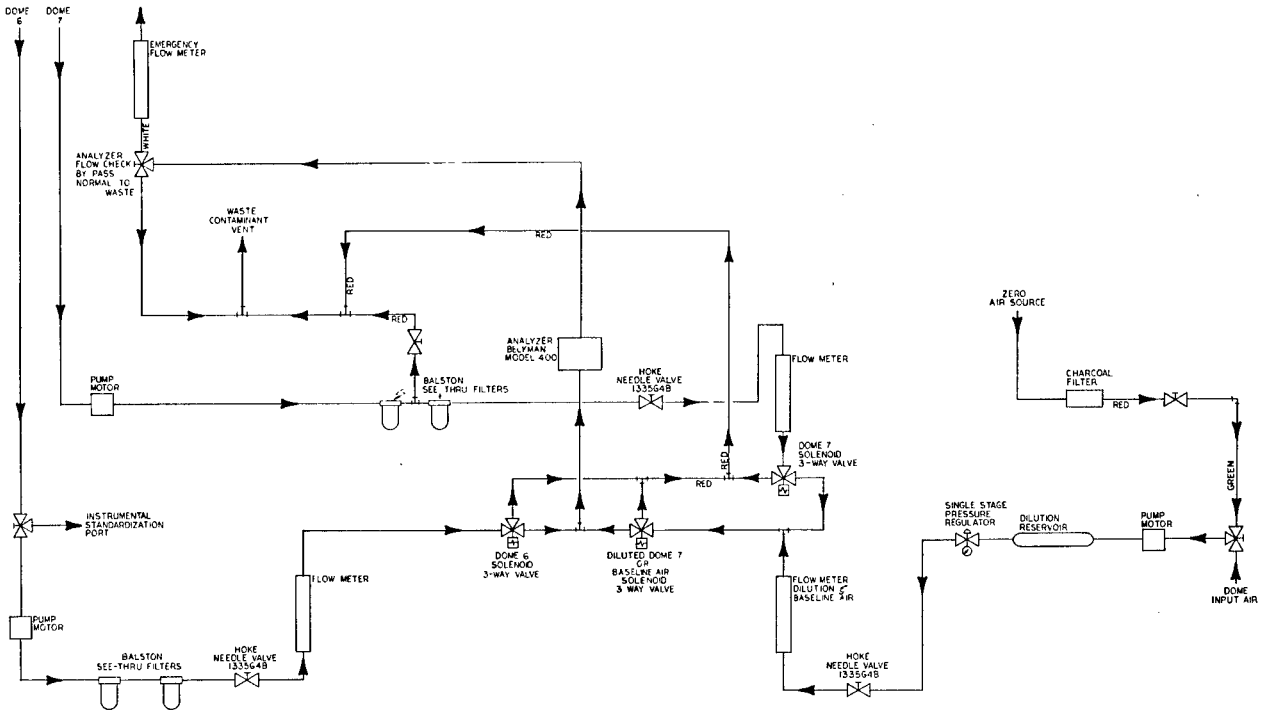


Figure 18. JP-5 sampling and analysis system.

Since instrumental response is directly related to the total carbon content of the sample, standardization was possible using a reliable defined system. Instrument grade propane (99 + % as C_3), diluted in 100 liter 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. The response was set to give the desired concentration a span of 60% full scale to maximize sensitivity while allowing for excursions in control of up to 50%.

Twenty-four hourly mean readings were used for daily concentration determinations. Use of the teletype and Base computer reduced calculation time to less than three minutes per chamber per day.

JP-5 Component Analysis - Gas Chromatography

In the first phase of this program, it was considered necessary to analyze for benzene concentration in the chamber. Of the column materials studied, SE30 as liquid phase gave the most promising separation. Two different columns have been used, one 12 ft. x 1/8 in. stainless steel, 10% SE30 on Chromosorb W-AW and the other 10 ft. x 1/8 in. stainless steel, 10% SE30 on Chromosorb W-HP.

Although the low benzene concentration in the fuel eliminated the need for continuous exposure chamber sampling for it, the columns proved satisfactory for (a) quality control checks of each drum before use, (b) spent fuel check for evaporation system operation, and (c) to give routine fingerprint analysis (along with benzene concentration) of the chamber as an operational check on the whole system.

The Varian 1200 was used for routine monitoring and was operated isothermally with the oven set at 40 C. Routine chromatographs were limited to peaks eluted in the first 20 minutes. A Spectra Physics Model I Computing Integrator was programmed to analyze the peak areas and give benzene concentration in ppm for the chamber samples.

The quality control study employed for selecting exposure concentrations used a head space sampling technique. One half ml of fuel was injected in a sealed sample vial. The vial was equilibrated for at least 15 minutes at 30 C before injection of a 20 µl head space sample from the vial. All JP-5 drums were found identical and a typical chromatogram is shown in Figure 19. The waste fuel not volatilized in the solvent evaporator tower was examined in a similar manner but the samples injected into the gas chromatograph was usually a 50 µl volume of vapor. A chromatogram of headspace vapor is shown in Figure 20. These 2 chromatograms illustrate the effective stripping of lower boiling fractions of the JP-5 fuel and is typical of what happens in human exposures from fuel spills, that is, that the resultant vapor exposures from solvent fuel mixtures are proportional to the specific vapor pressure of individual components.

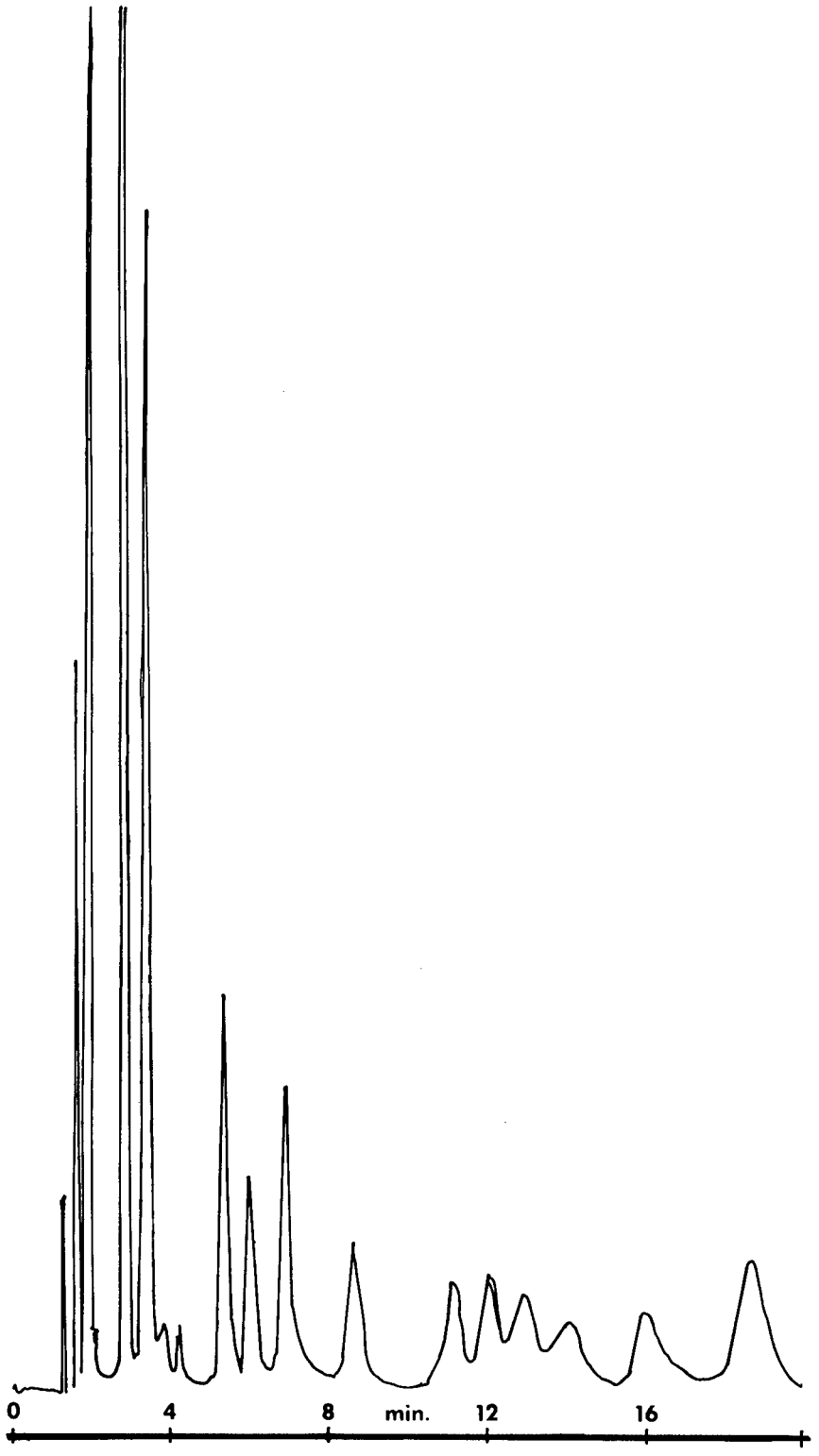


Figure 19. JP-5 drum quality control sample. (20 µl injection, headspace equilibrated at 30 C for 15 minutes. Varian 1200, FID, 12 ft. x 1/8 in., SS, 10% SE30 on Chromosorb W-AW, isothermal 40 C.)

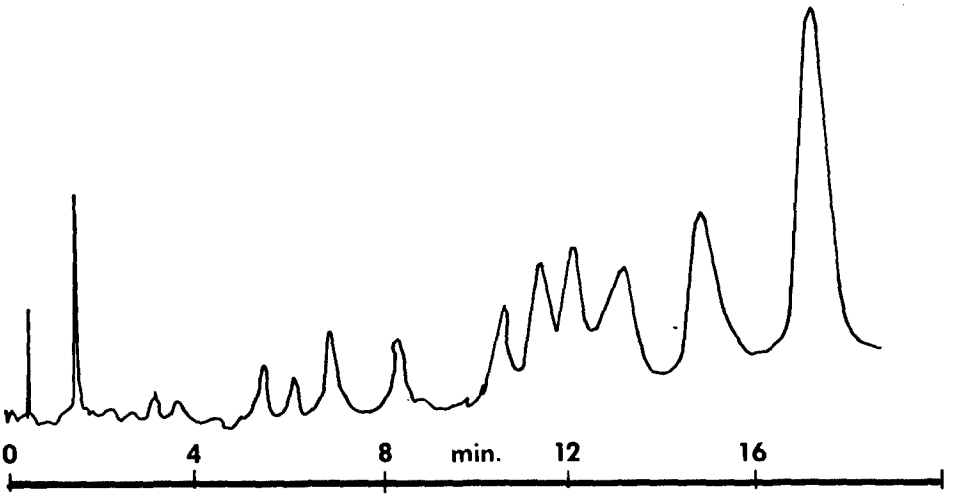


Figure 20. Waste JP-5 quality control sample. (100 µl injection, headspace equilibrated at 30 C for 15 minutes. Varian 1200, FID, 12 ft. x 1/8 in., SS, 10% SE30 on Chromosorb W-AW, isothermal 40 C.)

As stated previously animal exposures to JP-5 vapors were planned to be based on air concentrations which would result in chamber concentrations of benzene equal to 10 and 1 ppm. This contrasted with JP-4 exposures that had a benzene concentration of 25 ppm at the highest level and resulted from the reduction of the TLV for benzene from 25 to 10 ppm by the American Conference of Governmental Industrial Hygienists during the intervening period. In preliminary tests of 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 actual benzene content was very low. The highest benzene concentrations we were able to achieve in the exposure chamber ranged from 0.5 to 0.7 ppm and these levels were reached at a JP-5 vapor concentration of 1.5 mg/liter which also was the highest stable concentration attainable. At JP-5 vapor concentrations approaching 2.0 mg/liter recondensation of fuel occurred in the introduction system which required frequent draining of the air lines. Consequently the JP-5 concentrations selected for animal exposures were 1.5 and 0.15 mg/liter.

Experimental Results

Exposure of dogs to 1.5 mg/liter JP-5 began on 27 July and mice on 3 August 1977. Shortly after initiation, unexpected deaths occurred in the exposed mice. Seventy-five of the group of 150 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 1.5 mg/liter JP-5. The evidence was that generation of this concentration of JP-5 was producing an aerosol which was lethal to mice after a few days exposure.

Therefore, a Royco® particle counter was used to obtain some estimate of the aerosol concentration and distribution in the JP-5 exposure chambers. The resulting data are shown in Table 17. Over the range of particle sizes examined, chamber 7 had a much higher aerosol density than chamber 6.

TABLE 17. COMPARISON OF AEROSOL COUNTS OF AIR IN JP-5 EXPOSURE CHAMBERS

Particle Diameter, μm	Particles per Cubic Foot: Chamber 6 (0.15 mg/L JP-5)	Particles per Cubic Foot: Chamber 7 (1.5 mg/L JP-5)
0.5 - 0.7	272	2.1×10^5
0.7 - 1.4	100	1.5×10^5
1.4 - 3.0	75	1.3×10^5
3.0 - 5.0	12	4.4×10^3
> 5.0	11	1.3×10^3

The rate of introduction of JP-5 into chamber 7 was then lowered to give a chamber concentration of 0.75 mg/liter. After allowing time for equilibration, particle density was again measured with the results shown in Table 18. Also shown for comparison is the aerosol density found in laboratory air.

TABLE 18. COMPARISON OF AEROSOL COUNTS IN JP-5 EXPOSURE CHAMBERS AND IN LABORATORY AIR

Particle Diameter, μm	Particles per Cubic Foot: Laboratory Air	Particles per Cubic Foot: Chamber 6 (0.15 mg/L)	Particles per Cubic Foot: Chamber 7 (0.75 mg/L)
0.5 - 0.7	1400	740	3900
0.7 - 1.4	130	360	2300
1.4 - 3.0	41	310	900
3.0 - 5.0	7	200	33
> 5.0	4	3	11

Although no animals were in chamber 7 when measurements shown in Table 18 were made, it is obvious that the density of aerosol had been greatly reduced by decreasing the concentration of JP-5.

Based on the evidence that animal chamber JP-5 concentrations of 1.5 mg/liter produced an aerosol exposure along with the vapor exposures and was fatal to mice after several days of continuous exposure the initial study was terminated. The new JP-5 subchronic exposure study concentrations selected were 0.75 and 0.15 mg/liter. A new group of dogs was selected from the original stock group for use in the 0.75 mg/liter JP-5 exposure. The number of mice in each group had to be reduced to avoid delays from ordering new animals. Some spare mice were available and were incorporated into the pool for redistribution of the exposure groups. Each study group then consisted of 111 mice instead of the original 150. The animals were phased into the exposure chambers, which were being operated at the selected JP-5 concentrations, over a 3-week period to allow their sequential removal from exposure after 90 days for specific tests and necropsy. The animal introduction schedule used is shown below:

Chamber 6 - 0.15 mg/liter JP-5	Chamber 7 - 0.75 mg/liter JP-5
Dogs - Thursday, 28 July	Dogs - Thursday, 11 August
Mice - Friday, 5 August	Rats - Friday, 12 August
Rats - Wednesday, 10 August	Mice - Monday, 15 August

Exposure concentrations presented to the animals in the chambers were well controlled throughout the study. The maximum variation in the 0.75 mg/liter JP-5 exposure chamber was 7% high for a single daily mean concentration giving a value of 0.796 mg/liter. The lowest daily mean for this chamber was 0.716 mg/liter. In the 0.15 mg/liter JP-5 exposure chamber the variation in daily mean values was even smaller with a range of values from 0.148 to 0.157 mg/liter. Samples of chamber air for gas chromatographic examination of solvent peaks in the JP-5 vapors generated were collected from the pressure side of the hydrocarbon analyzer system. Usually 2 ml samples were drawn for injection into the gas chromatograph. The chromatograms made in this manner were used to determine benzene concentration in the chambers. A typical chromatogram of chamber air is shown in Figure 21 where the benzene peak used for analysis is eluted from the column at 10.5 minutes. This chromatogram differs from those shown of headspace samples from unused JP-5 and the waste JP-5 in that the vapors generated for chamber introduction are created at approximately 50 C while those used for headspace analysis are from raw or used fuel heated to 30 C. It should be noted that these chromatograms do not show all of the hydrocarbon peaks in the JP-5 fuel but are representative of the low boiling fractions which come off the gas chromatographic column in the first 20 minutes.

Benzene concentrations in the 0.15 mg/liter JP-5 exposure chamber range around 0.1 ppm with the lowest value measured 0.06 ppm and the highest 0.12 ppm. In the 0.75 mg/liter JP-5 exposure the benzene concentration was usually 0.5 ppm with a range of measured values of 0.33 to 0.57 ppm.

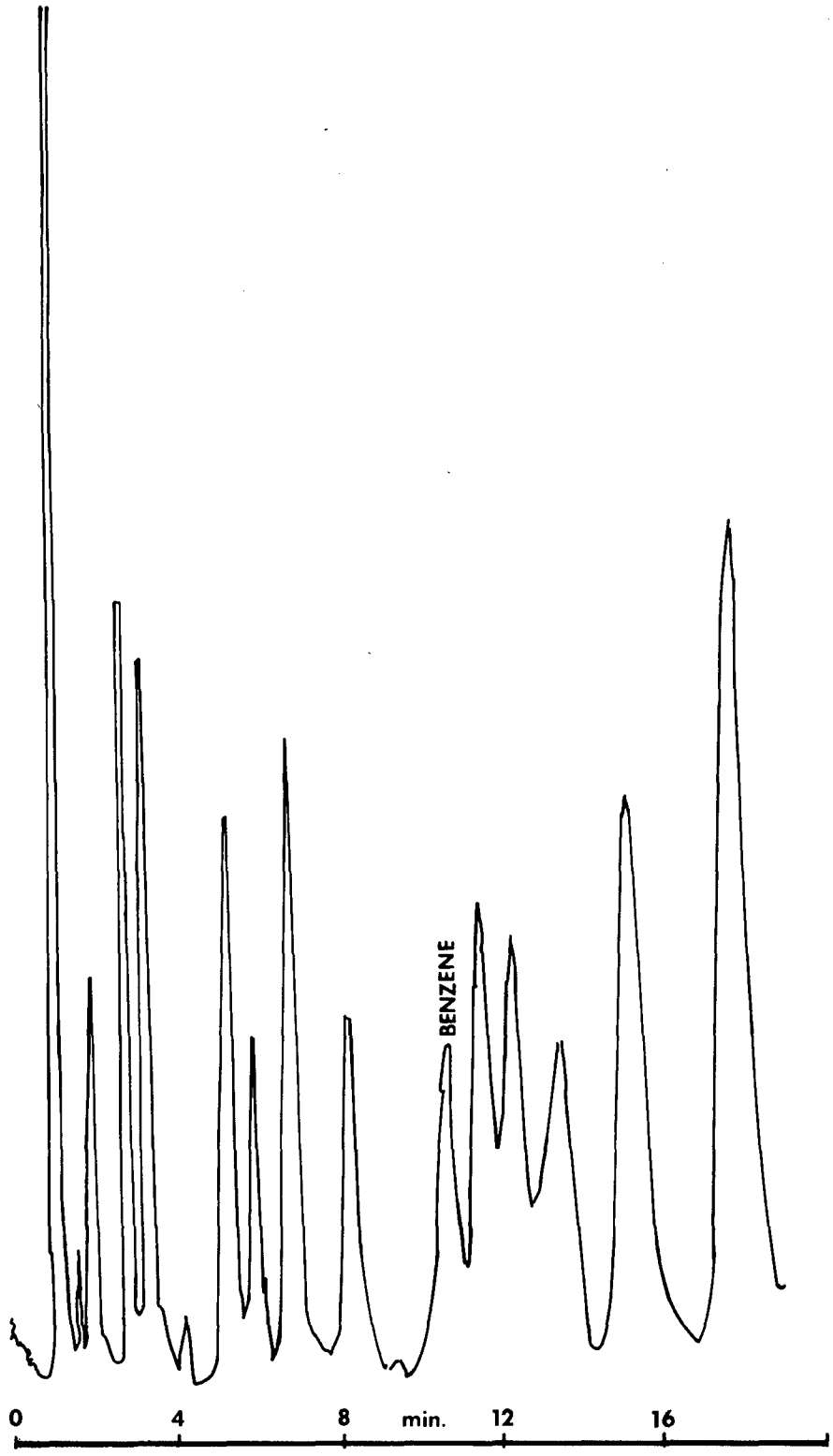


Figure 21. A typical chromatogram of chamber air containing 0.75 mg/L JP-5. (2 ml injection of dome atmosphere. Varian 1200, FID, 12 ft x 1/8 in., SS, 10% SE30 Chromosorb W-AW, isothermal 40 C.)

Effects on Animals

The growth of male Fischer 344 rats was retarded by exposure to JP-5 vapors as shown in Figure 22. The mean body weights of both the 0.15 mg/liter and 0.75 mg/liter JP-5 exposure groups were significantly different from unexposed control rats at the 0.01 confidence level throughout the exposure phase and until the eighth month of the study when the difference dropped to the 0.05 significance level. The observed effect was similar for both exposure groups with no apparent dose response. The growth of female rats, shown in Figure 23, was also affected but not as much as the changes seen in males. A period of weight loss observed in the controls decreased the apparent difference from exposed animals.

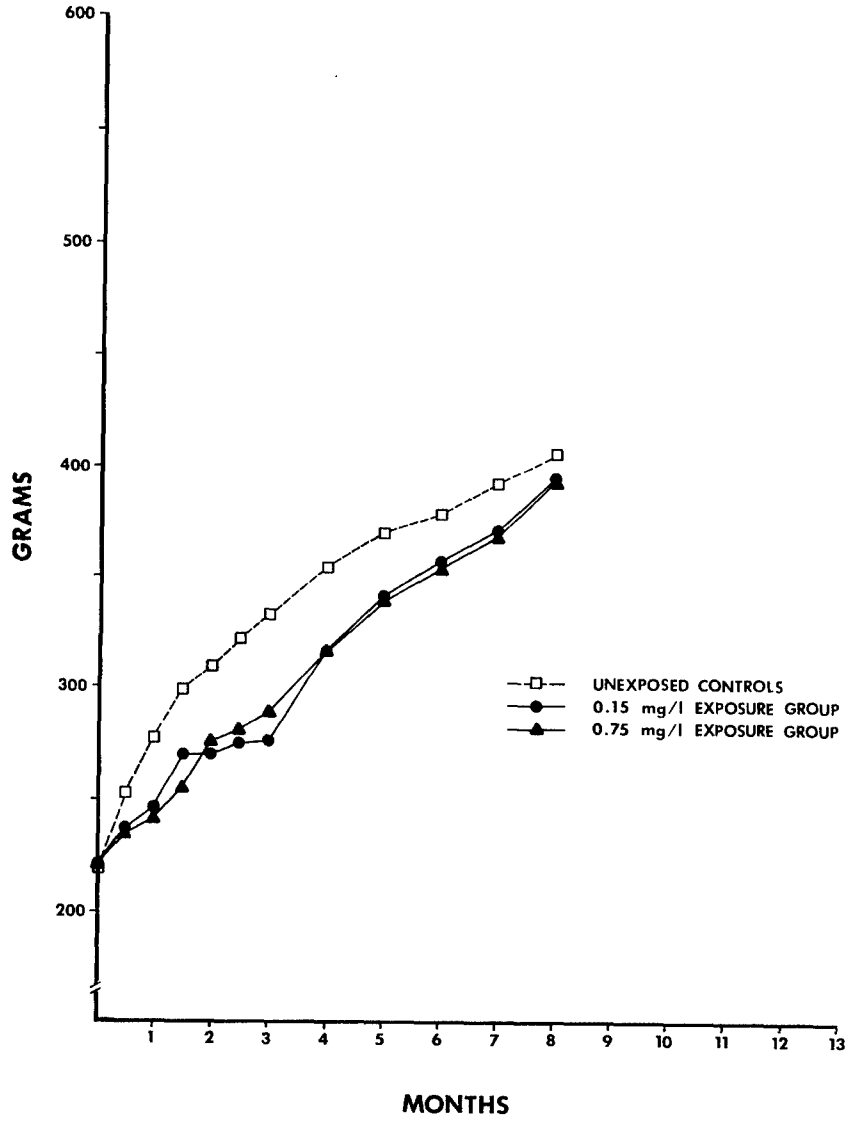


Figure 22. Growth of male rats exposed 90 days to inhaled JP-5 vapors.

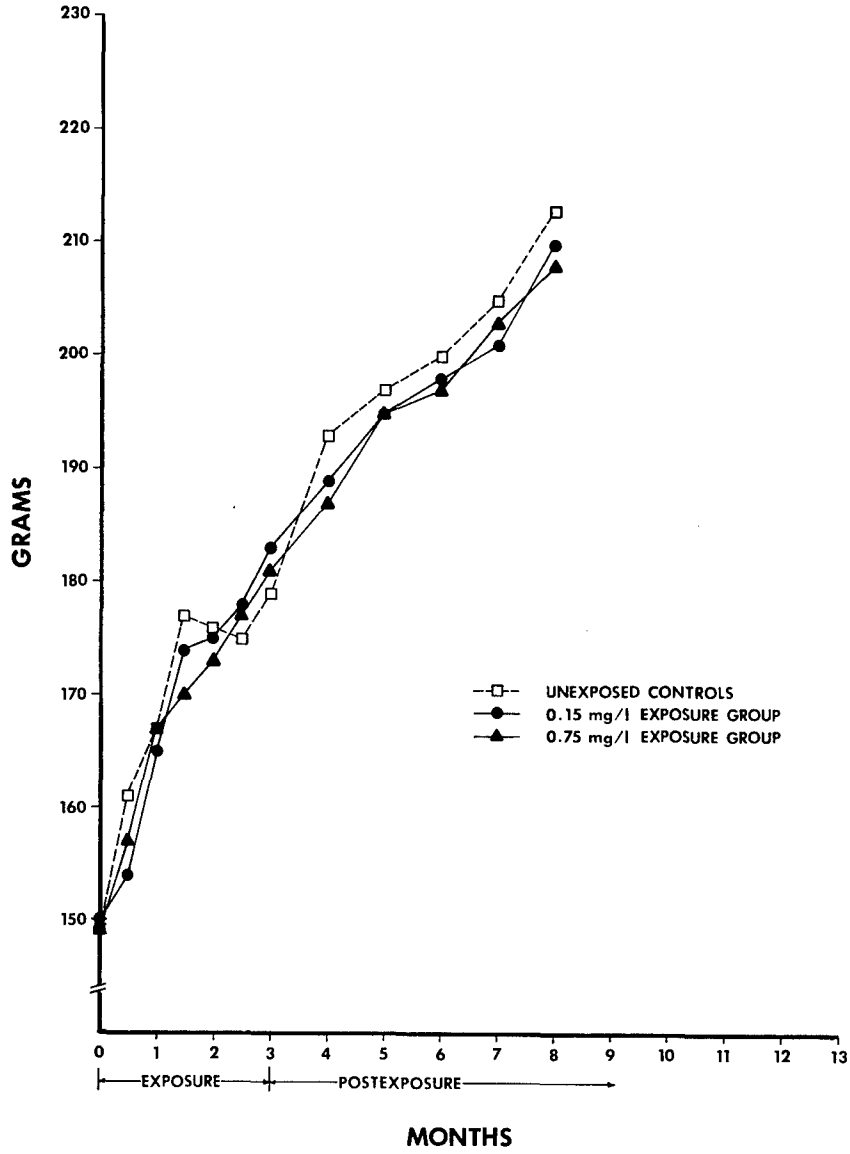


Figure 23. Growth of female rats exposed 90 days continuously to inhaled JP-5 vapors.

The growth of beagle dogs exposed to JP-5 vapors was unaffected after 90 day continuous exposure as shown in Figure 24. Clinical measurements made on blood samples taken from the dogs at biweekly intervals showed a slight but significant decrease in red blood cell counts and in hemoglobin levels in the 0.75 mg/liter JP-5 exposed group of dogs. A trend toward lower hematocrit levels was also observed. Also noted in this group of dogs were significant differences from control dogs in serum albumin levels which resulted in decreased A/G ratios as shown in Tables 19 and 20 respectively. These data show a trend toward lower values in the dogs exposed to the highest concentration of JP-5 but are still within normal biological limits except for one dog that had an inverted A/G ratio for the last two sampling periods. Blood urea nitrogen levels were also increased at various sampling periods but this change occurred only sporadically.

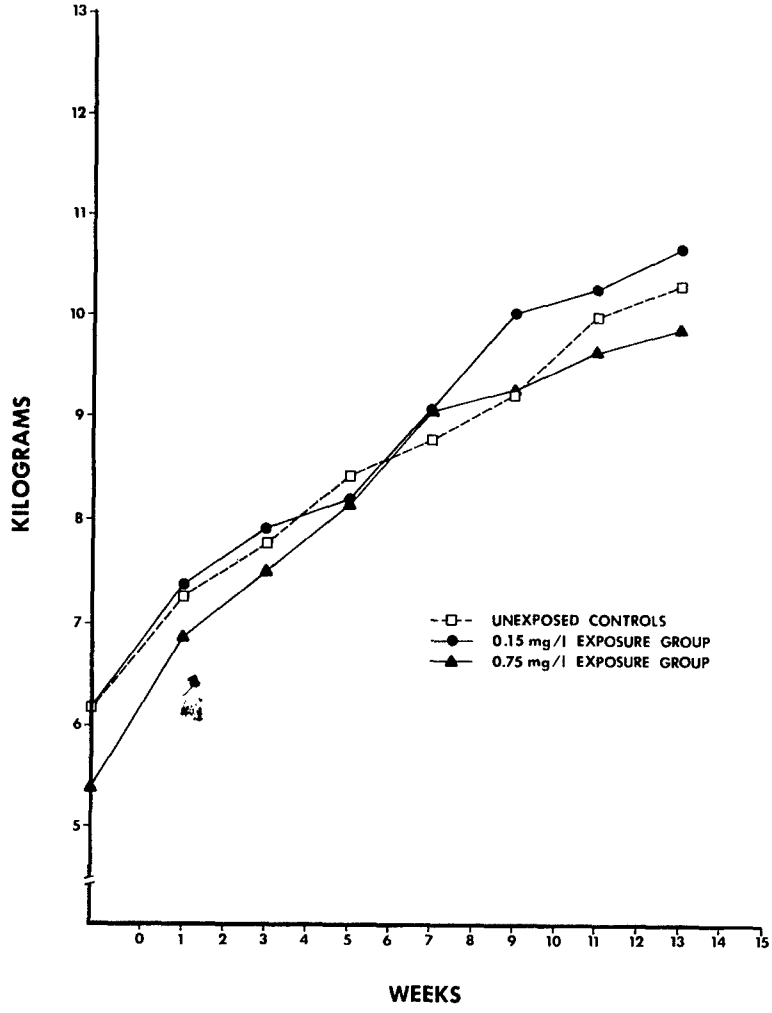


Figure 24. Growth of beagle dogs exposed 90 days continuously to inhaled JP-5 vapors.

TABLE 19. SERUM ALBUMIN DETERMINATIONS IN BEAGLE DOGS[a] EXPOSED CONTINUOUSLY TO JP-5 VAPORS FOR 90 DAYS

Weeks of Exposure	Unexposed Controls	0.15 mg/L Exposure Group	0.75 mg/L Exposure Group
-4	3.4 (.14)	3.2 (.14)	3.4 (.24)
-1	3.4 (.10)	3.2 (.19)	3.3 (.12)
1	3.4 (.11)	3.2 (.17)	[b]
3	3.5 (.12)	3.1[d] (.18)	3.2[c] (.14)
5	3.5 (.15)	3.2[c] (.23)	3.0[d] (.11)
7	3.5 (.14)	3.3 (.20)	3.1[d] (.12)
9	3.6 (.08)	3.3[d] (.15)	3.2[d] (.08)
11	3.5 (.10)	3.4[c] (.15)	3.2[d] (.14)
13	3.6 (.19)	3.5 (.14)	3.4 (.14)

[a] N=6
[b] Sample lost
[c] Significant at the 0.05 level
[d] Significant at the 0.01 level
( ) Standard Deviations

TABLE 20. ALBUMIN/GLOBULIN RATIOS DETERMINED IN BEAGLE DOGS[a] EXPOSED CONTINUOUSLY TO JP-5 VAPORS FOR 90 DAYS

Exposure	Unexposed Controls	0.15 mg/L Exposure Group	0.75 mg/L Exposure Group
-4	1.6 (.09)	1.5 (.03)	1.7 (.27)
-1	1.7 (.16)	1.6 (.19)	1.7 (.22)
1	1.6 (.05)	1.6 (.10)	-[b]
3	1.6 (.24)	1.5 (.20)	1.6 (.10)
5	1.6 (.04)	1.6 (.17)	1.5 (.15)
7	1.6 (.10)	1.6 (.23)	1.4[c] (.23)
9	1.7 (.07)	1.7 (.23)	1.4 (.23)
11	1.7 (.20)	1.6 (.19)	1.3 (.38)
13	1.6 (.07)	1.5 (.13)	1.3 (.29)

[a] N=6
[b] Sample lost
[c] Significant at the 0.01 level
( ) Standard Deviations

Red blood cell fragility was significantly increased in female beagles (Figure 25) exposed to the 0.75 mg/liter JP-5 vapor concentration and although not statistically significant was also increased in the lower exposure group. The increase in red blood cell fragility was significant for both exposure groups of male dogs and as seen in Figure 26 appears to be dose related. These findings may help to explain the decreases in red blood cell counts and hemoglobin levels measured in these animals.

Blood samples were collected from individual rats of the 25 from each study group sacrificed for examination upon completion of the 90 day exposure to JP-5 vapors. The samples were taken under anesthesia from the exposed brachial artery. Some difficulty was encountered in the collection of these samples and a large number of samples were partially hemolyzed. The data from hemolyzed and unhemolyzed samples were compared, and means found to be essentially identical. Therefore, data from hemolyzed samples were included for analysis of exposure effects.

The data are shown in Table 21 for female rats and in Table 22 for males. The elevation in potassium values might appear to be related to hemolysis but, as noted above, values from hemolyzed samples were not significantly different from controls. Probably the number of red cells hemolyzed was small even though serum color was visibly deepened. Statistical comparison within groups between hemolyzed and nonhemolyzed samples indicated that SGOT and total bilirubin determinations may have been affected by the degree of hemolysis present. Therefore, these determinations are not reported. Although a number of mean values for various clinical chemistry determinations are statistically significant the differences are within normal biological ranges and are not thought to be clinically important with the exception of BUN and creatinine changes which may be related to tissue changes observed.

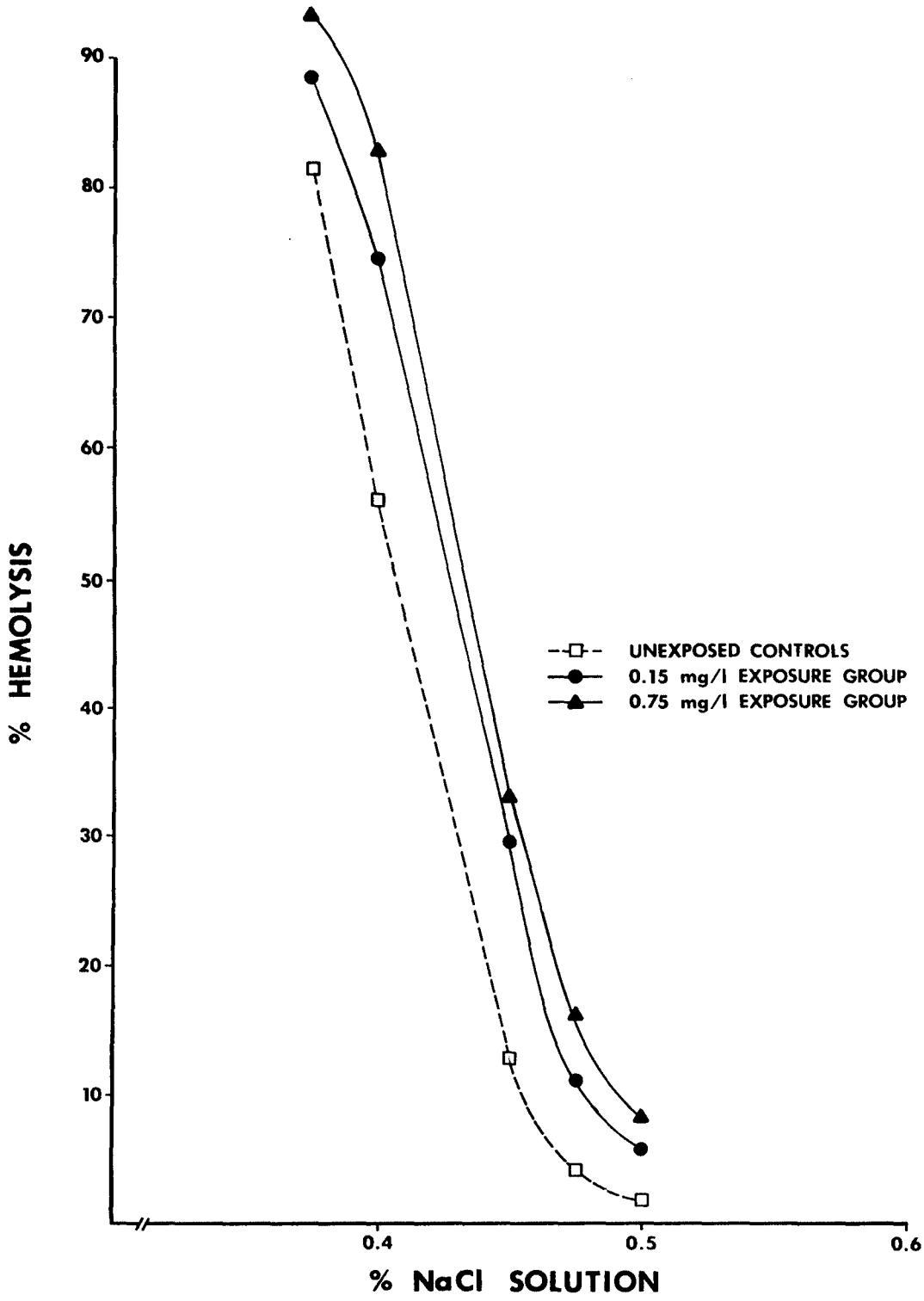


Figure 25. Effect of 90 day continuous exposure to JP-5 vapors on red blood cell fragility in female beagle dogs.

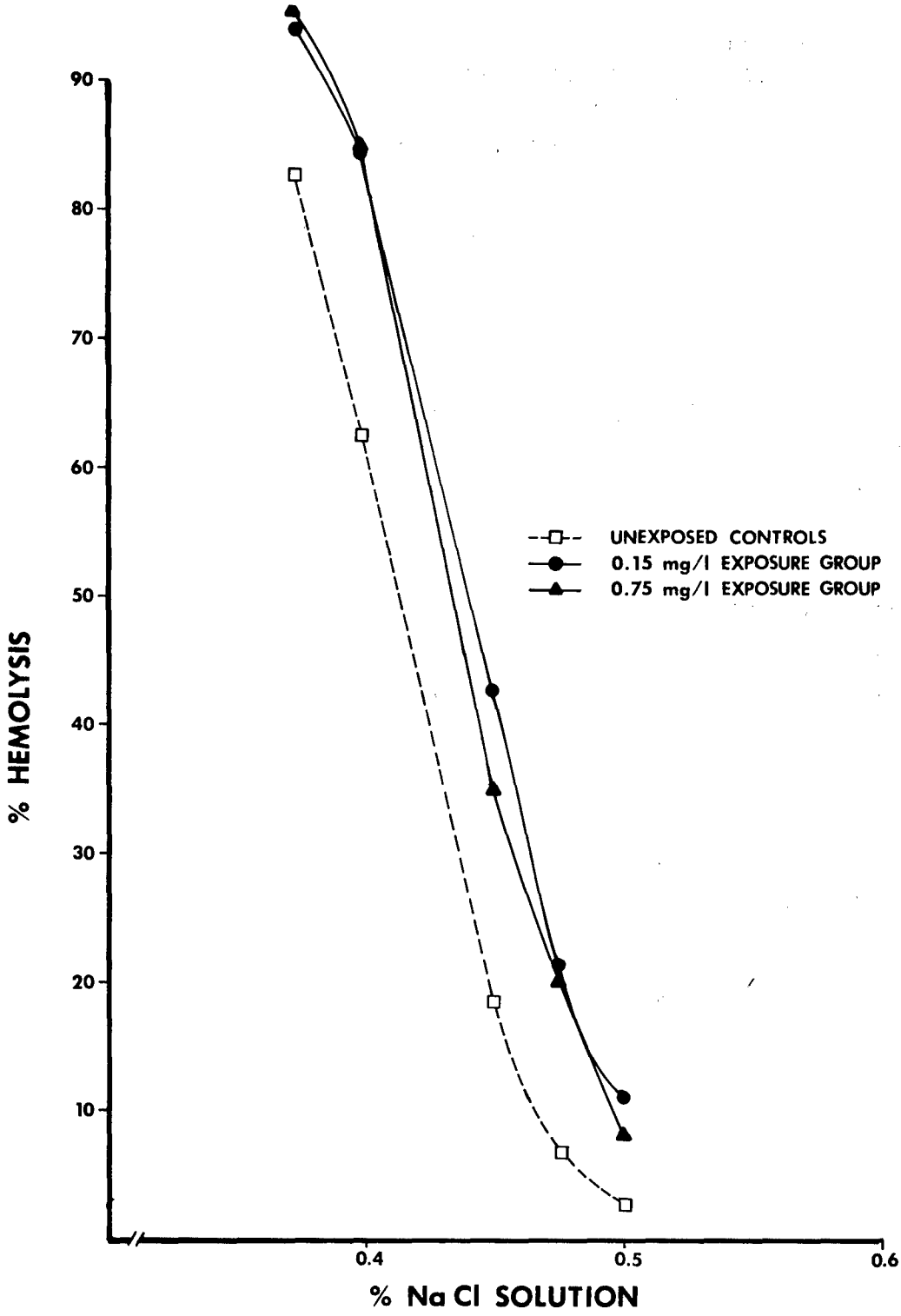


Figure 26. Effect of 90 day continuous exposure to JP-5 vapors on red blood cell fragility in male beagle dogs.

TABLE 21. HEMATOLOGY AND CLINICAL CHEMISTRY DETERMINATIONS ON FEMALE FISCHER 344 RATS AFTER 90 DAY CONTINUOUS EXPOSURE TO JP-5 VAPORS

(Group Mean Values)

Clinical Measurement	Unexposed Controls	0.15 mg/L Exposure Group	0.75 mg/L Exposure Group
RBC ($x10^6$)	7.65	7.91[b]	8.05[b]
WBC ($x10^3$)	4.0	5.2	5.1
HCT (Vols %)	42	42	42
HGB (g %)	14.0	14.5[b]	14.3[a]
Sodium (mEq/L)	144	150[b]	148[b]
Potassium (mEq/L)	5.5	6.6[b]	6.2
Calcium (mEq/L)	10.4	10.1	9.8[b]
Glucose (mg/dl)	139	130	114[a]
Total Protein (g/dl)	6.2	6.0	6.3
Albumin (g/dl)	4.5	4.2[b]	4.2[b]
Globulin (g/dl)	1.7	1.9[b]	2.1[b]
SGPT (IU/L)	54	50	49
Alkaline Phosphatase (IU/L)	10.0	13.5	11.8
BUN (mg/dl)	15.9	19.4[b]	19.6[b]
Creatinine (mg/dl)	0.43	0.46[a]	0.49[b]
A/G Ratio	2.8	2.3[a]	2.0[b]

[a]Significant at the 0.05 level.

[b]Significant at the 0.01 level.

TABLE 22. HEMATOLOGY AND CLINICAL CHEMISTRY DETERMINATIONS ON MALE FISCHER 344 RATS AFTER 90 DAY CONTINUOUS EXPOSURE TO JP-5 VAPORS

(Group Mean Values)

Clinical Measurement	Unexposed Controls	0.15 mg/L Exposure Group	0.75 mg/L Exposure Group
RBC ($x10^6$)	8.78	8.43[a]	8.41[a]
WBC ($x10^3$)	5.3	5.1	5.4
HCT (Vols %)	46	45[a]	42[a]
HGB (g %)	15.3	15.3	14.7[a]
Sodium (mEq/L)	148	147	149
Potassium (mEq/L)	5.7	6.3	6.3
Calcium (mEq/L)	11.0	10.4[a]	10.5[a]
Glucose (mg/dl)	171	147[a]	146[a]
Total Protein (g/dl)	6.4	6.2	6.5
Albumin (g/dl)	4.6	4.3[a]	4.3[a]
Globulin (g/dl)	1.9	1.9	2.1[a]
SGPT (IU/L)	52	58	53
Alkaline Phosphatase (IU/L)	16.1	16.4	13.8[a]
BUN (mg/dl)	14.9	17.0[a]	18.5[a]
Creatinine (mg/dl)	0.53	0.55	0.62[a]
A/G Ratio	2.5	2.3[a]	2.0[a]

[a]Significant at the 0.01 level.

Consistent with the changes seen in dogs are the slight reduction in RBC, HCT and hemoglobin in male rats and the decrease in serum albumin along with decreased A/G ratios seen in both male and female rats. In the rat there is also a significant increase in globulin levels. Blood urea nitrogen (BUN) and creatinine values are also significantly increased in both JP-5 exposure groups of rats. A similar increase was also noted in dogs but in that case the differences from unexposed control values were not statistically significant. The liver, spleen and kidneys of individual rats were weighed and the ratios of organ to body weights of JP-5 exposed rats were compared with their unexposed controls. The organ weights of the exposed rats were lower than controls consistent with decreased growth. The kidneys of male rats exposed to 0.75 mg/liter JP-5 were, however, as large as the control animals and the mean organ to body weight ratio for this group was significantly higher than the control value.

Histologic examination of rat tissues revealed some alveolar pneumonia and pulmonary congestion in female control rats which may have been related to the transient weight loss observed in this group of animals. Three female rats exposed to 0.15 mg/liter JP-5 for 90 days showed atypical hepatic hyperplasia but there were no remarkable changes noted in the higher exposure level group.

Kidney injury was seen in both exposed groups of male rats consisting of nephropathy characterized by multifocal tubular atrophy and of focal tubular necrosis at the cortico-medullary junction. Both lesions were more severe in the 0.75 mg/liter JP-5 exposure group and appeared to be related effects in that the more severe the nephropathy was the more severe the tubular necrotic lesions. These changes were not seen in unexposed controls or in female rats.

In mice, the lesions that appear to be related to JP-5 exposure were seen in liver and kidney. Mild, diffuse fatty change, consisting of numerous small cytoplasmic vacuoles within the hepatocytes was seen in one (2.7%) of the controls, 24 (72.7%) of the low dose, and eight (23.5%) of the high dose animals. The lesion was of approximately the same severity (mild) in most animals. Representative samples of this lesion were positive for fat with special stains. This lesion is probably the result of mild, reversible cell injury due to JP-5 exposure.

Mild, diffuse cytoplasmic vacuolization, consisting of "foamyness" or the presence of numerous minute, poorly defined vacuoles within the hepatocytes was present in seven (18.9%) of the controls, five (15.2%) of the low dose, and 15 (44.1%) of the high dose groups. Samples of this lesion were negative for both fat and glycogen with special stains. This change probably represents mild reversible injury to subcellular organelles, and may be the result of JP-5 exposure due to the increased incidence in the high dose mice. Transmission electron microscopy will be performed on representative samples of this lesion which may further clarify the nature of this change.

Mild, diffuse fatty changes, consisting of numerous large, clear, round, cytoplasmic vacuoles, occurred within the epithelium of the proximal convoluted renal tubules of four (11.7%) of the high JP-5 exposure group and in none of the others. Samples of this lesion were positive for fat with special stains. All of these animals also had fatty livers. The lesion probably represents a mild, reversible cell injury. The kidney changes seen in male rats were consistent with clinical chemistry findings of elevated blood urea nitrogen and creatinine levels as well as the increase in kidney to body weight ratio.

In both control female rats and mice there was a much greater uterine hydrometra than in the JP-5 exposed rodents. The difference appeared to be proportional to the dose level in the exposure groups. Diffuse endometrial gland cysts which appeared in moderately dilated endometrial glands occurred in 22% of the control mice and in neither of the exposure groups. We are not able to relate this difference to JP-5 exposure at this time but will examine the remaining female animals carefully as they die or are sacrificed to determine if JP-5 has some effect on ovarian function.

In dogs significant lesions were noted only in the liver. Diffuse, mild, cloudy swelling of hepatocytes occurred in all of the high level exposure, in two (33.3%) of the low dose dogs, and in none of the controls. Affected hepatocytes were moderately swollen, pale, and had a "foamy" cytoplasm. The cytoplasm was negative for both fat and glycogen with special stains. Transmission electron microscopy, to be performed later, may clarify the nature of this lesion. The lesion probably represents mild reversible damage to subcellular organelles. It appears to be dose related and is probably the result of JP-5 exposure.

Hemorrhagic necrosis was noted in the liver of one dog. This was a mild focal process probably unrelated to JP-5 exposure.

The study will be continued through the next year and further information will be given in succeeding annual reports.

A Subchronic Inhalation Toxicity Study of 90 Day Continuous Inhalation Exposure to Diesel Fuel Marine

Diesel Fuel Marine (DFM) is the standard fuel used by a large number of the ships in the U. S. Naval fleet. Because of the conditions on board a ship where DFM would be stored and used in confined spaces and personnel might be exposed for the entire length of a cruise, a continuous exposure for 90 days was chosen for this study.

Diesel Fuel Marine is derived from traditional petroleum sources and 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, leucocytes and platelets being found in humans after long-term exposure to benzene at high concentrations (Greenberg, 1939; Hardy and Elkins, 1948; Aksoy et al., 1972).

Preliminary to the subchronic exposure study, saturated vapor exposures were conducted. Five male rats were exposed to air saturated with DFM vapors (approximately 2.6 mg/liter) for 8 hours/day for 5 days. There were no deaths nor were there any overt signs of toxicity in animals.

Materials and Methods

Groups of 6 beagle dogs (3 male, 3 female), 150 Fischer 344 rats (75 male, 75 female), and 140 C57B1/6 female mice were continuously exposed to concentrations of 0.05 mg/liter or 0.30 mg/liter DFM FOR 90 days in inhalation chambers. Another group consisting of similar numbers of animals was housed in a separate facility and served as controls. The chambers were maintained at a slightly reduced operating pressure to prevent the escape of contaminant into the work area.

The DFM used in this study was supplied to the Toxic Hazards Research Unit by the U. S. Navy. The material was received from a major petroleum company in 55 gallon steel containers which were tested by quality control procedures to determine their uniformity.

DFM vapors were introduced into the domes by passing liquid DFM through a flowmeter to a heated glass evaporator column. The air passing through the evaporator then carried the DFM vapors into the main air supply for the exposure chamber. Concentration of DFM was continuously analyzed by pumping air samples from each chamber into a total hydrocarbon analyzer calibrated with known concentrations of propane. The systems used for generation of DFM vapors and for analysis of chamber concentrations were the same as those used for conducting JP-5 exposures for animals and were shown in

Figures 16 and 18 respectively. Because of the application of heat during the generation of DFM vapors and the subsequent cooling of the material as it entered the chamber air supply, a small amount of condensate aerosol was formed. A Royco® Particle Counter equipped with a 508 digital monitor was used to measure the aerosol production.

At the conclusion of the exposure phase of the study, all of the dogs, 1/3 of the rats and 1/3 of the mice were sacrificed for gross and histopathologic examination to detect any pathologic lesions caused by exposure to DFM. The remaining animals are being held for 19 months at which time 1/2 of the rodents will be sacrificed for examination. Any remaining rodents will be held until the cumulative mortality of the species reaches 90% of the original number of animals. At that time, all of the representatives of that species will be sacrificed.

The animals were observed hourly during the exposure phase of the study for signs of toxic stress. Dogs and rats were weighed individually every two weeks while mice were weighed in groups on a monthly schedule.

Blood samples were taken from fasted dogs at biweekly intervals and clinical determinations were made for the series of tests shown in Table 23. Red blood cell osmotic fragility tests were performed on dog blood using a modification of the method described by Davidsohn and Henry (1969). The clinical hematology and chemistry tests, shown in Table 23, were also performed on the rats sacrificed at the end of the exposure and will be performed on the rats sacrificed at 19 months postexposure.

Livers, kidneys and spleens taken from the dogs and rats that were sacrificed were weighed. The same organs will also be weighed during the scheduled rat sacrifice at 19 months.

Experimental Results

The exposure phase of the study was just concluded. No overt signs of toxicity were exhibited in any of the animals during the 90 day exposure period. One female control rat died on test day 84. A few scattered deaths in mice occurred during the exposure, but there appeared to be no direct relationship between these deaths and exposure to DFM vapors. A summary of the animal deaths during the exposure is presented in Table 24.

Gross pathology examination of the dogs and mice that were sacrificed at the conclusion of the 90 day exposure did not reveal any unusual or increased incidence of lesions when compared to control animals. The results of gross pathology examination of the sacrificed rats as well as the results of the histopathologic examination of all species were not available for this report.

TABLE 23. CLINICAL HEMATOLOGY AND CHEMISTRY TESTS PERFORMED ON DOGS AND RATS EXPOSED TO DFM VAPORS

Hematology	Chemistry
Hematocrit	Sodium
Hemoglobin	Potassium
Total RBC	Calcium
Total WBC	Albumin/Globulin
Differentials	Total Protein
Mean Corpuscular Volume (MCV)	Glucose
Mean Corpuscular Hemoglobin (MCH)	Alkaline Phosphatase
Mean Corpuscular Hemoglobin Concentration (MCHC)	SGPT
	SGOT
	Bilirubin
	Creatinine
	BUN

TABLE 24. MORTALITY RATIOS[a] OF ANIMALS DURING 90 DAY CONTINUOUS EXPOSURE TO DFM VAPORS

Species	Unexposed Controls	0.05 mg/L Exposure Group	0.30 mg/L Exposure Group
Dogs			
Male	0/3	0/3	0/3
Female	0/3	0/3	0/3
Mice	2/140	4/140	5/140
Rats			
Male	0/75	0/75	0/75
Female	1/75	0/75	0/75

[a]Number died over number exposed

The effects of DFM exposure on animal body weight were mixed. Dog body weights are shown in Figure 27. The exposed dogs were generally heavier and gained weight at a slightly faster rate than the control dogs. Male rat body weights are shown in Figure 28. The weights of exposed male rats were significantly ($p < 0.01$) depressed after two weeks of exposure. These differences remained constant throughout the exposure. After 6 weeks of exposure to DFM, a dose response relationship was apparent when the weights of the male rats exposed to 0.30 mg/liter were significantly ($p < 0.01$) less than the male rats exposed to 0.05 mg/liter. This relationship also continued

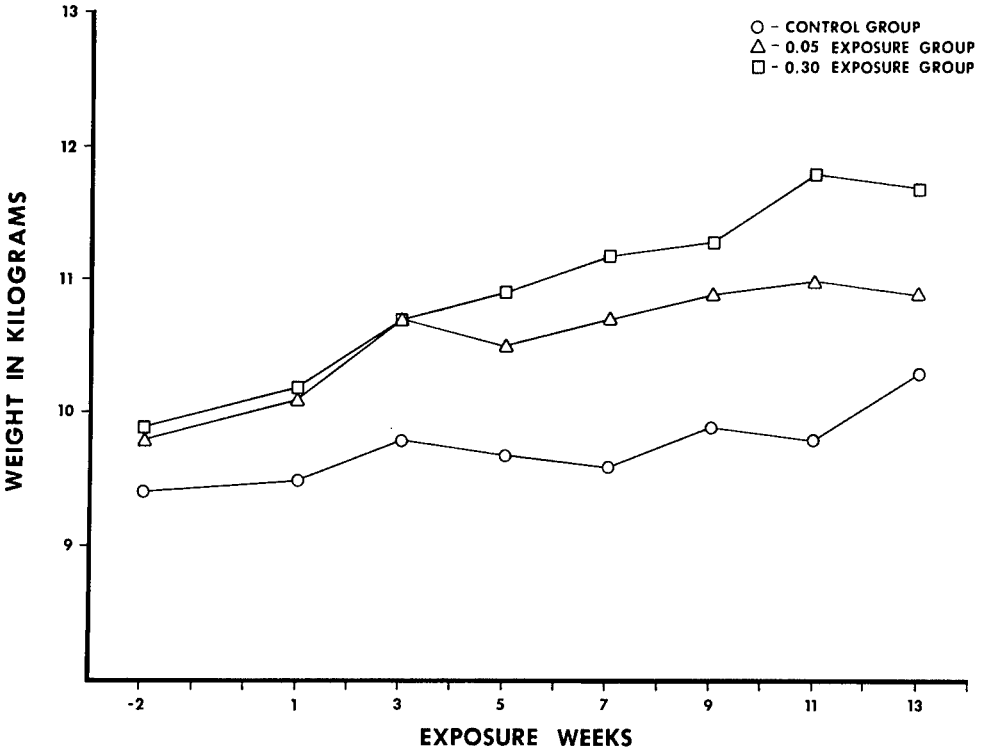


Figure 27. Effect of DFM exposure on dog body weight.

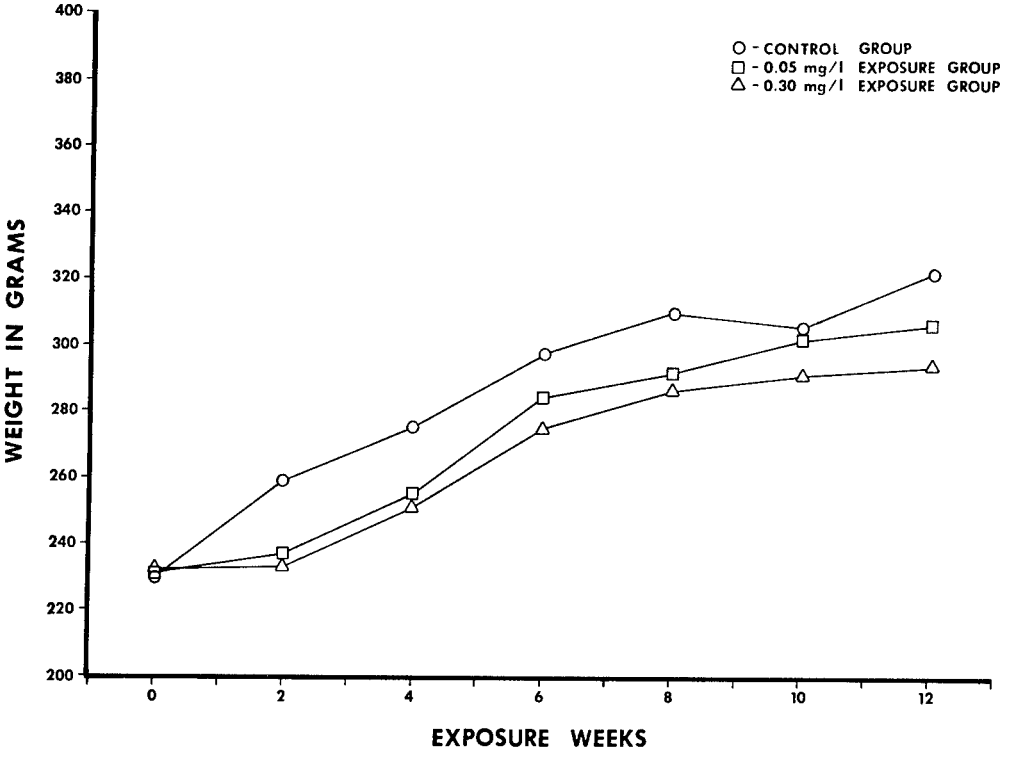


Figure 28. The effect of DFM exposure on male rat body weight.

through the exposure. The body weights of female rats are shown in Figure 29. From time to time, one of the test groups would show statistical significance when the weights were compared with the control group. However, these events were scattered and no trend toward an increase or decrease in female rat weight was evident. DFM exposure did not affect the weight of mice.

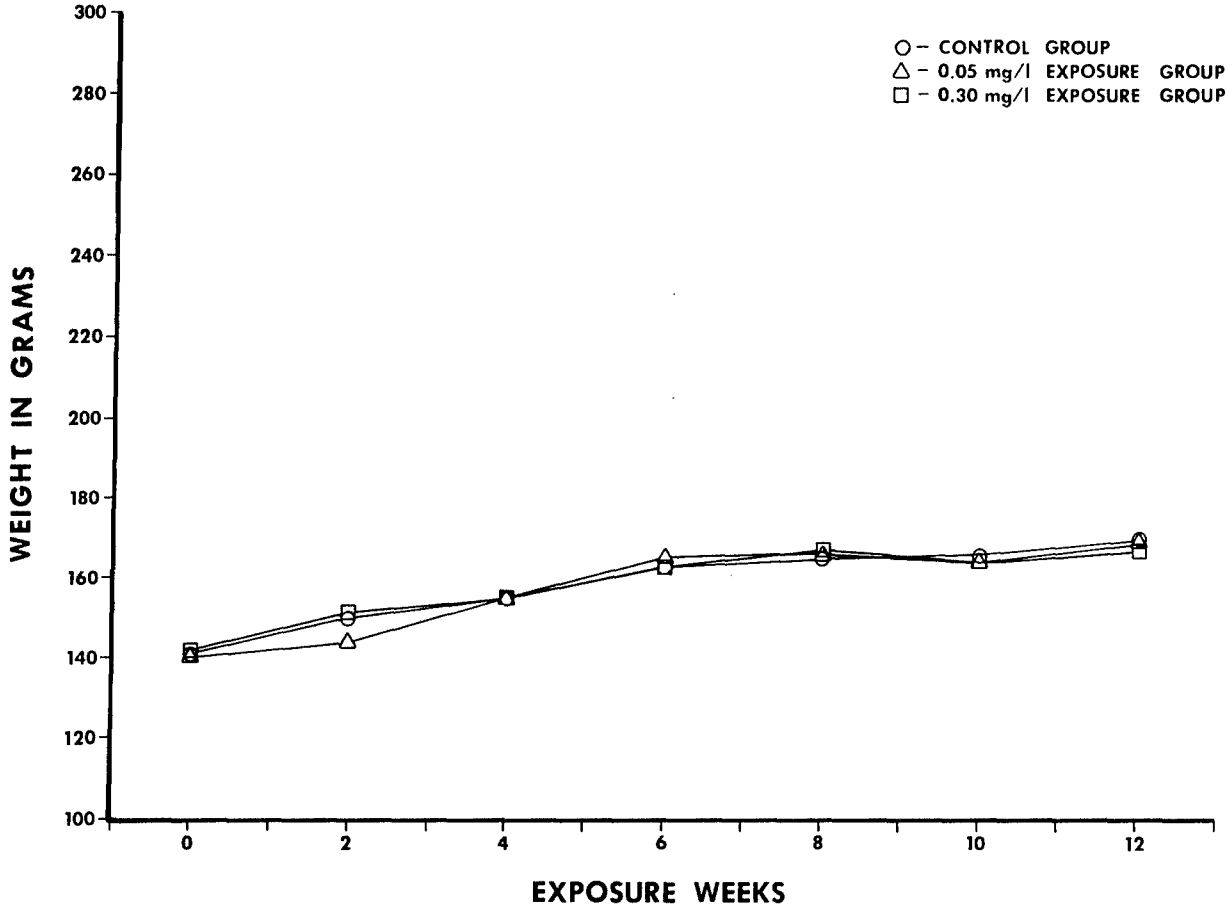


Figure 29. The effect of DFM exposure on female rat body weight.

Hematology and chemistry values in male and female dogs were essentially normal throughout the exposure period. One of the male dogs exposed to 0.30 mg/liter DFM did show an elevated SGPT level approximately one half way through the exposure. This increase subsequently returned to normal. Occasionally statistically significant differences would be found in some of the parameters being measured, but the differences between the test and control values were never large enough to be considered clinically significant.

Red blood cell osmotic fragility tests were conducted on dog blood on a biweekly schedule. The results of these tests are presented in Table 25. There appeared to be a very slight increase in the osmotic fragility of exposed dogs when compared to controls, particularly between the 0.475% and 0.40% salt concentrations.

TABLE 25. EFFECT OF 90 DAY CONTINUOUS EXPOSURE TO DFM ON DOG RBC OSMOTIC FRAGILITY[a]

Sample Period (Days)	Control	DFM 0.05 mg/l	DFM 0.30 mg/l	
14 pre	8.8 ± 6.3	7.9 ± 4.6	4.9 ± 3.4	
6	2.8 ± 1.2	4.4 ± 2.2	3.7 ± 1.2	
20	2.9 ± 1.0	6.5 ± 0.8[c]	4.5 ± 2.6	0.50% Salt
34	3.4 ± 3.3	6.1 ± 2.1	6.2 ± 4.8	Concentration
48	5.5 ± 4.3	8.8 ± 3.1	9.3 ± 8.5	
62	4.7 ± 4.2	7.7 ± 2.5	11.6 ± 13.5	
76	2.9 ± 0.9	8.3 ± 3.3	6.9 ± 6.3	
14 pre	20.9 ±11.2	16.9 ± 7.9	10.5 ± 7.8	
6	5.4 ± 2.1	9.9 ± 3.2	8.0 ± 4.5	
20	7.5 ± 3.4	13.7 ± 2.1	10.5 ± 6.8	0.475% Salt
34	9.4 ± 6.8	14.2 ± 3.1	13.2 ± 8.2	Concentration
48	12.3 ± 7.8	18.0 ± 5.3	19.3 ± 16.5	
62	9.0 ± 6.3	17.2 ± 4.7	25.1 ± 23.1	
76	9.1 ± 5.1	18.3 ± 7.9	19.8 ± 15.6	
90	6.9 ± 2.9	18.2 ± 7.7	16.2 ± 13.2	
14 pre	44.5 ±15.1	38.0 ± 13.1	29.9 ± 15.6	
6	14.3 ± 6.7	23.2 ± 5.5[b]	19.9 ± 12.4	
20	13.8 ± 6.7	28.7 ± 6.1[c]	23.6 ± 13.7	0.45% Salt
34	21.2 ±14.5	30.1 ± 3.9	28.8 ± 17.8	Concentration
48	27.8 ±13.1	39.3 ± 8.5	37.2 ± 22.3	
62	21.2 ±13.8	41.5 ± 9.7[b]	44.7 ± 27.9	
76	20.6 ± 9.5	38.8 ± 11.9[b]	38.1 ± 23.1	
90	14.8 ± 5.2	37.7 ± 13.9[c]	31.9 ± 19.3	
14 pre	89.9 ± 3.8	86.2 ± 8.4	75.9 ± 16.3	
6	57.9 ±16.6	70.6 ± 9.7	65.3 ± 17.2	
20	56.2 ±15.3	77.8 ± 6.7[b]	72.1 ± 18.1	0.40% Salt
34	62.2 ±16.8	80.6 ± 5.8[b]	75.7 ± 19.3	Concentration
48	72.9 ±13.7	86.9 ± 6.4[b]	78.5 ± 14.0	
62	67.2 ±19.1	86.4 ± 8.5[b]	85.4 ± 15.5	
76	66.4 ±15.7	84.7 ± 8.5[b]	80.1 ± 18.1	
90	55.6 ±12.7	82.8 ± 10.2[c]	73.9 ± 18.5	
14 pre	97.1 ± 1.2	94.9 ± 3.5	90.9 ± 6.8	
6	79.8 ±14.0	90.4 ± 3.2	84.1 ± 10.9	
20	80.5 ± 9.9	92.7 ± 3.4[b]	88.9 ± 11.3	0.375% Salt
34	82.3 ±11.6	93.4 ± 3.1	89.1 ± 10.5	Concentration
48	91.1 ± 6.4	95.7 ± 2.5	93.7 ± 5.4	
62	83.4 ±13.5	94.5 ± 4.8	93.2 ± 7.7	
76	84.0 ±11.1	93.5 ± 4.4	91.3 ± 9.5	
90	78.9 ± 8.9	91.9 ± 5.1[b]	87.4 ± 9.9	

a - % hemolysis, mean ± S.D.; N=6 dogs/group, 3♂, 3♀
b - significant test vs. control, $p < 0.05$
c - significant test vs. control, $p < 0.01$

Many of the hemolysis values obtained from the 0.05 mg/liter test group were found to be significantly different than controls, but the hemolysis values of the 0.30 mg/liter DFM test group, while being similar to the 0.05 mg/liter group, were not found to be significantly different from controls. This is obviously a reflection of the variance of the samples in the 0.30 mg/liter group.

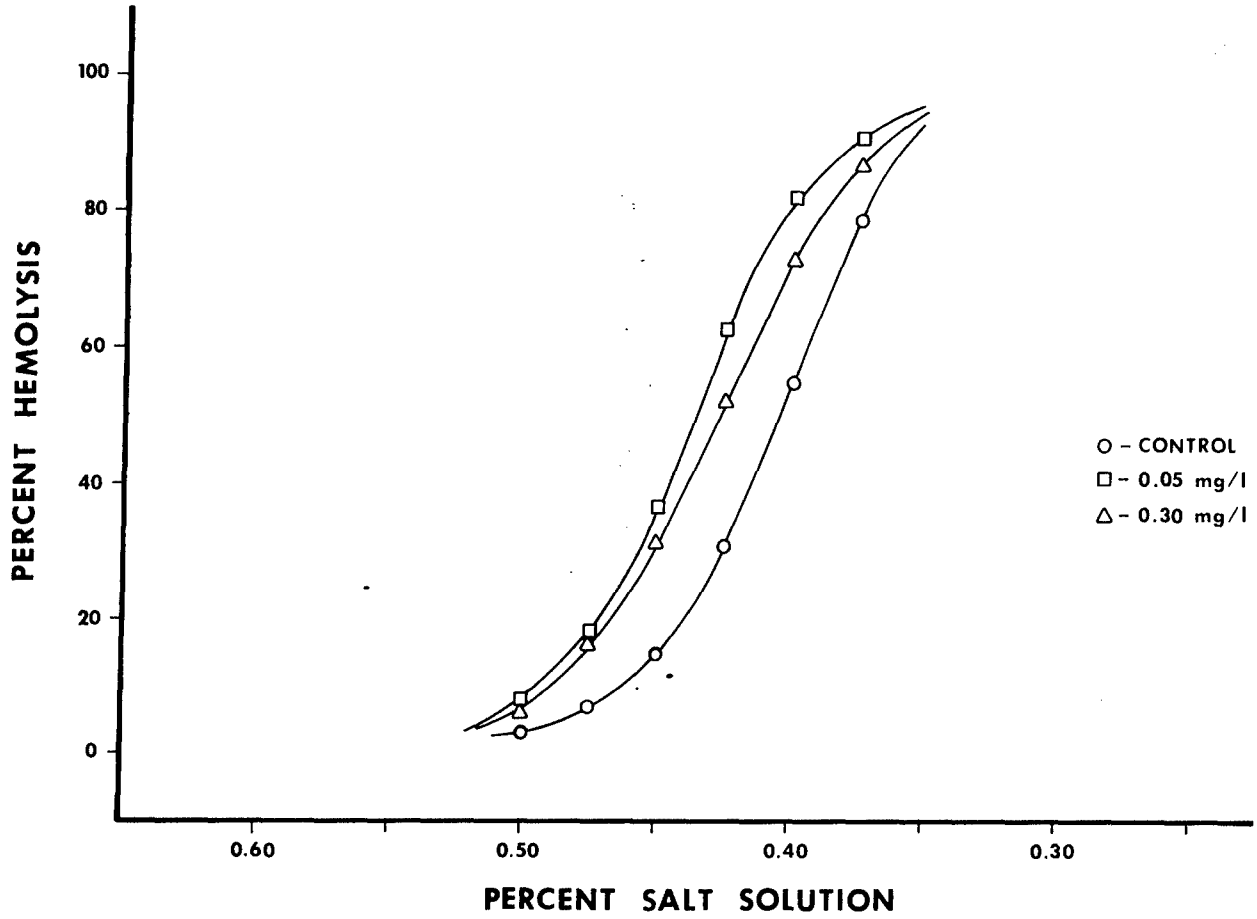


Figure 30. Mean RBC osmotic fragility in dogs continuously exposed to DFM vapors for 90 days.

Figure 30 is a plot of the hemolysis values obtained at the conclusion of the 90 day exposure. Here again, increased osmotic fragility is indicated by the fact that both of the exposure group curves are elevated over the control and that these curves are slightly flattened especially between the 0.50% and 0.45% salt concentration points. These data indicate a slight increase in the osmotic fragility of red blood cells of dogs exposed to DFM vapors. No dose response relationship was evident. The increases were minimal, variable and would not be considered clinically significant or detrimental to the health of the animal.

The mean hematology and chemistry values of rats are shown in Tables 26 and 27. The red blood cell counts of exposed rats were slightly lower than controls, but the values were well within normal limits. Serum glutamic pyruvic transaminase (SGPT) values were also reduced in the exposed rats when compared to respective controls. Leukopenia was evident in the exposed rats, especially in the 0.30 mg/liter exposure groups where the cell counts approached the lower end of the normal limits. The mean WBC of this exposure group was also greater than two standard deviations less than the means of the respective control groups, indicating that the reductions were of biological significance.

TABLE 26. MEAN HEMATOLOGICAL AND CLINICAL CHEMISTRY VALUES OF MALE RATS AFTER 90 DAY CONTINUOUS EXPOSURE TO DFM VAPORS

Clinical Measurements	Unexposed Controls	0.05 mg/L Exposure Group	0.30 mg/L Exposure Group
RBC ($x10^6$)	8.4	8.2	7.7[a]
WBC ($x10^3$)	7.8	5.6[a]	5.2[a]
HCT (Vol %)	48	47	45[a]
HGB (g/dl)	15.5	15.3	14.7[a]
Total Protein (g/dl)	6.9	6.8	7.0
Albumin (g/dl)	4.5	4.4	4.5
Globulin (g/dl)	2.3	2.4	2.5
Glucose (mg/dl)	175	144[a]	130[a]
Potassium (mEq/L)	6.7	6.2	6.6
Calcium (mg/dl)	12.2	11.9	12.1
Sodium (mEq/L)	152	149[a]	149[a]
Total Bilirubin (mg/dl)	0.44	0.44	0.44
BUN (mg/dl)	17	17	17
Creatinine (mg/dl)	0.69	0.65	0.67
SGPT (IU/L)	55	42[a]	34[a]
SGOT (IU/L)	96	87	86
Alkaline Phosphatase (IU/L)	16.0	17.1	16.2

[a]Significant at the 0.01 level.

TABLE 27. MEAN HEMATOLOGICAL AND CLINICAL CHEMISTRY VALUES OF FEMALE RATS AFTER 90 DAY CONTINUOUS EXPOSURE TO DFM VAPORS

Clinical Measurements	Unexposed Controls	0.05 mg/L Exposure Group	0.30 mg/L Exposure Group
RBC ($x10^6$)	7.2	6.8[a]	6.9
WBC ($x10^3$)	7.7	6.8	5.3[a]
HCT (Vol %)	43	41	42
HGB (gm/dl)	14.5	14.1	14.2
Total Protein (gm/dl)	7.0	6.3	6.7
Albumin (gm/dl)	4.7	4.2	4.2[a]
Globulin (gm/dl)	2.3	2.1	2.5
Glucose (mg/dl)	134	90[a]	99[a]
Potassium (mEq/L)	5.3	5.4	5.8
Calcium (mg/dl)	12.5	11.5[a]	11.5[a]
Sodium (mEq/L)	150	147[a]	149
Bilirubin (mg/dl)	0.62	0.62	0.51
BUN (mg/dl)	16.9	17.0	17.4
Creatinine (mg/dl)	0.56	0.49	0.56
SGPT (IU/L)	46	38	39
SGOT (IU/L)	96	103	90
Alkaline Phosphatase (IU/L)	9.2	10.9	14.4[a]

[a]Significant at the 0.01 level.

The weights of dog livers, spleens and kidneys measured at the sacrifice after 90 days of exposure along with the organ to body weight ratios are shown in Table 28. The mean kidney/body weight ratio in dogs exposed to 0.30 mg/liter DFM is less than the controls because of the increased body weights of the exposed animals and probably does not reflect a toxic effect on this organ. There was an increase in the liver size in dogs exposed to 0.30 mg/liter DFM. The liver/body weight ratio was also significantly higher in this group even though the body weights were greater than controls. These results may be an indication of liver sensitivity to continuous exposure to high levels of DFM.

TABLE 28. THE EFFECT OF INHALATION EXPOSURE TO DFM ON DOG ORGAN WEIGHT[a]

	Control	0.05 mg/L	0.30 mg/L
Body Weight (kg)	10.25 ± 1.45	10.92 ± 2.67	11.69 ± 2.69
Liver Weight (g)	288 ± 45	329 ± 58	377 ± 59[b]
Liver/100 g body	2.83 ± 0.34	3.01 ± 0.56	3.29 ± 0.38[b]
Spleen Weight (g)	78 ± 20	78 ± 29	61 ± 38
Spleen/100 g body	0.76 ± 0.14	0.72 ± 0.14	0.52 ± 0.27
Kidney Weight (g)	54 ± 7	49 ± 12	53 ± 9
Kidney/100 g body	0.53 ± 0.04	0.47 ± 0.12	0.46 ± 0.06[b]

[a]Mean ± S.D., N=6/group

[b]Significant test vs. control, $p < 0.05$.

Male rat organ weights are shown in Table 29 and female rat organ weights are shown in Table 30. Both male and female rats exposed to 0.05 mg/L DFM had decreased liver and kidney weights. Since the body weights of the female rats were not significantly different at the time of organ weight measurement, a comparison of organ/body weight ratios is acceptable. It can be seen from Table 30 that the liver and kidney body weight ratios are also significantly ($p < 0.01$) less than controls. Kidney and kidney/body weight ratios were also reduced in females exposed to the higher concentration of DFM. The mean liver weight of both sexes of rats exposed to 0.30 mg/L DFM appeared to be unaffected by exposure.

TABLE 29. THE EFFECT OF INHALATION EXPOSURE TO DFM ON MALE RAT ORGAN WEIGHT

	Control	0.05 mg/L	0.30 mg/L
Body Weight (g)	318.9 ± 16.1	302.8 ± 13.5[b]	302.5 ± 13.1[b]
Liver Weight (g)	8.8 ± 0.6	8.4 ± 0.7	8.8 ± 0.5
Liver/100 g body	2.77 ± 0.15	2.79 ± 0.22	2.91 ± 0.12[b]
Spleen Weight (g)	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.2
Spleen/100 g body	0.15 ± 0.04	0.16 ± 0.03	0.18 ± 0.07
Kidney Weight (g)	2.1 ± 0.2	1.9 ± 0.2[c]	2.0 ± 0.2
Kidney/100 g body	0.67 ± 0.06	0.66 ± 0.07	0.69 ± 0.04

[a]Mean ± S.D., N=25/group

[b]Significant at the 0.01 level.

[c]Significant at the 0.05 level.

However, when the decreased body weights of the males exposed to this high concentration of DFM are considered, the mean liver weight would reflect a slight increase. This increase is significant ($p < 0.01$) when the liver/body weight ratio is examined.

TABLE 30. THE EFFECT OF INHALATION EXPOSURE TO DFM ON FEMALE RAT ORGAN WEIGHT[a]

	Control	0.05 mg/L	0.30 mg/L
Body Weight (g)	168.6 ± 6.3	172.0 ± 6.2	172.2 ± 6.7
Liver Weight (g)	4.8 ± 0.4	4.1 ± 0.3[b]	4.8 ± 0.5
Liver/100 g body	2.84 ± 0.22	2.37 ± 0.15[b]	2.78 ± 0.26
Spleen Weight (g)	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
Spleen/100 g body	0.22 ± 0.04	0.22 ± 0.03	0.19 ± 0.04
Kidney Weight (g)	1.4 ± 0.1	1.1 ± 0.1[b]	1.2 ± 0.1[b]
Kidney/100 g body	0.80 ± 0.04	0.66 ± 0.04[b]	0.71 ± 0.04[b]

[a]Mean ± S.D., N=24/group

[b]Significant at the 0.01 level.

Organ weights can be used as an indicator of sensitivity of an organ to exposure to a chemical agent. The variations in the weights of the livers and kidneys of rats exposed to DFM vapors establish no clear cut pattern toward a specific target organ. The organ weights may become more meaningful when histopathologic examination of the tissues is complete.

Examination of the DFM in the drums obtained for the study revealed that several were different in coloration from the majority. Subsequent investigation of the supply of DFM delivered by the petroleum refiner disclosed that they had been unable to fill the entire order of DFM and had substituted several drums of a fuel identified as Diesel Fuel Supreme, a more expensive fuel. None of the latter fuel was used and since there was a sufficient quantity of DFM to complete the exposure phase of the study it was not necessary to obtain additional supplies.

The DFM routinely used was yellow colored and the diesel fuel supreme was red. Routine gas chromatographic quality control samples revealed the red fuel to be different in composition from the yellow fuel and therefore the yellow DFM was distilled to determine that it met military fuel specifications that 90% distillation be achieved below 357 C with an endpoint no greater than 385 C. In the distillation test conducted 90% distillation was reached at 319 C which was satisfactory for this fuel.

Headspace vapor samples of DFM equilibrated at 30 C for 15 minutes were injected into the gas chromatograph as described in the JP-5 section of this report. A typical chromatogram of raw DFM is shown in Figure 31. Waste DFM, chromatographed in the same manner is shown in Figure 32 which illustrates the change in character of the used fuel after volatilization of the majority of the lighter fractions. The chromatograph used was a Varian 1200 with a flame ionization detector. The column was a 12 foot long, 1/8 diameter inch stainless steel coiled tube using a 10% SE30 Chromosorb W-AW packing and the system was held isothermal at 40 C.

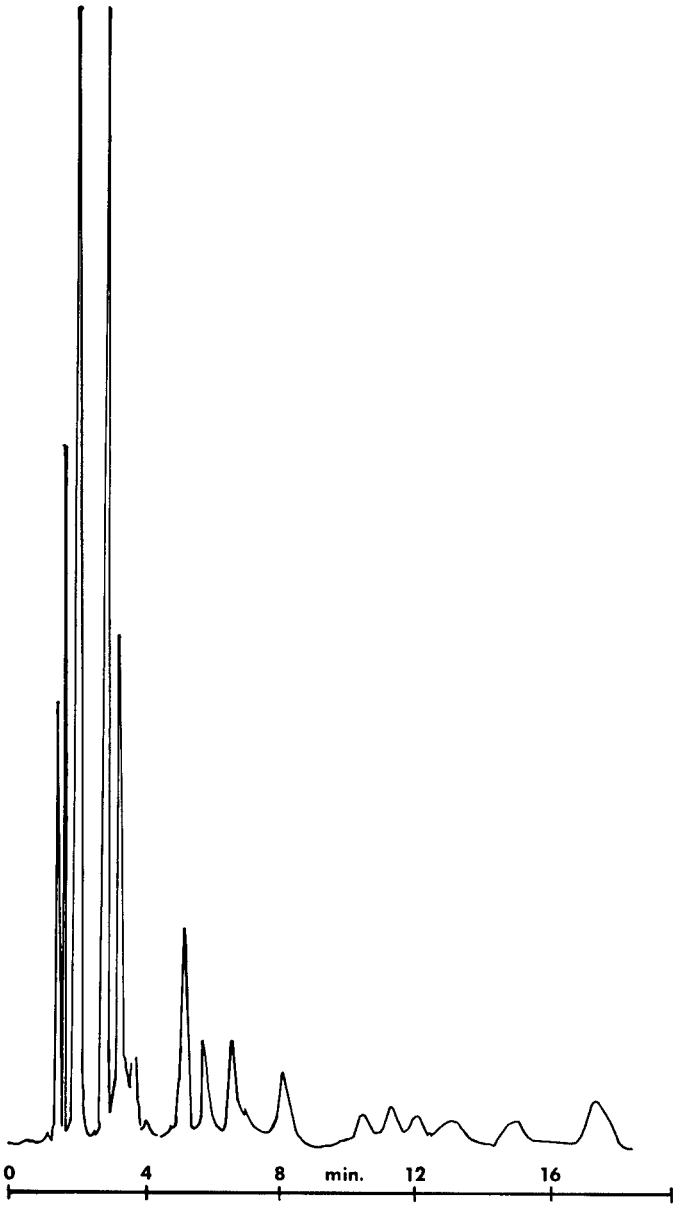


Figure 31. Chromatogram of headspace gas over raw DFM. Quality Control - DFM. 20 µl injection, headspace equilibrated at 30 C for 15 minutes. Varian 1200, FID, 12 ft. x 1/8 in. SS, 10% SE30 Chromosorb W-AW, isothermal 40 C.

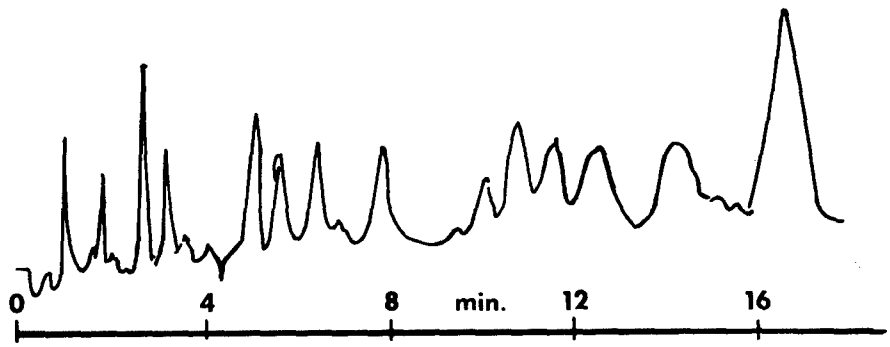


Figure 32. Chromatogram of headspace gas over waste DFM. Quality control - Waste DFM. 50 µl injection, headspace equilibrated at 30 C for 15 minutes. Varian 1200, FID, 12 ft. x 1/8 in., SS, 10% SE30 on Chromosorb W-AW isothermal 40 C.

The DFM study was operated with consistently lower aerosol concentrations than those obtained in the JP-5 study. With JP-5, fine control of chamber concentrations was achieved by adjustment of chamber air flow, leaving other controllable parameters fixed. The generator temperature varied with changes in ambient temperature in the chamber room area. During the DFM study, exposure chamber air flows were never allowed to drop below 40 CFM and a variable transformer was used to control temperatures and output of the DFM evaporator system. Since chamber residence time appeared to be a significant factor in growth of the JP-5 or DFM aerosol the faster air flow rates decreased the overall amount of particulate present in the exposure environment.

The condensation aerosol formed by components of diesel fuel marine generated into the exposure chambers was monitored on a daily basis using a Royco® 225 particle counter. Tables 31, 32 and 33 show the mean aerosol counts for the entire 90 day exposure period. Chamber 7 had a DFM vapor concentration of 0.30 mg/liter while Chamber 6 was operated at a level of 0.05 mg/liter DFM. The aerosol counts shown for Chamber 6 were about a factor of fifteen lower than for Chamber 7 while the vapor concentration was only a factor of six lower.

TABLE 31. SUMMARY OF CUMULATIVE MEAN AEROSOL COUNTS TAKEN IN THE 0.30 mg/L DFM EXPOSURE CHAMBER FOR 100 DAYS

Channel Number	Size Range (Microns)	Mean Count/Liter	Deviation
1	0.5-0.7	3884	± 5800
2	0.7-1.4	1780	± 3300
3	1.4-3.0	3256	± 8300
4	3.0-5.0	251	± 700
5	5.0-10.0	53	± 100

TABLE 32. SUMMARY OF CUMULATIVE MEAN AEROSOL COUNTS TAKEN IN THE 0.05 MG/L DFM EXPOSURE CHAMBER FOR 100 DAYS

Channel Number	Size Range (Microns)	Mean Count/Liter	Deviation
1	0.5-0.7	311	± 200
2	0.7-1.4	109	± 70
3	1.4-3.0	159	± 130
4	3.0-5.0	11	± 13
5	5.0-10.0	7	± 7

TABLE 33. SUMMARY OF CUMULATIVE MEAN AEROSOL COUNTS OF THE DFM CHAMBER INPUT AIR SYSTEM

Channel Number	Size Range (Microns)	Mean Count/Liter	Deviation
1	0.5-0.7	173	± 250
2	0.7-1.4	20	± 20
3	1.4-3.0	12	± 10
4	3.0-5.0	1	± 1
5	5.0-10.0	1	± 1

A typical gas chromatogram of DFM vapors in the 0.03 mg/L DFM exposure chamber is shown in Figure 33. The benzene peak used for routine analysis is eluted from the column in approximately 10.25 minutes and is identified as such. The mean benzene concentration during the DFM exposures was determined to be 0.26 ± 0.04 ppm in the high level exposure chamber and 0.04 ± 0.02 ppm in the low level exposure.

The methodology used for DFM generation into the animal exposure chambers resulted in excellent control throughout the 90-day exposure phase of the study. The 0.30 mg/L DFM exposure group received a mean concentration for 90 days of 0.299 ± 0.005 mg/liter and the 0.05 mg/liter exposure system operated for 90 days with a value of 0.050 ± 0.001 mg/liter. With the exception of chamber cleaning periods which lasted 30 minutes of each day excursions in concentration did not exceed 10% of the desired values.

Continuous exposure for 90 days to DFM vapors produced a marked dose related decrease in male rat weight gain, but did not affect the weight of female rats. DFM also produced a slight increase in dog red blood cell osmotic fragility and reduced the number of white blood cells in the rats. There were no reductions in erythrocyte numbers in either dogs or rats. Future rat blood analysis and tissue examination of all species should help to further characterize any possible toxicity of DFM.

The study is continuing and will be discussed in more detail in ensuing progress reports as more data become available.

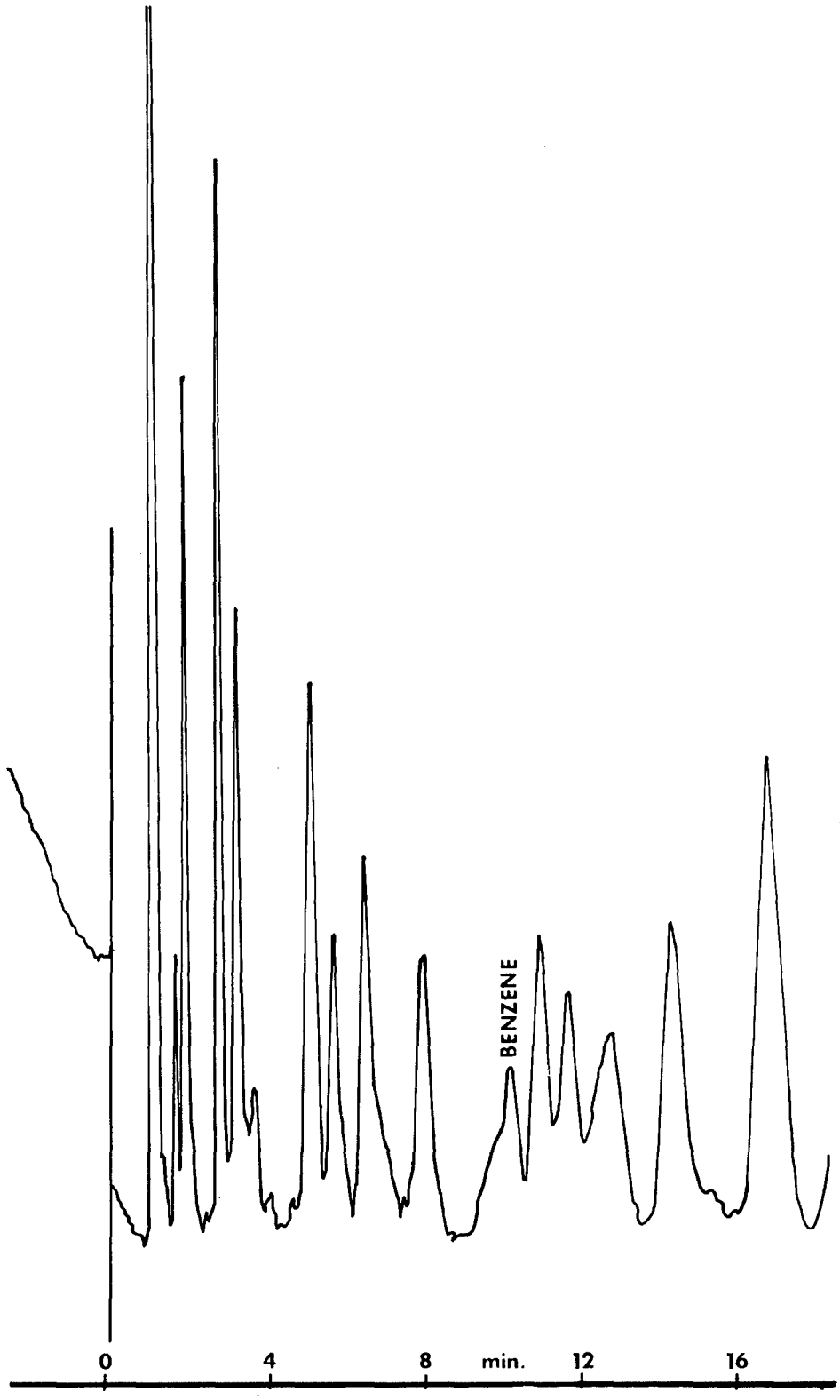


Figure 33. A typical chromatogram of chamber air containing 0.30 mg/m^3 DFM vapor.

Acute Toxicity Studies on Air Force, Navy and Department of Transportation Materials

Several compounds were submitted to the Toxic Hazards Research Unit for acute toxicity screening tests to determine the potential hazard of these materials in a manner that could be compared with other candidate materials. The specific toxicity tests conducted on each material were related to their use and physical characteristics but not all tests were conducted on every material submitted by the three participating agencies.

U. S. Navy Materials

(The Acute Toxicity of Two Antifouling Organometallic Polymers known as OMP-4 and OMP-5)

Organometallic polymers (OMP) have the potential of being long-lasting and effective antifouling agents against organisms such as barnacles, tubeworms, algae, hydroids, sponges and bacteria (Dyckman et al., 1973), and are under consideration by the Navy as additives to ship hull paints. These polymers containing trialkyltin moieties have been shown to exhibit some degree of mammalian toxicity, Miller et al. (1976) have found (tri-n-butyltin methacrylate/tri-n-propyltin methacrylate/methyl methacrylate) (OMP-1) to be irritating to the skin and eyes of rabbits and exhibit a single dose oral LD_{50} in rats of 230 mg/kg. A four-hour acute inhalation exposure resulted in a LC_{50} determination of 64 mg/m^3. Another polymer, tri-n-butyltin methacrylate/methyl methacrylate (OMP-2), was also found to be irritating to rabbit skin and eyes and to produce a single dose LD_{50} in rats at a level of 280 mg/kg (Naval Medical Research Institute letter report, 1976). Acute inhalation studies consisting of four-hour exposures resulted in a LC_{50} of 51 mg/m^3. Two other organometallic compounds designated OMP-4 and OMP-5 had no previous toxicity information; thus it was considered necessary that this information be obtained. The acute toxicity tests which were conducted are listed below:

1. Single oral dose LD_{50} in rats and mice.
2. Single intraperitoneal dose LD_{50} in rats and mice.
3. Primary skin irritation in rabbits.
4. Eye irritation in rabbits.
5. Skin sensitization potential in guinea pigs.

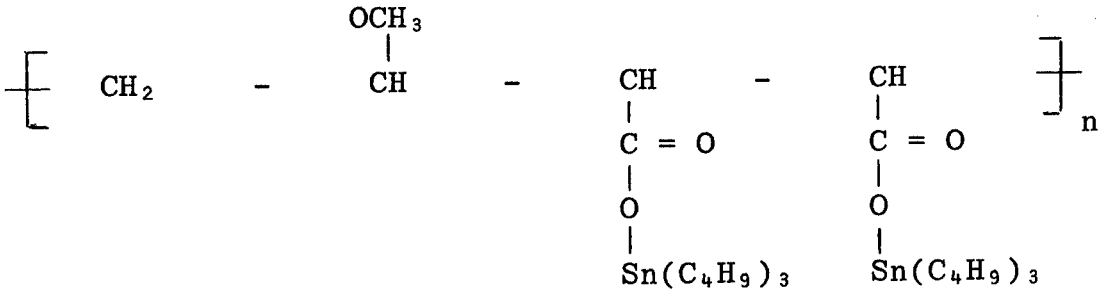
Materials and Methods

Male Sprague-Dawley rats weighing from 200 to 300 grams and male ICR mice weighing from 20-30 grams were purchased from Harlan Industries, Incorporated. These animals were used for the oral and intraperitoneal LD_{50} studies. Female New Zealand albino rabbits

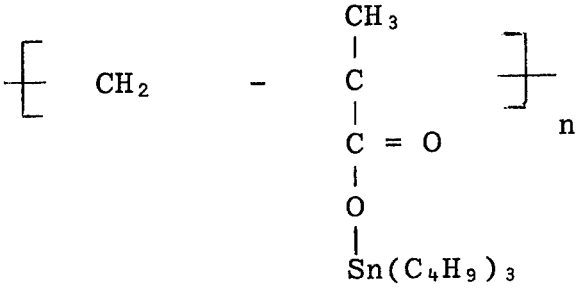
and male Hartley derived guinea pigs were obtained from Sweetwater Farms for use in the skin and eye irritation and skin sensitization studies.

The chemical structures of the polymers of OMP-4 and OMP-5 are as follows:

OMP-4 -- % Tin = 31.06



OMP-5 -- % Tin = 28



OMP-4 was prepared for the oral dosing of rats by making a solution of the material in cyclohexanone (J. T. Baker Chemical Company, Lot #623342). This solution was then added to a propylene glycol vehicle (Halsey Drug Company, Incorporated, Lot #30922). The cyclohexanone was then evaporated under vacuum and stirring. The resulting mixture was an emulsion of OMP-4 in propylene glycol. A solution of cyclohexanone and propylene glycol previously evaporated in the same manner was administered to animals that served as controls. Oral dosing of mice and intraperitoneal dosing of rats and mice were done using mineral spirit dilutions of OMP-4. Mineral spirit alone was given to animals that served as controls. Neat OMP-4 was used for eye irritation and skin irritation studies. Topical application of OMP-4 to guinea pigs was made using mineral spirit solutions.

OMP-5 was supplied as a coarse granular material, which was too large to pass through a syringe needle. Thus, it was necessary to grind the OMP-5 in a mortar and pestle so that it was fine enough to pass through a No. 140 wire mesh screen. The ground material was then suspended in Mazola corn oil for oral and intraperitoneal administration to rats and mice. A suspension of OMP-5 in peanut oil (Matheson, Coleman and Bell, Lot Number B12F02) was

used for the guinea pig skin sensitization studies. OMP-5 was applied as the coarse material for eye and skin irritation tests.

Acute Oral Toxicity - LD_{50} Determination

Syringes fitted with special oral intubation needles were used to administer a single dose of the compound to rats and mice that were fasted for 16 hours prior to the dosing. The fasted condition allows for a more uniform absorption of the material since the amount of food in the stomach of animals fed ad libitum varies. Groups consisted of five rats or mice per level. The animals were held for 15 days following administration of the material, and any animal that died during this time was included in final mortality calculations. Animals that survived the 14 days were weighed and sacrificed at that time. The LD_{50}'s with the 95% confidence limits were calculated using the moving average interpolation method of Weil (1952)

Acute Intraperitoneal Toxicity - LD_{50} Determination

Materials were administered as a single injection into the peritoneal cavity of rats and mice. Groups consisted of five animals per dose level. Deaths which occurred during the 14 day holding period were included in the final mortality tally. Animals that survived the 14-day period were weighed and sacrificed at that time and LD_{50} calculations made as described above.

Primary Skin Irritation

The primary skin irritation potential of OMP-4 and OMP-5 was measured by a patch test technique on intact and abraded skin areas of albino rabbits. Six rabbits were used for the evaluation of each compound. The dorsal area of each rabbit was clipped free of hair 24 hours prior to administration of the compound, thus allowing irritation from the clipping process to heal. Equal numbers of exposures were made on the intact and abraded skin. Abrasions were minor incisions through the stratum corneum which were not deep enough to disturb the derma or to produce bleeding. The materials were applied in quantities of 0.5 grams. Each site was covered with a 1 x 1 inch piece of surgical gauze two layers thick followed by a 4 x 4 inch piece of Elastoplast adhesive tape. The rabbits were then fitted with leather restraining collars to prevent disturbance of the patch area. After 24 hours, the collars, dental dam, and patches were removed. Any reaction resulting from the test material was evaluated at this time and again at 72 hours post application using the method of Draize et al. (1959).

Acute Eye Irritation

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

Skin Sensitization

Groups of test animals consisted of 20 male albino guinea pigs. OMP-5 was injected as a peanut oil solution, while OMP-4 was topically applied as a mineral spirit preparation because of its strong irritant properties. The sensitization test was started on a Monday when the guinea pigs were weighed and closely clipped on the scapular areas. A volume of 0.05 ml of a 0.1% dilution of the test material was administered at the upper right scapular area of each guinea pig. A similar control administration of the vehicle was made at the upper left scapular area. Readings were taken 24 hours and 48 hours later and recorded.

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

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

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

0 = needle puncture ("np") - no wheal

1 = very faint pink ("vfp") - no value for this reaction

2 = faint pink ("fp")

3 = pink ("p")

4 = red ("r")

5 = bright red ("R")

6 = edema - <1 mm in height ("e")

7 = edema - >1 mm in height ("E")

*8 = necrosis - <1 sq. mm ("n")

*9 = necrosis - >1 sq. mm ("N")

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

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

Experimental Results

Acute Oral Toxicity

The results of the acute oral toxicity studies of OMP-4 and OMP-5 are summarized in Table 34.

TABLE 34. ACUTE ORAL TOXICITY OF OMP-4 AND OMP-5

Compound	Species	Data in mg/kg (Number of Deaths)*	LD_{50} (95% C.L.) in mg/kg
OMP-4	Rat	Control (0), 125 (0), 250 (2), 500 (5), 1000 (5)	268 (191-376)
OMP-4	Mouse	Control (1), 31.25 (1), 62.5 (3), 125 (4), 250 (5), 500 (5)	56 (25.9-119.5)
OMP-5	Rat	300 (0), 600 (1), 1200 (1), 2400 (5)	1427 (947-2154)
OMP-5	Mouse	250 (0), 500 (4), 1000 (5), 2000 (5)	406 (300-549)

*N=5

Rats and mice exposed to OMP-4 were lethargic for approximately 24 hours after dosing. Fluid and bloody discharge from the nares and eyelids of the rats occurred in many of the animals receiving the higher doses. The intestinal tracts of the animals that died were distended and hemorrhagic areas were found in the stomachs of a few animals. Deaths resulting from exposure occurred within 72 hours of dosing. Animals that survived the 14-day holding period appeared in good condition with normal weight gains.

Treatment with OMP-5 produced symptoms similar to those of OMP-4. Lethargy and blood tinged fluid were observed in many of the animals. Deaths occurred up to 3 days postexposure in the rats and up to 7 days postexposure in the mice.

Acute Intraperitoneal Toxicity

The results of the acute intraperitoneal toxicity studies of OMP-4 and OMP-5 appear in Table 35.

TABLE 35. ACUTE INTRAPERITONEAL TOXICITY OF OMP-4 AND OMP-5

Compound	Species	Data in mg/kg (Number of Deaths)*	LD_{50} (95% C.L.) in mg/kg
OMP-4	Rat	Control (0), 0.98 (2), 1.95 (3), 3.90 (4), 7.81 (4), 15.6 (5), 31.2 (5)	1.4 (0.35-5.5)
OMP-4	Mouse	Control (0), 6.25 (0), 12.5 (3), 25 (3), 50 (5), 100 (5)	15.4 (9.1-25.9)
OMP-5	Rat	6.25 (0), 12.5 (0), 25 (5) 50 (4), 100 (5), 200 (5)	19 (15-25)
OMP-5	Mouse	7.8 (0), 15.6 (1), 31.2 (2), 62.5 (5), 125 (5)	29 (18-47)

*N=5

Intraperitoneal injection of OMP-4/mineral spirit in rats and mice produced irritation of the peritoneum. Serous fluid, scattered hemorrhagic areas of adhesions of the major abdominal organs were found in many of the animals injected with the OMP-4/mineral spirit solution. Control animals receiving mineral spirit alone exhibited some scattered adhesions along the intestinal tract. Deaths of exposed animals occurred within 48 hours of treatment with the majority of animals dying within 24 hours.

Intraperitoneal injection of OMP-5 produced lethargy and inhibition of the righting reflex. Deaths occurred within 48 hours of injection. Animals that survived the 14-day holding period exhibited normal weight gains. Small areas of adhesions were seen on the intestines of some animals.

Primary Skin Irritation

Undiluted OMP-4 was applied to intact and abraded rabbit skin and produced slight edema and blanching of the skin at the application site after 24 hours of contact. At 48 hours after removal of the material, the blanching was still present and fissuring and eschar formation had occurred. Examination at 7 days revealed severe necrosis of the patch site in all six rabbits. A primary irritation score of 2.4 was determined, thus classifying OMP-4 as a moderate irritant. Application of OMP-5 produced no irritation to rabbit skin.

Eye Irritation

Moderate swelling of the conjunctivae and discharge were seen in three of the six rabbits 24 hours after application of OMP-4. One of the rabbits had diffuse corneal opacity with slight swelling of the iris at 24 hours. The swelling and discharge decreased with the 48 and 72 hour readings. The eyes appeared normal when examined 7 days after application. OMP-5 produced slight redness and moderate chemosis in all six of the rabbits after 24 hours of contact. Corneal opacity, which was scattered, and swelling in the iris were seen in two of the rabbits. The iris swelling was still present in the two rabbits after 72 hours while damage to the conjunctivae had decreased in most of the animals. The eyes appeared normal after 9 days.

Skin Sensitization

Initial injections of a dilute solution of OMP-4/mineral spirit indicated that OMP-4 was too irritating to be introduced by injection and that topical application would be a more satisfactory procedure for determining sensitization potential. When scoring the reactions the areas of irritation were not measured due to the differences in the spreading of the material on the skin. Intensity of the challenge reactions to OMP-4 was mild and not noticeably different from those at the mineral spirit control sites. OMP-4 is therefore not considered to be a sensitizer by this method.

Injection of OMP-5 in a peanut oil solution established a slight sensitizing potential for this compound. One guinea pig on test died of circumstances unrelated to OMP-5 injections. Ten of the remaining 19 animals gave responses of between 25 and 100 when scored at 24 hours after the challenge injection. Examination at 48 hours showed 8 of 19 animals with scores of 25 to 100. The reactions at this time had increased slightly in color intensity.

Discussion

The acute oral LD_{50} of OMP-4 (268 mg/kg) in rats determined in this study is quite similar to the LD_{50} values determined for other compounds of this type. Miller et al. (1976) have determined the oral LD_{50} of OMP-1 to be 280 mg/kg, while the Naval Research Institute (letter report, 1976) has found the oral LD_{50} of OMP-2 to be 230 mg/kg. OMP-5 appeared to be somewhat less toxic with an oral LD_{50} in rats of 1427 mg/kg. All the above compounds should be considered in the toxic range. Data are not available on the intraperitoneal toxicity of OMP-1 or OMP-2; therefore comparisons cannot be made. OMP-4 was more toxic than OMP-5 (1.4 mg/kg as compared to 19 mg/kg) when administered by intraperitoneal injections.

OMP-4 was more irritating than OMP-5. Irritation was seen along the intestinal tract after oral dosing, and severe peritonitis was produced by OMP-4 after intraperitoneal injection. The dermal irritation potential of OMP-4 was also found to be greater than OMP-5. Evaluation of the dermal irritation depended on the erythema and edema produced by the material. OMP-4 caused slight edema in all the animals tested, and based on this finding, was classified as a moderate irritant. The eschar formation and injury to the dermal fascia would classify OMP-4 as a corrosive material. OMP-1 (Miller et al., 1976) and OMP-2 (Naval Medical Research Institute letter report, 1976) have both been found to be moderate skin irritants, but neither has been reported to elicit the corrosive action seen with OMP-4.

The eye irritation test places much of the scoring weight on corneal tissue evaluation, while damage to the conjunctivae produced by OMP-4 along with the one case of corneal injury established the material as an eye irritant. OMP-5 was also classified as an eye irritant. The dry granular nature of this compound made the material difficult to clear from the eyes and probably led to prolonged and increased irritation of the conjunctival tissue as well as corneal injury.

The skin sensitization test is designed to evaluate the potential of a material to act as an antigen. Applications of small quantities of the material over a period of time induces antibody synthesis. The induction potential is then evaluated by grading the irritation (area and intensity) of a single challenge administration. The irritation produced by intradermal injections of OMP-4 necessitated topical applications rather than an injection series. OMP-4 did not show a sensitizing potential by this method. OMP-5 administered intradermally gave a slight sensitization response.

The results of this study show that OMP-4 was similar to other compounds of this type in the level of oral toxicity, while OMP-5 was slightly less toxic. OMP-4 was found to be corrosive to the skin and irritating to the eyes. OMP-5 was not irritating to the skin, but was an eye irritant and exhibited sensitizing potential.

Air Force Materials

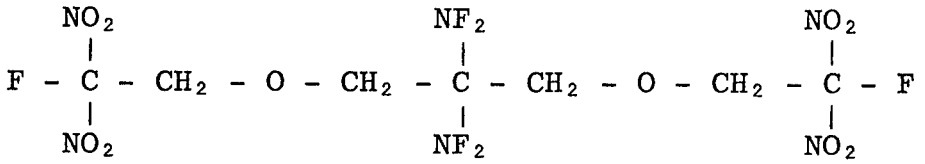
Acute toxicity tests were performed on a variety of miscellaneous materials submitted for testing by the Air Force. Among these materials were three samples of baby shampoos taken from a military base exchange. One of the commercial shampoo samples was suspected of producing eye irritation in military dependents and the THRU was asked to evaluate the potential of irritation to eyes. The samples were submitted without commercial labels and the manufacturers were unknown to the THRU to avoid commercial claims or other problems. Since the chemical composition and other identification information was unknown the details of the test results will not be described in this report other than to state that rabbit eye irritation tests showed one sample to be nonirritating,

one was borderline, and one gave irritant responses in 4 of 6 rabbits tested.

The Acute Toxicity of SYEP

The compound Bis(2,2-difluoroamino)-1,3-bis(dinitrofluoroethoxy) propane, known as SYEP, is a high energy plasticizer used by the Air Force for which no previous toxicity information existed. Therefore, to evaluate any toxic hazard of the compound acute oral LD_{50}, acute intraperitoneal LD_{50}, and skin sensitization studies were conducted.

SYEP was supplied to the THRU laboratory by Rockwell International Corporation, Rocketdyne Division. The chemical and physical properties of the material are as follows:



Chemical information: analyzed by liquid chromatography.

Purity in dichloromethane as shipped = 99.8%

Hydroxyl as FDNE = 0.01% by weight

Hydroxyl as Sec -OH = 0.01% by weight

Acid number = 0.01 mg KOH/g

Water = 0.04% by weight

SYEP is a shock sensitive compound, and in order to reduce the explosive hazard of the material during shipping, it was diluted with dichloromethane to a 30% w/w dilution. Prior to initiation of the toxicity tests, the dichloromethane was removed by passing a gentle stream of air over the diluted SYEP.

Male rats were fasted for 16 hours prior to administration of the single oral dose. SYEP was suspended in corn oil and given to the rats with syringes and oral dosing needles at an injection volume of 0.01 ml/g of body weight. Animals were individually weighed at the time of dosing to determine the proper dosing volume. Five rats were dosed at each concentration and observed for 14 days. Any deaths which occurred during this period were included in the final mortality results. The moving interpolation method of Weil (1952) was used to determine the LD_{50} and 95% confidence limits. All rats surviving the 14-day holding period were sacrificed.

Male mice were used in the intraperitoneal toxicity studies. SYEP was suspended in corn oil and injected into the peritoneal cavity at a volume of 0.01 ml/g of body weight. Groups consisted of five mice per concentration. Animals were observed for 14 days after the single injection of SYEP and deaths occurring during this period were included in the mortality data. The LD_{50} was estimated using the method of Weil (1952).

Gross pathology examinations were carried out on the animals that died during the 14-day holding period after the oral and intraperitoneal studies. In addition, a few of the animals surviving the 14-day period were also submitted for examination.

The method used for skin sensitization testing was the same as that used for OMP-4 and OMP-5 described earlier in this report.

Acute oral toxicity test results are presented in Table 36. Deaths which occurred in the high dose group took place within 24 hours of administration of SYEP. Deaths in the lower dose groups were spread out over a one to four day period. Gross examination of the Sprague-Dawley rats receiving 4000 mg/kg SYEP revealed distended stomachs, dark colored spleens and kidneys and accentuated lobular architecture of the liver. Animals receiving the lower doses of SYEP had reticulated livers and kidneys.

TABLE 36. ACUTE ORAL TOXICITY OF SYEP IN SPRAGUE-DAWLEY RATS

Concentration (mg/kg)	Mortality Ratio*
4000	5/5
2000	5/5
1000	4/5
500	0/5
250	0/5

LD_{50} and (95% C.L.) = 812 (600-1098) mg/kg

*Number died/number dosed.

In the acute intraperitoneal toxicity tests conducted in CF-1 mice, deaths occurred within two days after injection of SYEP. The mortality results are given in Table 37. Gross examination of the mice showed the same reticulation of liver and kidney tissues that was seen in the orally dosed rats.

TABLE 37. ACUTE INTRAPERITONEAL TOXICITY OF SYEP IN CF-1 MICE

Concentration (mg/kg)	Mortality Ratio*
400	5/5
317	5/5
252	4/5
200	0/5

LD_{50} and (95% C.L.) = 235 (211-261) mg/kg

*Number died/number dosed.

Fourteen of 20 Hartley guinea pigs receiving intradermal injections of SYEP showed severe reactions 24 hours after the challenge injection. The mean score of these 14 animals was 316. The irritation areas at the test sites were about the same size as control sites, but the color intensity was much greater. The reactions had moderated somewhat after 48 hours when 9 of the 20 guinea pigs showed reactions with a mean score of 158. The color intensity had subsided at the 48-hour examination.

The acute oral and intraperitoneal toxicity data indicate that Bis(2,2-difluoroamino)-1,3-bis(dinitrofluoroethoxy)propane also known as SYEP is a moderately toxic compound. SYEP has strong sensitization potential and should be handled with special precaution to prevent skin or inhalation contact. We were unable to evaluate the inhalation hazard of this compound because of its explosive character at temperatures very near its boiling point.

Miscellaneous Materials

A group of 21 miscellaneous Air Force materials was received for testing in 1977 and partial results were presented in the last annual report (MacEwen and Vernot, 1977). Acute oral toxicity data for two materials are presented here as are the results of skin sensitization testing. The 21 materials received for study along with the manufacturer or supplier are listed in Table 38.

Acute toxicity and irritation potential of the materials were evaluated by using the following tests:

1. Single dose oral LD_{50} in rats and mice
2. Primary skin irritation in rabbits
3. Skin sensitization in guinea pigs

Unfortunately, the supply of some materials was limited, necessitating a prioritization of tests performed. The methods for the oral LD_{50} determinations and primary skin irritation tests as well as the results of these tests on most of the compounds can be found in the last annual report (MacEwen and Vernot, 1977). The method used for evaluation of sensitization can be found in this report in the section on toxicity testing of U. S. Navy Materials.

Acute oral toxicity data are given for 1,2,3-benzotriazole and guanidino salicylamide salt in Table 39. When partial mortality or no deaths occurred at the 5000 mg/kg dose level no further testing was conducted since larger doses could not be loaded into the gastrointestinal tract. With guanidino salicylamide salt it was necessary to form a paste with corn oil, and we were unable to give a large enough dose to cause any deaths in CF-1 mice. The rats used for oral dosing were Sprague-Dawley strain received from Carworth Farms.

TABLE 38. LIST OF MISCELLANEOUS AIR FORCE COMPOUNDS SUBMITTED FOR ACUTE TOXICITY STUDIES

Material	Chemical Supplier
3-amino-1,24-triazole (solid)	Fairmont Chemical Company
Salicyl amino guanidine (liquid)	Mobil Chemical Company
2,6-di-tert-butyl-dimethyl-amino-p-cresol (solid)	Ethyl Corporation
N,N'-disalicylidene-1,2-propane diamine (liquid)	duPont
Silicone DC-200 (liquid)	Dow Corning
1,2,3-benzotriazole (solid	American Aniline Products, Incorporated
Tricresyl Phosphate (liquid)	Stauffer Chemical
1,4-dihydroxyanthraquinone (solid)	Aldrich Chemical
Sulfurized 9-octadecanoic acid (liquid)	Cincinnati Milacron Chemical Chemicals
Azelaic acid (solid)	Emery Industries
Dimer acid (liquid)	Emery Industries
N-benzyl-3,7-dioctyl (solid)	Geigy Chemical
Phenothiazine (solid)	West Agro, Incorporated
Dioctyl phenothiazine (solid)	Rohm and Haas
Sebacic acid (solid)	Rohm and Haas
Acryloid HF-866 (liquid)	Rohm and Haas
Acryloid HF-844 (liquid)	Rohm and Haas
Guanidino salicylamide salt (liquid)	Emery Industries
Nonyl phenol (liquid)	Rohm and Haas
Phosphonate salt (liquid)	Hanover Chemical Company
Tris(β-chloroethyl)phosphate (liquid)	Stauffer Chemical

TABLE 39. ACUTE SINGLE DOSE ORAL TOXICITY OF 1,2,3-BENZOTRIAZOLE AND GUANIDINO SALICYLAMIDE SALT

Species	LD_{50} (95% C.L.) in mg/kg	Data Used to Calculate LD_{50} in mg/kg (Mortality N=5)	Toxicity Classification
		1,2,3-Benzotriazole	
Rat	1072(725-1585)	2000(5),1000(2),500(0)	Toxic
Mouse	615(540-701)	794(5),630(3),500(0)	Toxic
		Guanidino Salicylamide Salt	
Rat		5000(2)	Borderline Toxic

Skin sensitization and sensitization potential data were determined for Hartley strain guinea pigs. The results of these determinations on 17 compounds are presented in Table 40. The categorization of 1,4-dihydroxyanthraquinone as a compound with "slight" sensitization potential resulted when only two of the 20 guinea pigs tested reacted when given the challenge injection. The mean score for each of the two guinea pigs that reacted was over 800 which indicates a severe response. The mean for the entire group response diluted out the severity of this reaction and while only a small portion of any population might react to this material the reaction for these individuals can be very intense. Special care should be taken in the handling of this compound to prevent skin contact and sensitization.

Nonyl phenol received a sensitization potential classification of severe since 18 of the 20 guinea pigs showed a reaction upon the challenge injection.

Sensitization tests were not conducted on one compound submitted by the Air Force identified as Silicone DC-200 since suitable information on this material has been published in the scientific literature (Rowe et al., 1948). The tests were not conducted on three other compounds, phosphonate salts, guanidino salicylamide salt and N,N'disalicylidene-1,2-propane diamine because the supply of material available was exhausted in conducting the other tests.

Department of Transportation Materials

(Percutaneous and Inhalation Studies for Classification of Toxicity Ratings for Transportable Chemical Agents)

Certain materials being transported have inadequate toxicology data which is necessary for proper classification by the Department of Transportation. These materials were tested in this laboratory to verify the suitability of proposed transportation health hazards classification criteria. This was done by determining experimentally the 14-day toxicity by skin absorption on rabbits, peroral LD_{50}'s on rats, and one-hour inhalation LC_{50}'s on male and female rats.

TABLE 40. SKIN SENSITIZATION TEST RESULTS ON 17 AIR FORCE MATERIALS DETERMINED IN GUINEA PIGS

Compound	Sensitization Response	Sensitization Potential
3-amino-1,2,4-triazole	None	None
Salicyl amino guanidine	Mild	Moderate
2,6-ditert-butyl-dimethyl-amino-p-cresol	Mild	Slight
1,2,3-benzotriazole	Mild	Slight
Tricresyl phosphate	None	None
1,4-dihydroxyanthraquinone	Severe	Slight
Sulfurized 9-octadecanoic acid	Mild	Moderate
Azelaic acid	Mild	Moderate
Dimer acid	None	None
N-benzyl-3,7-dioctyl phenothiazine	Mild	Slight
Phenothiazine	None	None
Dioctylphenothiazine	Mild	Moderate
Sebacic acid	Mild	Slight
Acryloid HF-866	Mild	Slight
Acryloid HF-844	Mild	Slight
Nonyl phenol	Moderate	Severe
Tris(B-chloroethyl)phosphate	Moderate	Moderate

The toxicity classification system published in a previous report by Back et al. (1972) was used to categorize the compounds in the present study. The following criteria were used to determine the category in which each compound was placed.

	Extremely Toxic	Highly Toxic	Toxic
Inhalation, 1-hour LC_{50}	500 mg/m^3 or less (50 ppm or less)	>500-2000 mg/m^3 (>50-200 ppm)	>2000-200,000 mg/m^3 (>200-20,000 ppm)
Skin Absorption (Dermal) LD_{50}	20 mg/kg or less	>20-200 mg/kg	>200-20,000 mg/kg
Oral, 14-day Single Dose LD_{50}	5 mg/kg or less	>5-50 mg/kg	>50-5000 mg/kg

During the current reporting period, a group of compounds was received and assigned code numbers prior to testing. These compounds, their THRU code numbers and the tests to be done on each are listed in Table 41.

Methods and procedures for dermal, oral and inhalation testing were described in a previous annual report (MacEwen and Vernot, 1975). The only change from the described methods was the fact that some inhalation exposures were done in a one cubic meter Rochester chamber with an air flow of 10-13 cfm. The exposure groups were increased to 10 rats of each sex per contaminant level for better statistical analyses.

The results of the completed acute inhalation toxicity tests and the assigned classification are shown in Table 42. The upper limit for the toxic classification for dermal absorption is 20 g/kg which is also an upper limit for practical testing of solvent. Above this level it is technically difficult to retain liquids in the gauze compresses under the dental dam used in the test procedure. Eight of the compounds tested were not absorbed through the skin in sufficient quantities or at sufficient rates to be classified as shown in Table 43.

The oral toxicity test results and the appropriate classification are listed in Table 44 for 7 compounds. This series of tests completes all outstanding studies requested by the Department of Transportation.

TABLE 41. LIST OF COMPOUNDS SUBMITTED BY THE DEPARTMENT OF TRANSPORTATION FOR ACUTE INHALATION, PERORAL AND PERCUTANEOUS TOXICITY STUDIES

Code No.	Compound	Route of Administration		
		Peroral	Inhalation	Percutaneous
279	Chloroacetyl chloride		X	
283	Sulfur chloride		X	
284	Sulfur dichloride		X	
292	Ammonium polysulfide	X	X	X
293	Carbon dioxide		X	
294	Lead acetate	X		
151	Carbon tetrachloride			X
295	Hexachloroethane	X		X
296	Naphthalene	X		X
298	Methyl chloroform	X		X
280	Trichloroethylene	X		X
299	Ammonium hydrosulfide	X		X
300	Bromochloromethane			X
302	Chloroform			X
225	Ethylene dibromide			X
168	Ferrosilicon			X

TABLE 42. ONE-HOUR INHALATION TOXICITY OF VARIOUS COMPOUNDS FOR MALE AND FEMALE RATS

Compound	Sex	LC_{50} (95% C.L.) in ppm	Data Used to Calculate LC_{50} in ppm (Mortality Response, N=10)	Classification
Sulfur dichloride	M	1266 (1085-1450)	621(0), 900(2), 1131(6)m 1444(3), 1712(8), 2111(10)	Toxic
Sulfur dichloride	F	1440 (1260-1646)	621(0), 900(2), 1131(2), 1444(1), 1712(9), 2111(9), 2575(10)	Toxic
Chloroacetyl dichloride	M	922 (754-1241)	347(0), 523(3), 641(1), 830(7), 926(6), 1106(3), 1341(8)	Toxic
Chloroacetyl dichloride	F	980 (838-1163)	347(0), 523(1), 641(0), 830(6), 926(5), 1106(7), 1341(6), 1500(8)	Toxic
Sulfur chloride	M	327 (203-430)	158(1), 313(5), 519(10), 610(7), 787(8), 910(9)	Highly toxic
Sulfur chloride	F	697 (558-846)	405(1), 610(5), 787(2), 852(9), 910(8),	Highly toxic
Carbon dioxide	M*	369,759 (330,596-434,417)	235,018(0), 274,571(1), 288,482(1), 315,339(2), 331,143(1), 368,500(2), 419,750(2), 432,500(4), 449,950(5)	Less than toxic
Carbon dioxide	F*	371,056 (336,418-422,537)	288,482(0), 315,339(1), 331,143(2), 368,500(2), 419,750(3), 449,950(5)	Less than toxic

*N = 5

TABLE 43. DERMAL TOXICITY OF VARIOUS COMPOUNDS TO FEMALE RABBITS

Compound	LD_{50} (95% C.L.) in mg/kg	Data Used to Calculate LD_{50}, mg/kg (N = 3)	Classification
Naphthalene	>20,000	20,000 (0)	Below Toxic
Ferrosilicon	>20,000	20,000 (0)	Below Toxic
Ammonium Hydrosulfide	1682(550-5147)	1000 (0), 2000 (2)	Toxic
Trichloroethylene	>20,000	20,000 (0)	Below Toxic
Carbon Tetrachloride	>20,000	20,000 (0)	Below Toxic
Ammonium Polysulfide	1790 (no range)	1260 (0), 1590 (0), 2000 (3)	Toxic
Ethylene Dibromide	1782(938-3384)	1000 (0), 2000 (2), 4000 (3)	Toxic
Hexachloroethane	>20,000	20,000 (0)	Below Toxic
Methyl Chloroform	>20,000	20,000 (0)	Below Toxic
Chloroform	>20,000	20,000 (0)	Below Toxic
Bromochloromethane	>20,000	20,000 (0)	Below Toxic

TABLE 44. ORAL TOXICITY OF VARIOUS COMPOUNDS TO MALE AND FEMALE RATS

Compound	Sex	LD_{50} (95% C.L.) in mg/kg	Data Used to Calculate LD_{50} (N = 5)	Classification
Naphthalene	M	1414 (804-2489)	1000(0), 2000(4), 4000(5)	Toxic
Naphthalene	F	1866 (1261-2760)	1000(0), 2000(3), 4000(5)	Toxic
Trichloro-ethylene	M	6727 (3790-11,940)	4000(0), 8000(3), 16,000(5)	Less than toxic
Trichloro-ethylene	F	5650 (4683-6817)	4000(0), 5040(1), 6350(4)	Less than toxic
Ammonium hydrosulfide	M	168 (146-193)	126(1), 159(1), 200(5)	Toxic
Ammonium hydrosulfide	F	214 (145-317)	100(0), 200(2), 400(5)	Toxic
Methyl chloroform	M	17,148 (11,593-25,366)	8000(0), 16,000(2), 32,000(5)	Less than toxic
Methyl chloroform	F	12,996 (9440-17,892)	8000(0), 16,000(4), 32,000(5)	Less than toxic
Hexachloro-ethane	M	4489 (2332-8640)	4000(2), 8000(5)	Toxic
Hexachloro-ethane	F	4489 2332-8640)	4000(2), 8000(5)	Toxic
Lead acetate	M	4665 (3153-6900)	2500(0), 5000(3), 10,000(5)	Toxic
Lead acetate	F	5610 (no range)	5000(0), 6300(5), 7940(5)	Less than toxic
Ammonium polysulfide	M	200 (118-339)	100(0), 200(2), 400(5)	Toxic
Ammonium polysulfide	F	162 (118-223)	100(0), 200(4), 400(5)	Toxic

SECTION III

FACILITIES

The support activities of the THRU essential to the operation of a research activity are usually not of sufficient magnitude to merit separate technical reports. Therefore, these activities are grouped together under the general heading "Facilities" to describe their contributions to the overall program of the laboratory. Included are special projects in analytical chemistry, training programs, and engineering modifications to the physical research facilities.

Environmental Health and Safety Programs

A sampling system was described in last year's annual report (MacEwen and Vernot, 1976) for detection and measurement of contamination of laboratory air by materials being introduced into inhalation toxicity chambers. Areas sampled included those adjacent to contaminant introduction hoods, the ambient and each altitude laboratory area and the basement chemistry laboratory. During the past year, this system was used in conjunction with the MMH exposures being conducted in the Thomas chambers. The analysis was carried out by the AutoAnalyzer iodimetric system and a commercial instrument which measured the change in color of a paper tape caused by small concentrations of MMH. Only once during the year was the detection threshold value of 10 ppb exceeded. That occurred on 5 January 1978 when the concentration of MMH in the chemistry laboratory reached a level of 15 ppb. Investigation revealed that the laboratory hood blower had burned out, and a small amount of MMH in the hood vaporized through bottle stoppers and leaked into the laboratory. Although 15 ppb is well below our action level of 50 ppb, personnel were evacuated from the laboratory until the hood blower was repaired and the laboratory concentration was no longer measurable.

An additional environmental sampling system has been set up for use with experiments being conducted for the Navy. These have been 3-month exposures to various hydrocarbon fuels (JP-5, DFM and decalin) conducted in the dome shaped animal chambers. Samples collected from the vicinity of the contaminant introduction hood and from the adjacent laboratory areas are pumped to a hydrocarbon analyzer for determination of laboratory air contamination. Initially, the system was set to sound an alarm at a concentration of 5 mg/m^3 which is equivalent to about 1 ppm of most hydrocarbons used in the laboratory. However, it was found that normal laboratory activities such as painting or refrigerant charging in the air conditioning system gave levels higher than this. Therefore, the action level was increased to 25 mg/m^3 which is still safe with regard to personnel exposure to fuel hydrocarbons, but is almost never exceeded by gasses from operations not associated with the exposure.

The system activated an alarm once during the third shift when a waste DFM receiver had filled up and was overflowing. Because of the alarm system, the leak was detected before a dangerous quantity of fuel had spread over the floor.

Computer Program Development

DATABASE for Dog Hematology and Clinical Chemistry

The data obtained from measurement of hematologic and clinical chemistry values in dogs exposed to MMH for one year, to UDMH for six months and to JP-5 continuously for 90 days, along with their controls, were organized and stored in the Wright-Patterson Air Force Base CDC 6600 computer memory. Through use of a software system provided by the Air Force, an individual wishing to retrieve information concerning individual or distribution parameters of selected test values may do so by communicating with the computer on an interactive basis i.e., the computer notifies the operator when the next question can be asked and what form it can take. The determinations which have been included in the file are listed in Table 45.

The codes shown in the table are typed into the computer terminal to identify the determinations being retrieved.

Information which can be retrieved for each test includes:

1. The number of data points available in memory for that test
2. Arithmetic means
3. Standard deviations
4. Ranges of extreme values

These can be retrieved for each experiment, for single sampling periods, over the whole course of the experiment or for selected groups of sampling periods. Control data from different experiments can be combined to provide population data for comparison purposes.

The groups listed in Table 45 are broader classifications which include a number of usually related determinations. The individual values may be retrieved by inputting the group classification, but means, standard deviations, and ranges are not retrievable using the group identification alone.

Design of the DATABASE required familiarization with COBOL, the computer language used in the Air Force software system, and also with Boolean algebra, the technique utilized to establish relationships and identifications for retrieval purposes.

TABLE 45. CLINICAL TESTS FOR WHICH DATA ARE INCLUDED IN DATABASE

Group	Determination	Code
HEMA		
	Red Blood Cell Count	RBC
	White Blood Cell Count	WBC
	Hematocrit	HCT
	Hemoglobin	HGB
	Sedimentation Rate	SEDI
	Reticulocytes	RETIC
MCORP		
	Mean Corpuscular Volume	MCV
	Mean Corpuscular Hemoglobin	MCH
	Mean Corpuscular Hemoglobin Concentration	MCHC
ELEC		
	Sodium	SOD
	Potassium	POT
	Chloride	CHLOR
GEN 1		
	Calcium	CAL
	Glucose	GLU
	Total Protein	TPRO
	Albumin	ALB
	Globulin	GLOB
	A/G Ratio	AGR
GEN 2		
	Iron	IRON
	Triglycerides	TRIGS
	Cholesterol	CLES
LIVER		
	Serum Glutamate Pyruvate Transaminase	SGPT
	Serum Glutamate Oxaloacetate Transaminase	SGOT
	Alkaline Phosphatase	ALKP
	Total Bilirubin	TBILI
	Bromsulfalein	BSP
KIDNEY		
	Blood Urea Nitrogen	BUN
	Creatinine	CREAT

After setup and debugging of the system, it was made available to the scientists of the THRU for their use. A few suggestions were made concerning improvement of certain portions of the system which did not respond as desired. Changes were made to accommodate the suggestions, and the system now appears to operate satisfactorily. As data are collected from dogs in ongoing experiments, they are inserted into the system memory so that the files will be kept continuously up-to-date.

Engineering Programs

Solid-State Electronics Development

Efforts continued during the past report period in expanding the department's expertise in designing and utilizing all phases of solid-state electronics technology. Acquisitions of equipment to provide for development capabilities in digital processing were accomplished. A microprocessor laboratory capable of all types of microprocessor development was installed in Building 79 adjacent to the laboratory chamber facilities. This laboratory will be involved in development of both software and hardware devices for utilization in laboratory operations. Equipment is available to provide program development capability for several different microprocessors, magnetic data storage, hard-copy documentation, and remote-hookup to a CDC time sharing computer. Adequate test-equipment is available for troubleshooting and repair of data systems.

Three major systems have been acquired as follows:

1. Primary Development Systems used for development of programs to be used in microprocessor systems, to provide a master library of programs to be used in various systems and to enable programs to be modified as requirements change.
2. Secondary Development System used for actual development of data acquisition, monitoring and control devices. This system provides a practical testing environment for application of programs to hardware devices.
3. General Purpose System. This system serves a dual-purpose of being a general-use terminal plus providing an interface to test devices which will utilize digital data communications in their operation, e.g. an instrument or device which may need to transmit data or information over telephone lines. This equipment has been checked out and evaluated for compatibility and interaction and is being utilized in the initial stages of equipment development.

Digital Data Communications Systems

As a continuation of efforts reported the last report period, additional terminal systems have been designed to aid in the application of programs using time-shared computer systems. An existing program for statistical analysis of chamber concentration is operated with a Model 33 Teletype and acoustical modem connnected to a remote time-sharing computer. Typical speed of this system is 10 characters per second. The system has been used for both analytical chemistry programs and as a hard copy printout and paper-type punch for animal-weighing system programs. Additional systems were designed with increased capabilities. These systems provide video-terminal displays, printer readout, magnetic disc program storage, remote communications capability and local programming using standard "Basic" language. Each remote terminal will consist of the following items connected as shown in Figure 34.

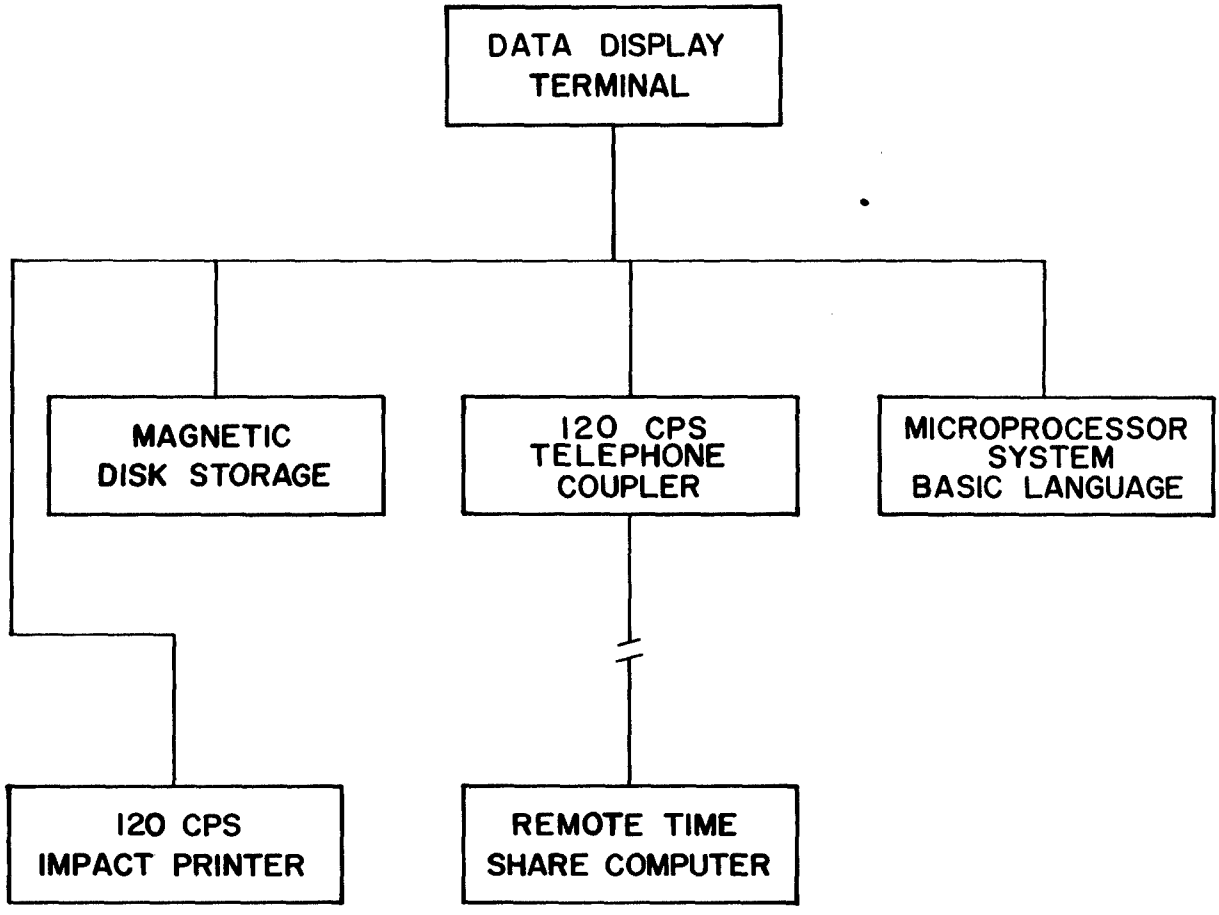


Figure 34. THRU remote data terminal system.

1. A video-terminal display unit for interactive control of programs and equipment.
2. A magnetic disc storage unit for permanent storage of programs and data. The units use an 8" square flexible magnetic disc for storage. Storage capacity is 250,000 characters for disc.
3. An impact alphanumeric printer for hard-copy documentation of programs and data. All units have a capacity of 120 characters per second printing rate.
4. A data modem for coupling the output of the remote terminal system to the telephone lines for accessing remote time-sharing computer facilities.
5. A microprocessor system with memory capabilities to run programs locally or from a remote computer.
6. Basic language programming capability either from disc-storage or hard-wired.

These configurations will allow maximum flexibility in supporting all areas of the THRU facility as shown in Figure 35.

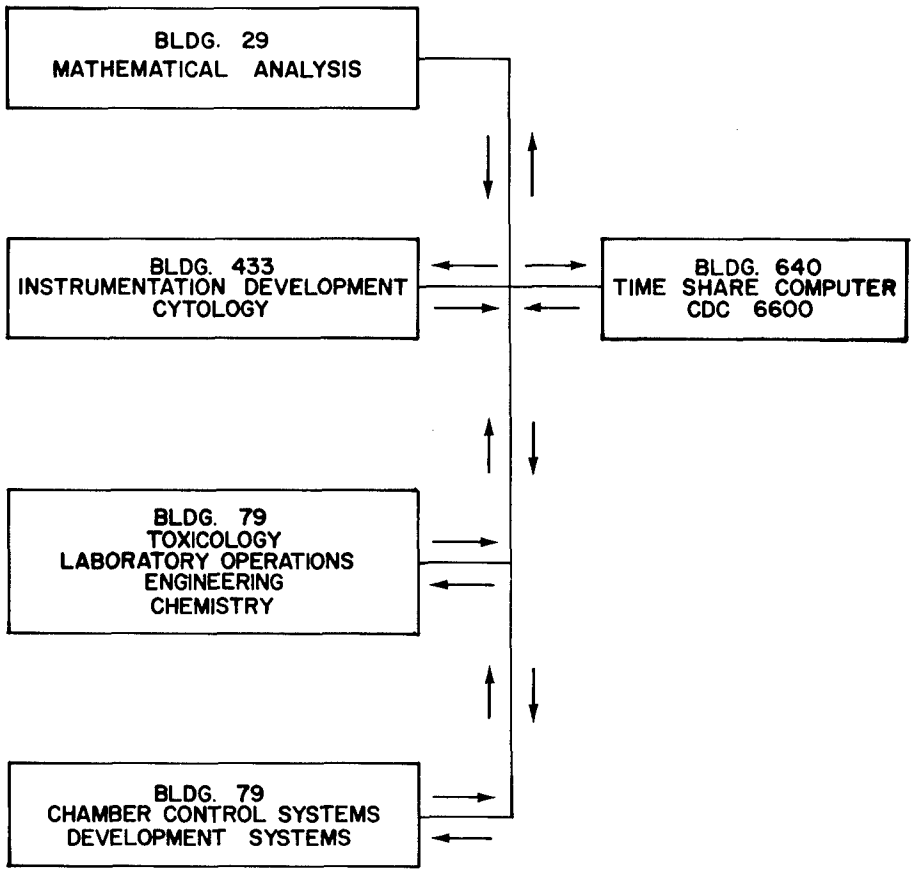


Figure 35. THRU remote data terminal locations.

New Facilities - Building 433

In late 1976, most of the bottom floor of Building 433 was made available for use by the THRU to provide space for a cytology laboratory and for portions of other THRU departments which did not have adequate work space available to them. For instance, the Instrumentation Laboratory of Development Engineering and general stock storage were required to move from Building 44 because of the planned demolition of that structure. The office area in Building 79 had become overcrowded so that the Administrative Services Officer and her secretary needed additional space. The drafting section had previously been confined to two small rooms which were inadequate for the amount of work being done. The machine shop and welding area had been located in a room adjacent to the Altitude Laboratory whose only access was through that laboratory, an unsatisfactory situation in view of the possible mutual contamination caused by these unrelated activities. In recent years, an increasing amount of work has developed in the area of non-inhalation toxicology including peroral, subcutaneous, intraperitoneal and intravenous administration. Performing these toxicological tests in the ambient toxicology area had become increasingly difficult particularly in view of the new Good Laboratory Practices Regulations. Therefore, these operations were transferred to a room in Building 433.

In order to bring these varied functions into Building 433, renovation was necessary. Approval was granted by Base Civil Engineering for a self-help project so that the THRU Development Engineering Department could perform all the tasks involved.

It was necessary to remove the existing electrical wiring, plumbing, floor and wall tile and partitions in most of the areas to prepare for building renovation. The work consisted of partitioning, painting, relocating lights, and tiling of floors. Necessary utilities, storage cabinets and work benches were installed.

The Cytology Laboratory has hot and cold water, natural gas, air, vacuum, carbon dioxide and electricity services located at the working areas. A Class II ventilating hood with a face velocity of 100 fpm for handling moderate to highly toxic materials and an internal clean room for growing animal cells were also provided. The clean room has germicidal lamps and auxiliary air conditioning for controlling the room environments.

The Toxicology Laboratory has work benches, hot and cold water, vacuum and electricity. Two Class I ventilating hoods are installed with an air flow of 150 fpm at the face openings for handling volatile carcinogens and other extremely toxic materials. The hood ventilating system has a water type scrubber for removing hazardous materials from the hood exhaust prior to entering the atmosphere.

The Instrumentation Laboratory has five work benches, necessary storage cabinets and utilities for the construction, repair, maintenance and testing of pneumatic, electric and electronic instruments and controls.

Compressed Air Supply System

Throughout the years, requirements have increased for the supply of compressed air necessary to the pneumatic operation of the test facilities. Existing equipment has provided relatively dependable service for a number of years. Despite good preventive and corrective maintenance, general wear and age have reduced the efficiency as well as the reliability of the existing equipment to provide a continuous source of clean air. The present equipment consists of two, vertical piston type compressors designated as primary and secondary units. They have 175 cfm capacity at 100 psi and 57 cfm capacity at 100 psi respectively. Both units are connected to a common aftercooler, air dryer, and supply line. Any malfunction of the common units of the system requires complete shutdown, resulting in loss of automatic facility operations. Each of the compressor units requires disassembly, inspection, parts replacement and re-assembly after approximately 2000 hours of operation. This maintenance operation usually takes four days, placing total reliability on the one remaining unit. In order to assure a minimum interruption of air supply to the facility, a portable unit is usually rented from a local vendor to place in service should the one remaining unit incur a malfunction. A rather large inventory of spare parts must be maintained for both primary and secondary units to insure minimum downtime during corrective maintenance operations. Often, the replacement parts have several months procurement lead time.

In order to alleviate the inefficient and costly problems associated with the existing compressed air supply, a new system was purchased and is presently being installed. The system consists of a compressor, refrigerated dryer, coalescing pre-filter unit, absorbing filter unit, automatic drain traps and differential pressure gauges. Upon installation completion, the new system will be used as a primary source and the existing system will be used as a secondary source, or back up. The available component units will provide a versatile combination of four different air flow systems.

The new compressor is a single stage, positive displacement, flood lubricated type unit using two screws rolling together to draw in atmospheric air, to efficiently compress the air with a minimum temperature rise and to discharge the air smoothly into the receiver. The drive screw turns the idler screw through a heavy synthetic lubricant film, so no timing gears are required. Long life, antifriction bearings accurately locate and support the rotors. The design is unique in mechanical reliability, with virtually no wear or inspection required. The synthetic lubricant does not oxidize, require changing, break down to shellac, varnish or sludge that would damage the unit. It is a completely self-contained package of compressor, receiver, and air-cooled aftercooler. The new aftercooler eliminates the inherent problems of leakage and corrosion associated with the water-cooled aftercooler of the existing system. Maintenance of the new compressor is quite minimal requiring only the observing of service indicators signalling when a specific area needs attention.

The new air dryer is one of conventional refrigerated design using standard R12 as the cooling medium and contains dual centrifugal separators to provide a most efficient moisture removal operation. Warm, wet air from the compressor enters an air to air heat exchanger, and proceeds from there to the first separator for preliminary moisture removal. The air progresses through a refrigerant to air heat exchanger into the second separator containing a filter, and out through the center of the air to air exchanger as clean dry, cool air. The non-cycling operation of the refrigerant section of the dryer is controlled by two modulating valves. One is the hot gas by-pass and the other is the expansion valve. Both valves automatically open and close depending on the amount of cooling required to maintain the desired dewpoint. The dryer unit is relatively maintenance free, requiring only periodic separator filter element replacement, condenser fin cleaning and inspection of automatic condensate traps.

The coalescing pre-filter unit is installed in the system at the discharge side of the air dryer. Air enters the inside of the filter element then flows through glass fibers where more than 99.9% of the submicron particles are filtered out, and where coalescing bulk liquid is formed. The formed liquid is carried by the air flow to the outer support core of the element where it appears as condensation. The element has an outer porous plastic covering and cannot be wetted by the coalesced liquid. Within the cellular structure of the covering, the emulsion of any oil and remaining water gravitates to the bottom of the filter bowl where the automatic drain discharges it. Air leaving the outlet of the pre-filter is suitable for the most critical instrumentation or control applications.

The absorbing filter unit is installed on the discharge side of the pre-filter unit. Air enters the inside of the filter element, flowing through the filtering media consisting of borosilicate glass fiber, cellulose pulp, and activated carbon, where any concentration of hydrocarbon vapors and/or odors present in the compressed air due to atmospheric pollution are removed. All waste material is discharged through the automatic drain trap. The compressed air discharged from this unit meets breathing-air quality standards. Maintenance requirements for both filtering units consist of periodic replacement of elements when indicated by excessive pressure drop between differential gauges installed in the system.

The addition of the newly installed system provides two completely separate, parallel systems, each independent of the other, capable of maintaining a constant uninterrupted supply of compressed air to the facility with reduced overall maintenance time and expenditures. Cooling water is not required for any unit of the new system, eliminating all associated water problems. Corrective maintenance and improvement of the existing system can be performed without interruption of air supply. Procurement of a rental unit will no longer be required. Spare parts inventory will be maintained at a minimum and excessive lead time for ordering of replacement parts will be reduced because of the inventory maintained by a local vendor.

In addition to the forementioned advantages, the system will provide breathing air for chamber entrants considerably cleaner than ambient air.

Upgrading of Laboratory Hood Classifications

Acute inhalation toxicity tests are conducted in a laboratory room located in Building 79 at Wright-Patterson Air Force Base which contains two Rochester type inhalation chambers of 2.5 m^3 size, two Longley type chambers of the same size and two smaller Rochester type chambers of 1 m^3 capacity. Between each pair of inhalation exposure chambers a hood was constructed to house contaminant generation and monitoring equipment to prevent exposures to laboratory personnel. Two additional laboratory fume hoods are also located in this room and are used for small chamber exposures and storage of chemicals used for inhalation toxicity studies.

The five hoods in this laboratory were upgraded to meet specific new Air Force laboratory hood use standards. This was accomplished by modifying the exhaust ducts and increasing the capacity of the exhaust blowers.

Prior to the change, one of the hoods met the Class II category requirements for a 100 fpm face velocity. The other four hoods failed to meet the minimum 75 fpm Air Force requirement of a Class III hood suitable for use with low toxicity materials.

The modifications resulted in one of the hoods having Class I, 150 fpm, category suitable for handling highly toxic and carcinogenic materials. Three of the hoods are in the Class II category suitable for moderately toxic materials. The fifth hood has a Class III status of 75 fpm for materials with low toxicity.

Safety provisions are incorporated in all of the hoods. A pressure sensor in the exhaust duct of each hood is connected to a pressure gauge and warning light. When air flow pressure exists in the duct, the warning light is illuminated and the gauge indicates a positive pressure. Laboratory personnel can detect a hazardous condition when a hood is being used and the warning lamp is not illuminated.

If the air flow stops, the light goes out and the gauge reads zero pressure. A burned out lamp can give a false warning. Therefore, the gauge must be checked when the light is out to confirm that there is no airflow and corrective measures are necessary.

Training Programs

Chamber Technicians

Phase I and Phase II formal training programs which involve the operation of the inhalation chambers were successfully completed by two chamber technicians hired in 1977. Both technicians have been

trained and are capable of drawing blood samples and handling and restraining all animal species used in our laboratory. Their training and proficiency also extends to the operation of the animal weighing system with the computerized output.

Additionally, both technicians have completed the Ralston Purina Animal Care Course which allowed for, along with one year of on-the-job experience, application for AALAS certification at the Assistant Animal Technician level. These examinations were received along with two others for certification as Laboratory Animal Technicians for more experienced chamber technicians.

Deliberate and simulated monthly emergency training procedures were conducted by all chamber technicians during this year. These procedures provide refresher training as well as insuring that the technicians will react properly in the event of an actual emergency. The technicians involved in this training are monitored by their supervisor to insure proper performance of the standard operating procedures.

The various emergency training procedures conducted in the past 12 months are shown below:

Date	Procedure	Personnel Participation
June 1977	Complete Power Failure	A
July 1977	Rescue of Incapacitated Dome Entrant	All
August 1977	Fire in the Dome During Dome Entry	A,B
September 1977	Air Supply Fan Failure	A,B
October 1977	Fire in the Airlock	A,B
November 1977	Fire in the Exposure Laboratory Area During Dome Entry	All
December 1977	Operation of the Scott Air Pak	All
January 1978	Fire in the Dome - No Entrant	A
February 1978	Complete Power Failure - Real	A,B
March 1978	Rescue of Incapacitated Dome Entrant	All
April 1978	Fire in the Dome During Entry	A,B
May 1978	Air Compressor Failure	A

A - Shift Operator
B - Safety Observer B
All - All Chamber Technicians on Duty.

Animal Technicians

Since last year's annual report, several animal technicians have become certified in the AALAS program. Three became certified at the second level, Animal Technician, while one was certified at the first level, Assistant Animal Technician. Applications for AALAS certification have been made by two other technicians at the assistant level and one at the animal technician level. UCI animal care personnel certification in the AALAS program is as follows:

1 - Laboratory Animal Technologist

6 - Laboratory Animal Technician

3 - Assistant Animal Technician

The basic course outline of certification by AALAS was described in detail in a previous annual report (MacEwen and Vernot, 1975).

Two animal caretakers have been hired in the past year and each has successfully completed the Purina Animal Care Course. This course, primarily a self-study course, lays a foundation for further study in the field of laboratory animal care. The caretakers completed the course at their own pace under the direction of a supervisor.

Upon satisfying the requirements for and successfully passing the first level AALAS examination, one of the above mentioned animal caretakers was promoted to the position of animal technician.

Advanced Practical Training

These exercises, as described in detail in a previous report (MacEwen and Vernot, 1977) were made available to the technician group. Technicians are assigned to assist the Research Support section on a weekly rotational basis. Each exercise must be signed by a staff member when that technician is able to do each procedure correctly and with confidence. This program is continuous as time permits and has added versatility to our animal technician staff.

Laboratory Animal Medicine and Audiotutorial Series

The Air Force Veterinary Medicine Division has provided approximately 50% of the Laboratory Animal Medicine and Audiotutorial Series and has made this available to the UCI animal technician group. Each subject within the series is directed by an expert in that particular field and includes audiotapes as well as film strips. The VS Division plans to obtain the remainder of this series in the near future.

The following lists the titles which are now available and being used by our technicians in the ongoing training program.

I. Introductory Series

A. Laboratory Animal Medicine/What It is and How It Relates to Veterinary Medicine

B. Diseases of Laboratory Animals as Complications of Biomedical Research

C. Legislation and Guidelines Pertaining to Laboratory Animals

II. The Mouse

A. Biology and Use in Research

B. Handling, Restraint and Other Techniques

C. Husbandry

D. Viral Diseases

E. Bacterial and Parasitic Diseases

F. Neoplastic, Non-infectious and Miscellaneous Diseases

III. Guinea Pig

A. Introduction and Husbandry

B. Biology

C. Disease

IV. The Rabbit

A. Introduction and Biology

B. Husbandry and Techniques

C. Pasteurellosis

D. Parasitic Diseases

E. Miscellaneous

Animal Care Training Videotapes

The videotapes of the animal care course conducted by the School of Aerospace Science at Brooks Air Force Base are being edited and transferred to cassettes. These will soon be available for training of animal and chamber technicians hired during the last two years. The course, outlined in detail in a previous annual report (MacEwen and Vernot, 1975), is a valuable training aid for technicians in their daily routine as well as preparing for AALAS examinations.

Physiological Fluids - Determination of Gas Chromatographic Peak Constituents

Work done in 1976 had shown that injection of carbon tetrachloride in rats at just below lethal levels had caused significant changes in three peaks in the gas chromatograms of urine volatiles. One peak increased significantly and the other two almost disappeared from the chromatograms indicating metabolic changes due to the carbon tetrachloride intoxication.

Similar procedures were carried out this year using MMH as the toxic compound. Two groups of male rats were fasted one day each week for four weeks with urine samples collected for 24 hours in metabolic cages. The urine from the three rats of each group were pooled and analyzed as one sample by the technique described in the 1977 annual report (MacEwen and Vernot, 1977). After sampling the rats 4 times for baseline data, the rats were injected with 4 µl MMH in 100 µl of water by the intraperitoneal route and fasted as before. The volatile compounds distilled from the pooled urine specimens were run on a gas chromatograph and significant changes were seen in 9 peaks of which 4 were reduced 50% or more, 3 were significantly increased and 2 were new peaks not seen in 4 sets of control runs. The data for each group are given in Tables 46 and 47.

Although the fingerprint approach to characterization of changes caused by intoxication is useful in showing differences between baseline and treated animals, it does not identify the chemical nature of the compounds affected. Knowledge of the chemical constitution of these compounds would be helpful in pinpointing the physiological system or systems acting as targets for the toxic effects of MMH. The instrument of choice for identification of gas chromatographic peaks is the mass spectrometer which is not available to the THRU laboratory. Therefore, commercial analytical laboratories were contacted to determine whether they were capable of gas chromatographic/mass spectrometric (GC/MS) analysis of our sample of urine volatiles trapped on Chromosorb 101.

One laboratory stated that they had the technical capability to perform this analysis, and were contracted to do so after investigation determined that their reputation in the field was good. The decision was made to send the laboratory urine from untreated rats for analysis and to specify those peaks for identification which demonstrated large increases or decreases after intoxication. The peaks selected were numbers 12, 17, 20 and 26 from Tables 46 and 47. Therefore, urine was sampled from a group of 3 untreated rats and a 2.5 ml portion chromatographed after trapping on a Chromosorb 101 precolumn to give the flame ionization detector gas chromatogram shown in Figure 36. Another 2.5 ml portion of urine was used to provide a volatiles sample trapped on the Chromosorb 101 precolumn which was sealed after trapping. A gas chromatographic column identical to the one used in our analysis was prepared and sealed. Both trapped sample and column were sent to the commercial laboratory for use in the identification of the 4 peaks mentioned.

TABLE 46. GAS CHROMATOGRAPHIC PEAKS SEEN IN RAT URINE VOLATILES AFTER MMH INJECTION - GROUP A

Peak No.	Baseline Values Week 1	Week 2	Week 3	Week 4	Injected 4 µl MMH
1	1260	612	1031	574	2379
✓2	0	0	0	0	442
3	427	206	169	207	222
4	151	67	89	168	202
5	12	0	0	0	0
6	3024	498	1413	1351	1023
7	7	0	0	0	0
✓8	6	0	1	0	33
9	140	252	156	455	159
10	2135	2100	1313	3402	1372
✓11	0	0	0	0	122
✓12	39	21	21	27	233
13	42	45	31	42	19
14	1470	1122	613	1386	775
✓15	2	1	1	0	4
16	11	16	9	7	8
17	256	147	116	170	80
18	4480	3438	3669	4935	2596
19	756	546	569	700	372
x20	158	153	127	137	50
x21	315	255	226	287	171
22	1680	1260	1375	2065	1937
23	3220	4260	3769	4725	3913
24	8302	8520	9063	7840	7029
25	238	240	325	140	335
*‡x26	1190	1368	2125	1120	736
28	118	168	188	140	78
29	2079	2670	4206	1876	3410
30	117	150	133	0	116
31	77	66	81	91	62
32	1309	1722	1894	406	1728
33	11	24	28	14	23
34	7	6	13	28	12
35	0	18	38	56	29
36	16	13	28	26	19
37	15	9	13	0	0
38	8	12	18	8	0
Volume	28 mls	27 mls	24 mls	28 mls	31 mls

✓ Increase

x Decrease

* This group of animals showed no peak at the retention time of Peak #27 found in Group B (Table 47).

TABLE 47. GAS CHROMATOGRAPHIC PEAKS SEEN IN RAT URINE VOLATILES AFTER MMH INJECTION - GROUP B

Peak No.	Baseline Values				Injected 4 µl MMH
	Week 1	Week 2	Week 3	Week 4	
1	1150	469	1211	1339	1199
✓2	0	0	0	0	205
3	223	300	236	228	382
4	155	125	510	82	157
5	0	0	13	0	0
6	655	2126	2600	1523	1301
7	0	0	0	53	0
✓8	5	13	8	11	31
9	265	219	585	604	92
10	2290	2300	2860	2677	871
✓11	0	0	0	0	115
✓12	35	33	25	25	790
13	40	43	48	53	82
14	1240	1343	943	1234	1199
✓15	5	1	5	2	15
16	13	17	16	11	19
x17	234	231	174	121	77
18	3750	4237	3725	2940	4100
19	650	813	748	546	595
x20	160	127	158	121	51
x21	293	302	311	260	229
22	1050	1644	2405	1050	2818
23	2525	3906	3640	3570	4459
24	6070	7850	10049	8190	7636
25	243	184	299	431	441
x26	1525	1156	1138	2137	646
27	75	0	293	53	379
28	138	250	195	200	82
29	1800	1544	1853	3281	3946
30	180	79	109	147	102
31	15	72	68	105	31
32	1365	713	1709	2031	2204
33	13	13	10	18	21
34	0	9	16	10	10
35	9	28	55	29	44
36	14	11	29	32	108
37	2	0	9	0	21
38	5	11	16	10	8
Volume	20 mls	25 mls	26 mls	21 mls	41 mls

✓ Increase

x Decrease

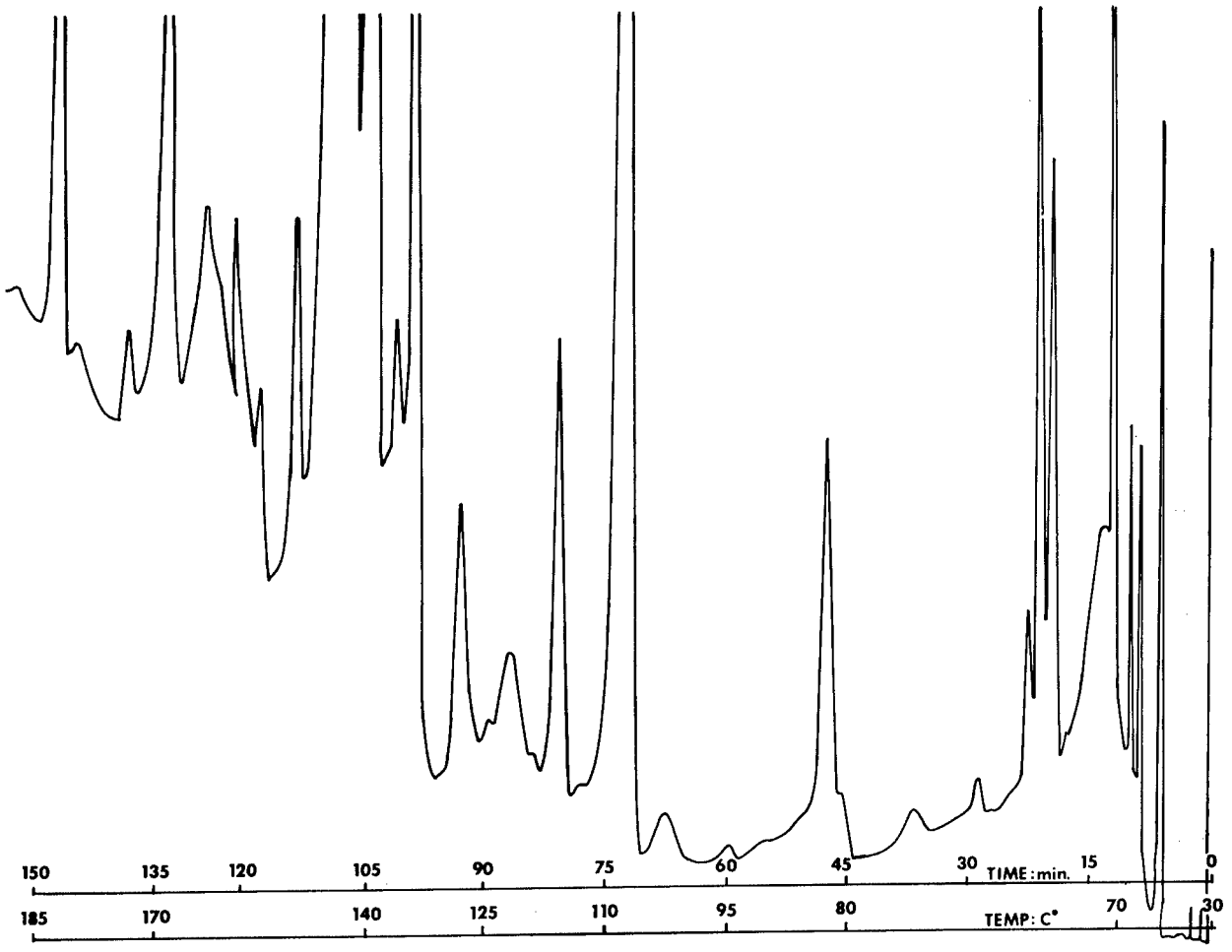


Figure 36. Peaks of rat urine volatiles seen in gas chromatograms prepared at the THRU.

After a month, the laboratory sent a report showing the gas chromatogram obtained in their GC/MS instrumentation (Figure 37) using the column we had sent. They also sent mass spectra corresponding to various points in the chromatogram and identifications based on a number of these spectra. Included in the identifications were supposedly 3 of the 4 peaks we had asked them to characterize. Peak 12 was identified as n-propanol, peak 17 as 3-methyl-2-butanone and peak 20 as dimethyldisulfide. However, because the correspondence between the chromatograms is poor and because of the high background in their chromatogram, it is not possible to show direct relationships between them. The high chromatographic background is reflected in the large number of high background peaks in the mass spectra which occur constantly and cannot be used for identification. Because of these ambiguities it was impossible to accept the peak assignments given by the commercial laboratory without a great deal of reservation.

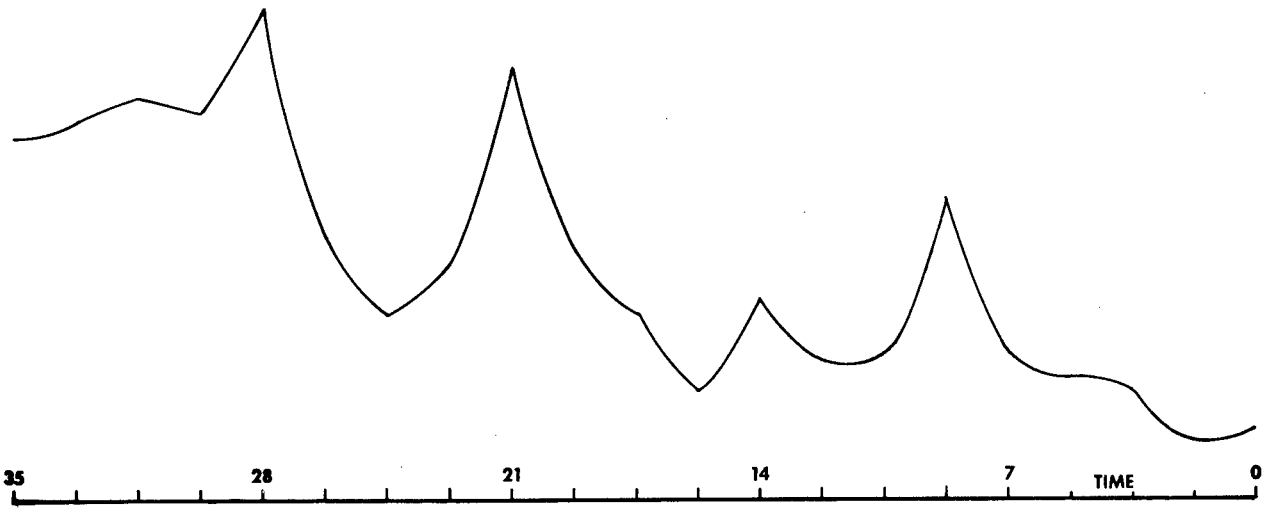


Figure 37. Peaks of rat urine volatiles seen in gas chromatograms prepared by a commercial analytical laboratory.

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