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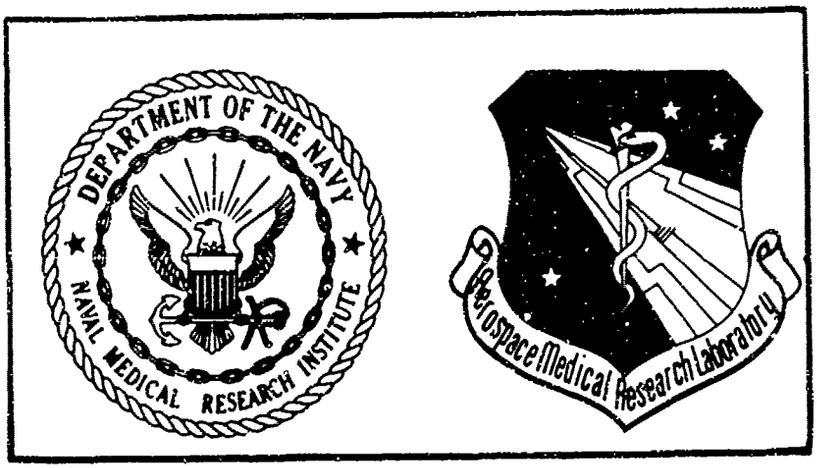

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TOXIC HAZARDS RESEARCH UNIT ANNUAL TECHNICAL REPORT: 1982

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TECHNICAL REVIEW AND APPROVAL

AFAMRL-TR-82-62

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

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

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

FOR THE COMMANDER

Roger C. Inman

ROGER C. INMAN, Colonel, USAF, BSC
Chief, Toxic Hazards Division

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Hydrazine	JP-10	DFM	Fyrquel 220
Otto Fuel II	JP-7	PGDN	Durad MP280
Jet Fuels	JP-TS	Shale Oils	Houghto-Safe 273
JP-4	RJ-5	Hydraulic Fluids	Toxicity
JP-5	Diesel Fuel	Triaryl Phosphate	Chronic (CONT'D)

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

The research program of the Toxic Hazards Research Unit (THRU) for the period of June 1981 through May 1982 is reviewed in this report. Chronic toxicity and oncogenic studies were carried out with hydrazine, Otto Fuel II, JP-4, JP-7, JP-10, JP-TS, and RJ-5. A series of acute toxicity studies was conducted on a variety of chemicals of interest to the Department of Transportation and chemical agents used by the Air Force and Navy. Neurotoxicity studies were conducted on hudraulic fluids containing triaryl phosphate compounds.

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BLOCK 19.

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Skin
Percutaneous
Oral
Inhalation
Sensitization
Dermal
3-Methylcholanthrene
Neurotoxicity
Metabolites
Petroleum Fuels

PREFACE

This is the nineteenth annual report of the Toxic Hazards Research Unit (THRU) and concerns work performed by the Department of Community and Environmental Medicine of the University of California, Irvine on behalf of the Air Force under Contract Number F33615-80-C-0512. This document constitutes the second report under the current contract and describes the accomplishments of the THRU from June 1981 through June 1982.

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

This is a co-sponsored U. S. Air Force/U. S. Navy research effort. That portion of the work effort sponsored by the U. S. Navy was under the direction of LCDR Morris J. Cowan, Jr. and LCDR L. Loring Pitts, MSC, USN, and identified as Navy Task Area Number MF58524001 "Chemical Hazards/Exposure Limits".

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

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SECTION I
INTRODUCTION

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

This document constitutes the 19th annual report of the Toxic Hazards Research Unit, a research team which operates a dedicated inhalation toxicology laboratory to investigate potentially hazardous chemicals and materials of interest to the U. S. Air Force, U. S. Navy, and other governmental agencies. The THRU research team is an interdisciplinary group of University of California, Irvine, toxicologists, chemists, statisticians and engineers. Support services in pathology, Veterinary Medicine and medical technology are provided to the contract by the Air Force.

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

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

ANNUAL CONFERENCE

As part of its contractual responsibilities, UCI/THRU presents an annual technical conference to disseminate new toxicologic information to the U. S. Air Force and other governmental and industrial scientists. This year's conference was chaired by Lutz A. Kiesow, M.D., Ph.D., Chief Scientist, Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland. Twenty-four

technical platform papers were presented covering a wide variety of occupational and environmental toxicology problems. The open forum discussions following each session resulted in significant contributions of additional technical information and scientific exchange. The conference, held 13 November through 15 November, 1981, drew 148 participants including speakers.

The welcoming remarks were presented by Anthony M. Thomas, M.D., former director of the Toxic Hazards Division, AFAMRL, recently retired, who was responsible for the initiation of this series of Conferences on Toxicology in Confined Spaces and Environmental Toxicology.

The Conference Program was submitted to the American Board of Industrial Hygiene and to the University of California, Irvine Continuing Education organization for evaluation. The ABIH awarded 2 1/2 points for recertification and 18 C.E.U.'s were awarded to attending physicians.

The papers presented at the conference were prepared for publication as the Proceedings of the 12th Conference on Environmental Toxicology which is a separate technical report (AFAMRL-TR-81-149).

Our next conference, currently in the development stage, will be held in November 1982 at the Holiday Inn Dayton Mall, Dayton, Ohio.

SECTION II
RESEARCH PROGRAM

Toxicology research conducted by the THRU during the past year was primarily concerned with the long-term tumorigenic or combined chronic toxic and tumorigenic effects of inhaled fuels. The one-year inhalation exposure phase of studies on high altitude aircraft fuels, JP-7 and JP-TS and a torpedo fuel, Otto Fuel II, were completed this year. Comparative studies on conventional and alternate fuels still in progress or undergoing evaluation of tissue changes include petroleum or shale JP-5 and Shale DFM. Animals from one-year inhalation exposures to petroleum derived JP-4 and a high density fuel JP-10 completed a one-year postexposure holding and observation period and were submitted for final tissue examinations. Final reports of the results of these studies will await completion of the tissue examinations.

Other research activities during the past year included a series of acute toxicity tests on a candidate composite material for aircraft construction, acetylene terminated sulfone (ATS). For information on ATS, see AFAMRL-TR-82-20. Acute and subchronic studies were also conducted on various hydraulic fluids including some triarylphosphates and an ethylene glycol based compound.

The current status of these ongoing studies is summarized in this report. A report of histologic evaluation of tissues from animals exposed to unsymmetrical dimethylhydrazine was received and is currently undergoing statistical examination. A final report is in preparation and will be published as a Technical Report in the coming year.

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

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

Background

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

Methods

A brief description of the protocol for preliminary studies was presented in the 1980 THRU Annual Report (MacEwen and Vernot, 1980). A complete description of the protocol along with the findings of

the Phase I and Phase II portions as well as the Phase III exposure data through May, 1981 was presented in the 1981 THRU Annual Report (MacEwen and Vernot, 1981).

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

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

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

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

The results of Phase II served adequately as a pilot study for Phase III in that it demonstrated that repeated weekly one-hour exposures to 750 ppm hydrazine were tolerated by rats and hamsters with no mortality. A total of 900 rodents were used in Phase III to evaluate the oncogenic potential of hydrazine following exposure to the selected concentrations of 750 or 75 ppm. The latter dose was chosen in an attempt to establish a no-effect level. The exposure regimen was the same as established in Phase II: one hour per week

for 10 weeks (total CT values 7500 and 750 ppm hours). The two exposure groups as well as an unexposed control group each consisted of 100 male rats, 100 female rats, and 100 male hamsters.

Results

Current body weight data for [illegible] III male rats, female rats, and male hamsters are presented in [illegible]gures 1, 2, and 3, respectively. The body weight gains of [illegible] and female rats from the high level exposure group were [illegible] affected during the exposure period and for a short time [illegible]eafter (Figures 1 and 2). Body weight gain of the male rats from [illegible]e low level exposure was also affected but to a lesser extent. [illegible] one-year postexposure, body weight means are essentially equal [illegible] all male rat groups and all female rat groups. The effects [illegible]razine exposure on male hamster body weight is less clear. [illegible]ere was an effect on body weight gain in the high level exposure group. However, for some unknown reason the rate of growth in control hamsters began to taper off during the third week on stud[illegible]. There was also a decline in the body weight of this control group toward the end of the exposure period which was never regained. At one year postexposure the control hamsters were significantly smaller than either the low level or high level exposure groups of hamsters. Current mortality ratios are presented in Table 1. Male and female rat mortality ratios are quite low. Hamster mortality has been higher than expected and mortality trends in this species were closely examined.

TABLE 1. MORTALITY IN PHASE III RODENTS AT 1 YEAR POSTEXPOSURE TO BRIEF HIGH LEVELS OF INHALED HYDRAZINE (7500 PPM-HOURS)

Species	Sex	Nominal CT (ppm/hours)	Mortality Ratio
Rats	Male	0	3/100
		750	2/100
		7500	3/100
Rats	Female	0	2/100
		750	1/100
		7500	4/100
Hamsters	Male	0	27/100
		750	23/100
		7500	17/100

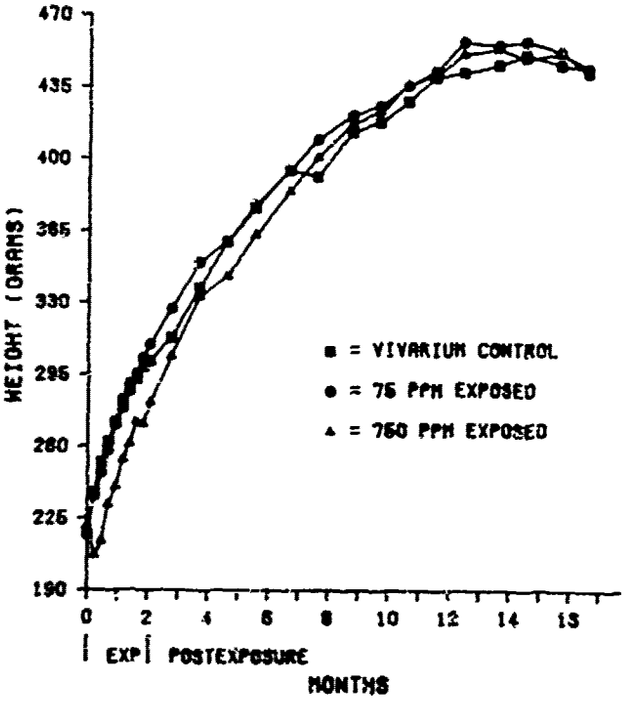


Figure 1. Effects of Phase III hydrazine exposure on male rat body weight.

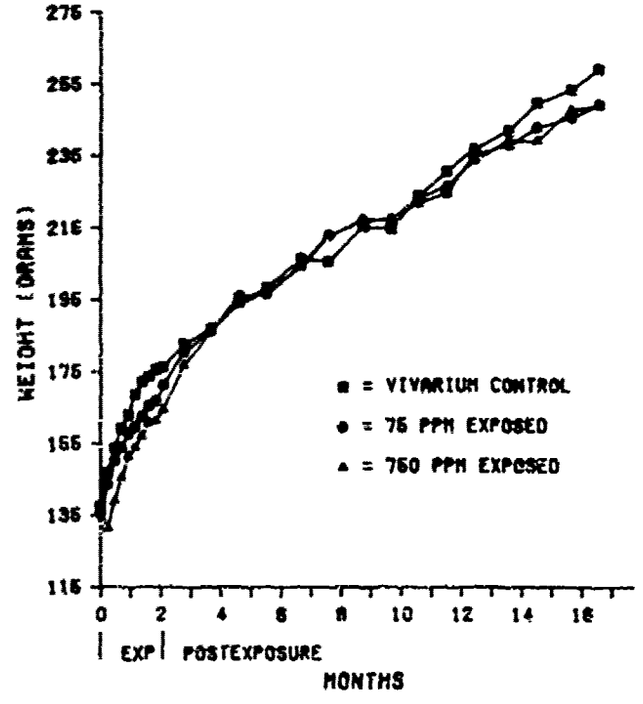


Figure 2. Effects of Phase III hydrazine exposure on female rat body weight.

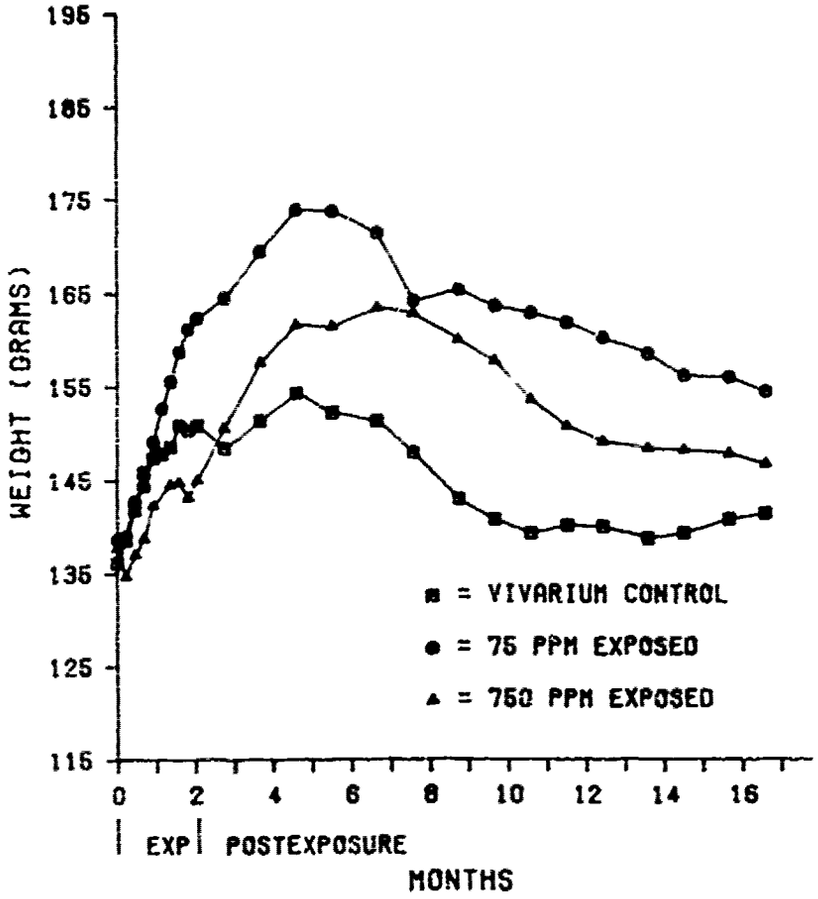


Figure 3. Effects of Phase III hydrazine exposure on male hamster body weight.

Hamster mortality curves are presented in Figure 4. These curves indicate a sharp rise in control group mortality starting 5 months postexposure followed more slowly in the other two groups. No specific cause for increased mortality or specific cause of death could be determined from gross pathologic or histologic examinations of the dead control hamsters nor could a pathogenic microorganism be cultured. Since that period, the differences in mortalities among the experimental groups have been decreasing.

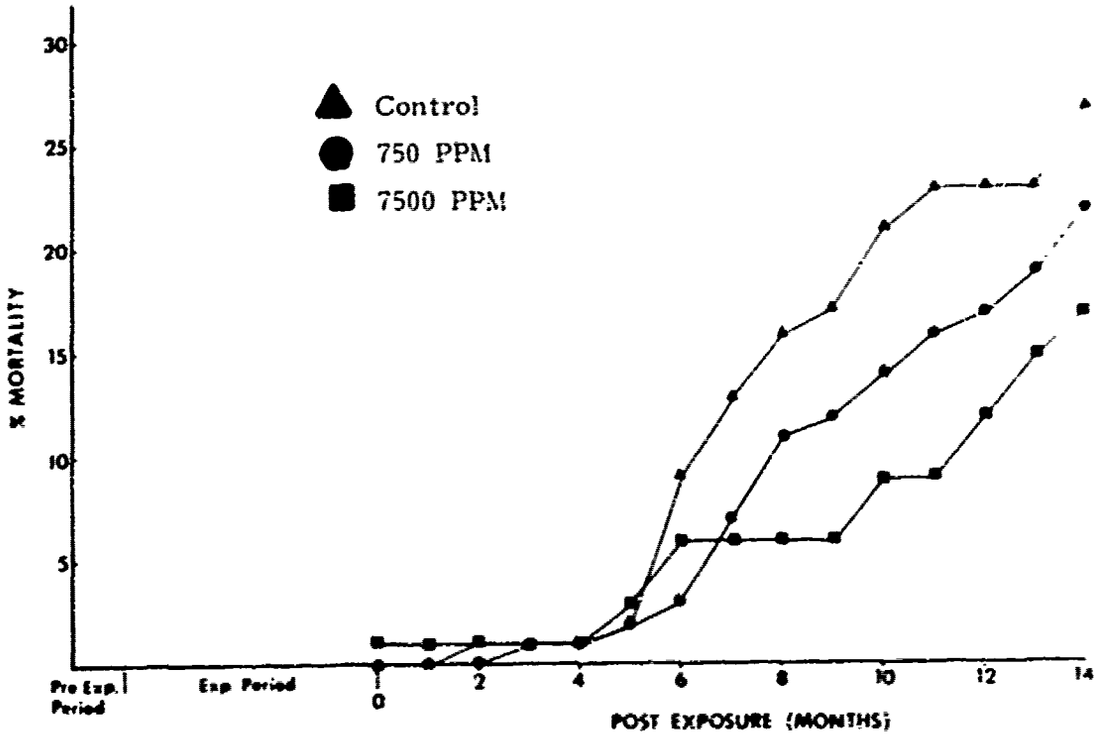


Figure 4. Cumulative mortality of hamsters exposed to brief high concentrations of hydrazine in Phase III.

The first sacrifice for the Phase III animal groups is scheduled for May 1983, 24 months after the last inhalation challenge with hydrazine. At that time all surviving hamsters will be sacrificed along with 10 male and 10 female rats from each of the exposed and control groups. The study concludes in November 1983, 30 months postexposure.

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

In 1979 the THRU conducted a 90-day continuous inhalation toxicity study of oil shale derived JP-5 Jet Fuel. This study, conducted for the Navy, was a companion to previous inhalation exposures with Petroleum JP-5. The results of the two studies will be used for the comparison of health hazards between conventional and alternate fuel sources.

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

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

Toxic effects observed at the conclusion of the 90-day exposure included increased kidney weights in male rats along with elevated blood urea nitrogen and creatinine levels (MacEwen and Vernot, 1980). Shale JP-5 exposure related tissue changes included nasal inflammation and hepatocyte cytoplasmic vacuolization in male and female rats, renal tubular necrosis in almost all male rats, and fatty livers in female mice (MacEwen and Vernot, 1981). With the exception of the nasal inflammation in rats exposed to shale JP-5, the results available at the exposure termination indicated the toxic effects of petroleum and shale JP-5 to be very similar.

The protocol, contaminant generation and monitoring methods for this study were detailed in a previous annual report (MacEwen and Vernot, 1980).

Results

Rat body weight curves are shown in Figures 5 and 6 for male and female rats, respectively. A dose related effect of exposure to shale JP-5 vapor is evident in the body weight of male rats. The effects on body weight were first noted after one month of exposure and continued through the entire 24-month study period. The effect of exposure to shale JP-5 vapor was not as pronounced in the female rats as in the males. Depressed growth was seen in the female rats exposed to 750 mg/m^3 when compared to unexposed controls, while exposure to 150 mg/m^3 had only minimal effect on female rat growth.

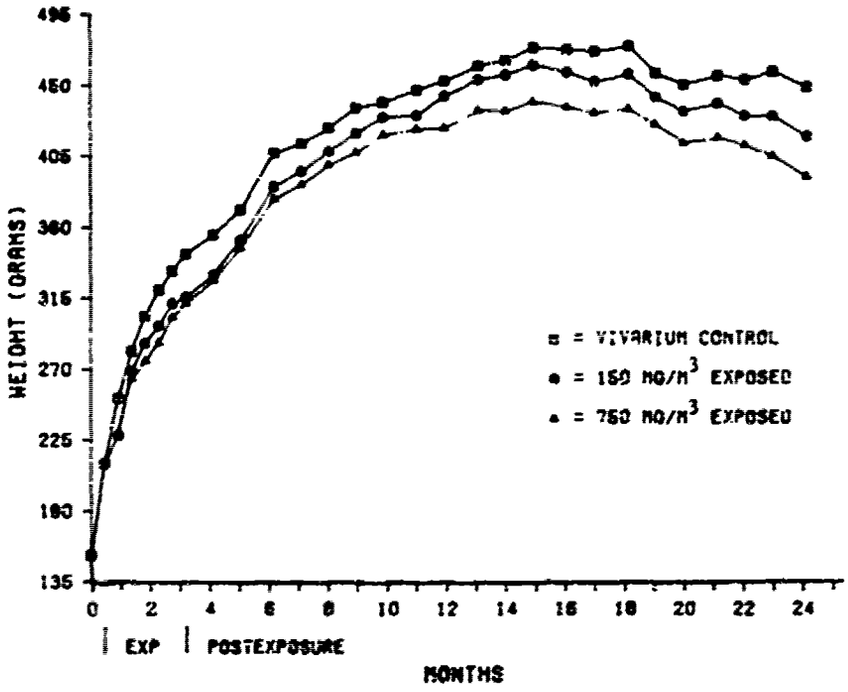


Figure 5. Effect of 90-day shale JP-5 exposure on male rat body weight.

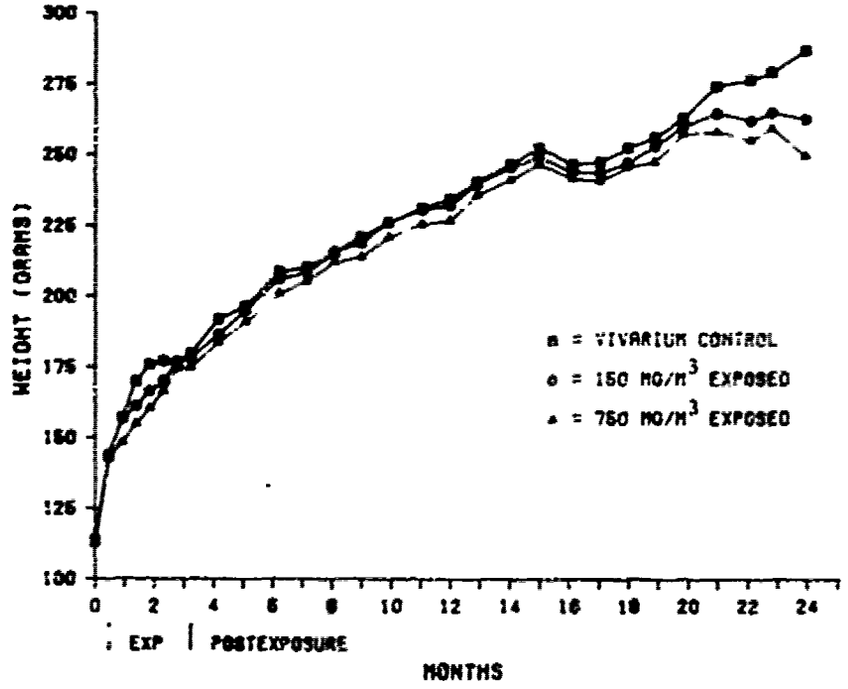


Figure 6. Effect of 90-day shale JP-5 exposure on female rat body weight.

The results of analysis of organ weights of rats sacrificed at 19 months postexposure are shown in Table 2. Kidney/body weight ratios of the male rats exposed to 750 mg/m^3 were significantly greater ($p \leq 0.01$) than kidney/body weight ratios of the unexposed control male rats. A similar difference between control and 750 mg/m^3 exposed male rats was also noted in the group of animals sacrificed at the conclusion of the exposure period. The decreased liver and kidney weights seen in the female rats exposed to shale JP-5 vapor may reflect the decreased body weights of these groups at the time of sacrifice. Organ/body weight ratios for the liver and kidney of exposed female rats are not statistically different from control values.

TABLE 2. ORGAN WEIGHTS OF RATS 19 MONTHS AFTER 90-DAY CONTINUOUS EXPOSURE TO SHALE JP-5 VAPOR (MEAN ± STD. DEV.)

	Male Rats		
	Controls	150 mg/m^3	750 mg/m^3
Body Weight, g	437	413[b]	403[b]
Liver Weight, g	11.57 ± 1.93	11.07 ± 1.60	11.61 ± 1.49
Liver/100 g body wt.	2.66 ± 0.49	2.69 ± 0.47	2.89 ± 0.41
Kidney Weight, g	2.89 ± 0.17	2.77 ± 0.37	2.90 ± 0.24
Kidney/100 g body wt.	0.66 ± 0.05	0.67 ± 0.11	0.72 ± 0.08[b]
Spleen Weight, g	1.50 ± 1.18	0.98 ± 0.21	1.56 ± 1.36
Spleen/100 g body wt.	0.35 ± 0.29	0.24 ± 0.05	0.39 ± 0.32

	Female Rats		
	Controls	150 mg/m^3	750 mg/m^3
Body Weight, g	268	252[a]	246[b]
Liver Weight, g	6.83 ± 0.63	6.58 ± 0.66	6.20 ± 0.65[b]
Liver/100 g body wt.	2.55 ± 0.25	2.61 ± 0.16	2.55 ± 0.17
Kidney Weight, g	1.89 ± 0.15	1.77 ± 0.10[b]	1.74 ± 0.13[b]
Kidney/100 g body wt.	0.71 ± 0.06	0.70 ± 0.04	0.71 ± 0.04
Spleen Weight, g	0.54 ± 0.14	0.51 ± 0.05	0.57 ± 0.37
Spleen/100 g body wt.	0.20 ± 0.05	0.20 ± 0.03	0.23 ± 0.16

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

Blood values of the male rats sacrificed at 19 months postexposure are shown in Table 3. Because of instrument malfunction, sodium and potassium analyses could not be conducted. A number of differences between control and shale JP-5 exposed rats are indicated; most of these are probably not associated with exposure. However, the elevated blood urea nitrogen levels in male rats exposed to 750 mg/m^3 shale JP-5 may indicate that the renal necrosis seen immediately following the 90-day continuous exposure has persisted. Blood values of female rats are shown in Table 4. The few statistically significant differences noted between control and exposed animals appear to be incidental findings unrelated to exposure. The BUN and creatinine levels of exposed female rats are not different from unexposed controls which is further indication of the sex specificity of the renal damage resulting from JP-5 exposure in male rats.

TABLE 3. MEAN BLOOD VALUES OF MALE RATS 19 MONTHS AFTER CONTINUOUS EXPOSURE TO SHALE JP-5 VAPOR FOR 90-DAYS

	Unexposed Controls	N	Exposed 150 mg/m^3	N	Exposed 750 mg/m^3	N
RBC (10^6)	8.1	22	8.1	24	8.2	22
WBC (10^3)	5.1	21	5.9[a]	24	5.9[a]	21
HCT (%)	50.5	22	50.9	24	48.7	22
HGB (g/dl)	16.9	22	17.1	24	16.2	22
Total Pro. (g/dl)	7.5	21	7.2[a]	24	6.7[b]	22
Albumin (g/dl)	4.3	21	4.2	24	3.9[a]	21
Globulin (g/dl)	3.2	21	2.9[a]	24	2.8[a]	22
A/G Ratio	1.3	21	1.4	24	1.5	22
Glucose (mg/dl)	116	21	136[a]	24	150[b]	22
Calcium (mg/dl)	11.4	21	11.2	19	10.9[b]	22
Bilirubin (mg/dl)	0.55	21	0.61	23	0.77	22
Creatinine (mg/dl)	0.5	20	0.5	21	0.6[a]	22
SGPT (IU/L)	53	22	41	24	40	22
SGOT (IU/L)	112	22	84	24	88	22
Alk. Phos. (IU/L)	9.5	22	7.9[a]	23	20.7[a]	21
BUN (mg/dl)	17.3	22	19.9	22	20.7[a]	22
MCV	62.8	22	64.2	24	60.1	22
MCH	21.1	22	21.6	24	20.0	22
MCHC	33.6	22	33.6	24	33.3	22

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

TABLE 4. MEAN BLOOD VALUES OF FEMALE RATS 19 MONTHS AFTER CONTINUOUS EXPOSURE TO SHALE JP-5 VAPOR FOR 90-DAYS

	Unexposed Controls	N	Exposed 150 mg/m^3	N	Exposed 750 mg/m^3	N
RBC (10^6)	7.2	18	7.5	20	7.3	22
WBC (10^3)	3.2	18	3.3	20	3.5	22
HCT (%)	43.2	18	42.3	20	42.7	22
HGB (g/dl)	14.9	18	14.5	20	14.9	22
Total Pro. (g/dl)	7.8	18	7.7	20	7.7	22
Albumin (g/dl)	4.5	18	4.4	20	4.3	22
Globulin (g/dl)	3.3	18	3.3	20	3.3	22
A/G Ratio	1.3	18	1.3	20	1.3	22
Glucose (mg/dl)	129	18	137	20	141	22
Calcium (mg/dl)	10.9	18	10.7	20	10.5[a]	22
Bilirubin (mg/dl)	0.46	18	0.49	20	0.48	22
Creatinine (mg/dl)	0.5	18	0.5	20	0.4	22
SGPT (IU/L)	38	18	42	20	38	22
SGOT (IU/L)	78	18	74	20	81	22
Alk. Phos. (IU/L)	6.9	18	15.1	20	15.1	22
BUN (mg/dl)	16.2	18	15.1	20	15.1	22
MCV	60.7	18	56.8[a]	20	58.7	22
MCH	20.9	18	19.5[a]	20	20.4	22
MCHC	34.5	18	34.3	20	34.8	22

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

Examination of the tissues of animals held for postexposure observation and killed at interim and final sacrifices is in process. Results of these examinations will be presented as they become available.

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

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

Beginning in February 1980, mice and rats were exposed to JP-4 concentrations of 5000 mg/m^3 and 1000 mg/m^3 by the inhalation route in Thomas Dome inhalation chambers for one year using a work week schedule of 6 hours/day, 5 days/week with holidays and weekends excluded to simulate a human industrial exposure regimen. Each exposure group consisted of 100 male and 100 female Fischer 344 rats and 100 male and 100 female C57BL/6 mice. Another group with the same numbers of animals was held at the Veterinary Sciences Division Building (Vivarium) to serve as controls. Anirals were caged in conformance with ILAR standards for laboratory animal care.

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

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

Results

Mean body weights for the rat groups obtained on a biweekly schedule through 12 months of exposure and monthly thereafter are shown in Figures 7 and 8. Both exposed groups of male rats showed a significant depression in mean body weight for the extent of the study. The female control group failed to gain weight at a rate equal to the exposed groups during the 12-month exposure period but surpassed the exposed groups during the postexposure phase of the study. Mouse mean weights revealed no exposure-related effects.

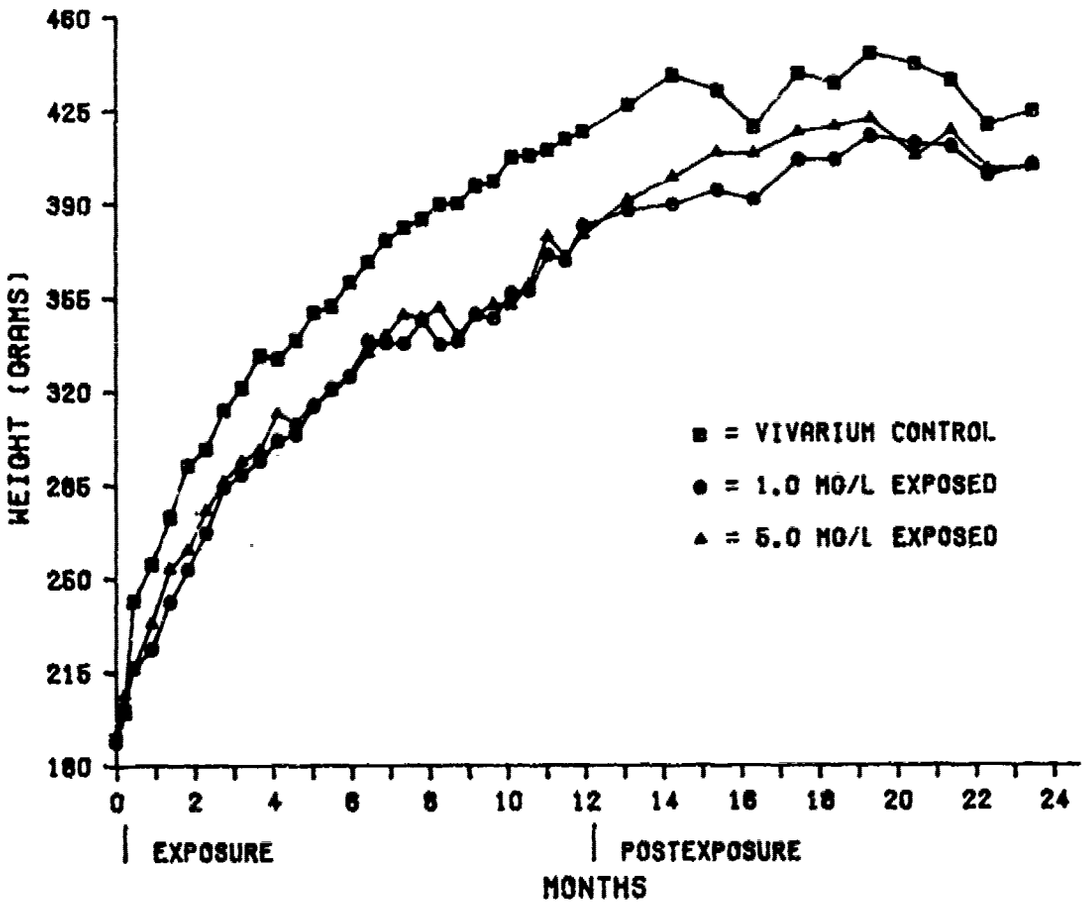


Figure 7. Mean body weights of male rats exposed to JP-4.

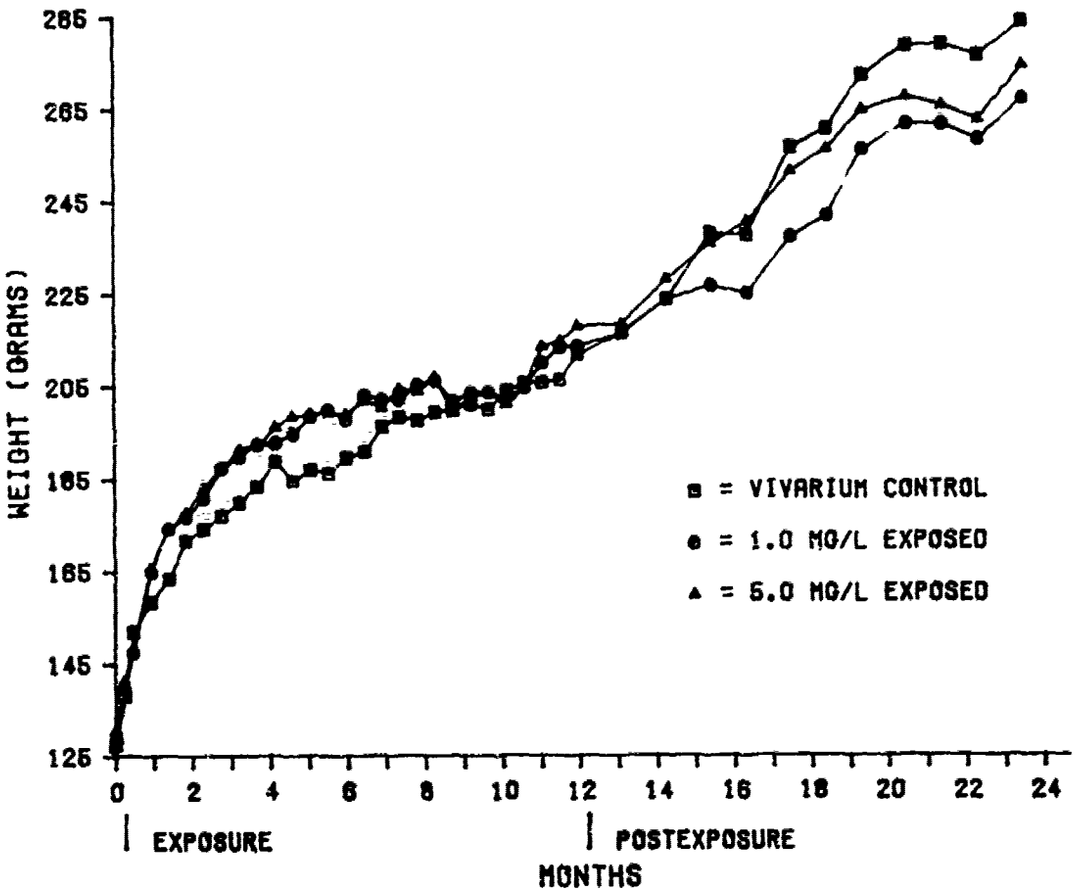


Figure 8. Mean body weights of female rats exposed to JP-4.

Organ weights and blood effects for male and female rats sacrificed at exposure termination can be found in a previous annual report (MacEwen and Vernot, 1981). Organ weights for the male and female rats sacrificed after one-year of postexposure observation are shown in Table 5. In all cases the mean body weights of the control rats were higher than their respective exposure groups, and there were some statistically significant differences in mean organ weights. In most cases these did not occur in the organ to body weight ratios and where they did, the responses were not dose related. We do not attribute any biological significance to these differences.

TABLE 5. MALE AND FEMALE RAT ORGAN WEIGHTS AT CONCLUSION OF JP-4 STUDY (MEAN ± STD. DEV.)

	Unexposed Controls N = 55	1000 mg/m^3 Exposure Group N = 57	5000 mg/m^3 Exposure Group N = 59
MALES			
Body weight, g	416 ± 29	401[a] ± 32	396[b] ± 29
Liver weight, g	14.20 ± 2.35	12.63[b] ± 1.63	13.26[a] ± 1.82
Liver/100 g body wt	3.43 ± 0.59	3.16[b] ± 0.41	3.35 ± 0.40
Spleen weight, g	1.68 ± 1.95	1.76 ± 1.90	1.48 ± 0.83
Spleen/100 g body wt	0.42 ± 0.58	0.43 ± 0.44	0.37 ± 0.21
Kidney weight, g	3.28 ± 0.45	2.95[b] ± 0.36	3.05[b] ± 0.46
Kidney/100 g body wt	0.79 ± 0.12	0.74[a] ± 0.11	0.78 ± 0.12
	N = 54	N = 66	N = 67
FEMALES			
Body weight, g	273 ± 27	260[b] ± 27	268 ± 30
Liver weight, g	9.36 ± 1.25	8.99 ± 1.69	9.14 ± 1.73
Liver/100 g body wt	3.44 ± 0.48	3.48 ± 0.66	3.45 ± 0.84
Spleen weight, g	1.13 ± 1.42	1.41[a] ± 2.65	0.69 ± 0.57
Spleen/100 g body wt	0.43 ± 0.57	0.57 ± 1.14	0.26 ± 0.25
Kidney weight, g	2.11 ± 0.18	2.03[a] ± 0.17	2.08 ± 0.21
Kidney/100 g body wt	0.78 ± 0.10	0.79 ± 0.09	0.78 ± 0.11

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

Examination of the animal tissues collected for histopathologic evaluation is incomplete. The results will be described in a future annual report.

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

A 12-month industrial type chronic exposure of rats, mice, hamsters, and dogs to 100 ppm JP-10 was conducted from June 1978 to June 1979. This experiment was designed to provide information to establish safe exposure levels and assess the oncogenic potential of JP-10 in rodents. Previous annual reports (MacEwen and Vernot, 1979, 1980, 1981) contain the experimental protocol, mortality results, body weight data, and clinical chemistry information collected from the start of the exposure to the termination of the rodent experiment in June 1980.

Maintenance of the dogs will continue for 5 years postexposure, until June 1984. Abnormally high SGPT and SGOT values were noted for one control dog following semi-annual measurements in December 1981. The reason for these elevations was not identified but the levels returned to normal within two weeks. One control dog died in July 1981. This animal had a short-term record of epileptic type seizures that may be related to the cause of death. Histopathologic results have not been completed. A review of the results of all quarterly physical examinations and semiannual clinical chemistry measurements indicate that the 15 surviving dogs are healthy at this time.

The absence of complete histopathologic examinations and results prevent the reporting of the information that is necessary to evaluate the oncogenic and non-oncogenic effects and recommend a safe exposure level for JP-10.

THE EVALUATION OF THE ONCOGENIC POTENTIAL OF OTTO FUEL II

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

Background information on this study was presented in the previous annual report (MacEwen and Vernot, 1981). Pertinent Otto Fuel II generation and monitoring data are also described in that report. Briefly, male and female dogs, rats, and mice were exposed to 1.4 mg/m^3 Otto Fuel II vapor. Male and female rats and mice were exposed to 240 mg/m^3 Otto Fuel II vapor. Exposures were conducted in

Thomas Dome Inhalation Chambers on a 6 hour/day, 5 day/week basis. Exposures were not conducted on weekends or holidays. At the completion of one year of exposure, 10 male and 10 female rats and 10 male and 10 female mice from each experimental group were randomly chosen for necropsy. All dogs were sacrificed at the conclusion of the exposure phase. Remaining rodents are presently being held for a one-year postexposure observation period. At the time of necropsy, tissues were collected for histopathologic examination. Rat blood was also collected for hematologic and clinical chemistry tests. Liver, kidney, and spleen weights were recorded on all rats and dogs sacrificed. Body weights will continue to be recorded regularly through the course of the study.

Otto Fuel II Generation and Monitoring

The chief component (≈75%) of Otto Fuel II is the nitrate ester 1,2-propylene glycol dinitrate (PGDN). The balance of Otto Fuel II is comprised of 2-nitrodiphenylamine (2%) added as a stabilizer and di-n-butyl sebacate (23%) added as a desensitizer.

Because of large differences in vapor pressures of the various constituents of Otto Fuel II, PGDN was the only compound vaporized into the inhalation exposure chambers in sufficient quantity to allow direct analysis. Therefore, exposure generation control and analysis was based on PGDN concentrations in the chambers.

The large difference in concentrations of Otto Fuel II between the two experimental atmospheres, as well as safety considerations, necessitated separate generation systems.

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

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

The contaminant introduction system for the 240 mg/m^3 exposures consisted of three large electrically heated evaporator towers. Each tower had a fuel flow of approximately 0.33 ml/min with a counter-current air flow of 5 cfm. The vapor passed through 1"

stainless steel lines to the dome input air line. Waste fuel was pumped from the bottom of the towers to a container for disposal.

Dome concentration was maintained by input heat as well as evaporator tower air flow rate. Fine control of concentration was obtained by adjustment of total chamber air flow.

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

In the process of vapor generation for the chambers, about 10-15% of the original Otto Fuel II was volatilized and the remaining fuel was returned from the generation system to a waste tank. At the completion of the exposure phase of this study, the unused fuel and the waste was returned to the U. S. Navy for ultimate disposal.

Although the study was initially designed as a one-year industrial type exposure for animals, it was continued for two additional months to observe a trend in the hematologic values of the PGDN exposed dogs. The mean analyzed chamber concentrations to which the animals were exposed are shown in Table 6.

TABLE 6. MEAN PGDN VAPOR CONCENTRATIONS FOR INDIVIDUAL EXPOSURE GROUPS

Species	Nominal Concentration mg/m^3	Days of Exposure	Measured Concentration* mg/m^3
Rats, Mice	240	249	241 ± 5.19
Rats, Mice	1.4	249	1.43 ± 0.08
Dogs	1.4	293	1.43 ± 0.08

* Mean ± S.D.

Quality control measurements were conducted on one of the ten canisters of Otto Fuel II received from the U. S. Navy. This container, identified as canister number 6, was tested three times with the results shown in Table 7. The analysis matched the percentage composition specified for Otto Fuel II.

TABLE 7. QUALITY CONTROL ANALYSIS OF OTTO FUEL II IN CANISTER NUMBER 6

Injection #	% PGDN	% DBS	% 2-NDPA
1	75.9	22.6	1.4
2	76.4	22.2	1.5
3	75.9	22.3	1.8
AVERAGE	76.1	22.4	1.6
STANDARD DEVIATION	±0.281	±0.240	±0.204

The presence of a trace contaminant, ortho-chloronitrobenzene (OCNB) was determined in the neat Otto Fuel II used for these exposures, in the distillate of volatilized Otto Fuel II, and in a vapor trap located in the PGDN introduction line of the low level exposure chamber. These analyses were conducted to estimate chamber concentrations and maximum probable dose of OCNB received by rats during the one-year exposure because this compound had been reported to be a tumorigen. OCNB had been shown to produce late life tumors in male rats and hepatocellular tumors in female rats after sustained 12-month doses of 500 milligrams per kilogram of body weight. Calculations based on maximum OCNB found in distillate condensate, using average rat ventilation rates and assuming 100% retention, show an estimated life dose for each rat to be 0.05 mg/kg, an insignificant amount compared to the 500 mg/kg dose found to be carcinogenic. The details of the OCNB analyses and computations were published in a separate technical report (Einhaus et al., 1982).

Results of Animal Exposures

Animals exposed to Otto Fuel II vapor for one year exhibited normal activity. Rodent mortality through the 20th month of the study is shown in Table 8. Exposure to Otto Fuel II vapor has to date not adversely affected the longevity of rats compared to unexposed controls. At 8 months postexposure, both male and female exposure group mortality values are less than respective controls. Mice exposed to Otto Fuel II vapor had slightly greater mortality rates when compared to rats. The values, however, are comparable to unexposed control mice.

TABLE 8. CUMULATIVE MORTALITY OF RODENTS EXPOSED TO OTTO FUEL II (CUMULATIVE %)

RATS

Study Month	Male (%)			Female (%)		
	Control	1.4 mg/m³	290 mg/m³	Control	1.4 mg/m³	290 mg/m³
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	1	0	0
4	0	0	0	1	0	0
5	0	0	0	4	0	1
6	0	0	0	4	0	1
7	0	0	0	4	0	1
8	0	0	0	4	0	1
9	0	1.4	0	4	0	1
10	0	1.4	0	6	0	1
11	1	1.4	0	7	0	2
12	1	1.4	0	7	0	3
13	1.2	1.7	0	11.1	3.1	5.6
14	1.2	3.3	0	11.1	3.1	5.6
15	1.2	3.3	1.1	11.1	3.1	5.6
16	3.5	3.3	1.1	13.3	4.6	5.6
17	5.8	3.3	1.1	13.3	10.8	6.7
18	7.0	3.3	1.1	13.3	10.8	6.7
19	7.0	3.3	4.5	15.6	12.3	6.7
20	8.1	5.0	6.8	22.2	12.3	12.2

MICE

Study Month	Male (%)			Female (%)		
	Control	1.4 mg/m³	290 mg/m³	Control	1.4 mg/m³	290 mg/m³
1	1	0	1	0	0	2.0
2	1	0	1	0	2.7	4.1
3	1	1.3	1	0	4.1	7.1
4	1	2.7	2	0	4.1	7.1
5	3	2.7	4	4	5.4	8.2
6	3	5.3	4	4	5.4	9.2
7	3	5.3	5.1	6	5.4	9.2
8	4	5.3	6.1	6	5.4	10.2
9	4	5.3	6.1	6	5.4	12.2
10	5	5.3	6.1	7	5.4	12.2
11	9	8.0	6.1	7	5.4	12.2
12	10	9.2	6.9	8	8.2	12.2
13	12.2	9.2	7.9	10	11.1	14.8
14	13.3	9.2	9.0	13.3	12.7	15.9
15	13.3	9.2	10.1	16.7	12.7	18.2
16	15.6	9.2	10.1	23.3	17.5	20.5
17	16.7	13.8	14.6	26.7	23.8	26.1
18	18.9	13.8	16.9	31.1	34.9	34.1
19	24.4	13.8	18.0	35.6	39.7	34.1
20	28.9	13.8	19.1	37.8	46.0	36.4

Male and female rat body weights are shown in Figures 9 and 10, respectively. Exposure to Otto Fuel II retarded the growth of male rats compared to unexposed control rats. This effect appeared to be dose related. Body weights of male rats exposed to 240 mg/m^3 were approximately 10% less than control male rat body weights through the exposure period. This difference continued into the first half of the postexposure observation period. Body weights of the female rats exposed to Otto Fuel II were comparable to unexposed controls through 7 months of exposure. Subsequent weighings during postexposure observation show both exposure groups weighing less than the unexposed control group until the 20th month when the control groups experienced a decreased rate of weight gain. The differences between the two exposure groups remained relatively constant.

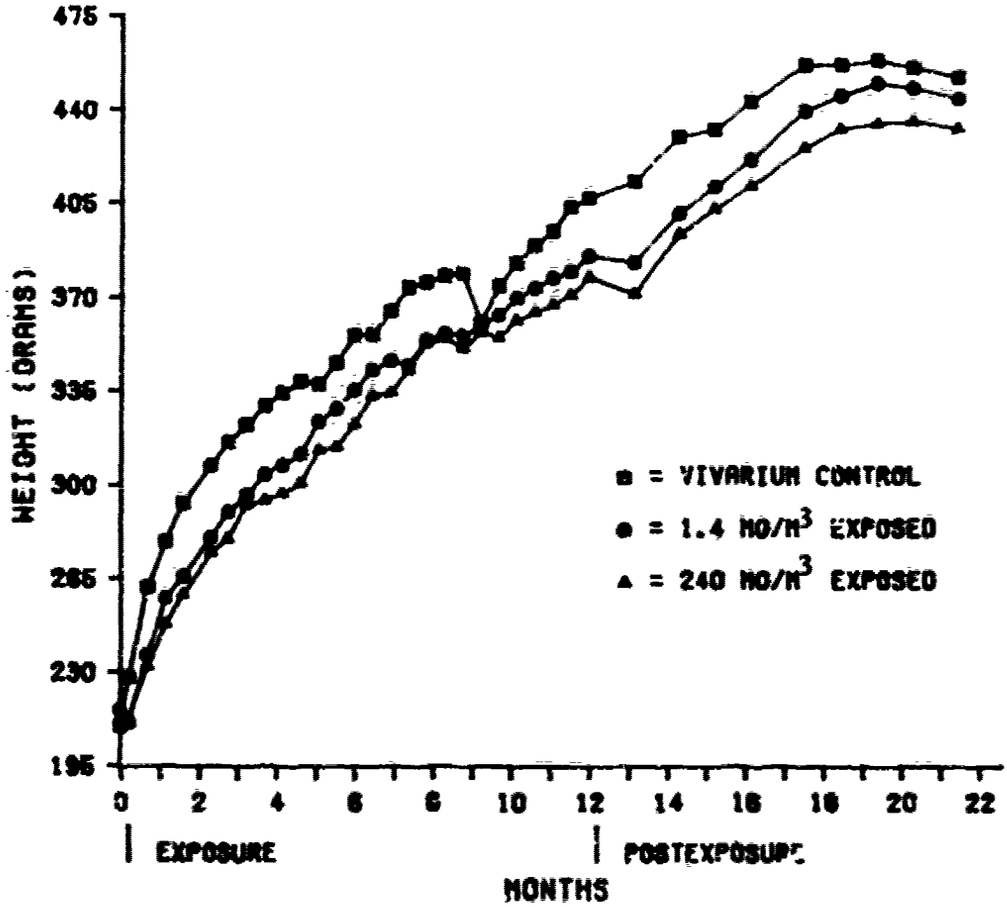


Figure 9. Effects of Otto Fuel II exposure on male rat body weight.

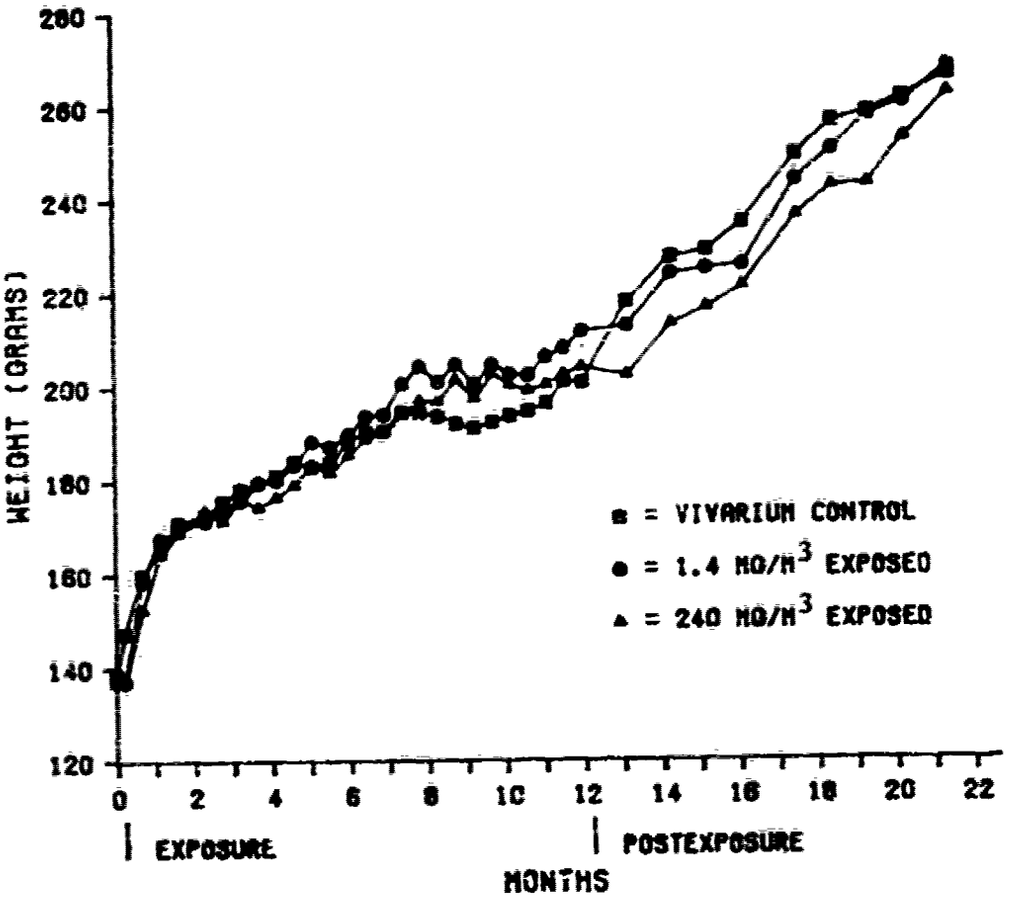


Figure 10. Effects of Otto Fuel II exposure on female rat body weight.

A number of hematology and clinical chemistry measurements were made on dog blood through the exposure period. The majority of these measurements revealed no significant trends. However, early in the exposure phase, the dogs exposed to Otto Fuel II developed signs of anemia. Red blood cell counts, hematocrit, and hemoglobin values are shown in Figures 11 through 13, respectively. These hematologic parameters were consistently lower than unexposed control dog blood values through 52 weeks of exposure.

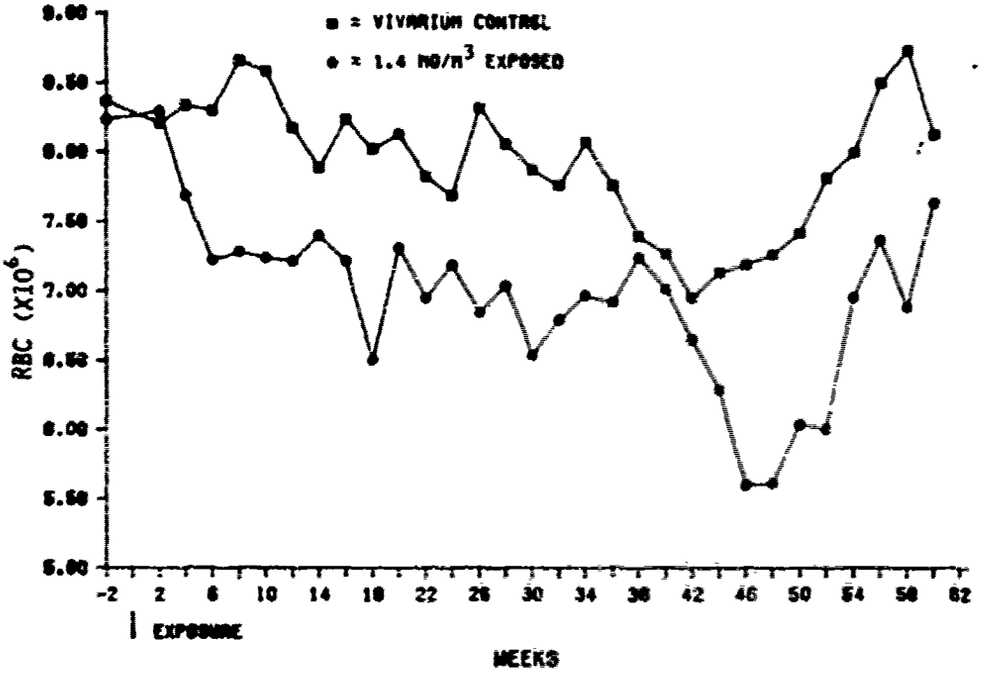


Figure 11. Effect of Otto Fuel II exposure on dog red blood cell count.

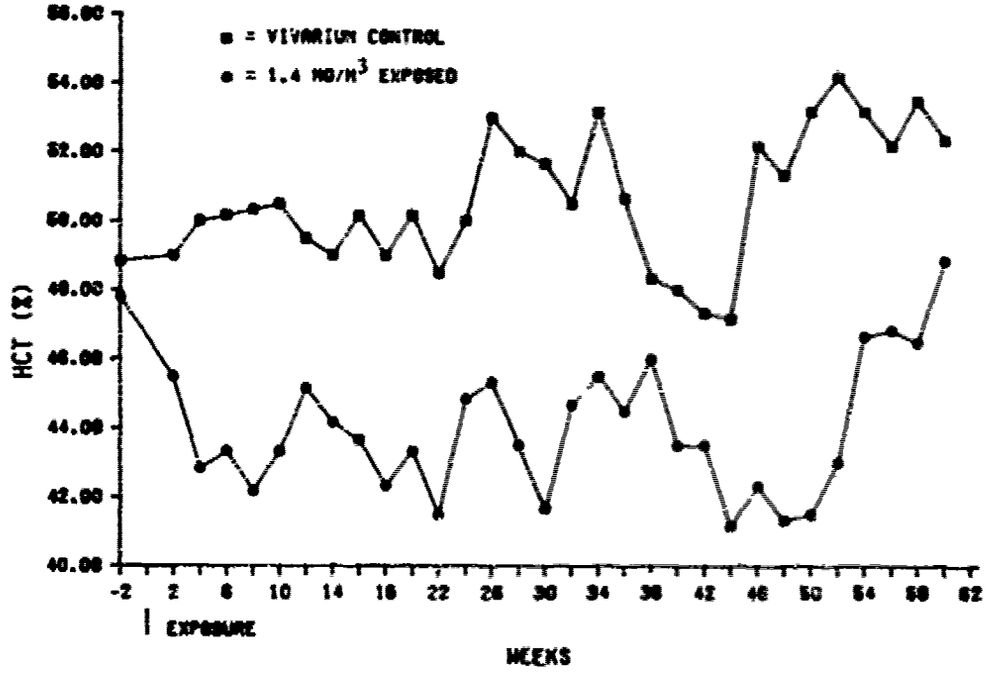


Figure 12. Effect of Otto Fuel II exposure on dog hematocrit.

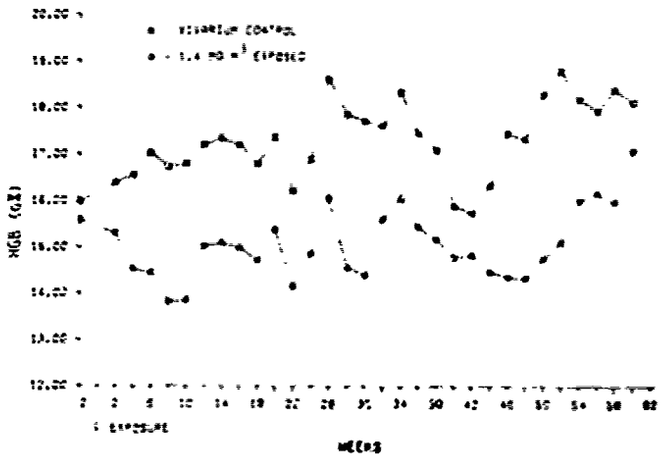


Figure 13. Effect of Otto Fuel II exposure on dog hemoglobin.

During the last quarter of the exposure period, a substantial steady decrease in RBC counts, hematocrit, and hemoglobin values of exposed dogs occurred. These decreases were also noted at a somewhat earlier time in the control dogs. It was not clear at the end of the scheduled year-long exposure if the changes were persistent or transient or if the changes were exposure related. Therefore, the exposure of dogs was extended an additional 60 days. During this exposure extension RBC, hematocrit, and hemoglobin values returned to previous levels. Values of dogs exposed to Otto Fuel II vapor continued to be subnormal when compared to control values and despite the apparent anemia present in the exposed dogs, the expected increase in reticulocyte counts did not occur; rather, a gradual decrease in reticulocyte counts appeared (Figure 14). The possibility that Otto Fuel II has adversely affected red cell production is being investigated. Special bone marrow smears were prepared from those animals sacrificed at exposure termination for myeloid:erythroid ratio determinations.

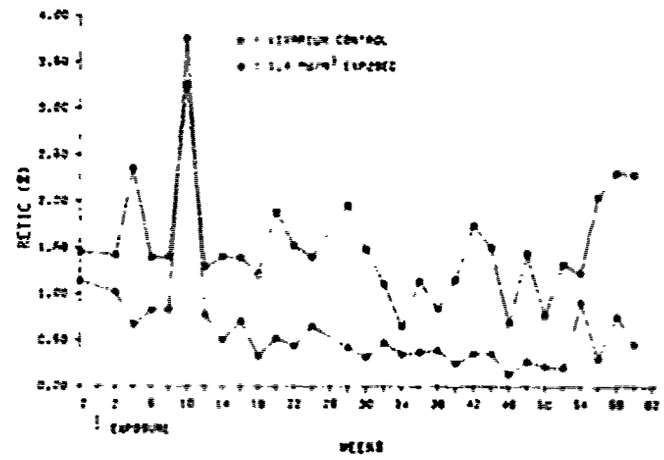


Figure 14. Effect of Otto Fuel II exposure on dog reticulocyte counts.

Red blood cell osmotic fragility tests were conducted during the seventh month of the exposure to investigate the possibility that the anemia present in the exposed dogs was a result of increased fragility. The results of these tests are shown in Figure 15. The fragility curves for exposed and control animals were very similar. The only difference is that the blood from exposed dogs is more completely hemolyzed at lower saline concentrations than the controls. The test was repeated at eight months exposure with similar results. The results may indicate that young red blood cells from the exposed animals are more fragile than a comparable population from controls.

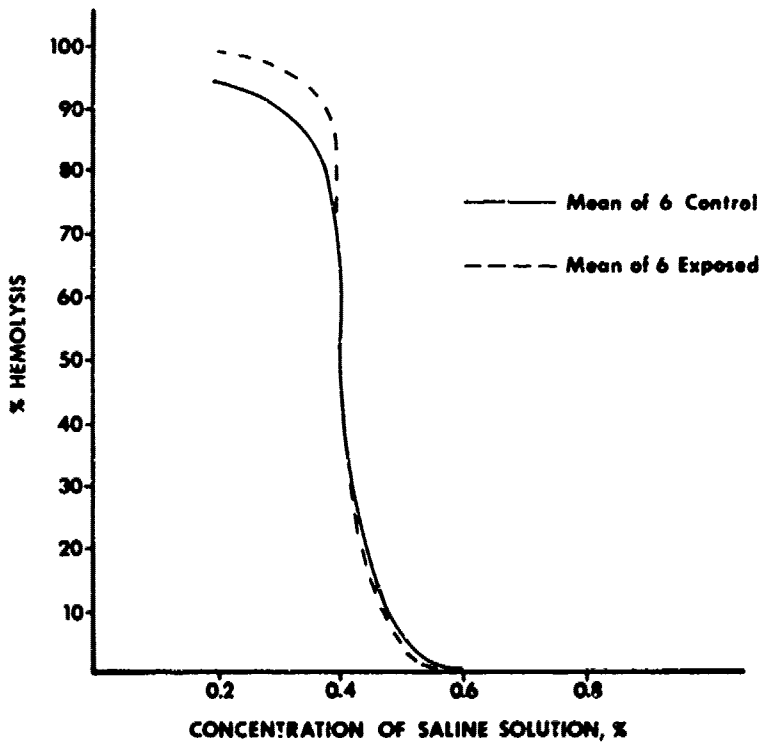


Figure 15. Osmotic fragility of dog blood after seven months intermittent exposure to 1.4 mg/m^3 Otto Fuel II.

Methemoglobin determinations were conducted periodically during the exposure on rats and dogs. The results are presented in Table 9. Increased methemoglobin concentration was evident in rats exposed to 240 mg/m^3 and dogs exposed to 1.4 mg/m^3. While these increased values were statistically significant compared to control values, they represent only mild elevations and were not physiologically detrimental.

TABLE 9. METHEMOGLOBIN DETERMINATIONS ON ANIMALS EXPOSED TO OTTO FUEL II

RAT

Exposure Day	Sex	Methemoglobin % Unexposed Controls Mean	S.D.	N	Methemoglobin % 240 mg/m^3 Exposed Mean	S.D.	N
3	M	0.97	0.92	7	1.84	1.10	10
8	F	0.81	0.70	7	2.00	1.70	9
13	M	0.40	0.31	8	1.59[a]	0.68	10
18	F	0.71	0.51	10	1.58[a]	0.64	10
23	M	0.62	0.35	10	2.9[a]	0.69	10
28	F	1.19	0.71	10	2.31[a]	0.95	10
33	M	1.20	0.71	10	2.49[a]	1.33	10
145	M	1.48	0.82	10	3.57[a]	0.52	10
170	F	2.51	0.52	10	3.18[a]	1.05	10
212	M	2.22	0.93	10	3.59[a]	1.47	10
241	F	1.39	0.42	10	3.08[a]	1.33	10
245	M	1.30	0.96	10	3.76[a]	0.67	10

DOG

Exposure Day	Methemoglobin % Unexposed Controls Mean	S.D.	N	Methemoglobin % 240 mg/m^3 Exposed Mean	S.D.	N
44	0.38	0.15	6	1.21[a]	0.48	6
136	1.79	0.87	6	4.6[a]	1.51	6
171	1.39	0.82	6	2.84[a]	0.57	6
231	1.37	0.30	6	3.09[a]	0.95	6

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

Examination of dog blood at 8 months of exposure failed to reveal the presence of Heinz bodies.

Organ weights obtained from the rats sacrificed at the conclusion of the exposure are shown in Table 10. Decreased liver weights were noted in the males and females exposed to Otto Fuel II vapor. However, although there is greater than 2 orders of magnitude difference in exposure concentrations, liver weights of animals in both exposure groups are equal, indicating that the liver weight decreases are probably not related to Otto Fuel II exposure.

TABLE 10. MEAN ORGAN WEIGHTS[a] OF RATS EXPOSED TO OTTO FUEL II VAPOR FOR ONE YEAR

	MALE RATS		
	Controls	1.4 mg/m^3	240 mg/m^3
Body Weight, g	407 ± 23	379[b] ± 29	378[b] ± 17
Liver Weight, g	10.54 ± 0.93	9.46[b] ± 0.93	9.39[b] ± 0.87
Liver/100 g body wt	2.59 ± 0.17	2.49 ± 0.09	2.48 ± 0.16
Kidney Weight, g	2.43 ± 0.17	2.29 ± 0.18	2.32 ± 0.13
Kidney/100 g body wt	0.59 ± 0.04	0.61 ± 0.02	0.62 ± 0.03
Spleen Weight, g	0.61 ± 0.09	0.55 ± 0.07	0.57 ± 0.08
Spleen/100 g body wt	0.15 ± 0.02	0.14 ± 0.01	0.15 ± 0.02

	FEMALE RATS		
	Controls	1.4 mg/m^3	240 mg/m^3
Body Weight, g	216 ± 29	206 ± 13	200 ± 14
Liver Weight, g	5.93 ± 1.09	4.89[b] ± 0.33	4.71[c] ± 0.32
Liver/100 g body wt	2.74 ± 0.26	2.38[c] ± 0.11	2.36[c] ± 0.18
Kidney Weight, g	1.49 ± 0.17	1.39 ± 0.06	1.43 ± 0.08
Kidney/100 g body wt	0.69 ± 0.03	0.68 ± 0.03	0.72 ± 0.04
Spleen Weight, g	0.43 ± 0.11	0.38 ± 0.05	0.35[b] ± 0.06
Spleen/100 g body wt	0.19 ± 0.03	0.18 ± 0.03	0.17 ± 0.02

[a] Mean ± S.D., N = 10
[b] Different from controls at 0.05 level of significance.
[c] Different from controls at 0.01 level of significance.

Blood was collected from the rats killed at the conclusion of the exposure period. The results of the hematology and clinical chemistry tests are shown in Tables 11 and 12 for male and female rats, respectively. There are a number of hematology and clinical chemistry parameters of the Otto Fuel II exposed rats which were statistically different from respective unexposed control rats. In all cases there was a lack of any dose response relationship, suggesting that the differences noted were not related to Otto Fuel II toxicity.

TABLE 11. MEAN BLOOD VALUES OF MALE RATS EXPOSED TO OTTO FUEL II VAPOR FOR ONE YEAR

	Control	N	1.4 mg/m^3	N	240 mg/m^3	N
RBC (10^6)	9.7	11	7.5[b]	11	8.8[a]	11
WBC (10^3)	4.9	11	2.9[b]	11	3.8[b]	11
HCT (%)	45.9	11	44.0[b]	11	46.5	11
HGB (g/dl)	14.8	11	14.6	11	15.0	11
Total Pro (g/dl)	7.3	11	6.9	11	7.1	11
Albumin (g/dl)	3.7	11	3.4[b]	11	3.5[a]	11
Globulin (g/dl)	3.6	11	3.5[b]	11	3.6	11
A/G Ratio	1.0	11	1.0	11	1.0	11
Glucose (mg/dl)	160	11	143	11	154	11
Tot. Bilirubin (mg/dl)	0.43	11	0.25[b]	11	0.26[b]	11
Dir. Bilirubin (mg/dl)	0.11	11	0.03	11	0.00	11
Creatinine (mg/dl)	0.5	11	0.4[b]	11	0.4[b]	11
SGPT (IU/L)	87	10	52[b]	11	53[b]	11
SGOT (IU/L)	121	11	98	11	90[a]	11
Alk. Phos. (IU/L)	9.5	11	8.8	11	8.9	11
BUN (mg/dl)	12.1	11	11.8	11	11.9	11
MCV	48.2	11	58.6[b]	11	53.0[a]	11
MCH	15.5	11	19.4[b]	11	17.1[a]	11
MCHC	32.2	11	33.1[a]	11	32.3	11
Glu. Trans.	10.0	11	10.2	11	10.0	11

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

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

TABLE 12. MEAN BLOOD VALUES OF FEMALE RATS EXPOSED TO OTTO FUEL II VAPOR FOR ONE YEAR

	Control	N	1.4 mg/m^3	N	240 mg/m^3	N
RBC (10^6)	6.3	10	7.6	10	7.4[a]	10
WBC (10^3)	4.5	10	2.9[a]	10	3.5	10
HCT (%)	41.2	10	44.4[a]	10	43.8	10
HGB (g/dl)	13.8	10	14.7	10	14.7	10
Total Pro (g/dl)	7.4	10	7.2	5	7.2	9
Albumin (g/dl)	4.0	10	3.6[b]	5	3.6[b]	9
Globulin (g/dl)	3.4	10	3.6	5	3.6[b]	9
A/G Ratio	1.2	10	1.0[b]	5	1.0[b]	9
Glucose (mg/dl)	143	10	149	7	145	9
Tot. Bilirubin (mg/dl)	0.41	9	0.29	3	0.32	10
Dir. Bilirubin (mg/dl)	0.16	2	0.02	2	0.08	10
Creatinine (mg/dl)	0.6	8	0.6	5	0.5[b]	10
SGPT (IU/L)	58	10	45[a]	7	47[a]	10
SGOT (IU/L)	121	10	83[a]	5	94	9
Alk. Phos. (IU/L)	7.4	9	6.7	7	6.8	10
BUN (mg/dl)	12.8	8	15.0	6	14.2	10
MCV	65.7	10	59.5[a]	10	59.3[a]	10
MCH	21.9	10	19.8[a]	10	19.9[a]	10
MCHC	33.4	10	33.3	10	33.5	10
Glu. Trans.	10.0	7	10.0	3	10.0	10

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

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

In comparing the rat blood values with those from dogs, the depression of hematologic parameters seen in Otto Fuel II exposed dogs is absent in the rats. While depressed RBC counts were evident in the exposed male rats, there was no dose response relationship. Hematocrit and hemoglobin values of male exposed rats were similar to the unexposed control rat values. Female rats exposed to Otto Fuel II vapor actually had higher hematology values than control animals.

The absence of depressed hematologic values in rats exposed to 240 mg/m^3 Otto Fuel II is surprising in view of the fact that these rats were exposed to an Otto Fuel II concentration almost 170 times greater than the dogs. This fact along with the absence of any major methemoglobin formation in rats exposed to 240 mg/m^3 Otto Fuel II suggests that rats are more resistant than dogs to the effects of Otto Fuel II on blood.

Dog organ weights obtained at the time of sacrifice are shown in Table 13. Reduced liver/body weight ratios were noted in dogs exposed to 1.4 mg/m^3 Otto Fuel II vapor.

TABLE 13. ORGAN WEIGHTS OF DOGS EXPOSED FOR 14 MONTHS TO OTTO FUEL II VAPOR (N = 6)

	Control	1.4 mg/m^3
Body Weight, kg	11.7 ± 2.9	13.2 ± 0.9
Liver Weight, g	336.9 ± 76.7	318.8 ± 30.9
Liver/100 g body wt	2.9 ± 0.2	2.4 ± 0.3[a]
Spleen Weight, g	117.9 ± 19.1	163.4 ± 53.3
Spleen/100 g body wt	1.1 ± 0.3	1.3 ± 0.4
Kidney Weight, g	56.6 ± 15.8	62.0 ± 6.7
Kidney/100 g body wt	0.5 ± 0.1	0.5 ± 0.1

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

Results of histopathologic examination of tissues collected from animals dying during the course of the study or at the scheduled sacrifice at exposure termination are not available for this report. Future annual reports will present histopathologic summaries as well as updates on postexposure observations.

THE EXPERIMENTAL DETERMINATION OF SAFE ATMOSPHERIC CONCENTRATIONS OF RJ-5

A study of the oncogenic potential of inhaled RJ-5, (norbornane dimer) was initiated in October, 1979 for four animal species. The exposure phase of the study was concluded in October 1980 and most of the animals were transferred to postexposure holding facilities. The animals of each species were divided into three groups: unexposed controls, and those exposed to either 0.03 or 0.15 mg/L RJ-5.

The experimental protocol designed to identify toxic effects and establish safe exposure limits as well as to identify the oncogenic potential of RJ-5 fuel can be found in previous annual reports (MacEwen and Vernot, 1980, 1981) which also contain information on the 12-months of industrial type exposure of the four animal species.

The numbers of animals of each species used are listed in Table 14 which also shows the distribution of animals in the Thomas Dome chambers along with RJ-5 exposure concentrations.

TABLE 14. ANIMAL DISTRIBUTION IN RJ-5 EXPOSURE CHAMBERS

	Chamber Concentration, mg/L				
	0.03	0.03	0.15	0.15	Unexposed Controls
Species and Sex					
Rats, Male	---	65	65	---	65
Rats, Female	---	65	65	---	65
Mice, Female	200	--	--	200	200
Hamsters, Male	100	--	--	100	100
Dogs, Male	---	4	4	---	4
Dogs, Female	---	4	4	---	4

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

Background

A six-month chronic inhalation toxicity exposure to 0.15 mg/L RJ-5 was conducted with animals in our laboratory and reported by

MacEwen and Vernot in 1975. A subnormal weight gain was noted in rats and particularly in dogs during the course of the experiment. Dogs and rats (CFE strain) sacrificed immediately following the conclusion of the exposure showed acute inflammation of the lungs as well as several cases of bronchopneumonia in the test groups.

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

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

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

Results

Mean body weights for groups of rats and hamsters obtained on a biweekly schedule through 12 months of exposure and monthly thereafter are shown in Figures 16 through 18. Weights of male rats and hamsters showed a depression in mean body weights as a result of RJ-5 exposure. The mean body weights of these groups continued in this pattern throughout the postexposure period. The mean body weights of exposed female rats outgained the control group during the exposure portion of the study. The control group subsequently outgained the test groups during the postexposure period. An examination of exposed dog and group mouse weights during and after exposure revealed no effect which could be attributed to RJ-5 exposure.

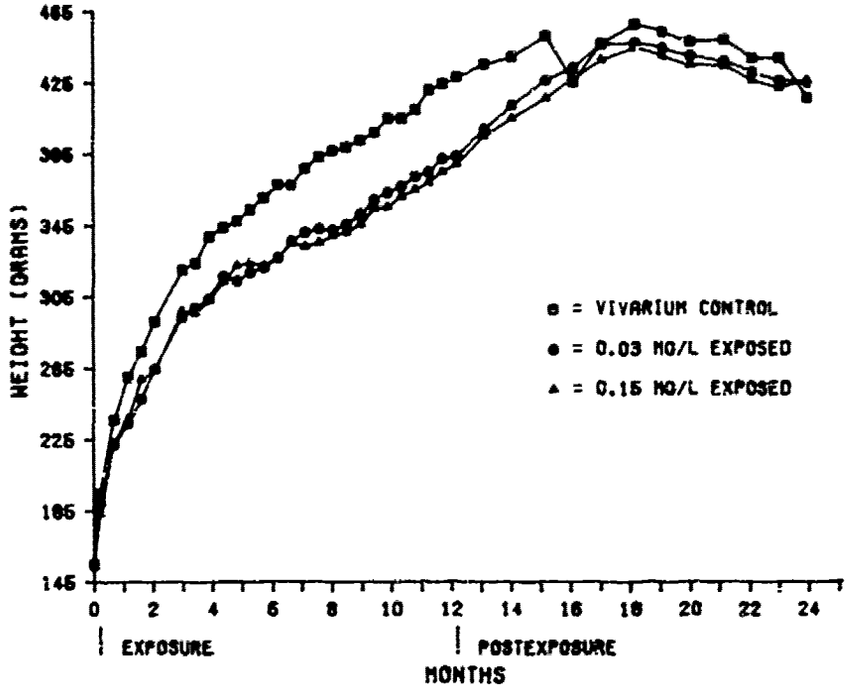


Figure 16. Final mean body weights for RJ-5 exposed male rats.

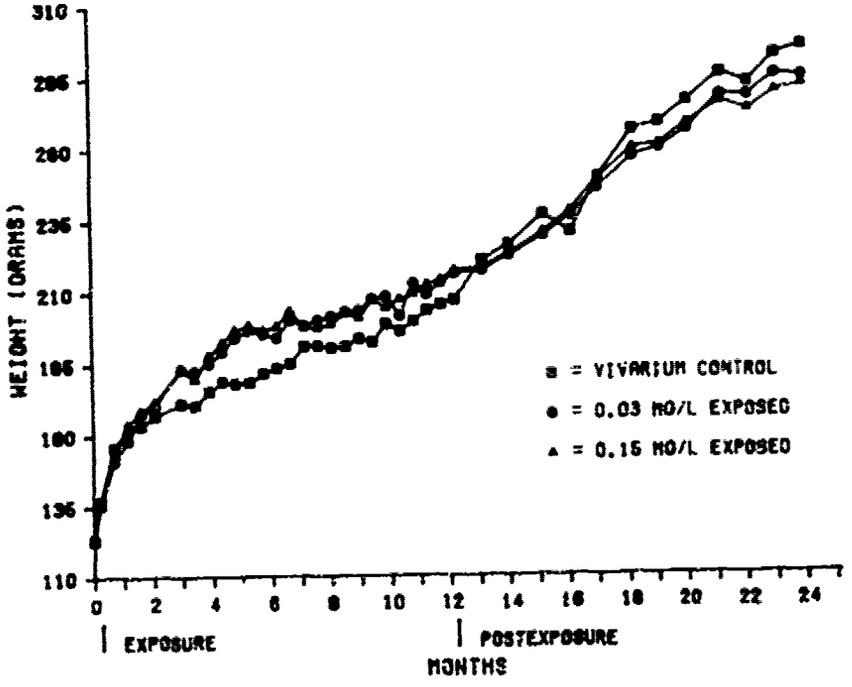


Figure 17. Final mean body weights for RJ-5 exposed female rats.

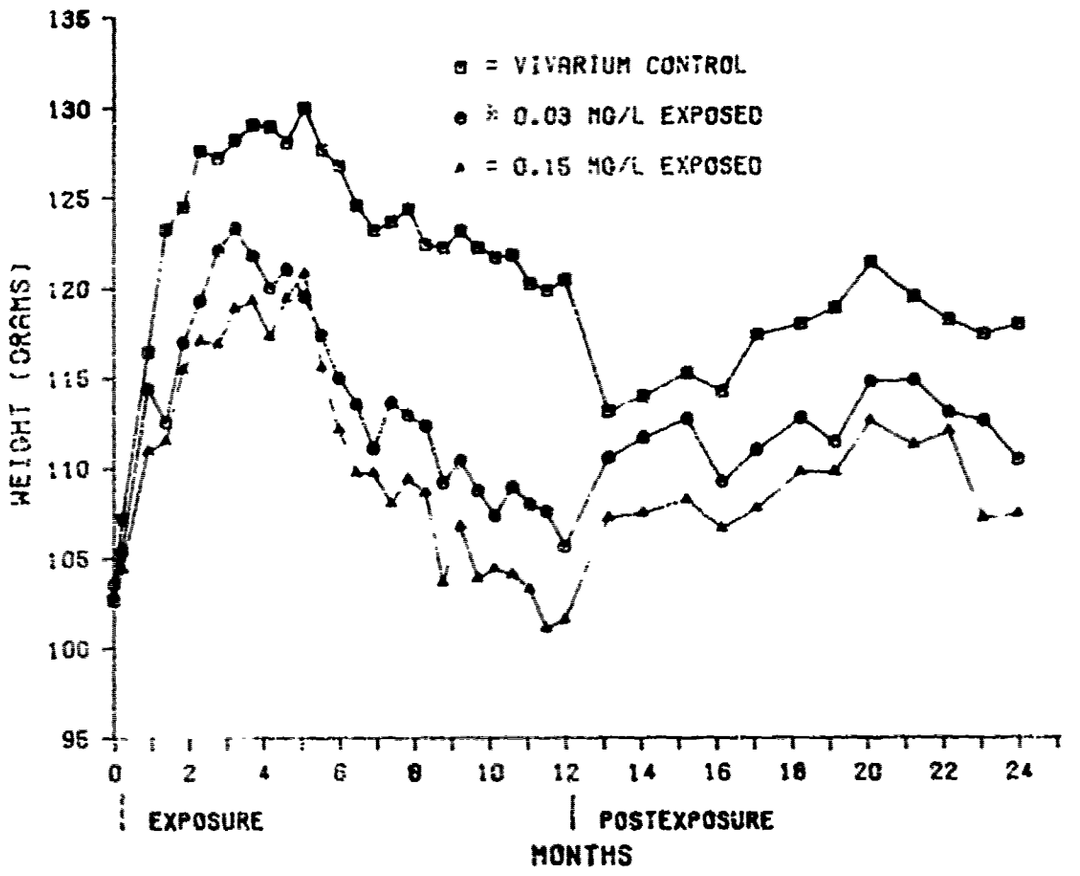


Figure 18. Final mean body weights for RJ-5 exposed hamsters.

The number of animal deaths that occurred during the study was not above expectations and was independent of exposure concentrations.

Effects on blood parameters and organ weights of male and female rats sacrificed at exposure termination can be found in a previous annual report (MacEwen and Vernot, 1981). Organ weights for the male and female rats sacrificed after one year of postexposure observation are shown in Tables 15 and 16. The only statistically significant finding was a decrease in mean weight of the kidneys of the exposed female rats reflected in non-significant decreases in the kidney to body weight ratios of the exposed groups. Mean kidney weights and ratios of the male test groups were lower than controls but not at a statistically significant level.

TABLE 15. MALE RAT ORGAN WEIGHTS 12 MONTHS AFTER ONE-YEAR EXPOSURE TO RJ-5 (N = 10)

	Chamber Concentration		
	Control	0.03 mg/L	0.15 mg/L
Body weight, g	384 ± 51	396 ± 39	400 ± 31
Liver weight, g	12.27 ± 2.94	11.61 ± 1.28	11.43 ± 1.49
Liver/100 g body wt	3.20 ± 0.78	2.95 ± 0.35	2.86 ± 0.30
Spleen weight, g	1.05 ± 0.39	1.30 ± 0.64	1.17 ± 0.38
Spleen/100 g body wt	0.27 ± 0.09	0.33 ± 0.16	0.29 ± 0.08
Kidney weight, g	3.01 ± 0.28	2.87 ± 0.23	2.89 ± 0.23
Kidney/100 g body wt	0.79 ± 0.10	0.73 ± 0.09	0.72 ± 0.05
Heart weight, g	1.26 ± 0.11	1.16 ± 0.09	1.25 ± 0.17
Heart/100 g body wt	0.34 ± 0.07	0.30 ± 0.03	0.31 ± 0.04
Lung weight, g	1.85 ± 0.26	2.03 ± 0.46	1.95 ± 0.28
Lung/100 g body wt	0.48 ± 0.06	0.52 ± 0.12	0.49 ± 0.07

TABLE 16. FEMALE RAT ORGAN WEIGHTS 12 MONTHS AFTER ONE-YEAR EXPOSURE TO RJ-5 (N = 10)

	Control	0.03 mg/L	0.15 mg/L
Body Weight, g	271 ± 42	264 ± 15	266 ± 39
Liver weight, g	7.44 ± 1.01	7.53 ± 1.41	6.48 ± 0.90
Liver/100 g body wt	2.86 ± 0.95	2.87 ± 0.65	2.44 ± 0.16
Spleen weight, g	1.47 ± 2.85	2.29 ± 4.08	0.51 ± 0.10
Spleen/100 g body wt	0.72 ± 1.65	0.93 ± 1.75	0.96 ± 0.03
Kidney weight, g	1.99 ± 0.20	1.80 ± 0.13[a]	1.78 ± 0.20[a]
Kidney/100 g body wt	0.76 ± 0.22	0.68 ± 0.08	0.67 ± 0.07
Heart weight, g	0.92 ± 0.10	0.87 ± 0.07	0.89 ± 0.10
Heart/100 g body wt	0.35 ± 0.11	0.33 ± 0.04	0.34 ± 0.03
Lung weight, g	1.39 ± 0.31	1.48 ± 0.23	1.32 ± 0.21
Lung/100 g body wt	0.53 ± 0.14	0.56 ± 0.10	0.50 ± 0.09

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

Mean organ weights of the dogs are shown in Table 17. The only significant difference noted is that of heart to body weight where the ratio is less for the high level dogs than for the controls.

TABLE 17. DOG ORGAN WEIGHTS 12 MONTHS AFTER ONE-YEAR EXPOSURE TO RJ-5 (N = 8)

	Chamber Concentration		
	Control	0.03 mg/L	0.15 mg/L
Body Weight, kg	10.3 ± 17.4	10.0 ± 20.8	12.6 ± 26.7
Liver weight, g	304.2 ± 71.3	305.7 ± 56.2	365.2 ± 73.3
Liver/100 g body wt	2.9 ± 0.4	3.1 ± 0.3	2.9 ± 0.3
Spleen weight, g	83.8 ± 44.1	56.3 ± 35.8	95.2 ± 71.5
Spleen/100 g body wt	0.8 ± 0.4	0.6 ± 0.3	0.7 ± 0.5
Kidney weight, g	52.3 ± 10.8	55.5 ± 16.2	59.9 ± 12.7
Kidney/100 g body wt	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.1
Heart weight, g	84.5 ± 11.2	81.1 ± 17.1	89.9 ± 16.9
Heart/100 g body wt	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.1[a]

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

Tables 18 and 19 show the mean hematology and clinical chemistry values of the male and female rats sacrificed at the end of the 12-month observation period. Although a few significant differences exist between control and exposed animal values, these appear to be random occurrences rather than due to exposure.

Histopathology information that is needed to assess the overall results of this study is incomplete at this time.

TABLE 18. MEAN HEMATOLOGIC AND CLINICAL CHEMISTRY VALUES OF MALE RATS 12-MONTHS POSTEXPOSURE IN INHALED RJ-5 (N = 10)

	Chamber Concentration		
	Controls	0.03 mg/L	0.15 mg/L
RBC (10^6)	8.2[a]	8.5[a]	9.5
WBC (10^3)	5.3[a]	4.7[a]	5.2
HCT (%)	50.0[a]	49.9[a]	52.1
HGB (gm/dl)	15.9[a]	16.9[a]	17.5
Total Protein (gm/dl)	7.7	7.7	7.9
Albumin (gm/dl)	3.5	3.6	3.7
Globulin (gm/dl)	4.2	4.0	4.2
A/G Ratio	0.85	0.90	0.87
Glucose (mg/dl)	143	147	148
Potassium (mEq/L)	5.0	4.7	4.9
Calcium (mg/dl)	11.8	11.7	11.9
Sodium (mEq/L)	157	159	159
Bilirubin (mg/dl)	0.57	0.55	0.60
Creatinine (mg/dl)	0.6	0.6	0.7
SGPT (IU/L)	52	36	33[b]
SGOT (IU/L)	104	82	86
Alk. Phos. (IU/L)	8.6	7.7	7.1
BUN (mg/dl)	19.0	17.3	18.8
MCV	61.5[a]	58.4[a]	55.2[c]
MCH	19.5[a]	19.8[a]	18.6
MCHC	31.8[a]	33.9[a,b]	33.7[c]

[a] N = 8

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

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

TABLE 19. MEAN HEMATOLOGIC AND CLINICAL CHEMISTRY VALUES OF FEMALE RATS 12-MONTHS POSTEXPOSURE TO INHALED RJ-5 (N = 10)

	Chamber Concentration		
	Controls	0.03 mg/L	0.15 mg/L
RBC (10^6)	7.5	7.1	7.9
WBC (10^3)	12.6	13.0	4.0
HCT (%)	43.4	41.1	44.3
HGB (gm/dl)	14.5	13.9	14.7
Total Protein (gm/dl)	7.1	7.7	8.0
Albumin (gm/dl)	3.9	3.8	3.9
Globulin (gm/dl)	3.8	3.9	4.0
A/G Ratio	1.00	0.96	0.99
Glucose (mg/dl)	147	134	146
Potassium (mEq/L)	4.8	5.1	5.0
Calcium (mg/dl)	11.3	11.4	11.3
Sodium (mEq/L)	150	152	152
Bilirubin (mg/dl)	0.49	0.51	0.51
Creatinine (mg/dl)	0.5	0.4	0.4
SGPT (IU/L)	50	65	35[b]
SGOT (IU/L)	101	166	67
Alk. Phos. (IU/L)	8.8	9.9	5.3
BUN (mg/dl)	15.5	12.8[a]	12.8[a]
MCV	60.4	60.6	56.0
MCH	20.2	20.6	18.6
MCHC	33.4	33.8	33.3

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

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

A SUBCHRONIC TOXICITY STUDY OF 90-DAY CONTINUOUS INHALATION EXPOSURE TO PETROLEUM DIESEL FUEL MARINE

A 90-day continuous inhalation toxicity study of diesel fuel marine (DFM) vapor was conducted by the Toxic Hazards Research Unit during 1978. A detailed discussion of the protocol and chemical generation and analysis methods can be found in the 1978 Annual Report (MacEwen and Vernot, 1978).

Beagle dogs, Fischer 344 rats, and C57BL/6 mice were continuously exposed to concentrations of 50 mg/m^3 or 300 mg/m^3 petroleum DFM vapor for 90 days in Thomas Dome inhalation chambers while unexposed controls were held in laminar airflow rooms in separate facilities. At the conclusion of the exposure, all dogs and 1/3 of the rodents were sacrificed for tissue collection and histopathologic examination. The results of this examination were reported in a previous report (MacEwen and Vernot, 1979). All rodents not sacrificed at the conclusion of the 90-day exposure were held for long-term observation. An interim sacrifice of 1/2 of the remaining rodents occurred 19 months postexposure. Animals remaining from this interim sacrifice were held until the 24th month of the study, at which time all surviving animals were sacrificed for tissue examination in December 1979.

Results of organ weighings and blood analysis obtained at the 19-month interim sacrifice were presented in the 1980 Annual Report (MacEwen and Vernot, 1980).

This report will present results of histopathologic examination of mice which were held for postexposure observation.

Non-neoplastic changes that occurred with some frequency in female C57BL/6 mice are shown in Table 20. The list does not include every lesion noted of which there were many single occurrences, not exposure related. There was a modest increase in the incidence of acute and chronic inflammation of the skin seen in the groups exposed to DFM vapor. The skin disease resulted in secondary changes in the lymphatic and hematopoietic systems. These changes are increases in bone marrow hyperplasia, hematopoiesis in the spleen and plasmacytosis of the mandibular lymph node. Inflammation of the skin is a common condition in aged female C57BL/6 mice, and even though its occurrence is slightly increased in both groups of exposed animals as compared to controls, it was probably not a significant effect of DFM exposure. There was a modest increase in the

presence of focal and multifocal fibrosis of the bone marrow of mice exposed to 300 mg/m^3. All other non-neoplastic lesions noted were considered incidental to exposure.

TABLE 20. SELECTED NON-NEOPLASTIC CHANGES SEEN IN FEMALE MICE HELD FOR POSTEXPOSURE OBSERVATION AFTER 90-DAY CONTINUOUS INHALATION EXPOSURE TO PETROLEUM DIESEL FUEL MARINE

	Control	50 mg/m^3	300 mg/m^3
Skin:			
Inflammation (Acute & Chronic)	15/87	20/87	24/87
Bone Marrow:			
Fibrosis	3/82	4/87	9/83
Hyperplasia	3/82	5/87	6/83
Spleen:			
Hematopoiesis	17/91	28/89	24/92
Liver:			
Cytoplasmic Vacuolization	3/93	2/91	6/94
Lymph Node:			
Plasmacytosis	1/82	6/87	4/83

Neoplastic changes observed in the mice from this study are shown in Table 21. An increase in benign liver tumor incidence in female mice exposed to 300 mg/m^3 was seen when compared to control values; however, this increase was not statistically significant.

TABLE 21. NEOPLASTIC CHANGES SEEN IN FEMALE MICE HELD FOR POSTEXPOSURE OBSERVATION AFTER 90-DAY CONTINUOUS INHALATION EXPOSURE TO PETROLEUM DIESEL FUEL MARINE

	Control	50 mg/m^3	300 mg/m^3
Skin:			
Fibrosarcoma	0/87	0/88	1/91
Lung:			
Adenoma	4/91	4/90	3/94
Carcinoma	0/91	0/90	2/94
Lymph Node:	0/82	0/87	1/83
Liver:			
Adenoma	4/93	6/91	9/94
Carcinoma	1/93	3/91	0/94
Duodenum:			
Adenoma	1/80	0/89	0/82
Anus:			
Papilloma	2/45	1/40	1/49
Uterus:			
Leiomyoma	1/90	0/89	0/94
Papilloma	1/90	0/89	0/94
Ovary:			
Adenoma	2/82	2/74	1/76
Pituitary:			
Adenoma	43/71	38/77	41/78
Adrenal Gland:			
Pheochromocytoma	1/86	0/90	0/94
Thyroid:			
Adenoma	9/82	7/84	4/82
Lacrimal Gland:			
Carcinoma	0/93	1/91	0/94
Cystadenoma	0/93	1/91	0/94
Malignant Lymphoma:	37/93	26/91	29/94
Circulatory:			
Hemangioma	1/93	0/91	2/94
Hemangiosarcoma	0/93	2/91	0/94

All other tumor incidence in petroleum DFM exposed mice was equal to or less than unexposed control values. Under the conditions of this study, petroleum DFM exhibited no carcinogenic potential in female mice. Histopathologic examination of rat tissue obtained during the postexposure holding is continuing. Results will appear in future reports.

A SUBCHRONIC TOXICITY STUDY OF 90-DAY CONTINUOUS INHALATION EXPOSURE TO SHALE DIESEL FUEL MARINE

This study was conducted as a companion to a previous subchronic inhalation study of Petroleum Diesel Fuel Marine to enable comparisons of toxic effects of alternate fuels. The protocol, contaminant generation and monitoring systems were described in the 1980 THRU Annual Report (MacEwen and Vernot, 1980). The exposure phase of the study began in March 1980. At the conclusion of the exposure phase a portion of the animals was killed for tissue examination. An interim sacrifice of a portion of the remaining rats was conducted in October 1981. Because of the limited number of mice alive at that time, none were examined. Instead they were held and they, along with all remaining rats, were sacrificed at the conclusion of the 24th month of the study.

During the course of this study, individual rat body weights were measured and recorded. Mean body weights of male rats are shown in Figure 19. Male rats exposed to 300 mg/m^3 shale DFM showed subnormal body weight compared to the unexposed control group. This trend was evident at exposure termination and continued through the postexposure observation period. Exposure to shale DFM vapor had little effect on female rat body weight gain (Figure 20).

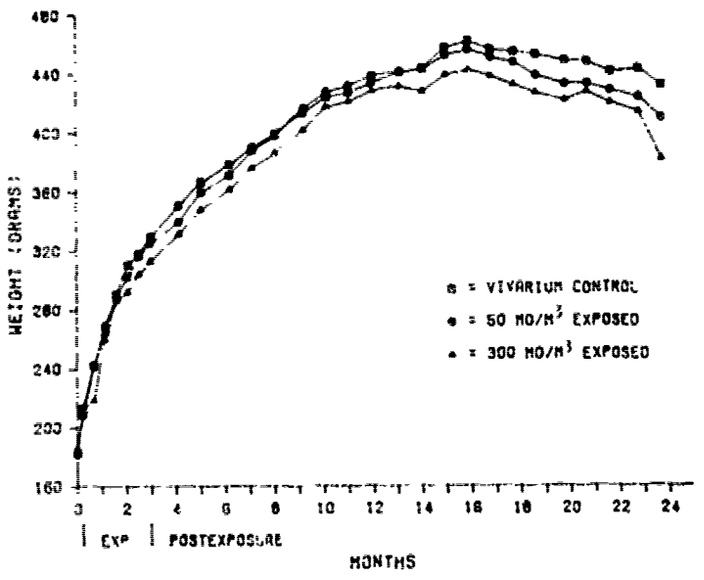


Figure 19. Mean body weights of male rats exposed to shale DFM.

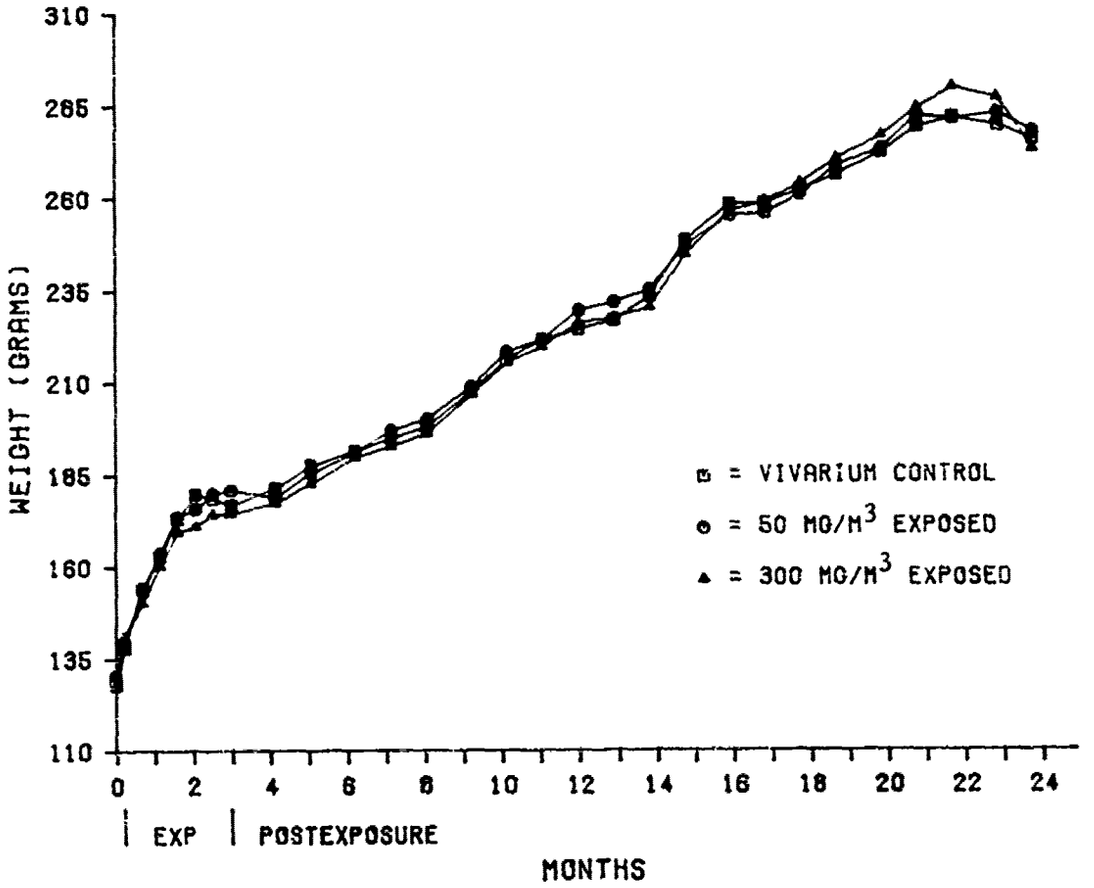


Figure 20. Mean body weights of female rats exposed to shale DFM.

Blood samples were collected from the rats at the interim sacrifice. Statistical analysis of blood values obtained from the rats are shown in Tables 22 and 23 for male and female rats, respectively. Data from one male and one female control rat were excluded from the analysis. These rats had unusually large spleen weights and many of the blood values were also obviously abnormal. Slightly increased BUN and creatinine levels were evident in male rats exposed to 300 mg/m^3. The group means were not statistically different from control values, however. Examination of individual values indicated the increased values were primarily due to two rats. Increased bilirubin levels were evident in female rats exposed to 300 mg/m^3. Five rats in this group had bilirubin levels in excess of 1.0 mg/dl. All other statistical differences noted were probably incidental and unrelated to exposure.

TABLE 22. MEAN BLOOD VALUES OF MALE RATS 19 MONTHS AFTER 90-DAY CONTINUOUS EXPOSURE TO SHALE DFM VAPOR

	Control	N	50 mg/m^3	N	300 mg/m^3	N
RBC (10^6)	8.01	20	7.4	18	7.4	19
WBC (10^3)	6.7	20	6.6	18	5.7	19
HCT (%)	49.2	20	46.8	18	48.2	19
HGB (g/dl)	16.2	20	15.4	18	15.9	19
Total Protein (g/dl)	7.7	20	7.7	18	7.7	16
Albumin (g/dl)	3.6	20	3.6	18	3.5	16
Globulin (g/dl)	4.1	20	4.1	18	4.2	16
A/G Ratio	1.0	20	0.9	18	0.8[a]	16
Glucose (mg/dl)	166	20	153	18	155	19
Calcium (mg/dl)	11.9	20	11.7	18	12.1	16
Potassium (mEq/L)	4.8	19	4.6	13	4.8	15
Sodium (mEq/L)	157	19	152[a]	13	153	15
Bilirubin (mg/dl)	0.54	20	0.56	18	0.58	19
Creatinine (mg/dl)	0.6	20	0.6	16	0.8	19
SGPT (IU/L)	44	20	56	18	40	18
SGOT (IU/L)	85	20	151	18	84	19
Alk. Phos. (IU/L)	7.5	20	8.2	18	6.7	19
BUN (mg/dl)	18.6	20	17.9	16	22.9	19
MCV	61.4	20	64.7	18	66.0	19
MCH	20.2	20	21.5	19	21.8	19
MCHC	32.8	20	33.2	19	33.1	19

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

TABLE 23. MEAN BLOOD VALUES OF FEMALE RATS 19 MONTHS AFTER 90-DAY CONTINUOUS EXPOSURE TO SHALE DFM VAPOR

	Control	N	50 mg/m^3	N	300 mg/m^3	N
RBC (10^6)	8.1	18	7.6	19	8.2	20
WBC (10^3)	3.5	18	4.1	19	3.7	20
HCT (%)	45.8	18	44.3	19	45.2	20
HGB (g/dl)	15.3	19	15.1	19	15.4	20
Total Protein (g/dl)	7.9	18	8.1	17	7.8	20
Albumin (g/dl)	3.9	18	4.0	17	4.1	20
Globulin (g/dl)	3.9	18	4.1	17	3.7[a]	20
A/G Ratio	1.0	18	1.0	17	1.1[a]	20
Glucose (mg/dl)	167	18	146	18	149	20
Calcium (mg/dl)	11.4	18	11.5	17	12.2[a]	20
Potassium (mEq/L)	5.2	18	5.1	16	5.5	19
Sodium (mEq/L)	153	18	154	16	152	19
Bilirubin (mg/dl)	0.45	18	0.48[a]	17	0.71[a]	20
Creatinine (mg/dl)	0.4	18	0.4	17	0.5	20
SGPT (IU/L)	53	18	51	18	50	20
SGOT (IU/L)	108	18	111	18	103	20
Alk. Phos. (IU/L)	7.4	18	6.5	17	6.3	19
BUN (mg/dl)	12.9	18	12.1	17	12.6	20
MCV	56.6	18	58.6	19	56.0	20
MCH	18.9	18	19.9[a]	19	19.1	20
MCHC	33.3	18	34.1[b]	19	34.1[b]	20

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

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

Rat organ weights were also measured at the interim sacrifice. The results are shown in Table 24. Statistical evaluation failed to reveal any differences between the shale DFM exposed and unexposed control male or female rat organ weights.

TABLE 24. MEAN RAT ORGAN WEIGHTS OBTAINED 19 MONTHS AFTER 90-DAY CONTINUOUS EXPOSURE TO SHALE DFM VAPOR

	MALE RATS		
	Control (N = 21)	50 mg/m^3 (N = 18)	300 mg/m^3 (N = 19)
Body Weight, g	422 ± 38	413 ± 30	400 ± 40
Liver Weight, g	12.81 ± 1.72	11.96 ± 1.39	12.12 ± 2.38
Liver/100 g body wt	3.06 ± 0.47	2.91 ± 0.39	3.03 ± 0.45
Kidney Weight, g	3.00 ± 0.32	2.87 ± 0.27	3.02 ± 0.49
Kidney/100 g body wt	0.72 ± 0.09	0.69 ± 0.05	0.76 ± 0.12
Spleen Weight, g	1.86 ± 0.53	1.14 ± 0.59	1.08 ± 0.37
Spleen/100 g body wt	0.49 ± 1.11	0.28 ± 0.16	0.27 ± 0.08

	FEMALE RATS		
	Control (N = 19)	50 mg/m^3 (N = 19)	300 mg/m^3 (N = 20)
Body Weight, g	265 ± 31.4	263 ± 31	278 ± 27
Liver Weight, g	6.83 ± 0.39	6.85 ± 1.23	6.99 ± 0.71
Liver/100 g body wt	2.62 ± 0.37	2.62 ± 0.38	2.52 ± 0.13
Kidney Weight, g	1.87 ± 0.11	1.86 ± 0.24	1.87 ± 0.19
Kidney/100 g body wt	0.72 ± 0.13	0.72 ± 0.11	0.67 ± 0.06
Spleen Weight, g	0.66 ± 0.70	0.52 ± 0.12	0.55 ± 0.14
Spleen/100 g body wt	0.28 ± 0.39	0.19 ± 0.04	0.19 ± 0.04

Histopathologic examination of tissues collected from the animals in this study is in progress. A report from the pathologist concerning results of examination of the animals sacrificed at exposure termination is expected in June 1982.

EVALUATION OF THE ONCOGENIC POTENTIAL OF JP-7 JET FUEL

Introduction

The Toxic Hazards Research Unit has been conducting a series of studies investigating the oncogenic potential of hydrocarbon fuels used by the Air Force. One of the fuels that had not previously been studied was the jet fuel designated JP-7.

JP-7 is a complex mixture of aliphatic and aromatic hydrocarbon compounds which closely resembles the jet fuel JP-5. In 1977, the Toxic Hazards Research Unit conducted an inhalation toxicity study of Petroleum JP-5. The concentrations used in the JP-5 study were 150 mg/m^3 and 750 mg/m^3. Because of the similarity of physical and chemical characteristics of JP-5 and JP-7, concentrations of 150 mg/m^3 and 750 mg/m^3 were chosen for the JP-7 exposures.

In previous long-term inhalation studies conducted by the THRU using Sprague-Dawley strain rats and Swiss ICR mice it has not been practical to house unexposed control groups in Thomas Dome exposure chambers. These strains had been studied for chamber effects and chamber controls were not found different from vivarium controls in any manner. In recent studies we have used different strains of rats and mice without retesting for difference between chamber housed and vivarium housed controls. Control groups, therefore, were housed in laminar airflow facilities. Since irregularity in housing conditions contributes an undesirable variable in a study, particularly when animal growth is monitored, two control animal groups, one housed in a Thomas Dome exposure chamber and one housed in a laminar airflow room, were included in this study for comparative purposes.

Methods

JP-7 is a complex mixture of hydrocarbon compounds refined from petroleum that is defined in terms of physical and chemical characteristics and includes various additives, all of which meet the requirements of Military Specification MIL-T-38219A. The specified physical and chemical parameters are detailed below:

Aromatics, vol. % max.:	5
Mercaptan sulfur, wt. %, max 1/:	0.001
Sulfur, total wt. %, max:	0.1
Distillation, °C (°F):	
Initial boiling point, min. temp.:	182 (360)
End point, max. temp.:	288 (550)
Flash point, min, °C (°F):	60 (140)
Density, kg/m^3, min at 15°C:	779
Density, kg/m^3, max at 15°C:	806
Vapor pressure, kPa (psi) at 149°C, max:	20.7 (3.0)
Freezing point, °C, max:	-43.5
Viscosity, at -20°C, centistokes, max:	8.0

Mice and rats were exposed to JP-7 vapor in Thomas Dome Chambers. The exposures were conducted from 15 April 1981 to 14 April 1982 on an industrial work week schedule of 6 hours/day, 5 days/week. Weekends and holidays were excluded to simulate a human exposure regimen.

Two chambers were utilized for the exposures; one contained a concentration of 150 mg/m^3 and the other contained a concentration of 750 mg/m^3. Animal groups consisted of 100 male and 100 female Fischer 344 rats and C57BL/6 mice, 9-11 weeks of age at exposure initiation, obtained from Charles River Breeding Laboratories. An additional group with the same numbers of animals was housed at the Veterinary Sciences Division Building (Vivarium) to serve as unexposed shelf controls. A fourth group with equal numbers of animals was housed in another Thomas Dome Chamber to serve as sham operated controls. All animals had food and water ad libitum during nonexposure hours. Food was removed during the exposure period.

The contaminant introduction and analysis system used for JP-7 vapor was similar to the systems used for other fuel studies. A detailed discussion of the system can be found in a previous annual report discussing an inhalation study of the Jet Fuel JP-4 (MacEwen and Vernot, 1980). The only major modification to that method was the use of heptane as the primary hydrocarbon calibration standard rather than propane.

Following the 1-year exposure period, 12 of the rodents, 6 of each sex from all groups, were killed for tissue collection and examination. The remaining rodents are being held for one year of postexposure observation. At the conclusion of this period (April 1983), all remaining rodents will be sacrificed for tissue collection and examination.

All animals were observed hourly during the exposure and are being observed 4 times daily during the postexposure holding period. More frequent examinations will be made toward the end of the study when natural mortality increases.

Rats were individually weighed at biweekly intervals during exposure and are weighed monthly during the post-exposure period. Mice were weighed in groups with the group mean weights followed on a monthly basis throughout the experimental period.

All animals that die or are sacrificed during the study are necropsied and tissues are collected for histopathologic examination in accordance with the NCI protocol. Electron microscopic examination is also being conducted on a small sample of rats from each group killed at the scheduled sacrifices.

JP-7 Generation Control and Monitoring

Twenty-five 55 gallon drums of JP-7 were obtained for this study. Quality control consisted of obtaining GC fingerprints from each drum of JP-7. Stability tests of the JP-7 were also run by taking a sample from a drum of JP-7 before use, and another sample when the drum was almost used up.

Table 25 shows the results of the initial quality control work. These samples were all run before the start of the study. The same ten largest peaks from each chromatograph were selected for comparison. The area under the peaks was measured with a computing integrator. Drum #017 was seen as the only sample with a significant number of peaks outside the range of the other samples. This drum was put aside and never used.

TABLE 25. COMPARISON OF THE TEN LARGEST GAS CHROMATOGRAPHIC PEAKS FROM EACH JP-7 DRUM

PEAK #	1	2	3	4	5	6	7	8	9	10	
PEAK RETENTION TIME (MIN)	21.52	25.88	28.52	29.92	30.53	32.77	35.67	35.93	36.45	37.16	
Drum #											
014	1.19	3.00	1.57	5.60	2.78	2.32	5.93	1.65	2.22	3.94	
015	0.54	3.20	1.54	5.91	2.88	2.00	6.14	1.56	2.24	3.97	
016	1.75	4.30	1.86	7.12	3.40	2.54	7.12	1.54	2.42	4.54	
017	1.02	2.70	5.62	5.16	2.33	2.37	4.44	1.95	1.18	2.85	
018	1.75	4.34	1.77	6.75	3.32	2.57	7.38	1.60	2.57	4.85	
019	1.86	4.32	1.68	7.05	3.50	2.78	7.52	1.54	2.49	4.80	
020	1.00	2.89	1.60	5.63	2.75	2.35	5.79	1.59	2.16	3.71	
021	1.68	4.27	1.47	6.68	3.32	2.59	7.45	1.65	2.61	4.96	
022	1.27	3.07	1.65	5.59	2.73	2.13	5.60	1.46	2.07	3.59	PEAK
023	1.76	4.13	1.85	7.18	3.36	2.62	6.92	1.48	2.39	4.69	% AREA
024	1.81	4.26	1.92	7.40	3.53	2.73	7.33	1.46	2.39	4.60	
025	1.42	3.46	1.70	6.30	3.07	2.51	6.40	1.47	2.13	3.89	
026	1.12	3.96	1.62	5.62	2.75	2.33	5.67	1.51	2.12	3.67	
027	1.17	2.96	1.60	5.52	2.68	2.26	5.51	1.49	2.03	3.66	
028	1.10	2.97	1.59	5.20	2.23	2.23	5.47	1.51	2.03	3.47	
029	1.79	4.17	1.86	7.18	3.39	2.56	7.38	1.58	2.53	4.17	
030	1.21	3.06	1.58	5.57	2.73	2.32	5.67	1.53	2.13	3.74	
031	1.81	4.34	0.99	7.08	3.46	2.56	7.33	1.52	2.42	4.63	
032	1.20	2.96	1.59	5.57	2.70	2.30	5.64	1.56	2.13	3.72	
033	1.45	3.36	1.69	6.49	3.13	2.66	6.66	1.55	2.27	4.12	
034	1.26	3.02	1.60	5.54	2.74	2.26	5.63	1.53	2.10	3.62	
035	1.89	4.49	1.87	7.09	3.46	2.70	7.46	1.56	2.55	4.95	
036	1.13	2.83	1.55	4.96	2.60	2.20	5.28	1.47	2.00	3.54	
037	1.52	4.06	1.76	6.40	3.28	2.81	7.45	1.56	2.39	4.49	
038	1.75	4.29	1.82	6.64	3.36	2.76	7.60	1.50	2.47	4.91	

Table 26 shows the results of a stability test that was performed. These samples were drawn from Drum #019. Repetitive runs were made from the initial sample in order to get a range of response from the computing integrator. The next sample was taken over a month later when the drum was almost empty. All the peaks had a good fit in the range of the initial sample.

TABLE 26. JP-7 STABILITY TEST RESULTS

		DRUM #19									
PEAK #		1	2	3	4	5	6	7	8	9	10
PEAK RETENTION TIME (MIN)		21.5	25.9	28.5	29.9	30.5	32.4	32.8	33.7	36.5	37.2
3 SEPTEMBER 1981											
RUN #	1	1.67	4.10	1.80	6.77	3.24	1.56	2.57	7.10	2.48	4.66
	2	1.61	4.30	1.87	6.82	3.46	1.72	2.83	7.49	2.48	4.67
	3	1.23	3.20	1.63	5.39	2.79	1.60	2.31	5.66	2.04	3.69
	4	1.56	4.30	1.95	7.51	3.60	1.44	1.95	7.64	2.40	4.44
	5	1.57	3.61	1.58	6.08	3.00	0.69	2.67	7.00	2.57	4.60
	6	0.97	4.23	1.69	5.60	2.88	1.65	2.34	5.68	2.07	3.64
8 OCTOBER 1981											
RUN #	1	1.26	3.21	1.62	5.71	2.82	1.63	2.35	5.70	2.10	3.58
	2	1.13	3.09	1.62	5.31	2.72	1.62	2.31	5.69	2.08	3.65

% AREA

JP-7 Introduction System

Figure 21 shows a schematic of the JP-7 dome introduction system using two evaporator towers. The JP-7 flowed down the heated glass coil inside the towers. As it flowed down the inside of the tower, a countercurrent airflow stripped off the volatile portion of the JP-7.

The output of each tower was combined and then split to give the proper proportion to each dome. The JP-7 was pumped from the drum by pressurizing the drum to 8.0 psi. Needle valves and flow meters controlled the liquid flow to each tower at 16 ml/min.

The towers were warmed to 50°C by using heating wires wrapped around the outside of the towers. The temperature was kept constant by using thermostats. The unvolatilized JP-7 was removed via the bottom of the tower where it flowed into a holding tank from which it was pumped to a waste drum.

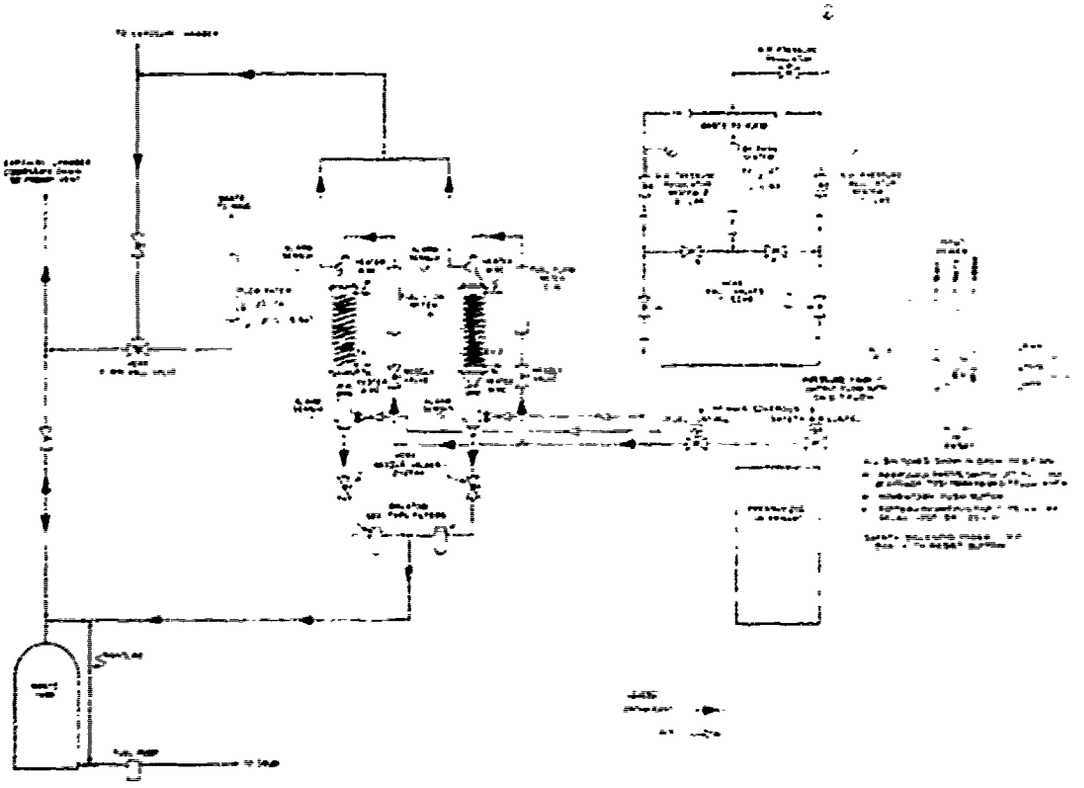


Figure 21. JP-7 introduction system.

Building compressed air was used to supply 5 cfm air to each tower. The flow was controlled by pressure regulators which were checked with a built-in flow meter.

Temperature sensors were used to monitor evaporator temperature at the top and bottom of each tower. If these temperatures exceeded 60°C, JP-7 flow and tower heat were automatically shut off using electrical solenoids. JP-7 flow and tower heat were also stopped in the case of chamber air flow loss.

The JP-7 supply drum and waste drums were kept inside safety cabinets. These cabinets were ventilated using the chamber vacuum system and the drums were grounded using cables inside the cabinets.

JP-7 Chamber Analytical System

Exposure chamber concentration analysis was accomplished by using the Beckman Model #400 hydrocarbon analyzer (HCA).

Exposure chamber air samples were pulled with a vacuum pump through the HCA to obtain a 3 L/min bypass flow and a 5.0 psi sample pressure. The measurements obtained from the HCA were plotted on a Heath stripchart recorder. An alarm box was used to monitor the recorder reading and set off an alarm signal if the chamber concentration reached preset high or low set values.

Figure 22 shows a schematic view of the analytical system. There was a separate system for each chamber with a common hydrogen and air supply.

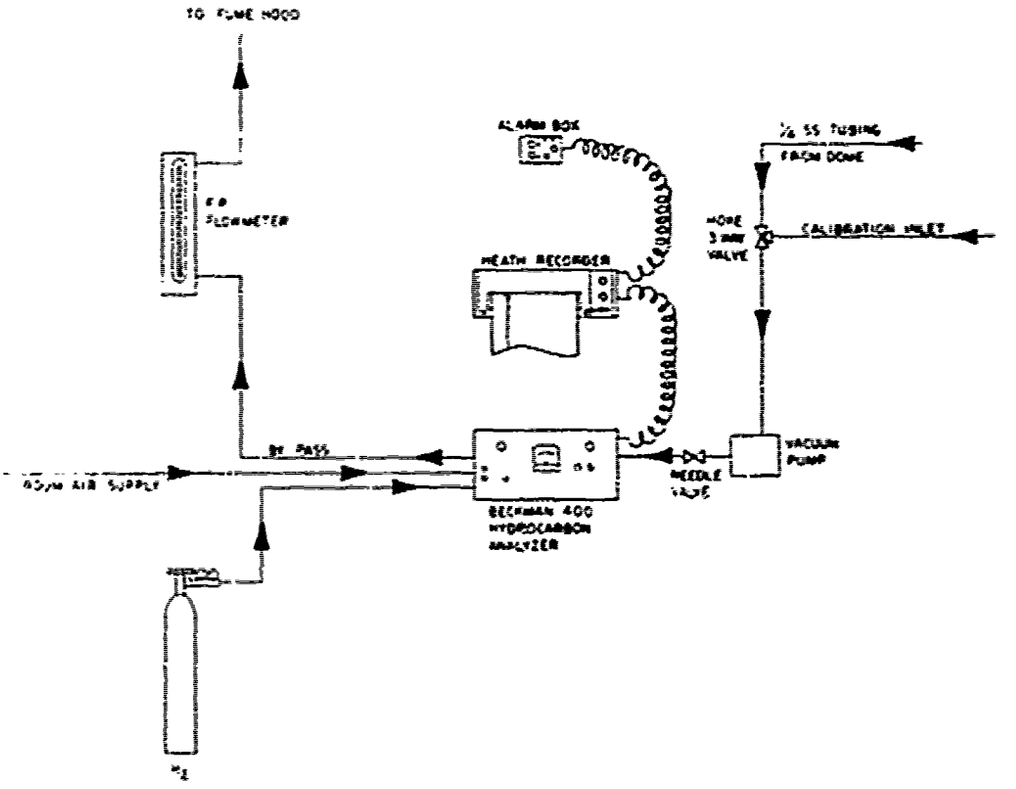


Figure 22. JP-7 analytical system.

Sample tubing in the analytical system was 1/4" OD stainless steel. A three-way valve in the sampling line allowed sampling from a calibration bag or a standard air source as well as from the chamber. HCA bypass flow was controlled with a needle valve and a Fisher-Porter flowmeter. Hydrogen gas was supplied to the HCA from a cylinder equipped with a regulator. The air supply to the HCA was from a dynamic house compressed air system with a back-up cylinder in the event of compressor failure.

Analytical Calibration

Calibration of the hydrocarbon analyzer (HCA) was accomplished by using n-heptane as a standard. The response of the HCA to heptane was found to be the same as to JP-7. Calibration standards were made by filling Mylar® bags with a known amount of air and injecting known amounts of liquid heptane. Initial calibration was accomplished by making three different concentration standards for each HCA. This was done to check linearity of response.

Results

Exposure Chamber Concentration Measurements

Chamber concentrations were calculated by recording average concentrations every half hour during a daily six-hour run. Statistical analysis of the experimental data was then accomplished by computer where the mean, range, and standard deviation were calculated. All JP-7 exposure concentration data from this study are now stored in the computer where they can be retrieved at any time.

Table 27 is a summary of the exposure concentration data. The overall mean exposure concentrations, ranges, and standard deviations were computed by taking averages of monthly readings. April 1981 and April 1982 were veraged as one month.

TABLE 27. JP-7 EXPOSURE CHAMBER CONCENTRATION SUMMARY (g/m^3)

Month	Mean	Range	S.D.	Mean	Range	S.D.
Apr 81-82	151.4	147.5-158.4	3.4	746.0	727.1-764.6	10.2
May 81	148.6	139.0-156.6	3.7	747.9	726.0-772.0	12.1
June 81	148.4	137.5-151.9	2.9	747.7	652.1-782.8	26.0
July 81	147.4	136.7-151.6	3.6	750.4	728.1-764.6	9.9
Aug 81	147.6	130.6-156.7	7.2	743.7	684.4-778.1	26.9
Sept 81	149.2	140.4-154.6	3.3	757.5	716.7-786.5	17.4
Oct 81	148.7	140.6-152.7	3.0	756.6	730.2-776.0	11.5
Nov 81	150.3	142.1-158.3	5.1	746.0	716.7-760.4	12.3
Dec 81	150.6	133.8-158.1	5.6	737.8	697.9-772.9	22.0
Jan 82	153.1	145.4-161.7	5.0	772.2	704.1-806.3	22.2
Feb 82	151.8	147.1-159.2	3.5	753.9	720.8-770.8	14.0
Mar 82	154.8	149.2-158.5	2.4	743.7	696.9-759.4	13.2
OVERALL MEAN	150.1	147.4-154.8	2.3	750.3	746.0-772.2	8.9

GC/MS Analysis of JP-7

A sample of neat JP-7 fuel was examined by gas chromatographic/mass spectrometric analysis. A typical chromatogram is shown in Figure 23 which illustrates the narrow range of components in this fuel. Five components have been identified but further identification of the mixture may be difficult because of overlapping and poor separation of peaks.

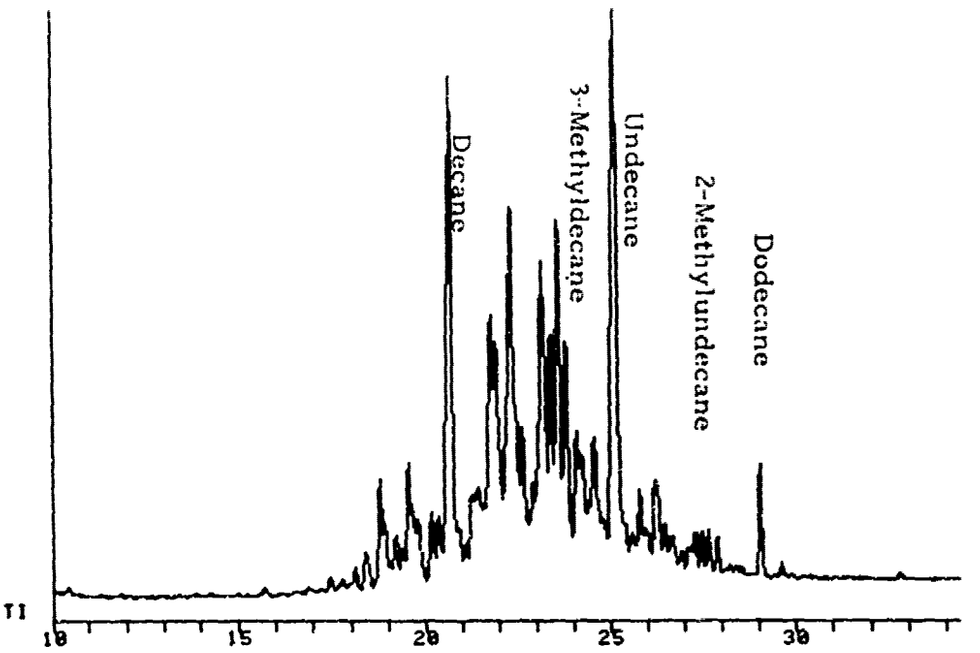


Figure 23. Gas chromatogram of JP-7.

Results of Animal Exposures

There were no overt signs of toxicity exhibited by the animals during the exposure. Mortality percentages are shown in Table 28.

TABLE 28. MORTALITY OF RODENTS AT COMPLETION OF ONE-YEAR EXPOSURE TO JP-7 VAPOR

		% Mortality			
Species	Sex	Vivarium Control	Exposure Chamber Control	150 mg/m^3	750 mg/m^3
Mouse	M	10	21	12	12
Mouse	F	5	15	19	9
Rat	M	2	1	0	0
Rat	F	4	1	1	1

Male rat body weights are shown in Figure 24. A noticeable difference between vivarium housed and chamber housed male rats is evident. Body weights of male rats exposed to 150 mg/m^3 were comparable to chamber housed controls through most of the exposure; however, a detrimental exposure related effect was seen in male rats exposed to 750 mg/m^3. Rats in this group consistently weighed less than chamber housed controls. These weight differences were significant at the 0.01 level.

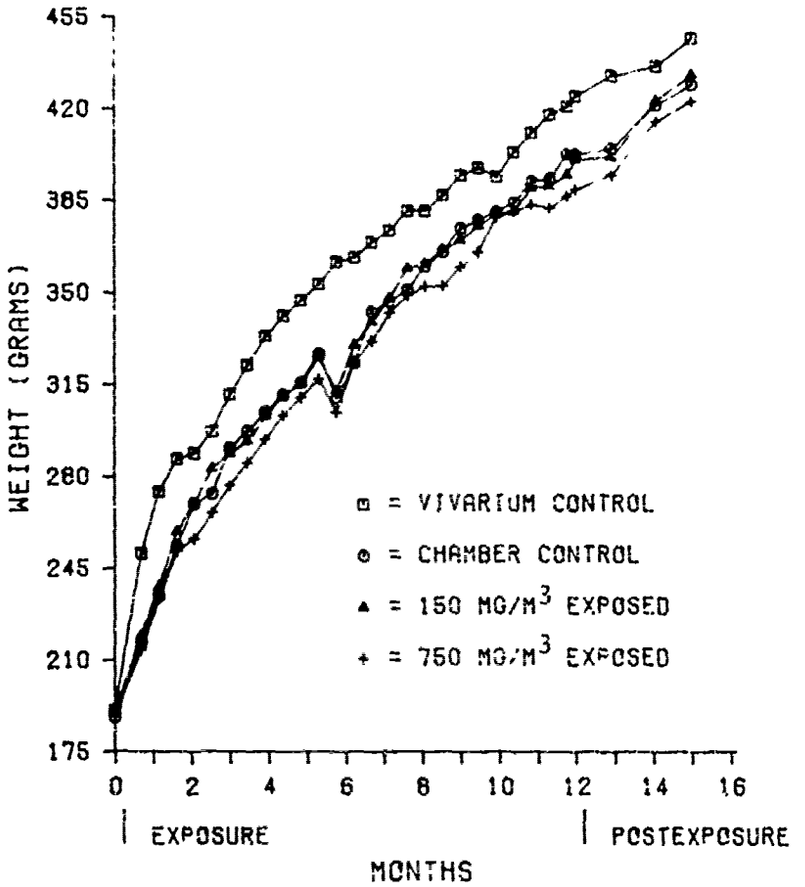


Figure 24. Effect of JP-7 exposure on male rat body weights.

Female rats exposed to either concentration of JP-7 actually gained more weight than either control group (Figure 25). The difference in body weights between chamber and vivarium housed female control rats was negligible during the exposure period. The growth rate of the vivarium controls increased during the 12th month and they are currently the heaviest group.

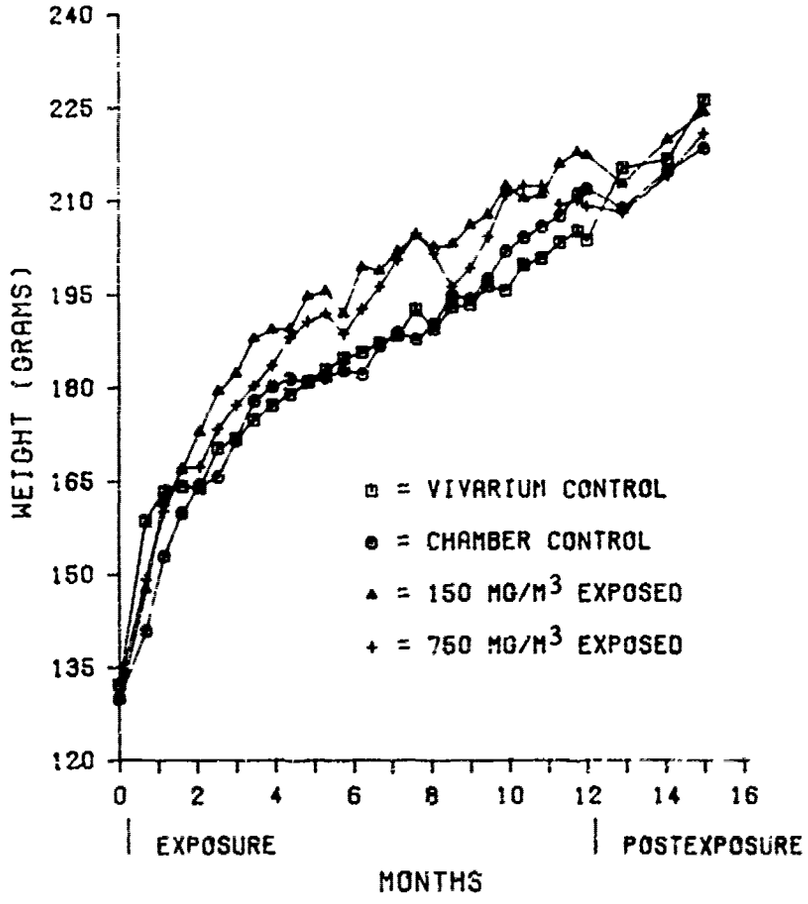


Figure 25. Effect of JP-7 exposure on female rat body weights.

Hematology and clinical chemistry values of male and female rats killed at the termination of the exposure period are shown in Tables 29 and 30, respectively. Although there were numerous statistically significant differences noted between JP-7 exposed rats and unexposed control rats, none of the values was outside normal biological variation for the species. Of possible note were the elevated creatinine and BUN values of male rats exposed to 750 mg/m^3. Previous 90-day continuous exposure studies conducted by the THRU wi ·drocarbon fuels similar to JP-7 have shown a correlation between . increase in these two values and kidney nephropathy. Whether this correlation exists ir the present study cannot be judged in the absence of histopathologic examination.

TABLE 29. MEAN HEMATOLOGY AND CLINICAL CHEMISTRY VALUES OF MALE RATS EXPOSED TO JP-7 VAPOR FOR ONE YEAR (N = 10)

	Vivarium Controls	Chamber Controls	Exposed 150 mg/m^3	Exposed 750 mg/m^3
RBC (10^6)	7.9	8.5	7.9	8.4
WBC (10^3)	4.9	5.1	4.3[b]	4.4
HCT (%)	46	49[a]	46[b]	49[a]
HGB (g/dl)	15.5	15.9	15.8	16.2[a]
Total Protein (g/dl)	7.6	7.6	7.7	7.9[a,b]
Albumin (g/dl)	4.1	4.0	4.0	4.1
Globulin (g/dl)	3.6	3.6	3.6	3.8[a,b]
A/G Ratio	1.2	1.1	1.1	1.1
Glucose (mg/dl)	194	198	193	196
Potassium (mEq/L)	5.4	6.5[a]	6.3	6.1
Calcium (mg/dl)	11.2	11.2	11.3	11.4
Sodium (mEq/L)	155	155	157[a]	156
Bilirubin (mg/dl)	0.48	0.40	0.51	0.43
Creatinine (mg/dl)	0.5	0.5	0.5	0.6[a,b]
SGPT (IU/L)	81	54	53	42[a]
SGOT (IU/L)	94	96	105	86
Alk. Phos. (IU/L)	12.5	8.6[a]	10.9[b]	10.3[a]
BUN (mg/dl)	14.7	11.4[a]	13.1[a,b]	13.0[a,b]
MCV	58.7	58.3	59.7	57.8
MCH	19.6	19.0	20.0	19.2
MCHC	33.4	32.6	33.7	33.3

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

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

TABLE 30. MEAN HEMATOLOGIC AND CLINICAL CHEMISTRY VALUES OF FEMALE RATS EXPOSED TO JP-7 VAPOR FOR ONE YEAR (N = 10)

	Vivarium Controls	Chamber Controls	Exposed 150 mg/m^3	Exposed 750 mg/m^3
RBC (10^6)	7.1	7.9[a]	7.2	7.8[a]
WBC (10^3)	3.4	3.4	3.2	3.3
HCT (%)	45	47	45	46
HGB (g/dl)	14.7	15.6	15.5	15.6
Total Protein (g/dl)	7.8	7.7	7.6	7.7
Albumin (g/dl)	4.2	3.8[a]	4.0	3.8[a]
Globulin (g/dl)	3.6	3.9[a]	3.6[b]	3.9[a]
A/G Ratio	1.2	0.9[a]	1.1[b]	0.9[a]
Glucose (mg/dl)	143	140	144	147
Potassium (mEq/L)	4.9	5.0	5.7	5.5
Calcium (mg/dl)	11.7	11.3	11.9	11.5
Sodium (mEq/L)	151	153	156[a]	156[a]
Bilirubin (mg/dl)	0.28	0.29	0.39	0.21
Creatinine (mg/dl)	0.4	0.5	0.5	0.5
SGPT (IU/L)	79	53	52	47
SGOT (IU/L)	120	103	108	97
Alk. Phos. (IU/L)	7.4	5.9	8.5[b]	8.1
BUN (mg/dl)	16.4	13.8[a]	13.1[a]	14.3
MCV	63.1	58.9	62.8	58.9
MCH	20.6	19.7	21.5	20.1
MCHC	32.7	33.4	34.2	34.1

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

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

Organ weights obtained on rats sacrificed at the exposure termination are shown in Table 31. Most of the statistically significant differences shown in this table are between chamber housed and vivarium control rat organ weights. The JP-7 exposure had little effect on rat organ weight.

TABLE 31. MEAN ORGAN WEIGHTS OF RATS EXPOSED TO JP-7 VAPOR FOR ONE YEAR

	Vivarium Control	Chamber Control	150 mg/m³	750 mg/m³
MALE RATS[A]				
Body weight, g	418 ± 16	400 ± 16[B]	396 ± 18[B]	392 ± 15[B]
Liver weight, g	11.46 ± 0.61	10.21 ± 0.81[B]	10.19 ± 0.65[B]	10.08 ± 0.58[B]
Liver/100 g body wt	2.74 ± 0.11	2.55 ± 0.19[B]	2.57 ± 0.08[B]	2.57 ± 0.13[B]
Kidney weight, g	2.44 ± 0.12	2.53 ± 0.16	2.51 ± 0.22	2.41 ± 0.08
Kidney/100 g body wt	0.58 ± 0.03	0.63 ± 0.03[B]	0.63 ± 0.03[B]	0.62 ± 0.02[B]
Spleen weight, g	0.72 ± 0.13	0.68 ± 0.15	0.64 ± 0.08	0.61 ± 0.02[B]
Spleen/100 g body wt	0.17 ± 0.03	0.17 ± 0.03	0.16 ± 0.02	0.16 ± 0.01
FEMALE RATS[A]				
Body Weight, g	197 ± 7	205 ± 9	217 ± 12[B,C]	205 ± 12
Liver Weight, g	4.92 ± 0.27	4.56 ± 0.24	5.01 ± 0.45[C]	4.84 ± 0.54
Liver/100 g body wt	2.49 ± 0.12	2.23 ± 0.13[B]	2.31 ± 0.19[B]	2.36 ± 0.22
Kidney Weight, g	1.44 ± 0.14	1.38 ± 0.08	1.38 ± 0.06	1.34 ± 0.09[B]
Kidney/100 g body wt	0.73 ± 0.06	0.67 ± 0.04[B]	0.64 ± 0.04[B]	0.65 ± 0.05[B]
Spleen Weight, g	0.42 ± 0.03	0.42 ± 0.05	0.39 ± 0.03	0.38 ± 0.03[B,C]
Spleen/100 g body wt	0.21 ± 0.02	0.20 ± 0.02	0.18 ± 0.01[B]	0.18 ± 0.01[B]

[A] N = 10 rats/group

[B] Different from vivarium controls at 0.05 level of significance.

[C] Different from chamber controls at 0.05 level of significance.

Histopathologic examination of tissue collected from animals at the exposure termination is not yet complete. The results of this examination along with further information collected during the postexposure observation period will be presented in future annual reports.

THE EXPERIMENTAL DETERMINATION OF THE ONCOGENIC EFFECTS OF JP-TS JET FUEL

The U. S. Air Force has requested a long-term inhalation study to determine the oncogenic effects of the high altitude jet fuel designated JP-TS. This jet fuel is similar in composition to the jet fuel JP-4 previously investigated by the Toxic Hazards Research Unit.

Intermittent exposure to JP-4 vapor at 5000 mg/m^3 for one year produced organ hypertrophy and bronchial irritation in rats and brought on CNS effects and osmotic erythrocyte fragility increases in female dogs. The reason for the organ hypertrophy in rats was not clear but appeared to be of little toxicologic significance as there was no tissue destruction or alteration. The increase in RBC osmotic fragility appears to have been a real effect of unknown etiology which was transient in nature. The central nervous system effect seen in dogs and respiratory irritation in rats are effects which could be considered relevant to possible human experience with chronic exposure to JP-4 vapor.

A 90-day continuous exposure study of rats, mice and dogs to 1000 and 500 mg/m^3 JP-4 has also been completed in our laboratory. The dogs in this study exhibited no CNS effects nor were any increases of osmotic fragility noted during the course of the study.

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

JP-TS is a broad mixture of aliphatic and aromatic hydrocarbon compounds defined in terms of physical and chemical characteristics and includes various additives, all of which meet the requirements of Military Specification MIL-T-25524B. Pertinent chemical and physical properties of the fuel detailed in the military specifications are listed below:

Sulfur, max.	0.3% (by wt.)
Mercaptan sulfur, max.	0.001% (by wt.)
Aromatics, max.	20.0% (by vol.)
Olefins, max.	3.0% (by vol.)
Distillation:	
Initial boiling point, °F	315
End point, °F	500
Freezing point, °F, max.	-64
Flash point, °F, min.	110
Viscosity, centistokes at -40°F, max.	12.0

A total ion chromatogram of JP-TS, obtained using gas chromatography/mass spectrometry (GC/MS), is shown in Figure 26 with identification of some of the major components. These constituents represent only a fraction of the total content of JP-TS jet fuel; the remainder consists of unspecified hydrocarbon compounds in the kerosene boiling range.

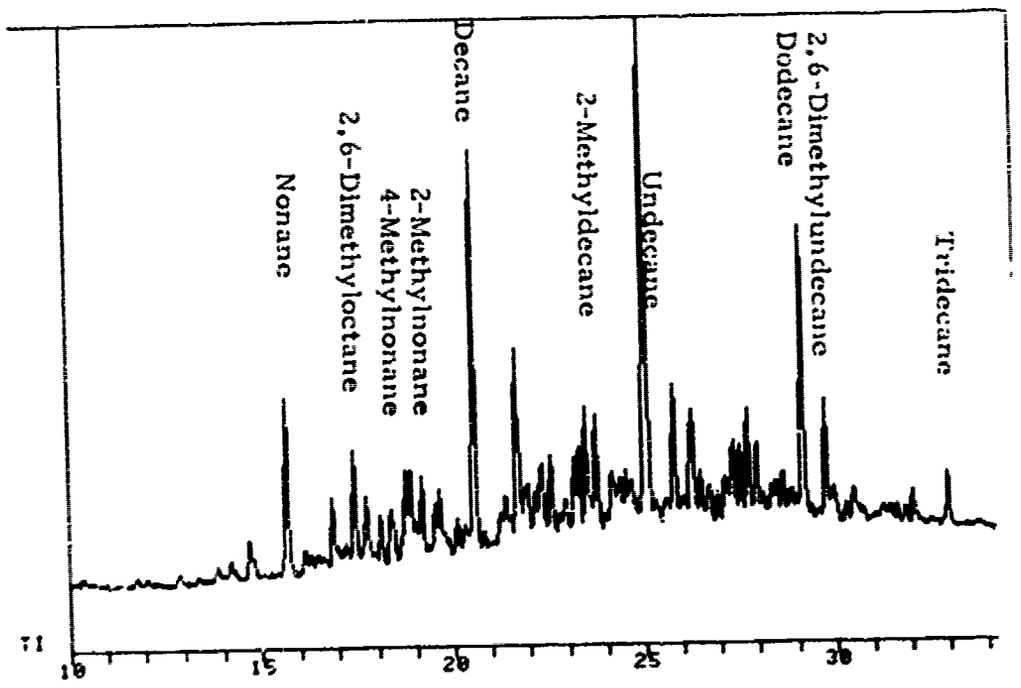


Figure 26. Total ion chromatogram of JP-TS by GC/MS.

Mice and rats were exposed to 200 and 1000 mg/m^3 JP-TS vapor by the inhalation route in Thomas Dome chambers for one year using an industrial work week schedule of 6 hours/day, 5 days/week with holidays and weekends excluded to simulate a human exposure regimen.

For these purposes, two chambers were utilized to provide exposure concentrations of 200 and 1000 mg/m^3 JP-TS. Each chamber housed 100 male and 100 female Fischer 344 rats and 100 male and 100 female C57BL/6 mice. Another group with the same numbers of animals were held at the Veterinary Sciences Division Building to serve as untreated shelf controls, and an additional control group was housed in a Thomas Dome chamber to serve as sham treated controls. These two sets of controls served in the same capacity for studies conducted concurrently with JP-7. The animals were caged in conformance with ILAR standards for laboratory animal care.

Following the exposure period, twelve rats or mice from each group of rodents were killed: two for electron microscopic and 10 for light microscopic examination. The remaining rodents are being held for postexposure observation for one year or until cumulative mortality reaches 90%. Order of sacrifice was determined by a randomization list prepared by a computer program.

The rats were randomized upon receipt into an appropriate number of cages after which quality control sampling and quarantine took place. First 20, then 10 rats and mice of each sex were randomly chosen on two successive weeks for necropsy and quality control examinations. All rodents had food and water ad libitum during nonexposure hours and during the postexposure holding period, but the food was removed during the 6-hour exposure periods.

The contaminant introduction system for JP-TS was similar to the system used for the concurrent JP-7 studies and described in that section of the report. Figure 21 illustrates the introduction system. The liquid material was pumped under low pressure from a 55-gallon supply drum.

Thermocouples were placed at the top and bottom of the glass evaporator to sense any hazardous increase in temperature and to activate both an alarm and a solenoid valve system which would cut off the fuel supply (Figure 27).

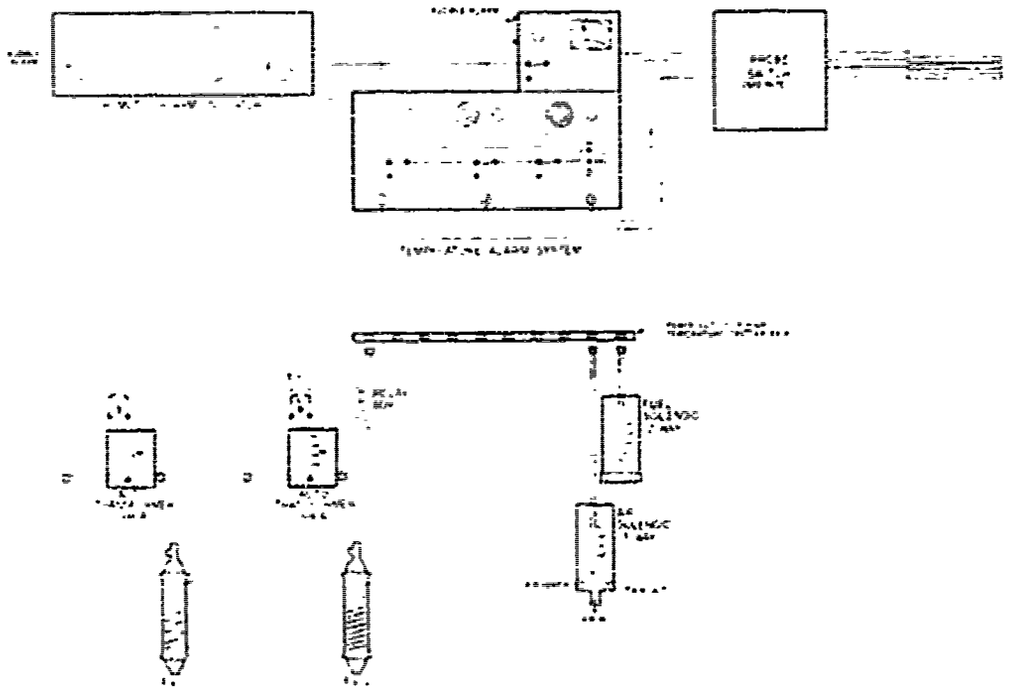


Figure 27. High temperature alarm system for JP-TS fuel vaporization into exposure chambers.

Analysis of the chamber concentrations was accomplished by pumping air samples from each exposure dome into a total hydrocarbon analyzer. The sampling system for analysis is detailed in the section on JP-7 and shown in Figure 22. During previous testing of fuels by this laboratory, it was found that heptane gave the same hydrocarbon detector sensitivity as the fuels. Therefore, known heptane concentrations were used as the calibration standards. A weekly span check of the hydrocarbon analyzer was made using a prepared tank of heptane.

A GC fingerprint of the vapor obtained from a liquid sample of each drum of JP-TS was obtained prior to the initiation of the study to insure that sufficient material was available for completion of the one-year exposure. GC fingerprints of the contaminant in the dome were also obtained periodically. A Royco® particle counter equipped with a digital monitor was used to detect vapor condensate aerosol formation.

All animals were observed hourly during the 12-month exposure and daily thereafter. This regimen will be followed until the mortality rate warrants more frequent examinations. At that time, cage group size will be reduced and observations will be increased to 6/day at 4-hour intervals. Animals found in a moribund condition are sacrificed. This is done to reduce cases of PMD and cannibalism as much as possible.

Rats were individually weighed at biweekly intervals during exposure and are being weighed monthly during the post-exposure period. Mice are weighed in groups with the group mean weights being followed on a monthly basis throughout the experimental period. Blood was taken via the portal vein from the rats sacrificed at the exposure phase termination for the tests shown in Table 32.

TABLE 32. CLINICAL HEMATOLOGY AND CHEMISTRY TESTS PERFORMED ON RATS EXPOSED TO JP-TS VAPOR

Hematology	Chemistry
Hematocrit	Sodium
Hemoglobin	Potassium
RBC	Calcium
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

All animals that died or were sacrificed in this study have been necropsied. If cannibalism or autolysis precluded the examination, a completed record containing this information was filed.

Histopathologic examination is routinely performed on 22 of approximately 40 tissues examined at necropsy from all rodents that died prior to or were sacrificed at the end of the one-year exposure period. Other tissues taken from these animals will be stored and will be examined only if warranted on the basis of postexposure or necropsy observations. Histopathologic examination will be performed on all of the tissues obtained from rodents that die or are sacrificed during the postexposure phase of the study.

With the exception of the rats scheduled for electron microscopic examination, liver, kidney and spleen weights were obtained for all rats killed at the one-year sacrifice.

Data from routine animal weigning, hematology, blood chemistry and organ weighing were analyzed for statistical significance using the Student's t-test. Pathologic lesion incidence will be analyzed using the Fisher Exact Test.

Results of Chemicals Analyses

Chamber concentrations of JP-TS were monitored continuously during the exposures and readings were recorded at 30 minute intervals for computation of daily and monthly mean concentrations. The overall mean concentrations for the nominal 200 and 1000 mg/m^3 exposures were 200.4 ± 3.0 and 1000.2 ± 16.1 mg/m^3, respectively. The monthly mean values are listed in Table 33.

TABLE 33. MONTHLY MEAN JP-TS EXPOSURE CONCENTRATIONS (mg/m^3)

Month	200 mg/m^3 Exposure Chamber	1000 mg/m^3 Exposure Chamber
April 1981	199.7	1003.1
May 1981	199.5	1010.0
June 1981	202.1	993.4
July 1981	201.6	1008.3
August 1981	199.2	994.4
September 1981	201.2	998.0
October 1981	198.6	995.6
November 1981	200.1	1005.1
December 1981	201.7	1004.2
January 1982	201.0	995.2
February 1982	200.2	993.8
March 1982	199.6	1001.5
April 1982	200.3	1005.5

Twenty-five barrels of JP-TS were received from the U. S. Air Force for use in these studies. Before initiating animal exposures, the JP-TS was subjected to quality control procedures. Samples of fuel were taken from all 25 barrels and injected into a gas chromatograph for fingerprint analyses. The percent of total area under the chromatogram was calculated for each of five major peaks as shown in Table 34 to determine that all barrels were supplied from the same production batch. All barrels were found suitable for use and the first fifteen were used in the animal exposures. The remaining barrels were returned to the Air Force for use as aircraft fuel.

TABLE 34. QUALITY CONTROL ANALYSIS[a] OF SAMPLES FROM JP-TS BARRELS

Barrel Number	GC Retention Time (Min.)				
	21.54	25.91	29.92	22.61	33.67
1	5.82	4.35	2.33	2.12	1.77
2	5.58	4.59	2.40	2.13	1.94
3	5.15	4.29	2.35	1.93	1.88
4	5.60	5.50	2.64	2.08	2.36
5	5.87	4.96	2.60	1.99	1.98
6	5.90	4.45	2.50	2.16	1.86
7	6.07	4.70	2.58	2.20	2.05
8	5.51	5.70	2.91	1.86	2.41
9	5.40	5.17	2.98	2.03	2.81
10	5.24	4.34	2.39	1.95	1.90
11	5.51	4.43	2.51	2.03	2.04
12	5.47	4.31	2.52	2.04	2.03
13	6.18	5.27	2.63	2.32	2.26
14	4.87	4.19	2.38	1.84	1.98
15	4.58	3.88	2.16	1.72	1.85
16	5.86	4.61	2.31	2.22	1.77
17	5.43	4.29	2.27	1.99	1.78
18	5.84	4.90	2.19	2.21	1.65
19	6.18	5.44	2.24	3.02	1.51
20	5.98	5.53	2.32	2.16	2.01
21	4.89	3.81	2.04	1.83	1.58
22	6.23	4.40	2.31	2.25	1.58
23	6.06	4.06	2.17	2.11	1.69
24	6.09	4.49	2.40	2.21	1.78
25	6.22	4.62	2.12	2.31	1.48

[a] Numbers are percent of total area from all gas chromatographic peaks recorded.

Results of JP-TS Exposure in Animals

The mean body weights of male and female rats are shown in Figures 28 and 29, respectively, through the exposure phase of the study. The chamber housed control rats gained weight in a similar manner to the chamber housed test rats. All exposure chamber housed male rats gained weight at a rate below vivarium controls and all chamber housed female rat groups outgained their respective female vivarium control group during the exposure period. The growth rate of the vivarium controls increased during the 12th month and they are currently the heaviest group.

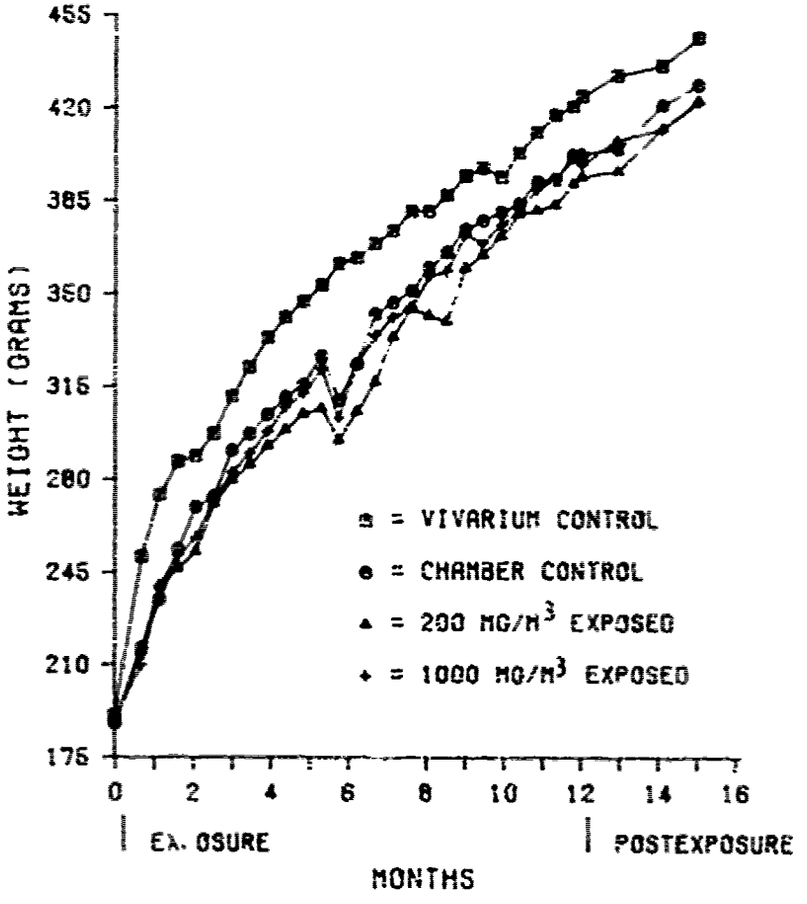


Figure 28. Mean body weight of male rats exposed to JP-TS.

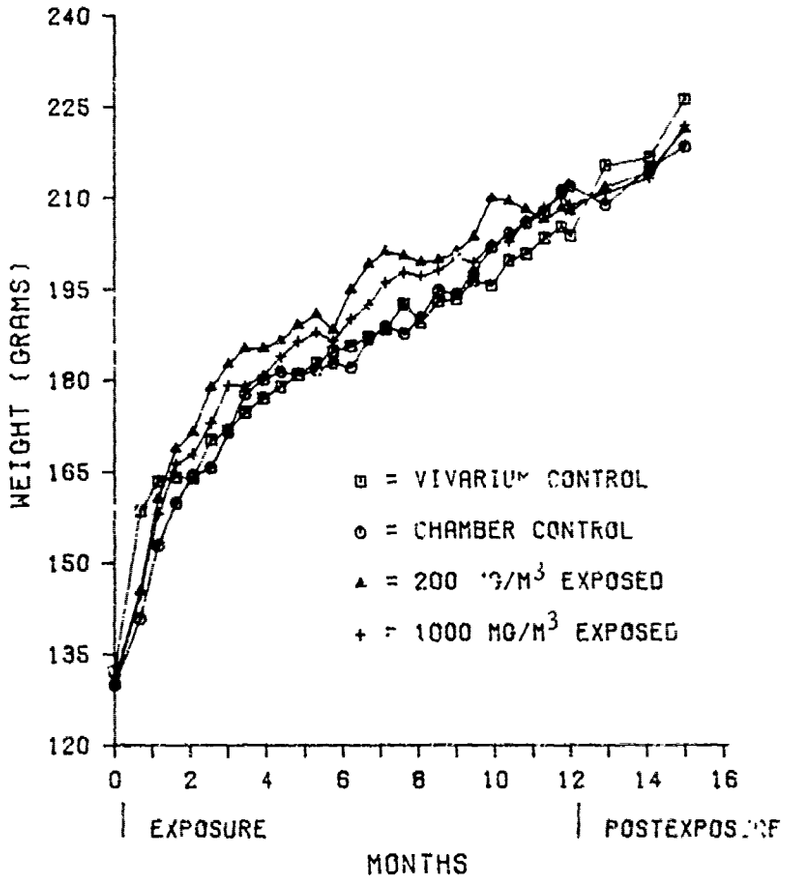


Figure 29. Mean body weight of female rats exposed to JP-TS.

Organ weights from rats killed at the exposure termination are shown in Tables 35 and 36. The mean liver weights and liver to body weight ratios of all chamber housed rat groups, both male and female, are less than the vivarium housed control groups. However, none of the test groups of rats differs from the chamber housed control groups. A similar effect is seen with the male rat test groups, kidney to body weight ratios being similar to the chamber housed control group but statistically different from the vivarium control group.

TABLE 35. MEAN ORGAN WEIGHTS OF FEMALE RATS AT TERMINATION OF ONE-YEAR EXPOSURE TO JP-TS (N = 10)

	Vivarium Control	Chamber Control	200 mg/m^3	1000 mg/m^3
Body weight, g	197 ± 7	205 ± 9	199 ± 15	202 ± 11
Liver weight, g	4.92 ± 0.27	4.56 ± 0.24	4.45 ± 0.42	4.37[A] ± 0.43
Liver/100 g body wt	2.50 ± 0.12	2.23[A] ± 0.13	2.23[A] ± 0.15	2.17[A] ± 0.25
Spleen weight, g	0.42 ± 0.03	0.42 ± 0.05	0.41 ± 0.04	0.40 ± 0.03
Spleen/100 g body wt	0.21 ± 0.02	0.20 ± 0.02	0.21 ± 0.01	0.20 ± 0.02
Kidney weight, g	1.44 ± 0.14	1.38 ± 0.08	1.36 ± 0.11	1.40 ± 0.10
Kidney/100 g body wt	0.73 ± 0.06	0.67 ± 0.04	0.69 ± 0.03	0.69 ± 0.06

[A] Different from vivarium controls at 0.01 level of significance.

TABLE 36. MEAN ORGAN WEIGHTS OF MALE RATS AT TERMINATION OF ONE-YEAR EXPOSURE TO JP-TS (N = 10)

	Vivarium Control	Chamber Control	200 mg/m^3	1000 mg/m^3
Body weight, g	419 ± 16	401 ± 16	404 ± 24	390[A] ± 22
Liver weight, g	11.46 ± 6.61	10.21[A] ± 0.81	10.05[A] ± 0.95	9.47[A] ± 0.94
Liver/100 g body wt	2.74 ± 0.11	2.55[A] ± 0.19	2.49[A] ± 0.17	2.43 ± 0.18
Spleen weight, g	0.72 ± 0.13	0.68 ± 0.15	0.66 ± 0.06	0.62 ± 0.05
Spleen/100 g body wt	0.17 ± 0.03	0.17 ± 0.03	0.16 ± 0.01	0.16 ± 0.01
Kidney weight, g	2.44 ± 0.11	2.53 ± 0.17	2.51 ± 0.19	2.40 ± 0.18
Kidney/100 g body wt	0.58 ± 0.03	0.63[A] ± 0.03	0.62[A] ± 0.03	0.61 ± 0.02

[A] Different from vivarium controls at 0.01 level of significance.

The hematology and clinical chemistry values of the rats killed at the termination of the exposures are shown in Tables 37 and 38. Both JP-TS exposed female rat groups demonstrated hypoglycemia. The glucose levels of the JP-TS exposed male rat groups were also less than the control levels; however, the differences were not statistically significant. An increase in serum creatinine was seen in the JP-TS exposed male rats but not in the females.

TABLE 37. MEAN BLOOD VALUES OF MALE RATS AT TERMINATION OF ONE-YEAR EXPOSURE TO JP-TS (N = 10)

	Vivarium Controls	Chamber Controls	200 mg/m^3	1000 mg/m^3
RBC (10^6)	7.9	8.5	8.3	8.9[a]
WBC (10^3)	4.9	5.1	4.7	4.5
HCT (%)	46.4	49.0[a]	47.6	48.4[a]
HGB (g/dl)	15.5	16.0	16.0	16.0[a]
Total Protein (g/dl)	7.6	7.6	7.8	7.8
Albumin (g/dl)	4.1	4.0	4.0	4.0
Globulin (g/dl)	3.6	3.6	3.8	3.8
A/G Ratio	1.2	1.1	1.1[a]	1.1
Glucose (mg/dl)	194	198	178	184
Calcium (mg/dl)	11.2	11.2	11.2	11.3
Potassium (mEq/L)	5.4	6.5[a]	6.2	5.9
Sodium (mEq/L)	155	155	157	156
Bilirubin (mg/dl)	0.48	0.40	0.46	0.45
Creatinine (mg/dl)	0.5	0.5	0.6[a,b]	0.6[a,b]
SGPT (IU/L)	81	54	51[a]	50
SGOT (IU/L)	94	96	92	152
Alk. Phos. (IU/L)	12.5	8.6[a]	9.9[a]	9.1[a]
BUN (mg/dl)	14.7	11.4[a]	13.0[a,b]	13.3[b]
MCV	58.7	58.3	57.7	54.7
MCH	19.6	19.0	19.3	18.1[a]
MCHC	33.4	32.6	33.5	33.1

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

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

TABLE 38. MEAN BLOOD VALUES OF FEMALE RATS AT TERMINATION OF ONE-YEAR EXPOSURE TO JP-TS (N = 10)

	Vivarium Controls	Chamber Controls	200 mg/m^3	1000 mg/m^3
RBC (10^6)	7.1	7.9[a]	7.0	7.4
WBC (10^3)	3.4	3.4	3.0	3.1
HCT (%)	44.9	46.6	45.6	46.1
HGB (g/dl)	14.7	15.6	15.1	15.8
Total Protein (g/dl)	7.8	7.7	7.6	7.7
Albumin (g/dl)	4.2	3.8	3.7	3.8
Globulin (g/dl)	3.6	3.9[a]	3.9[a]	3.9[a]
A/G Ratio	1.2	1.0[a]	0.9[a]	1.0[a]
Glucose (mg/dl)	143	140	107[a,b]	115[a,b]
Calcium (mg/dl)	11.7	11.3	11.5	11.4
Potassium (mEq/L)	4.0	5.0	5.3	6.0[a,b]
Sodium (mEq/L)	151	153	155[a]	157[a,b]
Bilirubin (mg/dl)	0.28	0.29	0.23	0.22
Creatinine (mg/dl)	0.4	0.5	0.5	0.4
SGPT (IU/L)	79	53	52	41
SGOT (IU/L)	120	103	96	90
Alk. Phos. (IU/L)	7.4	6.0	6.3	6.4
BUN (mg/dl)	16.4	13.8[a]	13.9[a]	13.7[a]
MCV	83.1	58.9	65.9	65.3
MCH	20.6	19.7	21.7[b]	22.4
MCHC	32.7	33.4	33.1	34.2

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

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

All surviving animals are being held for a one-year postexposure observation period. They are presently housed in laminar air flow animal facilities.

EVALUATION OF SKIN CORROSION POTENTIAL OF VARIOUS DEPARTMENT OF TRANSPORTATION CHEMICALS

The Department of Transportation (DOT) submitted a list of 22 chemicals to be evaluated for skin corrosion potential. The object of the test was to establish the minimum corrosive concentrations. Both solid materials and aqueous solutions were tested. The list of chemicals tested is shown in Table 39. Code numbers were assigned to each chemical for unique identification. All of the chemicals were obtained from commercial chemical supply companies.

TABLE 39. LIST OF DOT CHEMICALS SUBMITTED FOR SKIN CORROSION TESTING

DOT No.	Chemical Name	Physical State
310	Ammonium Hydrogen Fluoride	Solid
311	Bromoacetic Acid	Solid
312	Chloroacetic Acid	Solid
313	Chromic Acid	Solid
314	Dichloroacetic Acid	Liquid
315	Ferric Chloride	Solid
316	Fluorosulfonic Acid	Liquid
317	Formic Acid	Liquid
171	Hydrazine	Liquid
318	Hydrobromic Acid	Liquid
256	Hydrofluoric Acid	Liquid
319	Hydriodic Acid	Liquid
265	Maleic Anhydride	Solid
325	N,N'-di-sec-butyl-p-phenylenediamine	Liquid
320	Perchloric Acid	Liquid
321	Phosphoric Acid	Liquid
189	Potassium Fluoride	Solid
322	Potassium Hydrogen Fluoride	Solid
252	Propionic Acid	Liquid
255	Sulfuric Acid	Liquid
323	Titanium Sulfate	Solid
324	Zinc Chloride	Solid

The method of testing conformed to DOT requirements for testing corrosion to skin (49CFR173, Appendix A). In addition to examination at 4 and 48 hours postapplication, the rabbits were held for 1 week postapplication to further evaluate skin damage. Additional observation was helpful in evaluating skin damage for a variety of reasons. DOT regulations call for a determination of irreversible

alteration of skin. We found that 48 hours postexposure observation was often insufficient time to adequately determine irreversibility of damage. This was particularly true when trying to establish minimum corrosive concentrations to within 2%. Also, as in the case of dichloroacetic acid, skin damage did not appear within 48 hours of application but was visible at 1 week.

Corrosion of the skin was measured by a patch test on the intact skin of albino rabbits. All possible hair on the backs and flanks of the animals was clipped 24 hours prior to chemical contact to allow for recovery of the skin from abrasion resulting from clipping.

Six New Zealand white rabbits (2-3 kg) were used for each concentration of test material. Six areas on the back, three per side, were used as patch test sites. This allowed for the simultaneous testing of six compounds on each rabbit. Solid materials were tested both in the native state and as aqueous solutions. The test material was applied as 0.5 gm for solids and 0.5 ml for liquids and then covered by a 1 inch square of surgical gauze two single layers thick. The gauze patches were held in place with strips of adhesive tape. The entire area was covered with a rubber dental dam strip and secured with elastoplast tape. The patches remained on the rabbits for 4 hours during which the rabbits were fitted with leather restraining collars to prevent disturbance of the patch area. After 4 hours, the wrap and gauze patches were removed and the resulting reactions evaluated for corrosion. Following this initial reading, all test sites were washed with water to prevent further exposure. Readings were also made at 48 hours (44 hours after the first reading) and at one week postapplication.

Corrosion was considered to have resulted if the substance in contact with the rabbit skin caused destruction or irreversible alteration of tissue. Tissue destruction was considered to have occurred if, at any of the examinations, there was ulceration or necrosis. Tissue destruction did not include mere sloughing of the epidermis or erythema, edema, or fissuring. A concentration was considered corrosive if 2 of the 6 rabbits exhibited skin corrosion and was considered negative if 5 of the 6 rabbits exhibited no corrosive signs. Testing continued until the difference between the corrosive and noncorrosive concentration was 2% or less or until the lowest corrosive concentration was below 2%.

Solid materials were tested both as solids and as solutions. All solutions were prepared with distilled water with the exception of N,N'-di-sec-butyl-p-phenylenediamine which was diluted in corn oil because of solubility problems. Concentrations were prepared on a weight percent basis.

Results

The results of the skin corrosion tests are presented by material. For interpretation of the symbols consult Table 40.

TABLE 40. DEPARTMENT OF TRANSPORTATION SKIN CORROSION TEST RESULTS

LEGEND:

[a] Highest concentration obtainable in water.
[b] Material applied slightly warm.
[c] Material applied as a slurry.
\+ Caused visible destruction or irreversible alteration in skin tissue after 4 hours contact or at 44 hours after removal from skin.
0 Did not cause damage after 4 hours contact or at 44 hours after removal from skin.
* Skin damage after 4-hours contact or at 44 hours after removal from the skin was negative but found positive at 1 week postapplication.

TEST RESULTS:

Ammonium Hydrogen Fluoride

	Rabbit No.					
Conc., %	1	2	3	4	5	6
Solid	0	0	0	0	0	0
60[a,b]	0	0	0	0	0	0

Bromoacetic Acid

	Rabbit No.					
Conc., %	1	2	3	4	5	6
Solid	+	*	0	+	+	See Below
10	0	0	0	0	0	0
12	0	0	0	0	0	0
14	0	*	*	+	+	0
16	*	*	*	+	+	0

Comments - Reactions to bromoacetic acid were very severe. Major edema and hemorrhage were noted in most of the rabbits tested. In addition to these toxic signs, three of the first group of rabbits treated with 0.5 gm solid material died prior to obtaining the 48-hour reading. These animals were replaced. Rabbit No. 5 died 3 days postapplication. Additional animals were tested in an attempt to obtain scores for six rabbits as required by DOT. One animal

TABLE 40. DEPARTMENT OF TRANSPORTATION SKIN CORROSION TEST RESULTS (Continued)

died in this new series and two more rabbits were treated with a reduced concentration of 0.25 gm. Both of these rabbits died within 48-hours of dosing. Because of the severe toxicity, no further applications of solid bromoacetic acid were conducted.

Chloroacetic Acid

	Rabbit No.					
Conc., %	1	2	3	4	5	6
Solid	+	+	+	+	+	+
36	0	0	0	0	0	0
38	0	+	0	0	+	+
40	0	0	0	0	*	*
42	0	+	*	+	+	0

Chromic Acid

	Rabbit No.					
Conc., %	1	2	3	4	5	6
Solid	+	+	+	+	+	+
46	0	0	0	0	0	0
48	0	0	0	*	0	+
50	+	+	0	+	+	+

Dichloroacetic Acid

	Rabbit No.					
Conc., %	1	2	3	4	5	6
28	0	0	0	+	0	0
30	*	0	0	0	0	+
32	*	+	+	+	0	0
34	*	*	*	0	+	0
36	*	*	0	+	+	+

Ferric Chloride

	Rabbit No.					
Conc., %	1	2	3	4	5	6
Solid	0	0	0	0	0	0
60[a,b]	0	0	0	0	0	0

TABLE 40. DEPARTMENT OF TRANSPORTATION SKIN CORROSION TEST RESULTS (Continued)

Fluorosulfonic Acid

Conc., %	Rabbit No. 1	2	3	4	5	6
20	0	0	0	0	0	0
22	0	0	0	0	+	0
24	+	+	0	+	0	0
26	0	0	+	+	+	0
28	0	0	+	0	+	0
30	+	0	0	+	+	+

Formic Acid

Conc., %	Rabbit No. 1	2	3	4	5	6
17	0	0	0	0	0	0
19	0	0	0	0	0	+
21	0	*	0	0	0	+
23	*	+	0	+	+	+

Hydrobromic Acid

Conc., %	Rabbit No. 1	2	3	4	5	6
20	0	0	0	0	0	0
22	+	0	0	0	0	0
24	0	0	+	0	0	0
26	+	+	+	0	0	+

Hydrofluoric Acid

Conc., %	Rabbit No. 1	2	3	4	5	6
2	0	0	0	0	0	0
4	*	+	+	+	+	+
6	0	0	+	+	+	+
8	0	0	+	+	+	+

TABLE 40. DEPARTMENT OF TRANSPORTATION SKIN CORROSION TEST RESULTS (Continued)

Hydriodic Acid

Conc., %	Rabbit No. 1	2	3	4	5	6
34	0	0	0	0	0	0
36	0	0	0	0	0	0
38	+	+	+	+	+	+
40	0	0	0	*	0	*
42	+	+	+	0	+	+
44	0	0	+	+	+	+

Maleic Anhydride

Conc., %	Rabbit No. 1	2	3	4	5	6
Solid	0	+	0	0	0	0
55[a,b]	0	0	0	0	0	0

Comments - Solid maleic anhydride was previously tested for skin corrosion by the THRU (MacEwen and Vernot, 1975). Three of four rabbits were judged positive at that time. DOT requested that the tests be repeated. In the present test one of six rabbits was judged positive. Test sites were grey/white at 4 hours. Usually the grey/white area is a strong indication that the tissue is necrotic. By 48 hours, some of the rabbits showed some minor edema at the test site. However, examination one week postapplication showed the sites to be normal.

N,N'-di-sec-butyl-p-phenylenediamine

Conc., %	Rabbit No. 1	2	3	4	5	6
50	0	0	0	0	0	0
52	+	+	0	0	0	0
54	+	+	0	+	+	0

Comments - This material was diluted in corn oil since it was insoluble in water. The tissue damage that resulted consisted of major edema at 24 hours. By 48 hours the skin at the test site was coriaceous. At one week the hardened skin was sloughing and only the epidermal layer was damaged. Histopathologic preparations from one skin test site were examined microscopically. Findings included.

TABLE 40. DEPARTMENT OF TRANSPORTATION SKIN CORROSION TEST RESULTS (Continued)

acanthosis and mild ulceration of the epidermal layer. Mild inflammation, hemorrhage and fibrosis were noted in the dermal layer. The dermal layer was judged viable.

Additional tests with undiluted N,N'-di-sec-butyl-p-phenylenediamine produced similar skin lesions; damaged epidermis with viable dermis. While this type of skin damage produced by N,N'-di-sec-butyl-p-phenylenediamine is probably reversible, it is felt that it is severe enough to classify as corrosive.

Perchloric Acid

	Rabbit No.					
Conc., %	1	2	3	4	5	6
36	0	0	0	0	0	0
38	0	0	0	0	0	0
40	0	+	+	+	0	0

Phosphoric Acid

	Rabbit No.					
Conc., %	1	2	3	4	5	6
60	0	0	0	0	0	0
62	0	0	0	0	0	+
64	0	+	+	0	+	+
66	0	0	+	+	0	+
68	+	+	+	0	+	*

Potassium Fluoride

	Rabbit No.					
Conc., %	1	2	3	4	5	6
Solid	0	0	0	0	0	0
90[a]	0	0	0	0	0	0

Potassium Hydrogen Fluoride

	Rabbit No.					
Conc., %	1	2	3	4	5	6
Solid	0	0	0	0	0	0
40[a]	0	0	0	0	0	0

TABLE 40. DEPARTMENT OF TRANSPORTATION SKIN CORROSION TEST RESULTS (Continued)

Propionic Acid

Conc., %	Rabbit No. 1	2	3	4	5	6
44	0	0	+	0	0	0
46	+	0	*	+	0	0
48	0	0	0	0	0	0
50	0	0	0	+	+	0
52	0	+	+	+	0	0

Sulfuric Acid

Conc., %	Rabbit No. 1	2	3	4	5	6
34	+	0	0	0	0	0
36	0	0	+	0	0	0
38	+	0	+	+	0	0
40	+	+	+	0	+	+

Titanium Sulfate

Conc., %	Rabbit No. 1	2	3	4	5	6
Solid	0	0	0	0	0	0
70[c]	0	0	0	0	0	0

Zinc Chloride

Conc., %	Rabbit No. 1	2	3	4	5	6
Solid	+	0	+	+	+	+
44	0	0	0	+	0	0
46	0	0	0	+	0	0
48	+	0	+	+	0	0
50	*	0	0	*	*	0

TABLE 40. DEPARTMENT OF TRANSPORTATION SKIN CORROSION TEST RESULTS (Continued)

Hydrazine

Considerable problems were encountered in an attempt to establish a corrosive concentration for hydrazine. A review of the literature revealed several publications concerning the dermal toxicity of hydrazine.

Keller et al. (1981) conducted rabbit percutaneous toxicokinetic studies with hydrazine. They found hydrazine to be well absorbed by rabbits percutaneously. After application, hydrazine has sufficient vapor pressure to evaporate from the skin. This fact is important since the skin corrosion tests conducted by the DOT protocol require test site occlusion for a 4-hour period. Occlusion undoubtedly increases the total amount absorbed.

Through the use of kinetic calculations, Keller et al. (1981) estimated that removal of percutaneous hydrazine within 5 minutes of exposure would reduce the amount absorbed by greater than 75%. They also suggest that human skin contact with hydrazine will be very uncomfortable due to its highly irritative nature. Thus, the worker is likely to wash soon after exposure thereby reducing the amount absorbed.

The published dermal LD_{50} for rabbits is 93 mg/kg (Rothberg and Cope, 1972). This is approximately equivalent to an application of 0.5 ml of a 40% solution on a 2 kg rabbit. Application of this amount to 4 rabbits resulted in various sized areas of darkly discolored skin which were scored as necrotic. All of the rabbits were lethargic with limb weakness. One of the rabbits died within 24 hours of dosing. Application of 30% solution to 2 more rabbits resulted in areas of skin discoloration along with minor edema. One of these rabbits died within 24 hours of dosing. The other rabbit was lethargic but survived the 44-hour observation period. It was judged free of skin damage at that time.

The dermal toxicity of hydrazine is a much greater hazard than the skin corrosion potential. For this reason further skin corrosion tests with hydrazine were not conducted.

Summary results of the skin corrosion tests of 10 solid and 12 liquid chemicals are shown in Tables 41 and 42, respectively. The results of chromic, dichloroacetic, and formic acid solutions are based on the criterion of skin damage detected one week after application. The corrosive and noncorrosive solution concentration results for these chemicals would increase by 2% if the criterion of skin damage at 48 hours were used.

TABLE 41. SUMMARY RESULTS OF SKIN CORROSION TESTING MATERIALS OF SOLID PHYSICAL STATES

Chemical Name	Tested As	Corrosive @ % Solution	Non-Corrosive @ % Solution
Ammonium Hydrogen Fluoride	Solid	-----	Undiluted
	Solution	-----	60[a]
Bromoacetic Acid	Solid	Undiluted	-----
	Solution	14	12
Chloroacetic Acid	Solid	Undiluted	-----
	Solution	38	36
Chromic Acid[b]	Solid	Undiluted	-----
	Solution	48	46
Ferric Chloride	Solid	-----	Undiluted
	Solution	-----	60[a]
Maleic Anhydride	Solid	-----	Undiluted
	Solution	-----	55[a]
Potassium Fluoride	Solid	-----	Undiluted
	Solution		90[a]
Potassium Hydrogen Fluoride	Solid	-----	Undiluted
	Solution	-----	40[a]
Titanium Sulfate	Solid	-----	Undiluted
	Solution	-----	70[a]
Zinc Chloride	Solid	Undiluted	-----
	Solution	48	46

[a] Highest concentration obtainable in water.
[b] Based on the criterion of skin damage as late as one week post-application.

TABLE 42. SUMMARY RESULTS OF SKIN CORROSION TESTING MATERIALS OF LIQUID PHYSICAL STATES

Chemical Name	Corrosive @ % Solution	Non-Corrosive @ % Solution
Dichloroacetic Acid[a]	30	28
Fluorosulfonic Acid	24	22
Formic Acid[a]	21	19
Hydrazine	Lethal	Lethal
Hydrobromic Acid	26	24
Hydrofluoric Acid	4	2
N,N'-di-sec-butyl-p-phenylenediamine	52	50
Perchloric Acid	40	38
Phosphoric Acid	64	62
Propionic Acid	46	44
Sulfuric Acid	38	36

[a] Based on the criterion of skin damage as late as one week post-application.

PERCUTANEOUS, ORAL, AND INHALATION STUDIES FOR CLASSIFICATION OF TOXICITY RATINGS FOR TRANSPORTABLE CHEMICAL AGENTS

The Department of Transportation (DOT) has submitted a list of 10 compounds for toxicity testing in accordance with 49CFR173.343(a). Most of the compounds are classified highly toxic or extremely toxic in the neat form by Back et al. (1972). These materials were tested in this laboratory to determine the minimum concentrations of each compound that will meet the Class B poison definition. A group of compounds was received and assigned code numbers prior to testing. These compounds, the THRU code numbers, and the tests to be done on each are listed in Table 43. Methods and procedures for oral, dermal, and inhalation testing are described in a previous annual report (MacEwen and Vernot, 1975).

TABLE 43. LIST OF COMPOUNDS SUBMITTED BY THE DEPARTMENT OF TRANSPORTATION FOR ACUTE INHALATION, PERORAL AND PERCUTANEOUS TOXICITY STUDIES

Code No.	Compound	Oral Toxicity	Inhalation Toxicity	Percutaneous Toxicity
001	Acetone cyanohydrin solution	X	X	X
006	Arsenic acid solution	X		
014	Arsenic trioxide, solid	X	X	
325	Calcium cyanide, solid	X	X	
037	Cyanogen bromide, solid	X	X	
050	Hydrocyanic acid, solution	X	X	X
326	Potassium cyanide, solid	X	X	
327	Potassium cyanide, solution	X		
307	Selenium oxide, solid	X	X	
116	Sodium azide, solid	X	X	

Each compound was tested at various dilutions to determine the concentration which would meet the minimum requirements for the Poison B definition. All materials were prepared to allow for peroral administration at one percent of body weight. The rats were fasted for a minimum of eight hours prior to administration of the oral dose. Dry mixtures were diluted using diatomaceous earth and dosed in corn oil while solutions were diluted and dosed with distilled water. All suspensions were kept in a turbulent state while in use by maintaining them on a magnetic stirring platform.

Groups of 10 male Sprague-Dawley rats weighing between 200 and 300 grams received a single dose of 50 mg/kg of the dilutions administered orally. Mortality was recorded at 48 hours as well as at 14 days. Various concentrations were tested until that dilution was achieved which killed less than half the group within 48 hours. LD_{50} calculations were made using the probit analysis method of Finney (1952).

Groups of 10 rabbits, evenly divided by sex, received doses of 200 mg/kg of the compounds held in continuous contact with the skin for 24 hours. Various concentrations were tested until the dilution was found which killed less than half the group within 48 hours. All dilutions were made in distilled water and the dermal LD_{50}'s calculated by the Finney (1971) method. The rabbits used in these studies were New Zealand White obtained from J & J Research Farms, Hamilton, Ohio and Willoughby Rabbitry, Sabina, Ohio.

Inhalation exposures to a total dust concentration of approximately 1 mg/L (1000 mg/m^3) were conducted for one hour for male Sprague-Dawley rats weighing between 200 and 300 grams. The solid compounds were diluted in diatomaceous earth and dispersed as a dust using a Wright Dust Feeder®. All dust exposures were conducted in a 120 liter Plexiglas® chamber with an airflow of 5 liters per minute through the dust feeder.

Dust concentrations were measured gravimetrically using a Gelman Model 1220 filter holder and 47 mm diameter 0.45 µm filter.

The results of the acute oral toxicity tests are shown in Table 44. Arsenic trioxide did not kill any rats when dosed undiluted at 50 mg/kg. Arsenic acid solution, dosed as received (75%) also did not kill rats when dosed at the prescribed level. Because of the steep mortality slope produced by hydrocyanic acid solution, it was not possible to produce sufficient partial mortality responses to allow for calculation of an LD_{50}. Rats that died did so within one hour of dosing. Those that survived the initial chemical anoxia survived the 14-day observation period.

TABLE 44. ORAL TOXICITY OF VARIOUS COMPOUND MIXTURES TO MALE RATS

Compound	48-Hour LD_{50} (95% CL) in % Conc.	Data used to Calculate 48-hour LD_{50} in % Concentration, N = 10	14-Day LD_{50} (95% CL) in % Conc.	48-Hour LD_{50} Equivalent Dose of Neat Compound mg/kg
Acetone cyanohydrin solution	49.6 (43.6-56.2)	20(0)[A], 40(1), 50(6), 60(8)	[B]	24.8 (21.8-28.1)
Arsenic acid solution	-----	75[C](0)	-----	-----
Arsenic trioxide	-----	100(0)	-----	-----
Calcium cyanide	40.0 (36.6-43.5)	35(0), 37.5(5), 40(7), 50(8), 60(10)	[B]	20.0 (18.3-21.8)
Hydrocyanic acid solution	[D]	13.5(0), 14.3(0), 15.7(10), 16.7(8), 17.8(10), 19.5(10)	[D]	-----
Potassium cyanide, solid	32.4 (26.2-53.0)	15(1), 20(2), 25(3), 30(7), 35(3), 40(7)	[B]	16.2 (13.1-26.5)
Potassium cyanide, solution	21.1 (19.2-25.0)	15(0), 17.5(1), 20(7), 25(6), 27.5(9), 30(10)	[B]	10.6 (9.6-11.5)
Selenium oxide	77.4 (72.4-82.5)	60(0), 70(3), 75(6), 80(5), 85(7), 90(8)	75.7 (69.4-81.6)	38.7 (34.7-40.8)
Sodium azide	96.0 (91.7-100.0)	75(0), 80(1), 85(2), 90(1), 95(7), 99(5)	[B]	48 (45.9-50.0)

[A] Number of deaths.
[B] LD_{50} is the same as for 48-hours.
[C] Dosed as received.
[D] Because of the steep mortality curve an LD_{50} could not be calculated.

An attempt was made to dose rats orally with cyanogen bromide, but the vapor pressure of the compound at room temperature caused it to gas off faster than the rats could be dosed. Dosing results were erratic and not reproducible so no further attempt at oral dosing was made. A similar problem with gassing off occurred when preparing cyanogen bromide for dust and dermal testing. As a result, it was not possible to conduct valid oral LD_{50} or inhalation tests with this compound in the manner prescribed by the Department of Transportation.

In the last column of Table 44, the 48-hour LD_{50} values were recalculated as dosages of neat compound given to the rats. Comparison of these data may be made directly with other sources of LD_{50} data. For example, selenium oxide as a neat compound in distilled water was tested in this laboratory and reported in the 1980 THRU Annual Report. At that time an LD_{50} of 54 mg/kg with a confidence range of 36 to 79 mg/kg was reported for male rats. This compares well with the current determination of 38.7 mg/kg with confidence limits of 34.7 to 40.8 mg/kg.

The inhalation toxicity data presented in Table 45 are not as precise as the oral toxicity data and any use made of these values must be given careful thought. The tests were conducted with reluctance to fulfill requirements set by the DOT. To illustrate this problem, we want to point out that selenium oxide generated as an aerosol from solution resulted in a one-hour LC_{50} of 100 mg/m^3 (MacEwen and Vernot, 1980) while an 86% mixture of selenium oxide dust mixed with diatomaceous earth (the LC_{50} value reported in Table 45) is equivalent to an 860 mg/m^3 concentration. This gross difference in lethal concentrations may be due to a variety of problems encountered in generation of mixtures with diatomaceous earth. Difference in particle size may have resulted in lower actual exposures or affected clearance mechanisms to reduce retained doses.

Exposures to the two liquid materials, acetone cyanohydrin and hydrocyanic acid, were less than satisfactory because the generation procedures allowed most of the compounds to be volatilized from the liquid at a rapid and uncontrolled rate. Although the exposures were of one-hour duration, most of the rats killed in these experiments died within the first 5 minutes and it is therefore possible that the LC_{50} values should be recalculated on the chamber flows for the first 2-3 minutes of exposure.

A dust exposure of arsenic trioxide at the higest concentration capable of being generated killed less than half the rats. A 90% arsenic trioxide dust at a total concentration of 700 mg/m^3 for one hour resulted in one of 10 rats dead at 48 hours and 14 days.

TABLE 45. ONE-HOUR INHALATION TOXICITY OF VARIOUS COMPOUNDS FOR MALE RATS

Compound	48-Hour LC_{50} (95% CL) in % Conc.	Data used to Calculate LC_{50} in % Concentration, N = 10	MMD, μ (σg)
Arsenic trioxide (Dust)	B	75(0)[A], 90(1)	3.6 (2.3)
Calcium cyanide (Dust)	9.0% (8.53-9.64)	6(0), 8(2), 9(8), 10(9)	4.5 (1.6)
Selenium oxide (Dust)	86[C]	30(0), 50(2), 60(0), 75(1), 90(5), 100(9)	3.8 (2.1)
Sodium azide (Dust)	B	75(0)[D]	6.2 (1.3)
Potassium cyanide (Dust)	6.7[C]	4(0), 5(3), 6(2), 7(9), 10(9), 20(9), 40(10)	6.0 (1.3)
Acetone cyanohydrin solution	16.2[C]	10(1), 15(1), 16(2), 17(9), 20(9)	0.9 (2.3)
Hydrocyanic acid solution	2.12 (1.93-2.23)	1(0), 2(2), 2.25(9), 2.5(9), 3(10)	E

A Number of deaths.
B Data are insufficient for LC_{50} calculation.
C Confidence limits could not be calculated.
D Highest concentration tested due to explosive hazard.
E Evaporation occurred rapidly, no droplet detected within animal breathing area.

The highest concentration of sodium azide tested, 75%, resulted in no mortality. Prior to dispersal of the dust by the Wright Dust Feeder® the material must be packed under pressure in a brass cylinder. Because azides present a severe explosion hazard when exposed to brass or heat (Pobiner, 1982), higher concentrations were considered to be a laboratory safety hazard.

Aqueous aerosol exposures were run at a total liquid concentration between 3 and 5 mg/L. Anything less than this concentration resulted in an all-vapor exposure with no discernible droplets. Even at this concentration very few droplets were detected at the animal breathing level in the acetone cyanohydrin exposures. Those that were detected were extremely small (less than 1 μ). No droplets were found at the rat breathing level in the hydrocyanic acid exposures.

Dermal studies have not been completed at this time. Results of these studies will be included in the next annual report.

EVALUATION OF THE ACUTE AND SUBCHRONIC TOXICITY OF FYRQUEL 220, DURAD MP280, AND HOUGHTO-SAFE 273

The U. S. Navy presently uses phosphate ester based fluids in many of its shipboard hydraulic systems. The hydraulic fluids in use are desirable because of their flame retardant characteristics. However, phosphate ester based hydraulic fluids pose a potential toxic hazard to Naval personnel since they contain small amounts of neurotoxic o-cresyl phosphate esters.

The Navy is presently considering discontinuing use of the phosphate ester based hydraulic fluids and using water-glycol based fluid mixtures instead. The water-glycol mixtures are also fire retardant; however, they, too, present a potential health risk since they contain small amounts of nitrosoamines.

Changing hydraulic fluids would necessitate major equipment modifications at great expense. Studies involving evaluation of the toxic effects of the various hydraulic fluids are necessary prior to equipment modification in order to properly compare the health risks associated with the various hydraulic fluids.

NMRI/TD requested that the THRU conduct a series of acute and subchronic toxicity studies with three hydraulic fluids. Two of the fluids, Fyrquel 220 and Durad MP280, are phosphate ester based while the third, Houghto-Safe 273 is water-glycol based.

The most significant routes of industrial exposure to the hydraulic fluids are expected to be dermal, due to spills or leaks, and aerosol inhalation, from pressurized system leaks. The list of proposed studies, shown below, reflects these routes of exposure.

1. Eye Irritation
2. Skin Irritation
3. Skin Sensitization
4. Acute Neurotoxicity
5. Acute Oral Toxicity
6. Acute Intraperitoneal Toxicity
7. Acute Inhalation Toxicity
8. Acute Dermal Toxicity
9. 21-Day Repeated Dose Dermal Toxicity
10. 21-Day Repeated Dose Inhalation Toxicity
11. 90-Day Continuous Dose Inhalation Toxicity

As of this writing, tests numbered 1-8 are complete. The subchronic inhalation studies are currently in progress. This report presents the results of the completed studies.

EVALUATION OF THE IRRITATION POTENTIAL OF FIRE RESISTANT HYDRAULIC FLUIDS

Animals

Female New Zealand albino rabbits weighing approximately 2.3 kg and male Hartley derived, albino guinea pigs weighing between 300 and 500 grams were used in these studies. The rabbits were purchased from Willoughby's Rabbitry, Sabina, Ohio and Sweetwater Farm, Incorporated, Hillsboro, Ohio. The guinea pigs were supplied by Murphy Breeding Labs, Plainfield, Indiana and Sweetwater Farm, Incorporated, Hillsboro, Ohio. Food and water were available ad libitum.

Test Materials

Samples of the three hydraulic fluids were supplied by the Navy Medical Research Institute/Toxicology Detachment, Wright-Patterson Air Force Base, Ohio. Lot number identification and some physical and chemical properties of the hydraulic fluids are as follows:

Houghto-Safe 273 (MIL-H-22072B)

MANUFACTURER:	E. F. HOUGHTON & COMPANY
NMRI/TD Sample Number:	1257-1
Lot Number:	B9A
Specific Gravity:	1.08
Boiling Point (°F):	220
Odor:	Slight Ammoniacal
Chemical Composition:	33-35% ethylene glycol; 43.5-51.5% water and 14-24% polyglycol

Pyrquel 220 (MIL-H-19457C)

MANUFACTURER:	STAUFFER CHEMICAL COMPANY
NMRI/TD Sample Number:	1257-2
Lot Number:	0820-E-11
Specific Gravity (60°/60°F):	1.150
Boiling Point (°F):	735
Odor:	Very Slight
Chemical Composition:	Tertiary butyltriaryl phosphate

Durad MP280 (MIL-H-19457B)

MANUFACTURER:	FMC CORPORATION
NMRI/TD Sample Number:	1257-3
Lot Number:	67-5
Specific Gravity (20°/20°):	1.10-1.40
Boiling Point (°C):	220-270
Odor:	Odorless
Chemical Composition:	Phenol, isopropylated phosphate (3-1) 100%

Primary Skin Irritation

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

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

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

After 24 hours, the wrap and patches were carefully removed, and the test areas were evaluated for irritation using the Draize (1959) table as a reference standard. Readings were also made at 72 hours (48 hours after the first reading). The total score of both readings for all six rabbits was divided by 24 to yield a primary irritation score.

Primary Eye Irritation

A 0.1 ml sample of hydraulic fluid was applied to one eye of each of six to nine albino rabbits. The opposite eye was untreated and served as a control. The treated eyes of three rabbits were flushed with lukewarm water approximately 30 seconds after instillation of the fluid. Examinations for gross signs of eye irritation were made at scheduled observation periods following application. Scoring of the irritative effects was according to the method of

Draize (1959). In this scoring system, injuries to the cornea and iris may represent as much as 80% of the total score because of their essential role in vision.

Skin Sensitization

A modified Landsteiner (Landsteiner and Chase, 1937) guinea pig sensitization test was routinely used by the THRU at the time two of the hydraulic fluids were tested. Fyrquel 220 and Houghto-Safe 273 were both tested by this method. The THRU has since adopted an alternate sensitization procedure (Horton et al., 1981) which is a modification of the Maguire (1973) guinea pig sensitization test. The latter test was the method used to examine the sensitization potential of Durad MP280. So that comparisons can be made, sensitization tests were repeated with Fyrquel 220 and Houghto-Safe 273 using the modified Maguire method.

Modified Landsteiner Test

Twenty male albino guinea pigs, Hartley strain, six to eight weeks of age were used for each hydraulic fluid. The Fyrquel was injected as a 0.1% dilution in peanut oil while the Houghto-Safe 273 was injected as a 0.1% dilution in distilled water. The test substance (0.05 ml) was injected intradermally into the upper right scapular area and a similar injection of the vehicle alone was injected into the upper left scapular area serving as a control site. Readings were made 24 and 48 hours later and recorded.

Doses of 0.1 ml of freshly prepared dilutions were injected into the clipped dosal lumbo-sacral areas of the guinea pigs on the following Wednesday, Friday, Monday, etc. until seven intradermal injections were administered. Care was taken to insure that the repeated doses were not injected into the same site.

The guinea pigs were rested for three weeks (incubation period) and given a challenge dose of the test substance into the lower right scapular area. A control injection of the vehicle alone was also administered into the lower left scapular area at this time. The reactions were read after 24 and 48 hours and recorded. The numerical scores were determined according to the criteria shown in Table 46. Sensitization response and potential of the test agent may be inferred from the numerical grade and numbers of animals affected (Kinkead et al., 1981).

TABLE 46. GRADING OF SKIN REACTIONS IN THE GUINEA PIG SENSITIZATION TEST

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

0 = needle puncture, no wheal
1 = very faint pink
2 = faint pink
3 = pink
4 = red
5 = bright red
6 = edema, <1 mm in height
7 = edema, >1 mm in height
* 8 = necrosis, <1 square mm
* 9 = necrosis, >1 square mm

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

Modified Maguire Test

Ten male albino guinea pigs, Hartley strain, six to eight weeks of age, were used for each hydraulic fluid. An area on the back of each animal directly above the forelegs was clipped with electric clippers and the fur chemically removed with a commercial depilatory on the morning of the first insult exposure as recommended by Maguire (1973). Test solutions, 0.1 ml at each application, were applied to this area on a 1/2 x 1/2 inch cotton gauze square, covered with dental dam, and held in place with adhesive tape. The first insult patch was allowed to remain in place for two days, then removed, and a second application of 0.1 ml was made. Two days later, this patch was removed, a total of 0.2 ml of Freund's adjuvant per animal injected intradermally, using 2 or 3 points adjacent to the insult site, and then a new patch containing 0.1 ml of the test material applied. Three days after the third application a fresh patch of 0.1 ml of the material was applied. The last patch was removed two days later, then the animals were allowed to rest for two weeks. Each time the insult patches were removed, the condition of the skin at the application site was evaluated and recorded. When the last patch was removed, the toes on the hind feet of the guinea pigs were taped to prevent the animal from scratching the irritated area.

After the two-week rest period, the right flanks of the same animals were clipped and challenged with the test solution. The challenge application was not occluded. The skin response at these sites was recorded at 24 and 48 hours after application according to

the evaluation method of Draize (1959) and shown in Table 47. Any animal eliciting a score of 2 or more at the test solution challenge site would be rated as a positive responder.

TABLE 47. GRADING OF SKIN REACTIONS IN THE MAGUIRE GUINEA PIG SENSITIZATION TEST

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

Results

Primary Skin Irritation

None of the three hydraulic fluids, when applied undiluted to intact and abraded rabbit skin, produced a primary irritation response and, therefore, they are not considered primary skin irritants for rabbits.

Primary Eye Irritation

None of the three hydraulic fluids caused any ocular irritation in the rabbits. No differences could be noticed when comparing the exposed eyes, washed or unwashed, with the respective control eyes at the scheduled observation periods.

Skin Sensitization

The results of the modified Landsteiner tests showed negative responses for all twenty guinea pigs injected with Houghto-Safe 273 at the 24 and 48 hour observation periods. The reactions of the guinea pigs injected with Pyrquel 220 were erratic and inconsistent. Slight reactions were found in four of twenty guinea pigs 24 hours after the challenge injection. Examination of the guinea pigs at 48 hours showed five guinea pigs with reaction scores greater than 25. The evaluation of the sensitization responses was hindered somewhat by the reaction of the guinea pigs to the peanut oil vehicle.

None of the hydraulic fluid samples tested by the Maguire method caused a dermal reaction in guinea pigs. The responses to the challenge applications of the hydraulic fluids to ten guinea pigs following a two-week incubation period were negative in all three cases.

Discussion

Application of the three hydraulic fluids to the intact and abraded skin of rabbits produced a primary irritation score of zero. Application of the undiluted fluids to eyes of rabbits produced no ocular irritation through the scheduled observation periods.

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 may induce immune cell synthesis. The induction potential is then evaluated by grading the irritation (area and intensity) of a single challenge administration of the test material.

Under the conditions of the Landsteiner test, Houghto-Safe 273 proved to be negative. Although the numerical values obtained for Fyrquel 220 indicated that the material had minimal sensitizing potential, the irritating potential of the peanut oil vehicle casts uncertainty on this finding. At most, the sensitizing potential of Fyrquel 220, determined by this method, is extremely low. None of the three fluids demonstrated a sensitizing potential by the Maguire testing method.

All three compounds would be acceptable hydraulic fluids with respect to the irritation and sensitization properties.

EVALUATION OF NEUROTOXIC EFFECTS OF HYDRAULIC FLUIDS

Introduction

The Toxic Hazards Research Unit was requested to evaluate the neurotoxic potential of two triaryl phosphate containing hydraulic fluids, Durad MP280, and Fyrquel 220. Durad MP280, an isopropylated triphenyl phosphate and Fyrquel 220, a tertiarybutyl triaryl phosphate, are currently in use as fire resistant hydraulic fluids.

Many organophosphorus compounds, including TOCP, have been found to cause delayed neurotoxic effects in man (Doull et al., 1979). A single exposure to a neurotoxic organophosphorus compound has been reported as capable of producing axonal damage after a delay of eight to ten days. Low level nerve injury may occur in humans after chronic exposure to these compounds. Similar neurotoxic effects have been demonstrated in adult chickens and cats after exposure to TOCP (Beresford and Glees, 1963).

Siegel et al. (1965) demonstrated the neurotoxic effect of a triaryl phosphate hydraulic fluid previously thought to be nontoxic in animals. Johansen et al. (1977) demonstrated that phosphates prepared from ortho alkyl substituted phenols are often neurotoxic

if the ortho alkyl group has at least one hydrogen atom on the α carbon for activation. They also demonstrated that increased substitution or branching of that group resulted in decreased neurotoxicity. These findings were also demonstrated by Bondy et al. (1960).

This study was designed to determine if delayed neurotoxic effects would result from exposure of adult chickens to the two hydraulic fluids. A vehicle control (corn oil) as well as a TOCP positive control were tested concurrently with samples of Durad MP280 and Fyrquel 220. The final determination of injurious effect was based on a comparison of the hydraulic fluid dosed chickens with the TOCP dosed control chickens. Both compounds must meet Navy specifications that the fluids have a triorthocresyl phosphate (TOCP) neurotoxicity equivalent of less than three percent.

Materials and Methods

Animals

Leghorn hens (Gallus domesticus) 12 to 14 months of age and weighing between 1.2 and 1.8 kilograms were purchased from Carey Farms, LaRue, Ohio. The debeaked hens were group housed in 3 by 6 foot cages to allow for freedom of movement. Food and water were available ad libitum.

Peroral Administration

The hydraulic fluids, as well as the positive control TOCP, were administered to unfasted hens as solutions in corn oil. Gastric intubation was accomplished employing a syringe fitted with a 6-inch infant catheter. The injection volume for the hens was 0.001 ml/gm which resulted in the average chicken receiving a volume of 1.5 ml. The chickens were weighed individually to determine the proper dosage volume.

The following regimen of dosing was performed on five consecutive days:

Durad MP280 - Groups of four hens each treated with the following doses: 240, 300, 360 and 420 mg/kg/day.

Fyrquel 220 - Groups of four hens each treated with the following doses: 240, 300, 360 and 420 mg/kg/day.

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

Corn Oil - Twelve hens given 1 ml/kg/day.

Grading by three observers began seven days after the first dose and continued 3 times/week (Monday, Wednesday and Friday), until 30 days after the initial dose. The following point score system was used:

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

During observation and grading, the chickens were removed from their enclosures and placed on a rubber mat to provide sure footing. Symptoms noted on the twenty-first day after the first dose were used for calculating the TOCP equivalent.

The calculation was done as follows:

$$\text{TOCP Equivalent (\%)} = \frac{\text{mg/kg TOCP}}{\text{mg/kg Test Material}} \times \frac{\text{Total Points for Test Material}}{\text{Total Points for TOCP}} \times 100$$

Following the final observation day, nerve tissue was sampled from four chickens from each of the following groups: Vehicle Control, TOCP (2-75; 2-90 mg/kg/day), Durad MP280 (240 mg/kg/day), and Fyrquel 220 (420 mg/kg/day). Nerve tissue was taken from the cervical, thoracic and lumbar regions of the spinal cord as well as a sample of peripheral nerve (sciatic).

Results

The resultant mean scores of three observers 21 days after the initial peroral dose are compiled in Tables 48 through 51. The vehicle control (corn oil) resulted in all negative scores. Positive neurotoxic symptoms were observed in all chickens that received 75 or 90 mg/kg of TOCP. Three of the four chickens that received 60 mg/kg TOCP showed minor symptoms while the fourth demonstrated advanced paralytic symptoms at 21 days and ultimately died at 22 days.

TABLE 48. SCORING OF NEUROTOXIC EFFECTS OBSERVED 21 DAYS FOLLOWING THE INITIAL PERORAL DOSE OF CORN OIL

Animal Number	Dose (ml/kg)	21-Day Mean Observation Score	Total Score Per Group
18219	1	0	
18224	1	0	
18227	1	0	
18232	1	0	
18235	1	0	
18240	1	0	0
18201	1	0	
18213	1	0	
18241	1	0	
18256	1	0	
18257	1	0	
18259	1	0	

TABLE 49. SCORING OF NEUROTOXIC EFFECTS OBSERVED 21 DAYS FOLLOWING THE INITIAL PERORAL DOSE OF TRIORTHOCRESYLPHOSPHATE (TOCP)

Animal Number	Dose (mg/kg)	21-Day Mean Observation Score	Total Score Per Group
18208	60	4	
18215	60	2	22
18217	60	4	
18238	60	12	
18239	75	8	
18250	75	10.7	38.7
18209	75	12	
18210	75	8	
18216	90	12	
18223	90	10.7	38.7
18242	90	8	
18258	90	8	

TABLE 50. SCORING OF NEUROTOXIC EFFECTS OBSERVED 21 DAYS FOLLOWING THE INITIAL PERORAL DOSE OF FYRQUEL 220

Animal Number	Dose (mg/kg)	21-Day Mean Observation Score	Total Score Per Group
18202	240	0	
18204	240	0	0
18211	240	0	
18212	240	0	
18214	300	0	
18230	300	0	0
18243	300	0	
18260	300	0	
18218	360	0	
18220	360	0	0
18222	360	0	
18225	360	0	
18226	420	0	
18244	420	0	0.7
18247	420	0.7	
18248	420	0	

TABLE 51. SCORING OF NEUROTOXIC EFFECTS OBSERVED 21 DAYS FOLLOWING THE INITIAL PERORAL DOSE OF DURAD MP280

Animal Number	Dose (mg/kg)	21-Day Mean Observation Score	Total Score Per Group
18203	240	0	
18205	240	4	12
18228	240	0	
18229	240	8	
18231	300	8	
18233	300	8	44
18246	300	16	
18252	300	12	
18206	360	8	
18207	360	8	34
18221	360	12	
18236	360	6	
18251	420	12	
18253	420	8	44
18254	420	8	
18255	420	16	

One observer recorded minor symptoms for one chicken dosed at the highest level of Fyrquel 220; otherwise all scoring of the Fyrquel 220 dosed chickens was negative. Significant neurotoxic symptoms were seen in chickens at all but the lowest dose level of Durad MP280. The 300 and 420 mg/kg dose levels each had one chicken dead at 21 days. At that time all surviving chickens at the three high dose levels demonstrated positive to advanced neurotoxic symptoms. Final mortality of the Durad MP280 treated hens, 30 days following the initial peroral dose, was 3/4 at the 420 mg/kg level and 2/4 at the 360 and 300 mg/kg levels. No deaths occurred at the 240 mg/kg level.

The groups chosen for calculation of TOCP eqivalent were 300 mg/kg Durad MP280 and 75 mg/kg TOCP. These were the lowest concentration groups showing significant neurotoxic signs. Using the standard formula, Durad MP280 has a TOCP equivalent of 31.2 percent. Fyrquel 220, of course, has a TOCP equivalent of zero percent.

Discussion

Under the conditions of this test, Fyrquel 220 does not present a hazard as a neurotoxic agent. Durad MP280, however, could present a health hazard as the TOCP equivalent is 31.2 percent, ten times the maximum established by military specification for fire resistant hydraulic fluids. Fyrquel 220 is acceptable under the standards established by the U.S. Navy for TOCP equivalent while Durad MP280 is not.

ACUTE ORAL, DERMAL, INTRAPERITONEAL, AND INHALATION TOXICITY OF HYDRAULIC FLUIDS

The samples of hydraulic fluids provided by NMRI/TD for these studies are the same as those previously described.

Methods

Acute Oral and Intraperitoneal Toxicity

Male and female Sprague-Dawley rats weighing between 200-300 grams and 150-250 grams, respectively, were used for determination of the acute oral and intraperitoneal LD_{50} values.

Syringes equipped with special oral dosing needles were used to administer the materials to the rats. Syringes with needles were used for intraperitoneal injections. Rats used for oral dosing studies were fasted 12 hours prior to dosing. Solutions of the

hydraulic fluids were prepared in distilled water (Houghto-Safe 273) or corn oil (Fyrquel 220 and Durad MP280). A single dose at a volume equivalent to 1% of the animal's body weight was given.

Testing was initiated by dosing 5 rats of each sex at a concentration of 5 ml/kg body weight. This concentration was used as an upper limit cut off level. If no toxicity was evident during a 14-day observation period, no further testing was conducted. If mortality was produced, testing continued with 10 animals of each sex per dose level. Mortality was recorded for 14 days after dosing. A 14-day LD_{50} with 95% confidence limits was calculated using the probit analysis method of Finney (1971).

Acute Dermal Toxicity

Male and female New Zealand White rabbits weighing between 2-3 kg were used for determination of the acute dermal LD_{50}. All rabbits were clipped as closely as possible with an Oster clipper having surgical blades and vacuum attachment. The backs of the rabbits and the sides down to about half way to the stomach area were clipped from the shoulders to the top of the rear leg area.

The materials were applied in equal amounts to both sides of the rabbit's back and remained in contact with the skin for 24 hours. The dose was kept in place by applying 4" x 4" 8 ply gauze patches over the compound on each side of the back. Latex rubber dental dam was then applied over the entire clipped area and elastoplast tape used to wrap the entire midsection of the rabbit, keeping the dose in place. Specially designed restraining harnesses were fitted to each rabbit at the time of dosing and kept in place during the entire dosing period. These harnesses prevented excessive movement of the rabbits and prevented them from chewing on the taped area. The harnesses, however, allowed the rabbits to eat and drink during the dosing period. Upon removal of the wrapping, the skin of the rabbit was wiped (not washed) in order to remove excess test material.

Testing was initiated by dosing 5 rabbits of each sex at an upper limit cut off concentration of 2 ml/kg body weight. If no toxicity was evident during the 14-day observation period, no further testing was conducted.

Acute Inhalation Toxicity

Male and female Sprague-Dawley rats weighing between 200-300 grams and 150-250 grams, respectively, were used for determination of the acute 4-hour LC_{50}.

One three port Collison® nebulizer operated at 30 psi was used for generation of the aerosol. A three neck round bottom flask was used to contain the hydraulic fluid sample pool. A single sample pool was used without replacement during the 4-hour exposures that were conducted in a 60 liter plastic chamber. Air flow through the chamber was maintained at about 10 L/min. Aerosol was sampled using three midget impingers connected in series. Isopropanol was used as a diluent and catch material for Fyrquel 220 and Durad MP280. Distilled water was used for Houghto-Safe 273. Total sample air volume was 1 liter. Particle sizing was accomplished with an Aries Impactor (seven stage). Respective stages were pooled and eluted in isopropanol or water for analysis (3 samples per hour for Fyrquel 220; 4 samples every 2 hours for Durad MP280 and Houghto-Safe 273). A McPherson UV/VIS spectrophotometer was used for analysis of Fyrquel 220 (260 nm, 1 cm cell) and Durad MP280 (265 nm, 1 cm cell). A Waters Liquid Chromatogram M-6000A with an R401 differential refractometer was used for Houghto-Safe 273 analysis.

Testing was initiated by exposure of 5 rats of each sex to an upper limit cut off concentration of 5 mg/L. If no toxicity was evident during a 14-day observation period, no further testing was conducted.

Observation

Animals were observed frequently during the day of dosing and twice daily during the 14-day holding period. Visible signs of toxicity were recorded. Body weights of all animals were obtained at the time of dosing and periodically during the 14-day holding period.

All animals, whether dying by sacrifice at the conclusion of the 14-day observation period or during the test, were subjected to gross necropsy following death. Histopathologic examination was performed on any abnormal tissue observed in animals from the acute oral, intraperitoneal, and dermal toxicity tests. Lung, trachea, liver, kidney, and abnormal tissue was examined for histopathologic alterations from animals exposed via inhalation.

Results and Discussion

Oral Toxicity

Doses of 5 ml/kg of any of the three hydraulic fluids failed to produce mortality during the 14-day observation period. Diarrhea was evident in rats given the Fyrquel 220 or Durad MP280. This was first noted about 6 hours after dosing and lasted for 1 or 2 days. The diarrhea coincided with a weight loss in the majority of rats

dosed with either of these two triaryl phosphate materials (Table 52). When the diarrhea subsided, normal and steady weight gains were evident. Rats dosed with Houghto-Safe 273 exhibited neither diarrhea nor weight loss.

TABLE 52. BODY WEIGHTS[a] OF RATS GIVEN A SINGLE ORAL DOSE OF HYDRAULIC FLUID (N = 5)

MALES

Day	Fyrquel 220	MP280	Houghto-Safe 273
0	272 ± 16	[illegible] ± 22	275 ± 27
1	268 ± 11	[illegible] ± 20	289 ± 28
2	278 ± 17	[illegible]46 ± 12	298 ± 17
4	294 ± 23	358 ± 13	310 ± 28
7	309 ± 25	[illegible] ± 17	333 ± 31
14	342 ± 30	[illegible] ± 18	369 ± 31

FEMALES

Day	Fyrquel 220	Durad MP280	Houghto-Safe 273
0	174 ± 8	165 ± 7	172 ± 6
1	167 ± 11	168 ± 10	186 ± 11
2	182 ± 9	168 ± 13	188 ± 11
4	191 ± 7	174 ± 12	194 ± 11
7	197 ± 10	192 ± 7	199 ± 11
14	213 ± 11	202 ± 5	212 ± 7

[a] Grams, Mean ± S.D.

Gross necropsy failed to reveal any significant exposure related lesions. Because no mortality resulted at a concentration of 5 ml/kg, no further oral toxicity studies were conducted.

Intraperitoneal Toxicity

Rats injected with Fyrquel 220 or Durad MP280 were mildly lethargic for 5 to 10 hours and a clear oily discharge was noticed around the anus of the rats. Reflexes appeared normal. One male rat injected with Fyrquel 220 died within 24 hours of dosing. All other male and female rats given Fyrquel 220 or Durad MP280 survived the 14-day observation period and no overt signs of toxicity were noted during that time. Rats injected with Houghto-Safe 273 became severely lethargic. Signs of toxicity included slow, labored respiration, sprawling of the hindlimbs, and extremely depressed righting and placement reflex. These symptoms lasted from 24 to 48 hours. Despite the initial depressed state, all rats survived the 14-day observation period.

Body weights are shown in Table 53. Weight loss was seen 1 or 2 days after injection of any of the hydraulic fluids. By one week the rats began to gain weight and subsequently showed normal weight gain.

TABLE 53. BODY WEIGHTS[a] OF RATS GIVEN A SINGLE INTRAPERITONEAL INJECTION OF HYDRUALIC FLUID (N = 5)

MALES

Day	Fyrquel 220	Durad MP280	Houghto-Safe 273
0	265 ± 19	254 ± 10	256 ± 7
1	242 ± 16	240 ± 15	247 ± 10
2	237 ± 20[b]	241 ± 16	239 ± 5
4	250 ± 20[b]	249 ± 18	244 ± 7
7	273 ± 21[b]	264 ± 22	261 ± 8
10	301 ± 23[b]	283 ± 20	278 ± 11
14	321 ± 22[b]	305 ± 20	300 ± 14

FEMALES

Day	Fyrquel 220	Durad MP280	Houghto-Safe 273
0	200 ± 20	109 ± 13	194 ± 7
1	182 ± 18	181 ± 14	183 ± 9
2	180 ± 15	180 ± 14	190 ± 10
4	190 ± 18	177 ± 14	195 ± 11
7	205 ± 25	188 ± 13	203 ± 8
10	213 ± 28	201 ± 13	212 ± 11
14	221 ± 21	205 ± 17	221 ± 12

[a] Grams, Mean ± S.D.
[b] N = 4

Since mortality was absent at intraperitoneal doses of 5 ml/kg of Durad MP280 or Houghto-Safe 273, no further intraperitoneal tests were conducted with these materials. However, the death of the one male rat at the 5 ml/kg dose of Fyrquel prompted further intraperitoneal tests. Results of the tests along with calculated LD_{50} values are shown in Table 54. The LD_{50} values for male and female rats are very comparable and both are greater than 10 ml/kg indicating a low toxicity hazard. It is interesting to note that no deaths occurred in the group of 10 male or female rats given 5.0 ml/kg. Injections at doses greater than 14.28 ml/kg were not attempted since a 50% kill point was obtained and dosing at higher concentrations becomes impractical because of large volumes.

TABLE 54. INTRAPERITONEAL TOXICITY OF FYRQUEL 220

Dose (ml/kg)	Mortality Ratios[a]	
	Male Rats	Female Rats
Corn Oil[b]	0/10	0/10
5.0	0/10	0/10
6.5	0/10	0/10
8.45	2/10	4/10
10.985	6/10	3/10
14.28	7/10	6/10
LD_{50} (C.L.)	11.21 ml/kg (9.70 to 13.65)	12.41 ml/kg (10.30 to 19.35)

[a] Number dead/Number dosed.
[b] Not used in LD_{50} calculation.

Table 55 shows the time to death of the combined male and female rats. The majority of deaths occurred between 48 and 72 hours post-dosing.

TABLE 55. EFFECT OF INTRAPERITONEALLY INJECTED FYRQUEL 220 ON TIME OF DEATH IN RATS

Dose (ml/kg)	Number of Deaths									
	24 hrs.		48 hrs.		72 hrs.		95 hrs.		120+ hrs.	
	M	F	M	F	M	F	M	F	M	F
Corn Oil	0	0	0	0	0	0	0	0	0	0
5.0	0	0	0	0	0	0	0	0	0	0
6.5	0	0	0	0	0	0	0	0	0	0
8.45	0	0	2	0	0	2	0	0	0	2
10.985	0	0	4	1	2	2	0	0	0	0
14.28	0	1	2	1	3	3	1	1	1	0

Body weights are shown in Figures 30 and 31 for male and female rats, respectively. All of the male rats given Fyrquel 220 experienced an initial weight loss lasting for 1 to 2 days. Male rats dosed at the higher concentrations had a more prolonged weight loss lasting up to 4 days. A dose related effect on weight recovery was evident in the male rats. In fact, the male group given 14.28 ml/kg never did attain its preexposure body weight during the 14-day observation period.

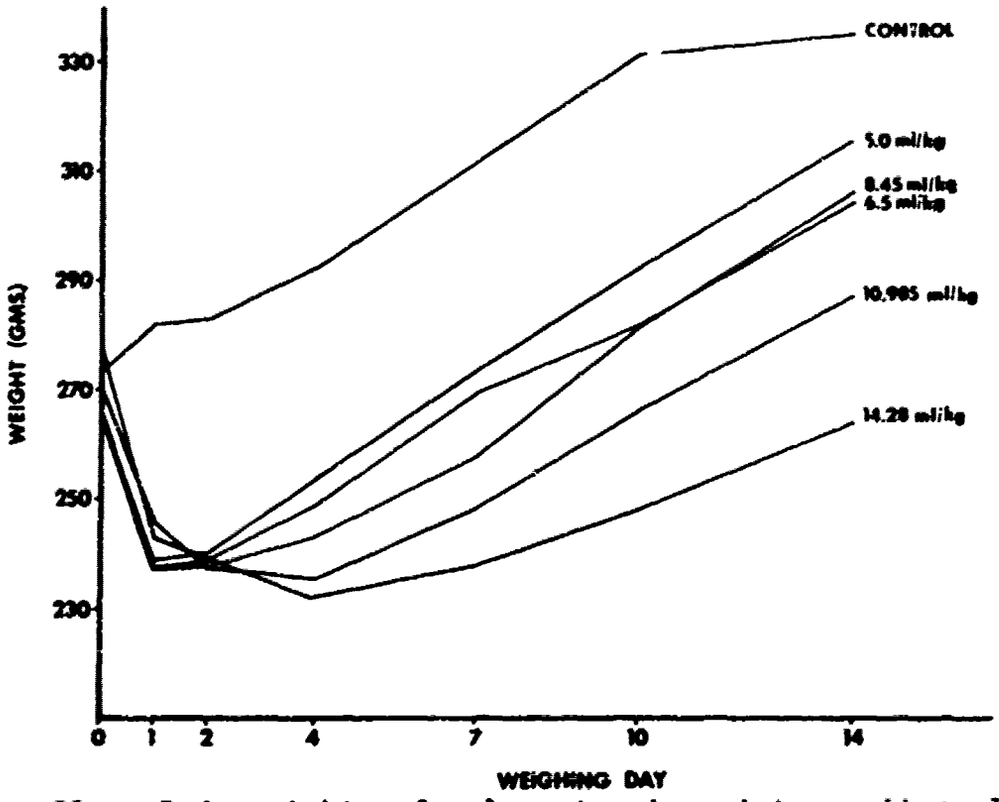


Figure 30. Body weights of male rats given intraperitoneal injections of Fyrquel 220.

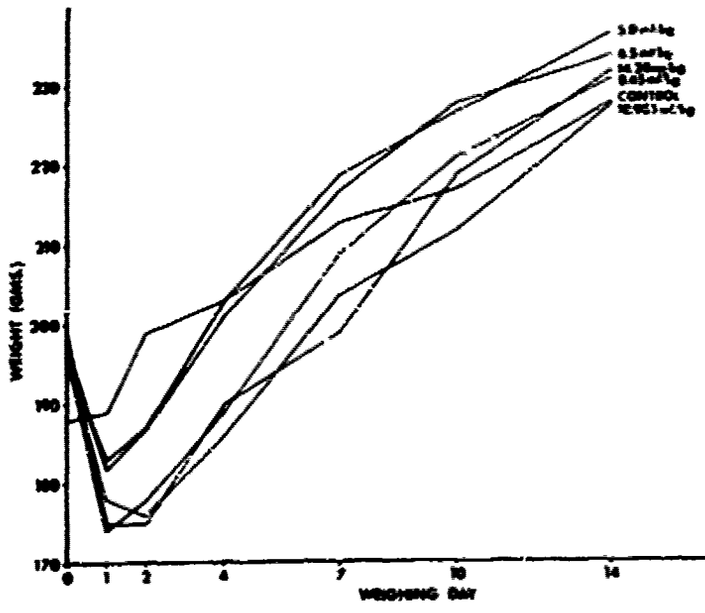


Figure 31. Body weights of female rats given intraperitoneal injections of Fyrquel 220.

Female rats also lost weight immediately after dosing. However, weight recovery during the 14-day observation period was more complete than seen with male rats. At the end of 14 days, the body weights of all female rat exposed groups were equal to or greater than corn oil treated controls.

Gross necropsy revealed a white plaque-like material on the abdominal organs. A similar type material was also present in the control animals, suggesting that the corn oil vehicle was responsible.

Acute Inhalation Toxicity

No mortality occurred in any of the groups of male and female rats exposed to the hydraulic fluid aerosols. In all cases mild lethargy lasting 1 to 3 hours postexposure was noted in the rats immediately upon removal from the chamber. The fur of animals exposed to Fyrquel 220 and Durad MP280 was unkempt and wetted with the hydraulic fluid. The fur of the animals exposed to Houghto-Safe 273 was normal.

The mass concentrations and particle size of aerosol measured in the animal exposures are shown in Tables 56 and 57, respectively.

TABLE 56. MASS ANALYSIS OF AEROSOL IN ACUTE 4-HOUR AEROSOL INHALATION TESTS

Material	Sex	Mass (mg/L)[a]
Fyrquel 220	M	5.79 ± 0.25 (5.20 - 6.20)
	F	6.31 ± 0.37 (5.74 - 7.07)
Durad MP280	M	6.19 ± 0.20 (5.84 - 6.53)
	F	6.35 ± 0.24 (5.63 - 6.61)
Houghto-Safe 273	M	6.84 ± 0.36 (6.10 - 7.24)
	F	7.56 ± 0.50 (6.55 - 8.54)

[a] Mean ± S.D. (Range)

TABLE 57. PARTICLE SIZE[a] OF AEROSOLS USED IN ACUTE 4-HOUR AEROSOL INHALATION TESTS

Material	Sex	Median Diameter Aerodynamic	Median Diameter Density Corrected	σ_g
Fyrquel 220	M	2.58	2.24	2.12
	F	2.58	2.24	2.01
Durad MP280	M	2.45	2.13	2.12
	F	2.45	2.13	2.09
Houghto-Safe 273	M	1.65	1.5	1.9
		2.3	2.1	2.0
	F	1.7	1.6	1.7
		2.3	2.1	2.1

[a] Size measured as diameter, μm.

Two sets of results are presented for Houghto-Safe 273. The first set represents sampling over the first two hour period. The second set represents sampling over the last two hours of exposure. There was an apparent increase in particle size which occurred during the course of the exposure. We believe this was due to selective vaporization of the relatively volatile aqueous component of Houghto-Safe 273 (ethylene glycol). Particles which are recycled into the generation pool by impaction on the generation vessel walls and introduction lines have less volatile components than the initial material. As the volatile composition of the Houghto-Safe 273 generation pool decreased, less particle shrinkage occurred with aging of the aerosol in the exposure chamber.

Body weights are shown in Table 58. During the first 24-hour period, little or no weight gain was observed in male or female rats exposed to Fyrquel 220 or Durad MP280. Male or female rats exposed to Houghto-Safe 273 lost a small amount of weight during this period. Subsequent weighings show normal and steady weight gains. Gross necropsy of the animals failed to reveal any significant exposure related lesions. Since there was no mortality at exposure concentrations in excess of 5 mg/L, the upper limit for testing under EPA guidelines, no further acute aerosol tests were conducted.

TABLE 58. BODY WEIGHTS[a] OF RATS EXPOSED TO THE INHALATION OF HYDRAULIC FLUID (GRAMS)

MALES

Day	Fyrquel 220	Durad MP280	Houghto-Safe 273
0	214 ± 9.2	254 ± 6.4	259 ± 8.3
1	214 ± 8.2	257 ± 10.5	256 ± 6.8
2	222 ± 9.9	262 ± 8.9	259 ± 13.0
4	239 ± 11.5	274 ± 8.2	273 ± 13.4
7	262 ± 13.7	290 ± 11.5	292 ± 10.5
10	282 ± 16.9	311 ± 7.6	309 ± 12.5
14	304 ± 15.6	338 ± 9.2	330 ± 13.7

FEMALES

Day	Fyrquel 220	Durad MP280	Houghto-Safe 273
0	179 ± 4.4	196 ± 2.9	190 ± 9.9
1	179 ± 6.9	200 ± 1.9	186 ± 9.3
2	184 ± 5.7	203 ± 4.1[b]	191 ± 8.8
4	189 ± 8.4	209 ± 4.8	191 ± 7.7
7	200 ± 8.6	212 ± 4.4	199 ± 8.9
10	205 ± 11.4	213 ± 4.6	207 ± 8.8
14	220 ± 13.4	227 ± 3.9	210 ± 8.3

[a] Mean ± S.D., N = 5
[b] Weighed on Day 3

Acute Dermal Toxicity

No signs of toxicity were noted in the rabbits receiving a single dermal application of 5 ml hydraulic fluid/kg body weight. Body weights of the rabbits for the 14-day observation period are shown in Table 59. A slight weight loss was seen shortly after dosing in male rabbits treated with Durad MP280 and in male and female rabbits dosed with Houghto-Safe 273. Overall body weight gain through the 14-day observation period was slightly less in rabbits given Durad MP280 when compared to rabbits receiving either Fyrquel 220 and Houghto-Safe 273. There was no evidence of skin irritation resulting from dermal contact with any of the three hydraulic fluids and gross necropsy of the rabbits on Day 14 failed to reveal any exposure related lesions.

TABLE 59. BODY WEIGHTS[a] OF RABBITS DERMALLY EXPOSED TO HYDRAULIC FLUID (kgs)

Day	FYRQUEL 220 Male	Female
0	2.21 ± 0.26	2.15 ± 0.24
2	2.28 ± 0.24	2.21 ± 0.29
4	2.28 ± 0.23	2.26 ± 0.26
7	2.44 ± 0.25	2.30 ± 0.34
10	2.54 ± 0.25	2.42 ± 0.38
14	2.70 ± 0.22	2.53 ± 0.28

Day	DURAD MP280 Male	Female
0	2.43 ± 0.27	2.73 ± 0.25
2	2.32 ± 0.32	2.79 ± 0.41
4	2.36 ± 0.33	2.67 ± 0.22
7	2.45 ± 0.33	2.80 ± 0.19
10	2.49 ± 0.29	2.87 ± 0.14
14	2.58 ± 0.29	2.94 ± 0.15

Day	HOUGHTO-SAFE 273 Male	Female
0	2.66 ± 0.32	2.69 ± 0.17
1	2.56 ± 0.29	2.56 ± 0.18
3	2.75 ± 0.29	2.69 ± 0.20
6	2.79 ± 0.29	2.79 ± 0.17
9	2.87 ± 0.27	2.85 ± 0.22
14	2.96 ± 0.27	3.06 ± 0.21

[a] Mean ± S.D., N = 5

THE DEVELOPMENT OF A RAT LUNG TUMOR MODEL: DOSE RESPONSE STUDIES ON THE INTRATRACHEAL INOCULATION OF 3-METHYLCHOLANTHRENE

Traditional methods of carcinogenesis testing in animals have used tumor development as the endpoint. Less attention has been paid to more subtle changes which may occur in the course of tumor development, such as immunologic or cytogenetic changes. This has

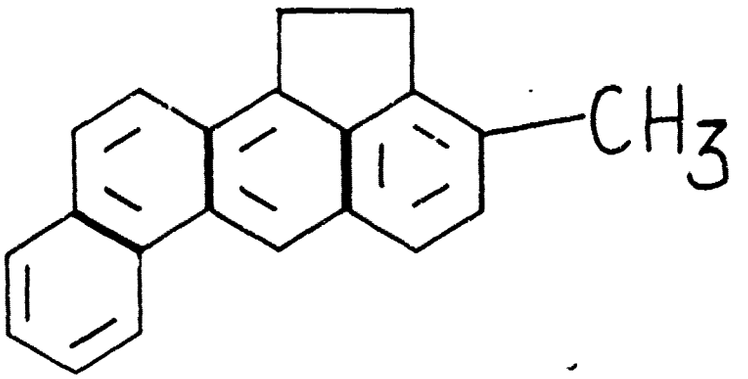
not been for lack of recognition that such changes may occur, but rather that economic pressures usually limit the range of observations that can be made in any given test or experiment. In order to meet the obvious need for determining the biological and biochemical events surrounding the carcinogenic process, a number of animal models have been developed which usually focus on carcinogenesis in a specific organ. These models include the mouse lung adenoma (Shimkin and Stoner, 1975) system, the mouse hepatocarcinoma (Ward et al., 1979) and a variety of mouse skin painting models. Several more models could be cited, among which the induction of lung tumors in the hamster by intratracheal inoculation of PAH (Saffiotti et al., 1968) has been especially useful.

This experiment will require a reasonably well defined animal model for lung cancer. The model chosen is the rat lung tumor induced by intratracheal inoculation of 3-methylcholanthrene (MCA). Although similar models exist in the mouse and hamster, the rat was chosen because it is the most frequently used animal in carcinogenesis studies at the Toxic Hazards Research Unit.

The Fischer 344 male rat is the strain of choice. This strain has been used for some years at the Toxic Hazards Research Unit, and a body of experience on the response of this animal to various materials has been accumulated. The tumor model is the induction of bronchioalveolar squamous cell carcinoma in the lung by intratracheal inoculation of MCA as described by Schreiber et al. (1972). In this model, tumors appear in nearly 100% of the animals. By choosing the appropriate dosage of MCA, reasonably well defined stages of tumor initiation, growth, and finally metastasis can be observed. This will permit the correlation of biological changes during carcinogenesis with identifiable pathological states.

Published experiments (Schreiber et al., 1972) indicate that high doses of MCA will produce lung tumors in rats after only a few weeks. In this study, it is the intervening period between initiation and malignancy that is of most importance. Therefore, it is desirable to determine a dose of MCA that will eventually give a high tumor incidence, but also provide a relatively long "latent" or tumor-free period during which selected biologic parameters may be measured. Therefore, this dose-response study will be conducted to establish the intratracheal dose of MCA which will produce the first tumor at about 5-6 months after beginning treatment, and lead to 80-90% tumor incidence at 12 months.

3-Methylcholanthrene was purchased from Sigma Chemical Company in one-gram samples. The structure and physical properties of MCA are shown below:



CAS:	000056495
SYN:	20-Methylcholanthrene
Molecular Weight:	268.34
Boiling Point (°C):	280
Melting Point (°C):	179-180
Density:	1.28
Physical State:	Pale yellow, slender prisms

The MCA was recrystallized from benzene and finely chopped with a spatula to produce rods 10 to 100 microns in length and 5 to 15 microns in thickness. The MCA was then suspended in physiological saline and 0.2% (w/v) gelatin (Sargent-Welsh, bacteriological grade) to give the desired concentrations of MCA.

Prior to intratracheal instillations, the rats were anesthetized by inhalation with 2 bromo-2-chloro 1,1,1-trifluoroethane (Halothane, Halocarbon Industries, Incorporated, Hackensack, New Jersey). Concentrations of MCA were adjusted so that inoculation volumes were 0.1 ml for both the test and the control rats. The control rats received 0.1 ml inoculations of the saline-gelatin solution.

Five dose levels and a control group received five bi-weekly treatments. Group size and concentrations are shown below. The complete treatment and sacrifice schedule is shown in Table 60.

Dose Level, mg	No. of Rats
5.0	25
1.0	25
0.5	25
0.25	25
0.1	25
0.0	25

TABLE 60. TREATMENT AND SACRIFICE SCHEDULE OF MCA INTRATRACHEALLY INJECTED RATS

	Weeks on Test									
	0	2	4	6	8	10	16	22	28	36
Intratracheal Injection	X	X	X	X	X					
Dose Level, mg	Animals Sacrificed									
5.0						2	5	5	5	5
1.0						2	5	5	5	5
0.5						2	5	5	5	5
0.25						2	5	5	5	5
0.1						2	5	5	5	5
0.0						2	5	5	5	5

Fischer 344 male rats were purchased from Charles River Breeding Laboratories, Wilmington, Massachusetts. They were received at a time which would allow for the beginning of the experiment when the rats were 8 weeks of age.

The rats were randomized upon receipt into an appropriate number of cages after which quality control sampling and quarantine took place. The rats had food and water available ad libitum throughout the course of the study.

Food: Purina Formulab #5008
Water: Softened water not to exceed 1 grain/gallon hardness measured as calcium carbonate.

The rats are housed in laminar flow Porta-Rooms for the duration of the study, in conformance with the Institute of Laboratory Animal Resource standards. All cages and bedding are changed twice per week.

The various concentrations of MCA were administered in saline-gelatin suspensions by intratracheal instillation. Before each treatment the rats were anesthetized with Halothane. As soon as the animal was anesthetized, a speculum was inserted into the rat's mouth which holds down the tongue. The upper incisors were retained on a wire which also held the mouth open. A light attached to the speculum provided a clear view of the pharnyx and vocal cords. An eight centimeter Teflon® (Hamilton Company) needle was then inserted between the vocal cords and gently pushed into the tracheal lumen. A light, but definite bumping against the tracheal rings could be

felt by the technician when the needle was properly inserted. The needle was pushed to approximately the tracheal bifurcation, the suspension injected and the needle withdrawn. Following the injection, the animals were placed in their home cages where they rapidly recovered from the anesthesia.

All rats that died or were sacrificed in this study were necropsied. At the intervals noted, rats from each treatment group will be anesthetized with Halothane and killed by exsanguination. The lungs will be excised en bloc and fixed by inflation via the trachea with 10% buffered formalin at a pressure head of 30 cm of water. Paraffin sections of 5 µm will be prepared and examined for evidence of tumor formation. No other organs will be examined nor tissue taken.

The injections were started on 26 January and continued through 23 March. The final sacrifice for the rats is scheduled for 5 October 1982 which will be 36 weeks from the initial injection.

Three rats from the high dose level, 5 mg, lost weight and appeared moribund prior to the final intratracheal injection. Two of the three failed to survive the trauma of anesthesia and injection while the third died four days later. Gross examination of the three rats revealed obvious tumors in the left lung lobe. Histopathology showed the tumors to be well differentiated squamous cell carcinomas in all three cases.

The first scheduled sacrifice (10 weeks after the first intratracheal dose) took place on 6 April 1982. Two rats were examined from each dose level. The two rats from the highest dose level had multiple tan masses in all lung lobes which could be observed on gross examination. The masses ranged in size from 0.1 to 0.2 centimeters in diameter. No masses were grossly observed in the rats from the remaining dose levels.

Orbital blood samples were taken from the rats immediately prior to sacrifice. The mean hematologic values are shown in Table 61. No statistically significant differences were observed between dose groups.

TABLE 61. SELECTED HEMATOLOGY ON 3-MCA TREATED RATS SACRIFICED AT 10 WEEKS (N = 2)

Blood Determinations	Conc. (mg/animal/dose)					
	0.0	0.1	0.25	0.5	1.0	5.0
WBC (10^3)	6.4	6.4	5.5	5.7	6.1	5.3
HCT (%)	48.0	48.0	48.5	47.5	47.0	48.5
Neut. (%)	18.0	17.5	24.0	15.5	22.5	22.0
Lympho. (%)	81.5	82.5	75.0	83.5	76.5	78.0

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

In 1978 the Toxic Hazards Research Unit conducted a 90-day continuous inhalation exposure to the alicyclic hydrocarbon, decalin (decahydronaphthalene). The protocol, experimental methods, and available results have been presented in previous annual reports (MacEwen and Vernot, 1979, 1980, 1981).

The purpose of the study was to develop data that could be used in establishing a threshold limit value for decalin. No threshold limit value exists for decalin, and the experimental data available are insufficient for establishing a limit. Gage (1970) described the exposure of 8 rats to 200 ppm decalin for 20 days on a 6 hour/day schedule with no toxic signs and grossly normal visceral organs at necropsy. Cardini (1942) reported lung congestion, kidney, and liver damage in guinea pigs exposed to 319 ppm decalin for up to 23 days.

Previous studies of decalin have been conducted by the THRU. Rats, mice, and guinea pigs were exposed to 50 or 250 ppm decalin on an industrial work schedule for 22 consecutive working days. Respiratory tract irritation was evident in the decalin exposed rats. Hydropic changes in the hepatocytes and hyalin droplet formation within the proximal tubular epithelial cytoplasm were seen with increased incidence and severity in the rats exposed to decalin vapor. Mice and guinea pigs exposed to decalin also had signs of respiratory tract irritation.

Exposure to decalin vapor for 90-days resulted in toxic effects similar to those seen with other hydrocarbon fuels tested in this laboratory. Nephropathy and decreased weight gain were evident in male rats continuously exposed to decalin vapor. Additional evidence of kidney injury was indicated by increased kidney weights in male rats exposed to 50 ppm decalin when compared to control rats. At 19 months postexposure there was still a body weight difference between control and decalin exposed rats. Kidney weights and clinical chemistry indices of kidney function were similar to control values. Evaluation of decalin induced renal damage was hindered by a high incidence of nephropathy that commonly occurs in aged rats. The overall incidence of nephropathy was slightly greater in the exposed male rats when compared to control rats, however. Hyperplasia of the mucosal lining of the renal pelvis and kidney mineralization were also noted with increased frequency in decalin exposed male rats. The mineralization was found in 6% of the controls, 30% of the 5 ppm exposed and 94% of the 50 ppm exposed males. It was thought that this was a sequela of the original toxic insult to the kidney by decalin exposure.

Liver fatty change and hepatocytic cytoplasmic vacuolization were the major exposure related lesions noted in mice at the conclusion of the 90-day exposure.

The most frequently noted postexposure non-neoplastic lesions are shown in Table 62. Most of the lesions observed were those that commonly occur in aged C57BL/6 mice. The increased incidence of many of these lesions noted in the decalin exposed mice are probably not exposure related in view of this fact and general absence of dose responses. Notable is the lack of liver fatty change and hepatocyte vacuolization in the mice exposed to 50 ppm. The absence of these lesions indicates the reversibility of these lesions noted in the decalin exposed female mice at the conclusion of the 90-day exposure.

TABLE 62. SELECTED NON-NEOPLASTIC LESIONS NOTED IN FEMALE MICE HELD FOR 21-MONTHS OBSERVATION AFTER 90-DAY CONTINUOUS EXPOSURE TO DECALIN VAPOR

	Control	5.0 ppm	50.0 ppm
Skin:			
Inflammation & ulceration	22/96	24/95	10/92[a]
Lungs:			
Crystals & macrophages	10/98	40/100[b]	37/98[b]
Perivascular cuffing	10/98	10/100	32/98
Lymphoid hyperplasia	8/98	21/100[b]	16/98
Liver:			
Fatty change	32/99	25/100	9/98[b]
Vacuolization	8/99	7/100	8/98
Kidney:			
Hydronephrosis	7/98	5/99	13/97
Perivascular cuffing	14/98	6/99	22/97
Reproductive:			
Cysts - Uterus	55/99	45/99	56/93
Cysts - Ovary	15/98	16/97	16/93
Mammary Gland:			
Cysts	3/68	1/66	12/65[a]
Thyroid:			
Hyperplasia	30/98	49/100[b]	54/97[b]
Cysts	2/98	0/100	16/97[b]

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

Neoplastic lesions found in the female mice are shown in Table 63. An increased incidence of pituitary carcinomas was noted in the mice exposed to 50 ppm decalin when compared to unexposed controls. As with the non-neoplastic lesions noted in the mice, pituitary

tumors are also common in aged C57BL/6 mice. There were a number of secondary lesions which were associated with the pituitary tumors. These were thyroid hyperplasia, thyroid primary tumors, and cystic ovaries. Thyroid hyperplasia and thyroid adenomas were slightly increased in the 50 ppm decalin exposed mice.

TABLE 63. NEOPLASTIC LESIONS NOTED IN MICE HELD FOR 21-MONTHS AFTER 90-DAY CONTINUOUS EXPOSURE TO DECALIN VAPOR

	Control	5.0 ppm	50.0 ppm
Skin:			
Adenocarcinoma	0/96	0/95	1/92
Hemangioma	1/96	0/95	0/92
Lung:			
Adenoma	0/98	0/100	1/98
Carcinoma	2/98	0/100	1/98
Stomach:			
Papilloma	0/98	1/97	0/95
Liver:			
Adenoma	0/99	0/100	1/98
Malignant histiocytoma	1/99	0/100	0/98
Hemangiosarcoma	2/99	0/100	0/98
Mammary:			
Malignant histiocytoma	1/95	0/95	0/96
Fibroma	1/95	0/96	0/96
Liposarcoma	1/95	0/96	0/96
Adenoma	5/95	0/96[A]	8/96
Colon:			
Leiomyosarcoma	1/94	0/90	0/95
Anus:			
Papilloma	1/51	0/71	0/87
Adenoma	1/91	0/71	0/87
Uterus:			
Sarcoma	1/99	0/99	0/93
Ovary:			
Luteoma	0/98	0/97	1/93
Harderian Gland:			
Carcinoma	0/68	0/66	1/65
Lipoma	0/68	1/66	0/65
Pituitary:			
Carcinoma	0/77	3/81	8/80[B]
Adenoma	34/77	55/81	35/80
Adrenal:			
Carcinoma	0/98	1/100	0/97
Pheochromocytoma	0/98	0/100	1/97
Thyroid:			
Adenoma	4/94	3/98	9/94
Carcinoma	3/94	0/98	2/94
Muscle:			
Malignant histiocytoma	1/96	0/97	0/94
Multiple Organs:			
Malignant thymoma	0/99	0/100	1/98
Plasmacytoma	0/99	1/100	0/98
Leukemia	4/99	0/100	1/98
Malignant Lymphomas (All Types/All Tissues)	40/99	39/100	28/98

A Different from controls at 0.05 level of significance.
B Different from controls at 0.01 level of significance.

Exposure to decalin for 90 continuous days did not cause any detrimental damage to body tissues. The lesions noted were, for the most part, those which are associated with aged mice with some sex specific changes in females. There were some changes that had incidence values of undetermined cause. Nevertheless there were no findings that would indicate that the exposure levels of decalin in this study are hazardous to the health of female C57BL/6 mice.

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.

MODIFIED TEMPERATURE AND HUMIDITY MEASUREMENT SYSTEM

Last year's annual report detailed the electronic control and data acquisition systems installed in the Thomas Dome Inhalation exposure chambers. Since that report, the sensor portion of the chamber temperature and relative humidity monitoring system has been modified to overcome the following deficiencies in the original design:

1. Fans blowing air over wet and dry temperature sensing bulbs ran continuously and were not able to be turned off individually. This led to continuous operation of the blowers even when no experiments were being performed in the chamber causing excessive wear and failure of the blowers.

2. Buildup of animal hair and dust on the blower air filter caused large pressure drops increasing the power load and decreasing blower life. No procedure for detecting this pressure drop had been designed for the temperature and relative humidity system.

3. There was no warning given of blower failure. Failure could be detected only by unrealistic increases in wet bulb temperature, conditions not always recognized by the chamber technicians on duty.

To rectify these deficiencies, separate on-off controls were provided for each chamber. These permit each system to be shut off when not operational, providing increased reliability and life to

the air blowers. To remedy the other problems, an electronic circuit was designed to monitor DC current drawn by each air blower. Clogged filters lead to easily detectable increases in current levels, and the initial phases of blower malfunction cause much larger increases in current. The protective circuit detects the smaller increases in current caused by filter clogging and activates an amber warning light indicating that a filter change should be made. A red indicator light is activated when blower motor current exceeds a critical value indicating blower replacement is required.

Concurrently with the above improvements, the circuits transmitting temperature readings to the system data logger were also improved. There were some difficulties in calibrating the original system and some signal interaction between the digital displays and the datalogger input circuits. The modified temperature measurement system has removed these deficiencies and has been in operation for approximately two months. It has greatly improved RH temperature measurements in Chambers 5 through 8 and will be installed in Chambers 1 through 4 in the near future.

ANIMAL WEIGHING SYSTEM

Animal weights are collected at the THRU by means of a disc-based micro-processor system. This system is part of a computer based system manufactured by the Toxicology Systems Division of Beckman Instruments, Incorporated. The total system includes local data-collection systems connected to a large time-shared computer system providing statistical manipulation of data and generation of reports. The system in use at the THRU includes the local data collection stations only. Custom software was provided by Beckman Instruments, Incorporated to allow the units to operate without the functions provided by a main-frame computer system. The local data-collection stations include a touch-screen entry terminal, a bar-code reader for reading experimental and animal encoded identification information, dual floppy-discs for the temporary storage of information, a printer output connector for local printing of data, and an electronic scale for weighing animals. This system has been in use for approximately 18 months collecting animal weight data from on-going experiments. These weights are limited to small animals by the 4 kg capacity of the electronic scale. The scale supplied with the stations was manufactured by Arbor Scales, Incorporated. Weight information from the electronic scale is routed to the data collection station by means of a binary-coded-decimal data input.

Animal weights in the range 0-4 kg are obtained using the Arbor® scale located on the data-collection station which can be placed adjacent to one of the chambers with the dome cap raised. This is possible with studies operated as six-hour daily exposures since the chamber caps are raised during evening hours. For weights which

exceed the 4 kg scale capacity and to obtain weights when continuous studies are in progress, the locally positioned electronic scale is unsatisfactory. To provide for obtaining weights under these conditions, the Beckman Animal Weighing System is being modified to accept weight information from remotely-located load-cell devices. These load-cell devices are located inside each chamber. They were installed originally as part of a separate weighing system developed by the THRU and used for obtaining animal weight data prior to the acquisition of the Beckman System. One 10 pound and one 50 pound load cell were installed in each of 8 chambers. These load cells were then connected to a centrally located readout and control console.

The readout control portion of this console is being interfaced to the Beckman TOXSYS™ data-collection station. This is being accomplished by providing local plug-in connections at each chamber. The data-collection station with the load-cell readout control device attached may now be connected to the load-cells by means of connector cables. Animal weights may then be obtained on the TOXSYS™ data collection station by placing animals on the load cells. Weights may be obtained for both small and larger animals exceeding 4 kilograms.

One problem with the operation is a peculiarity of the Arbor electronic scale design. Occasionally during the operation, the scale will not cycle to the next weight; in effect "locking up". If this condition is not detected by the operator, multiple incorrect weights may be entered. The data-collection station operating software is being modified to provide messages to the operator when this condition is encountered. In addition, control signal interlocks are being included to prevent further weighing until the condition has been corrected by retaring the scale or turning the power off momentarily. Completion of these software modifications will prevent the input of incorrect information to the data collection system. Additional improvements to the software are planned after successful completion of the first phase of modifications to improve the accuracy of animal weight inputs. Software procedures are to be added to eliminate errors which may be present due to drift and animal movements during the process of weighing.

LUNG FIXATION APPARATUS

Optimum fixation of rat lungs is necessary for micropathologic examination after exposure to inhaled materials. A major requirement is that perfusion of the lungs with formalin be accomplished rapidly and at a constant pressure, determined empirically to be 30 cm of water. A design obtained from the Air Pollution Health Effects Laboratory, UCI was modified to perfuse up to thirty rat lungs simultaneously with a constant 30 cm of water pressure head.

Acrylic plastic material was selected for fabrication of the unit because of its resistance to formalin. A rectangular tank 12 inches wide by 19 inches long and approximately 3 1/2 inches high was constructed. Both ends were extended well above and below the main tank to serve as handles for carrying the unit and also to serve as supports for other components. Two fittings were installed into the bottom of the tank, one for draining and the other for an overflow tube.

Another tank was constructed of the same width and length, but approximately 5 1/2 inches high. This container was located above the tank described previously. Thirty holes were drilled into the bottom of this tank to accomodate commercial cannula and stopcock assemblies. A fitting for an overflow tube was also installed. Partitions with holes near the bottom surface were mounted to divide the tank into three separate compartments. The holes in the partitions may be plugged with rubber stoppers. This permits the option of using ten or twenty of the cannula assemblies, thus preserving fixation fluid. By removal of the rubber stoppers, all thirty cannula assemblies can be used.

Between the two end supports and under the smaller tank, another sheet of acrylic material was installed to form a support for a mini peristaltic pump which provides highly accurate pumping at extremely low flow rates. The pump operates at 115 volts and is capable of pumping against pressures of up to 5 PSI. Next to the pump is mounted another smaller rectangular container to serve as a reservoir for the formalin. The reservoir is partitioned to form a filter chamber packed with glass fibers at the intake end.

In operation, the entire unit forms a tier configuration. Formalin is pumped from the reservior into the uppermost tank, draining down into the lungs mounted on the end of the cannula assemblies. The liquid level is maintained in the upper tank by means of an overflow tube set at a predetermined height. This overflow drains into the main tank where the lungs are suspended, completely submerged in formalin. The level in this tank is also maintained by an overflow tube that drains into the filter of the reservoir, trapping any tissue before it can reach the pump. The liquid is continually recirculated and filtered in this manner.

This unit provides constant pressure on the inside of the lungs by controlling the head pressure of the liquid, virtually eliminating rupturing of the lungs. In addition, a large number of lungs can be preserved with fixative in a single operation. The apparatus can be moved from one location to another. Figure 32 depicts the finished unit in diagrammatic fashion.

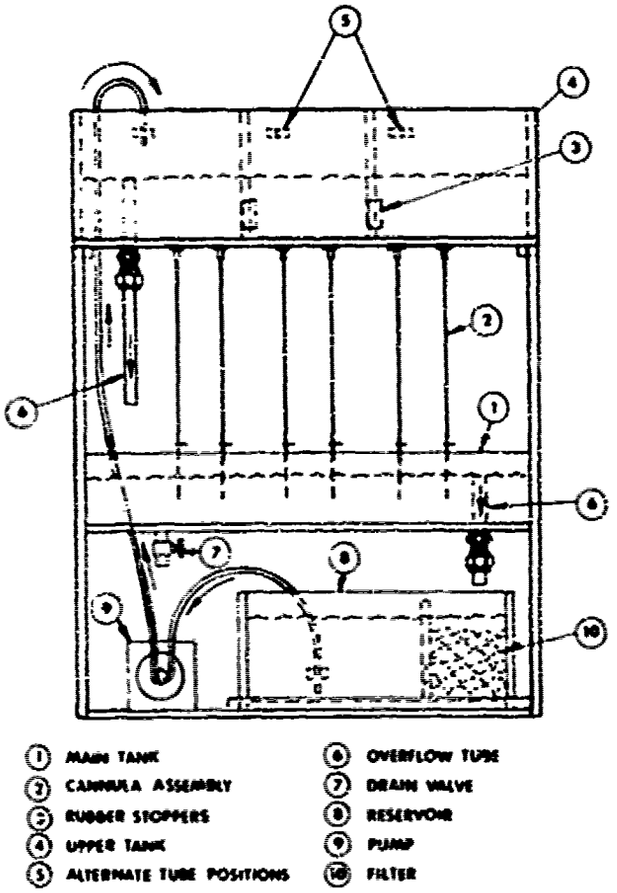


Figure 32. Diagram of lung fixation apparatus.

SMALL ANIMAL EXPOSURE UNIT, SECONDARY CONTAINMENT SYSTEM

The Respiratory Toxicology Section presented requirements for the design and fabrication of a secondary containment system to be used during exposure of small animals to radioactive aerosol. The requirements were that the primary exposure unit be located within the secondary system to contain any leakage of radioactive aerosol, and that the radioactive aerosol be collected on an exhaust High Efficiency Particulate Air Filter or flushed down with water which would be held for disposal. Additional specifications included:

1. An internal 110 volt electrical power source controlled through an external variable voltage control.

2. Clean, filtered and regulated compressed air.

3. Access for water to be used for flushing down internal components.

4. A main floor capable of supporting the primary exposure unit and other components, yet permitting free air flow and water drainage.

5. A sloped floor beneath the main floor to provide for collection and drainage of internal flush water.

6. Two glove ports for internal access from the outside to contact the internal flush water system and manipulation of other internal components.

7. Access to make equipment adjustments and to insert animals into or remove them from the primary exposure unit.

Unit construction consisted of fabricating a weldment of stainless steel angle iron with a flat sheet stainless steel top and a sloped stainless steel sheet bottom. All four sides of the bottom were angled down to a central point for drainage. In the front portion of the bottom an access hole, 5 inches by 10 inches, was cut and sealed with a plate and rubber gasket to prevent leakage. Clear acrylic plastic sheets were attached to the angle iron frame to form each side and back and front panels. Rubber gaskets were installed between the angle iron frame and plastic panels to prevent leakage. An access opening was cut in the front panel, and a vertical sliding door was installed that could be locked in several positions, including fully open. An exhaust hole was cut into the top plate to provide access to a stainless steel box containing a pre-filter and HEPA filter elements. On top of the filter box was mounted a high volume, direct drive wheel blower capable of delivering 1510 CFM of air at 1725 RPM. An exhaust hose was attached to the exit side of the blower and run into the adjacent exhaust hood. Input water was connected through the top plate to two flexible hoses used for flushing down the inside of the unit. Glove port holes were installed into each end panel to permit use of the flushing hoses and other various functions. A special manifold with multiple regulated ports for dispensing compressed air was fabricated and installed in the unit. Legs were welded onto the stainless steel frame locating the unit high enough off the countertop to permit installation of a shut-off valve and attachment of a flexible hose for draining of flush water.

The complete secondary containment system was installed, secured to the wall, and all electrical and plumbing connections were completed. The primary exposure system components were assembled within the secondary containment chamber, and the entire system was placed in service. Figure 33 is a diagram of both primary and secondary units.

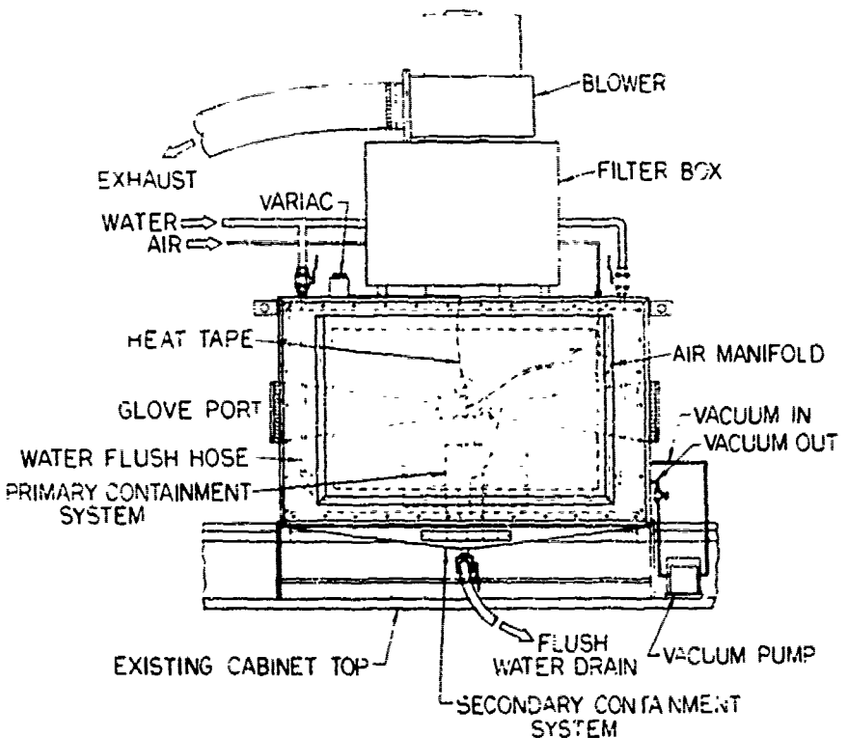


Figure 33. Diagram of primary and secondary containment systems.

RESPIRATORY VALVE MODIFICATION

The Respiratory Toxicology Department requested construction of a respiratory face mask for use on unsedated beagle dogs, shaped to fit the animal's muzzle. It was necessary to modify two commercially available pulmonary valves and combine them into one unit for separating inspiratory and expiratory flows. The valves and valve foundation were fabricated from two bidirectional pulmonary valves (Hans-Rudolph, Model 2600, Kansas City, Missouri) outlined in Section A of Figure 34. One valve is used for inspiration and the other for expiration. The acrylic valve bodies were cut into two segments as shown in Section B, the cut surfaces were then covered with 2 mm thick acrylic sheet bonded and contoured to the shape of the valve body. The shaded portions shown in Section B were discarded.

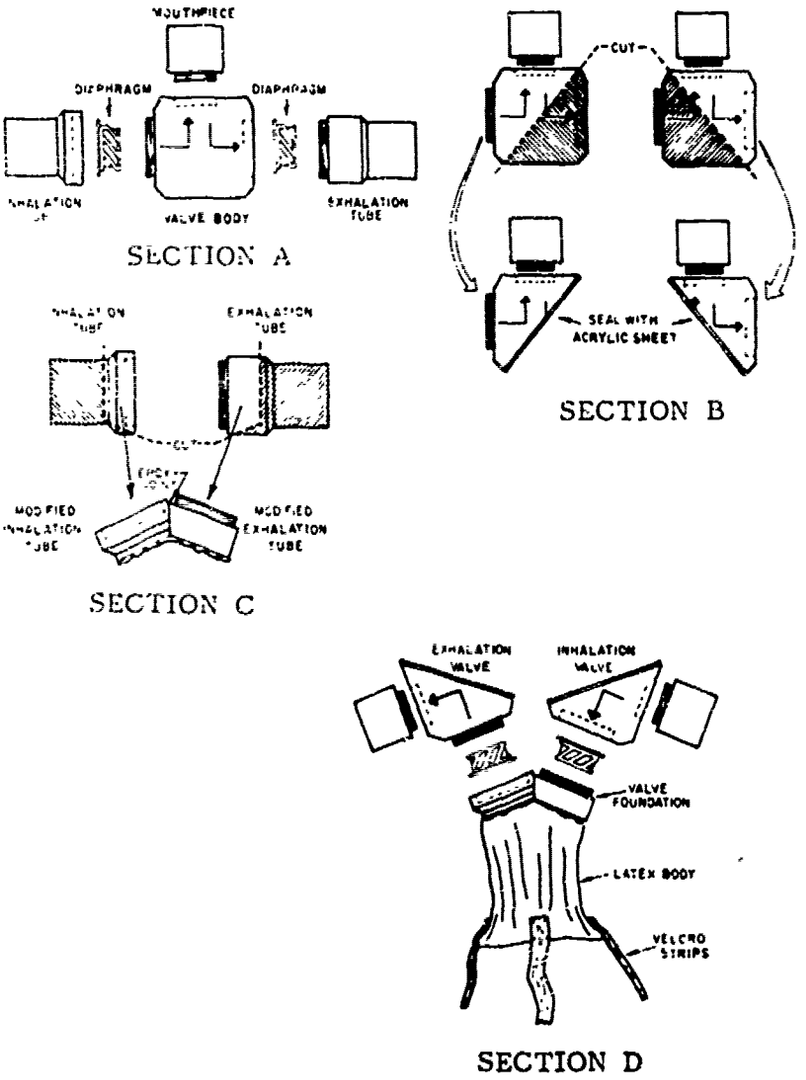


Figure 34. Respiratory valve for measurement of inspiratory and expiratory flows.

Attachment of the valve bodies to the mask was accomplished by use of the valve foundation shown in Section D. To fabricate this valve foundation, the inhalation and exhalation tubes of the bidirectional valve were machined from their original length of 6 and 4 cm, respectively, to 2 and 1.5 cm leaving the threaded portions intact. The tubes were then attached to the valve bodies to determine the correct location, marked for beveling and removed. One side of each tube was then machined at 50°, and the two tubes were bonded together to form a unit with the threaded portions facing outside at an included angle of approximately 100°. The assembled

tubes were then cemented to the mask, the original silicone rubber diaphragms re-installed, and the valves attached in the correct position to form an integral unit. The unit can be easily disassembled, cleaned and reassembled as often as required.

PHENOLIC ESTERS IN HYDRAULIC FLUIDS

The present military specification for triarylphosphate hydraulic fluids, MIL-H-19457C(SH), does not require testing for delayed neurotoxicity using a sensitive species such as chickens. Instead, the specification prescribes that triorthocresylphosphate (TOCP) content be one percent or less. After testing of Durad MP280 revealed it to be a delayed neurotoxin, NMRI/TD requested that the TOCP content of this hydraulic fluid and Fyrquel 220 be determined using the technique outlined in MIL-H-19457C(SH) given below:

Summary of the Method

A sample of the phosphate ester is saponified overnight in a Parr bomb containing aqueous caustic solution. After neutralization, the phenolic fraction is extracted with diethyl ether. The ether solution is chromatographed and o-cresol is determined by external standardization using known mixtures of o-cresol in diethyl ether.

MATERIALS AND APPARATUS

Materials

(a) Sodium hydroxide, American Chemical Society (A.C.S.) reagent grade, pellets.
(b) Diethyl ether, A.C.S. reagent grade.
(c) Sodium sulfate, A.C.S. reagent grade.
(d) Hydrochloric acid, 'Baker Analyzed' reagent, J. T. Baker Company or equivalent.
(e) Reference o-cresol: Aldrich Chemical, Milwaukee, Wisconsin or Eastman Chemicals.

PROCEDURE

Tare the Parr Bomb, add 5 g sample and reweigh. Add 5 g NaOH and 10 ml distilled H_2O.

Seal the bomb and place in oven at 140-150°C for 16 hours. Note that much longer saponification times could result in lowered results.

Cool to room temperature and transfer contents to a 150 ml beaker. Rinse bomb with distilled water, adding rinsings to beaker.

Cool beaker in ice bath and slowly add, while stirring, 1:1 HCl to pH less than 5.

Transfer contents to a separatory funnel with a distilled water washing and an ether washing.

Extract twice with 20 ml portions of diethyl ether. Combine ether extracts in a dry 150 ml beaker. Add 1 g anhydrous sodium sulfate and stir thoroughly to remove traces of water.

Decant the ether solution into a 50 ml volumetric flask. Rinse the sodium sulfate with small portions of diethyl ether and transfer rinsings to the volumetric flask. Adjust to final volume of 50 ml.

Chromatograph 1 µl solution and obtain area for o-cresol.

To obtain weight percent o-cresol, prepare a weighed standard in 50 ml diethyl ether. Select a weight corresponding to the expected concentration based on a preliminary run.

Chromatograph 1 µl standard solution to obtain area for o-cresol isomer.

Calculations

$$\text{Weight percent o-cresol} = \frac{\text{wt standard} \times \text{area sample} \times 100}{\text{area standard} \times \text{wt sample}}$$

Chromatographic conditions shown in Table 64 were adjusted slightly from those given in MIL-H-19457C(SH) to protect the sample from pyrolysis. Rapid programming rise of temperature was also found to be unnecessary. Peak separation was similar to that given in the military specifications.

TABLE 64. CHROMATOGRAPHIC CONDITIONS FOR MEASUREMENT OF ORTHO-CRESOL

Varian 3700, Flame Detector
6' x 1/8" Nickel Column, packed with Carbopack C/0.1%
SOP 1000 Lot #M06080, 80/102 mesh
He Carrier - 20 ml/min
Column Temp. 220°C; Temp. 220°C; Detector Temp. 240°C
H.P. 3388A Printer Plotter
Sample - 1 µl

Table 65 is a listing of the o-cresol standards run to determine quantitative response.

TABLE 65. STANDARDS USED FOR DETERMINATION OF TOTAL ORTHO-CRESOL IN HYDRAULIC FLUIDS

% o-cresols	Number of Samples	Area Units Mean
0.001	3	8,932
0.015	3	66,403
0.020	18	86,264
0.040	3	174,550
0.100	6	434,150

Figure 35 is the chromatogram obtained with 0.02% o-cresol in ether, equivalent to 0.2% o-cresol in the hydraulic fluid. Figures 36 and 37 are chromatograms obtained from ether extracts of the saponified phosphate esters Fyrquel 220 and Durad MP280. The Durad MP280 contained only a small amount of o-cresol, and the Fyrquel 220 contained hardly any. Durad MP280 was a more complex mixture than Fyrquel 220 containing 8 substantial peaks while Fyrquel 220 had only 2 or 3.

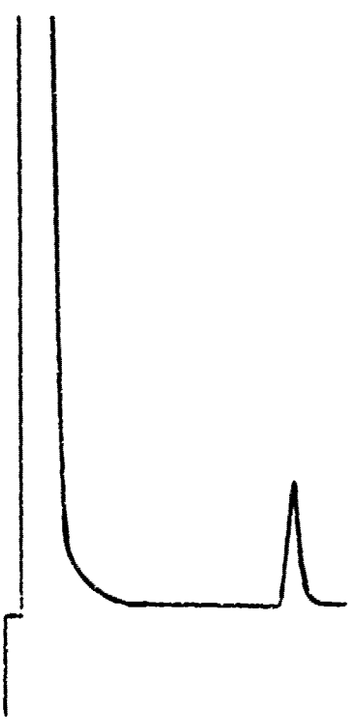


Figure 35. Gas chromatogram of 0.02% o-cresol in ethyl ether.

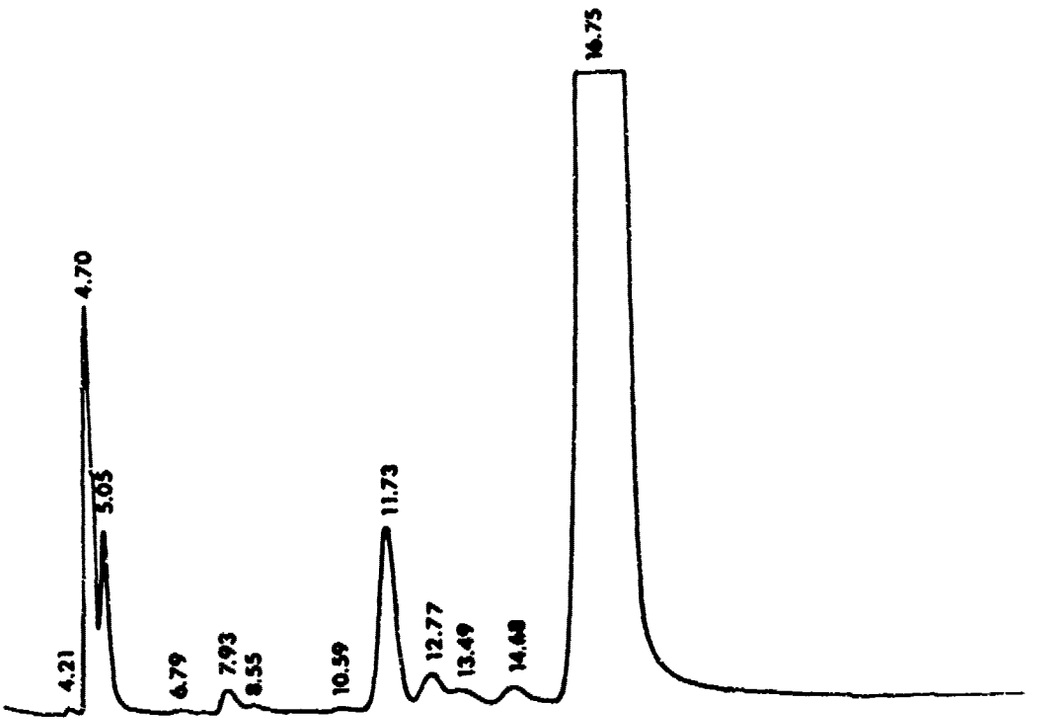


Figure 36. Gas chromatogram of Fyrquel 220.

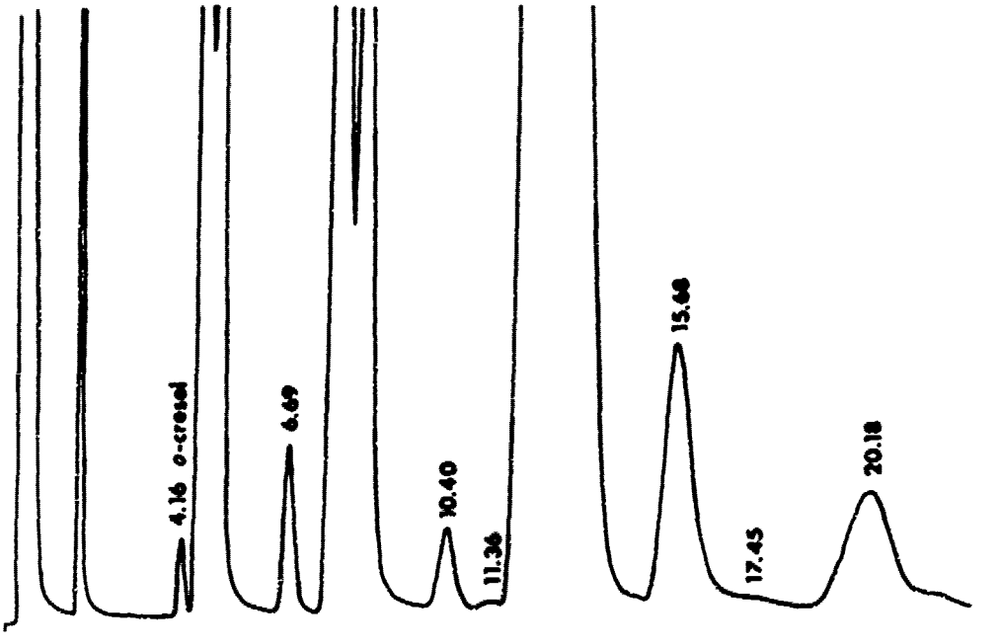


Figure 37. Gas chromatogram of Durad MP280.

Duplicate samples of the Durad MP280 and Fyrquel 220 were analyzed. The calculated o-cresol content of Durad MP280 is 0.15% and that of Fyrquel 220 is 0.015%. Both formulations meet the standards set forth in MIL-H-19457C(SH).

PHYSIOLOGIC FLUIDS - DETERMINATION OF JP-4 METABOLITES BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS)

Identification of urinary metabolites in previous years was accomplished by direct injection of urine into the GC/MS without previous treatment. This method was simple, direct and rapid, and its use led to the identification of all positional and geometric isomers of methylcyclohexanol in the urine of rats exposed to methylcyclohexane and identification of benzene, 2,5-dimethylfuran and 2-hexanone in the urine of rats exposed to JP-4. The method suffered the disadvantage of detecting only non-conjugated metabolites since the conjugated compounds were not volatile. Therefore, in an effort to broaden the applicability of the procedure, methods were developed for hydrolysis of urinary β-glucuronides and sulfates. These are detailed in Table 66.

TABLE 66. URINE TREATMENT METHODS DEVELOPED FOR GC/MS

Method A for Extraction of Ether Soluble Materials from Urine

1. Place 3 ml of urine in extraction well of Kontes Bantamware ether extractor.
2. Place 20-25 ml of 2X water extracted ethyl ether in flask.
3. Use Teflon sleeves and starch gel to seal joints.
4. Reflux slowly for 20 hours.
5. Remove water phase at room temperature from bottom of Bantamware flask.
6. Using dry nitrogen, evaporate at room temperature to 2 ml in flask.
7. Place in 3 ml TR-Vial and store in refrigerator or evaporate to 0.2 ml using a dry nitrogen stream.

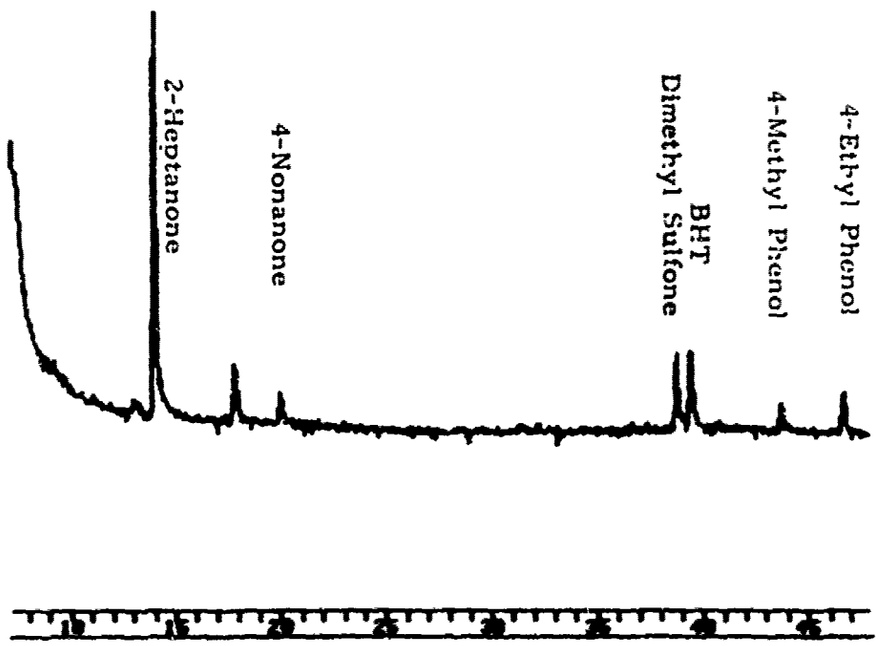


Figure 38. Total ion chromatogram of ether extract of untreated control rat urine.

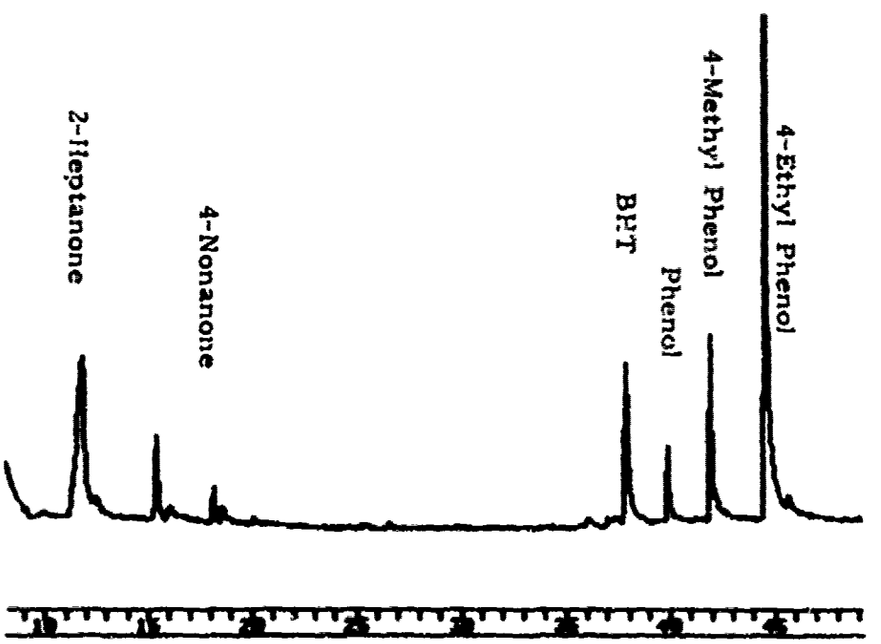


Figure 39. Total ion chromatogram of ether extract of control rat urine treated with β-glucuronidase.

TABLE 65. URINE TREATMENT METHODS DEVELOPED FOR GC/MS (CONTINUED)

Method B for Hydrolysis of Urine Glucuronides

1. Make up 6.86 pH buffer: weigh 0.34 g KH_2PO_4 and 0.355 g N_2HPO_4 into a 10 ml volumetric flask and make up to volume.

2. Add 0.3 ml of 6.86 pH buffer to 3 ml urine in 4 ml TR-Vial.

3. Weigh 0.010 g of β-glucuronidase into vial, swirl to dissolve, and place in block heater at 37°C overnight.

4. Extract with ethyl ether as detailed in Method A.

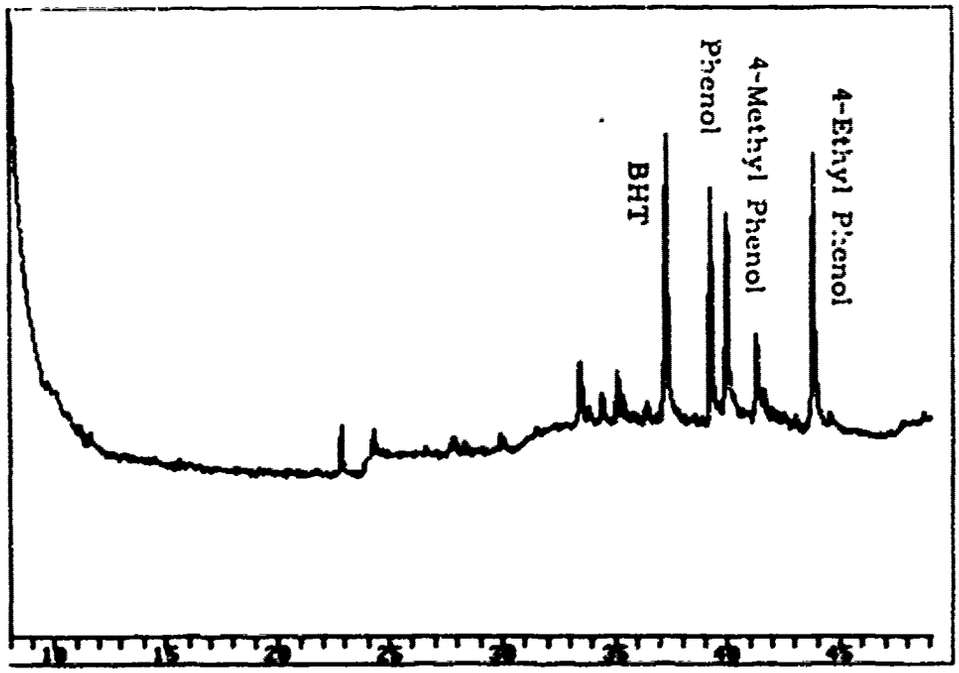
Method C for Hydrolysis of Urine Sulfates

1. Make up 3N HCl by diluting 250 ml of concentrated HCl to one liter.

2. Add 1.5 ml 3N HCl to 1.5 ml urine, place quickly in 4 ml TR-Vial and heat in block heater at 95°C for 30 minutes, cool.

3. Extract with ethyl ether as detailed in Method A.

These methods were applied to urine from male rats that were being exposed to 5000 mg/m^3 and 1000 mg/m^3 of JP-4 along with their controls. The exposure schedule was 6 hours/day, 5 days/week for one year. Animals were sampled during the last month of exposure. Control urine gave the total ion chromatograms shown in Figures 38, 39, and 40. Identifications were made using the mass spectrometer library of mass spectra. In Figure 38, peaks more volatile than 2-heptanone are lost in the ether tail, but this ketone and 4-nonanone, both seen in the urine after direct injection, have been extracted into the ether phase. In addition, there are peaks due to dimethyl sulfone, 4-methyl-2,6-ditertiarylbutylphenol (BHT), p-cresol, and p-ethyl phenol eluting at long retention times. When the urine is treated with β-glucuronidase and extracted with ether, the chromatogram shown in Figure 39 is obtained. The phenolic peaks have increased dramatically demonstrating that more of the phenols were present as glucuronide conjugates than in the free state. A new peak appeared and was identified as phenol by the library search system.



[a] Urine previously treated with ß-glucuronidase and extracted with ethyl ether.

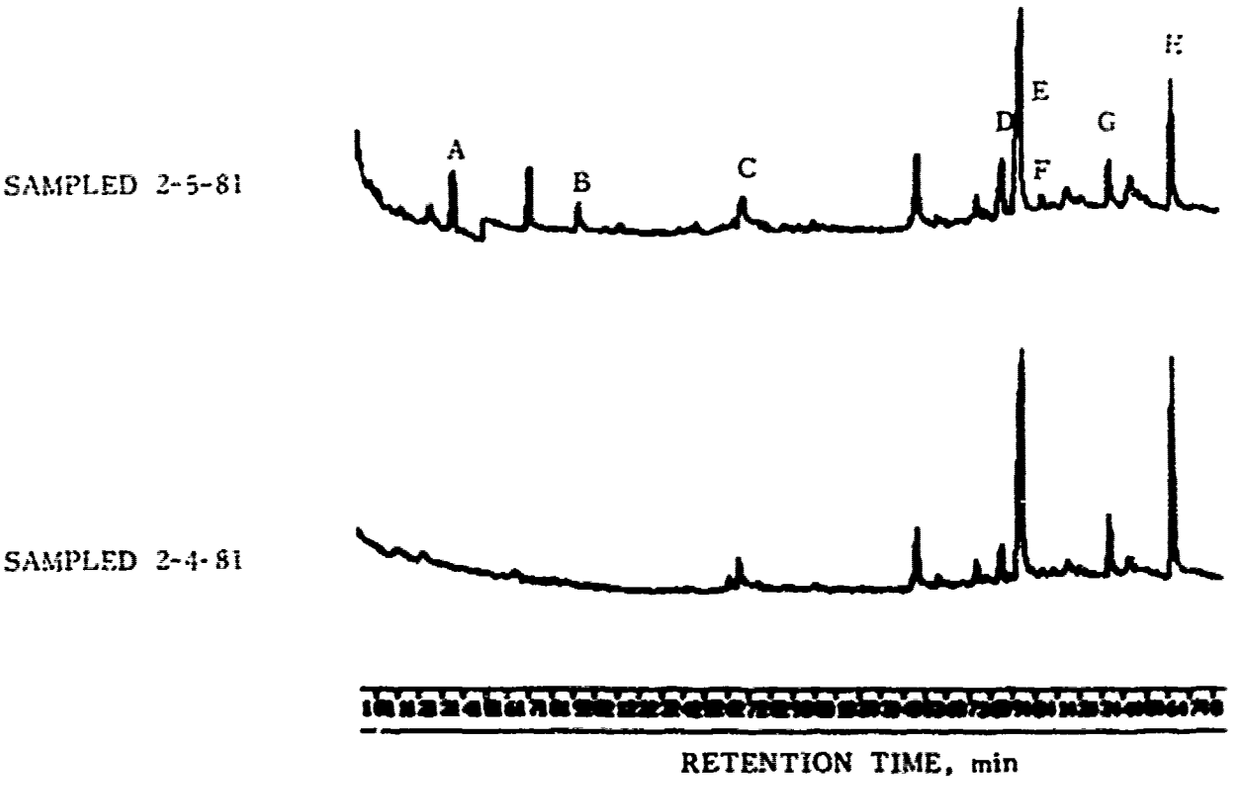
Figure 40. Total ion chromatogram of ether extract of control rat urine treated with HCl.[a]

The control male rat urine which had previously been treated with ß-glucuronidase and extracted with ether was treated as in Method C to hydrolyze sulfate conjugates. The hydrolyzate was extracted with ether, concentrated, and injected into the GC/MS to provide the total ionization gas chromatogram shown in Figure 40 with identification made by the GC/MS library program as before. All phenolic peaks previously seen in untreated and ß-glucuronidase-treated urine were present in this chromatogram along with a new unidentified peak between phenol and p-cresol. Therefore, the normal rat metabolites, p-cresol and p-ethyl phenol, are present in the urine in the unconjugated form, as glucuronides and as sulfates.

Phenol, however, is present only in conjugated form. 4-Methyl-2,6-ditertiarybutylphenol, also known as butylated hydroxytoluene (BHT), is a commonly used animal feed antioxidant and is probably a component of the food being given to the rats. It is present free and as both glucuronide and sulfate.

Urine samples taken from rats exposed to 5000 and 1000 mg/m^3 JP-4 were then subjected to the treatment given in Table 66 and chromatographed. Figures 41 through 44 show the chromatograms obtained from all the treatments on urine sampled from rats chronically exposed to 5000 mg/m^3 petroleum JP-4 and from Method B treatment

on urine from rats exposed to 1000 mg/m^3. Each figure contains chromatograms from urine samples taken on successive days. Except for the extracts from unhydrolyzed urine, there are no significant differences between chromatograms given by samples treated identically but taken on different days.



A – 2-Heptanone
B – 4-Nonanone
C – Benzaldehyde
D – Dimethylsulfone
E – BHT
F – 1,2-Cyclohexanediol
G – 4-Methylphenol
H – 4-Ethylphenol

Figure 41. Total ion gas chromatograph of ether extract of untreated urine from male rats exposed to 5000 mg/m^3 petroleum JP-4.

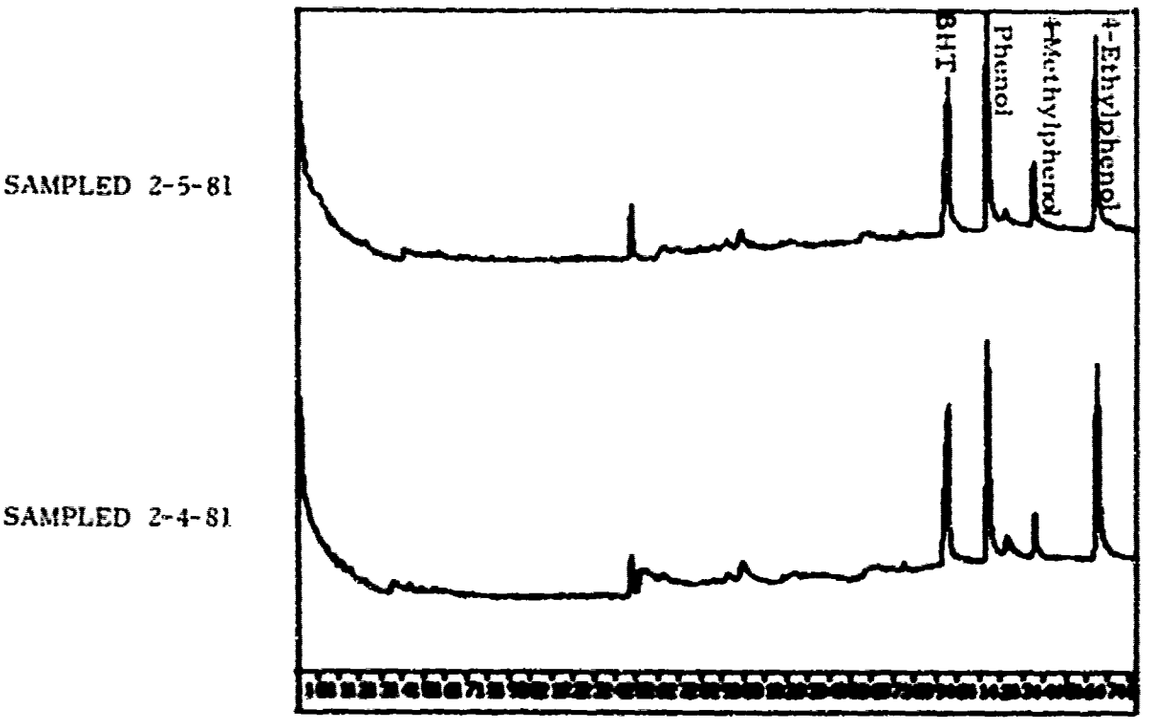


Figure 43. Total ion chromatogram of ether extract of HCl treated urine from rats exposed to 5000 mg/m^3 petroleum JP-4.

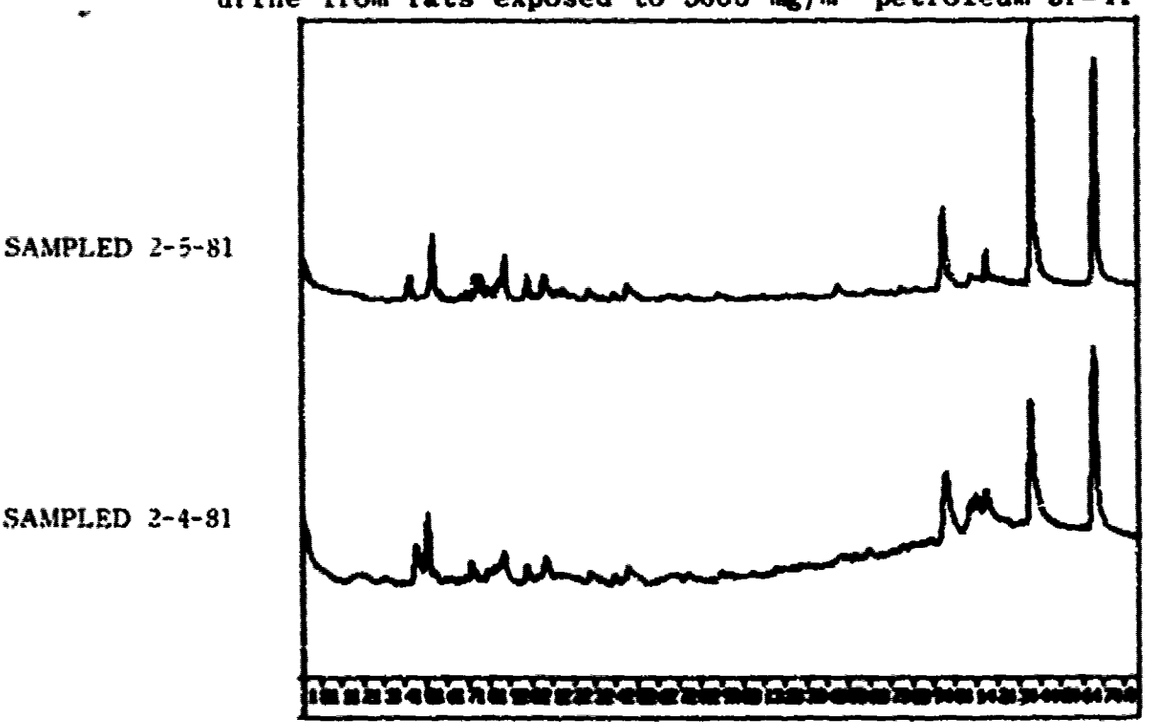


Figure 44. Total ion chromatogram of ether extract of β-glucuronidase treated urine from rats exposed to 1000 mg/m^3 petroleum JP-4.

Comparison of results given by different treatments demonstrates that peaks appearing late in the GC (mostly phenolic compounds) occur in chromatograms shown by all treatment extracts indicating that these compounds occur free in urine and as glucuronide and sulfate conjugates. The presence of phenolics is not exposure related since control samples gave the same peaks. The large increase in the number of peaks after β-glucuronidase treatment is not unexpected since the major metabolic pathway for the aliphatic hydrocarbons making up the bulk of the fuel should be hydroxylation to form alcohols which are normally conjugated as glucuronides. The chromatogram given by the extract of β-glucuronidase treated urine from rats exposed to 1000 mg/m^3 JP-4 (Figure 38) is qualitatively very similar to that from 5000 mg/m^3 exposed animals. Quantitatively, the peaks in the earlier portion of the chromatogram are much lower in the 1000 mg/m^3 derived sample. Metabolic pathways are probably quite similar in both cases and rates are not obviously saturated at the higher level. The fact that a number of peaks appearing late in the chromatograms have the same peak height in samples from both 1000 and 5000 mg/m^3 exposures supports the proposition that these are due to normal urine metabolites, phenolic in character. There is one obvious qualitative difference between the chromatograms shown in Figure 42 and Figure 44. In the samples from the higher exposure animals, a peak appears at a retention time of 33 minutes which is completely absent from the lower concentration samples. This may be due to a different metabolite appearing at higher levels of exposure, or it may represent a release of some physiological component by the rat in response to the stress of higher exposure.

The compounds which have been tentatively identified in each chromatogram from exposed urine are shown in the figures. Identification was made by analysis of the mass spectra or comparison with the GC/MS library system. In some cases, these have been checked by injection of the identified compound into the GC/MS, giving retention time as well as mass spectrometric matches. The components specific to urine from exposed rats which have been tentatively identified are shown in Table 67.

TABLE 67. METABOLITES OF PETROLEUM JP-4 TENTATIVELY IDENTIFIED IN RAT URINE

Unconjugated Metabolites

2,5-Dimethylfuran[a]
2-Hexanone[a]
Benzaldehyde[a]
1,2-Cyclohexanediol

TABLE 67. METABOLITES OF PETROLEUM JP-4
TENTATIVELY IDENTIFIED IN RAT URINE (CONTINUED)

β-Glucuronides[b]

2,3-Dimethyl-2-pentanol
3-Methyl-2-pentanol
2-Hexanol[a]
1-Methylcyclohexanol[a]
trans-2-Methylcyclohexanol[a]
cis-3-Methylcyclohexanol[a]
trans-3-Methylcyclohexanol[a]
Dimethylcyclohexanol[c]
2-Phenyl-2-propanol[a]

Sulfates

No sulfate conjugates different from those found in control urine have been detected.

[a] Retention time and mass spectrum checked against known.
[b] Most of the peaks remain unidentified.
[c] Two peaks have mass spectral characteristics of dimethylcyclohexanol.

Although the greater part of the metabolites remain unidentified, some conclusions concerning the products of rat metabolism of JP-4 can be made:

1. Most of the metabolites are β-glucuronides of secondary or tertiary alcohols formed by ω-1 oxidation of alkanes or ring oxidation of cycloalkanes.

2. Some further oxidation of the alcohols may occur as shown by the presence of 2-hexanone and benzaldehyde (using our techniques, it was not possible to detect benzoic or other aromatic acid derivatives which may be present).

3. Few, if any, purely aromatic compounds are represented by metabolites, since the phenolic materials so formed should lead to sulfate conjugation which we were not able to detect.

With our limited resources, it does not appear productive to try to identify the remaining peaks in the GC/MS of petroleum JP-4 metabolites. We will retain all GC/MS information on our computer disks for possible future retrieval and analysis.

ANIMAL TECHNICIAN TRAINING PROGRAM

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

6 - Laboratory Animal Technologists
4 - Laboratory Animal Technicians
1 - Assistant Animal Technician

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

This year's training program consisted of weekly presentations of lectures and films by UCI animal technicians, Air Force, and civilian personnel. This program provides information beneficial to everyone and created the opportunity for all to develop skill and experience in presentation of technical information.

A training program on the subject of Good Laboratory Practice was presented which consisted of a combination of lectures and reading with a series of tests. The basic course outline is as follows:

Module One - The Background
Module Two - Organization of People
Module Three - Internal Controls, Audits, and Your Master Schedule
Module Four - Protocols, SOP'S, and Other Forms of Defining Procedure
Module Five - Record Keeping Retention/Storage
Module Six - An Inspector's Checklist
Module Seven - When the Inspector Calls
Freedom of Information - Boon or Bane?
Protection of FOI
Animal Rights "The Next Common Cause?"

Additional training programs were utilized for training new animal care personnel and as refresher courses for experienced technicians.

CHAMBER TECHNICIAN TRAINING

The Thomas Dome Standard Operating Procedures Training Program which was revised in 1980 (MacEwen and Vernot, 1981) was given to one new technician trainee. This technician has completed the program as required for advance to regular chamber operator status.

The routine monthly emergency training procedures have been revised since the last annual report. Training in a planned procedure is accomplished by the Senior Technician on each shift. Periodic written examinations are given by the Principal Technician to all Chamber Technicians and revision of any procedure and/or retraining is made by the Principal Technician as the need arises. Listed below is the schedule for the training procedure and examinations given during the past year. Documentation of all practical, oral, and written examinations is also maintained to meet the standards of the Good Laboratory Practice regulations.

January	-	Vacuum Pump Failure
*February	-	GLP Procedures and Toxicology SOP's
March	-	Supply Air Fan Failure
*April	-	Operation of Scott Air Pak
May	-	Complete Power Failure
*June	-	Air Compressor Failure
July	-	Fire in Dome During Entry
*August	-	Vacuum Pump Failure
September	-	Fire in Airlock During Entry
*October	-	Fire in Exposure Laboratory During Entry
November	-	Air Compressor Failure
*December	-	Rescue of Incapacitated Dome Entrant

*Written Examinations

Since last year's report, one technician was hired with the Laboratory Animal Technician Level certification. One technician moved up to the Technologist Level.

A long-standing goal of the THRU has been achieved during the past year. All UCI Laboratory Operations personnel are now certified in the AALAS Program. The numbers of Chamber Technicians certified at each level are shown below:

2 - Laboratory Animal Technologists
5 - Laboratory Animal Technicians
3 - Assistant Laboratory Animal Technicians

While the Toxicology Operations and Animal Care groups of the THRU are pleased to achieve this goal of complete certification, efforts will continue to upgrade the skills and knowledge of these people.

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Hydrazine	JP-10	DFM	Fyrquel 220
Otto Fuel II	JP-7	PGDN	Durad MP280
Jet Fuels	JP-TS	Shale Oils	Houghto-Safe 273
JP-4	RJ-5	Hydraulic Fluids	Toxicity
JP-5	Diesel Fuel	Triaryl Phosphate	Chronic (CONT'D)

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

The research program of the Toxic Hazards Research Unit (THRU) for the period of June 1981 through May 1982 is reviewed in this report. Chronic toxicity and oncogenic studies were carried out with hydrazine, Otto Fuel II, JP-4, JP-7, JP-10, JP-TS, and RJ-5. A series of acute toxicity studies was conducted on a variety of chemicals of interest to the Department of Transportation and chemical agents used by the Air Force and Navy. Neurotoxicity studies were conducted on hudraulic fluids containing triaryl phosphate compounds.

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