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TOXIC HAZARDS RESEARCH UNIT ANNUAL TECHNICAL REPORT: 1976

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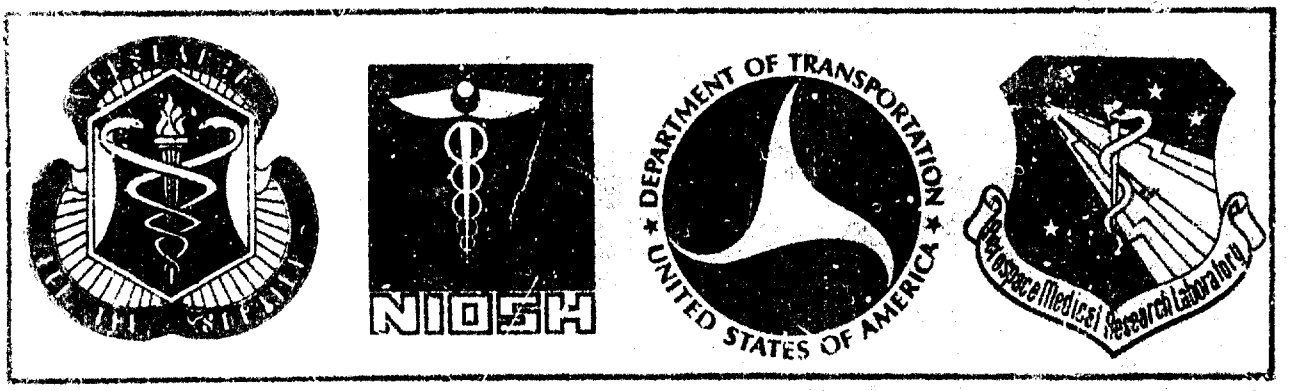

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TECHNICAL REVIEW AND APPROVAL

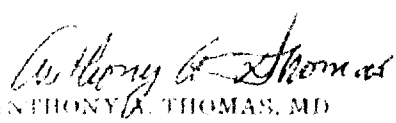
AMRL-TR-76-57

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

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

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

FOR THE COMMANDER



ANTHONY A. THOMAS, MD
Director
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Aerospace Medical Research Laboratory

AMRL-TR-76-57 September 1976

ERRATUM - November 1976

The following correction applies to Technical Report No. AMRL-TR-76-57, Toxic Hazards Research Unit Annual Technical Report 1976.

On Page 160, sixth line should read -- eight prosectors have been hired and placed in training to alleviate this problem.

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The research programs of the Toxic Hazards Research Unit (THRU) for the period of June 1975 through May 1976 are reviewed in this report. Chronic toxicity experiments were conducted using JP-4, RJ-4 (perhydromethylcyclopentadiene) and RJ-5 (reduced dimers of bicyclopentadiene) jet fuels. Studies were carried out on the oncogenicity of hydrazine, 1,1-dimethylhydrazine and coal tar aerosol. The hepatotoxicity of dimethylnitrosamine was investigated by the oral route and, in conjunction with 1,1-dimethylhydrazine, by the inhalation route.

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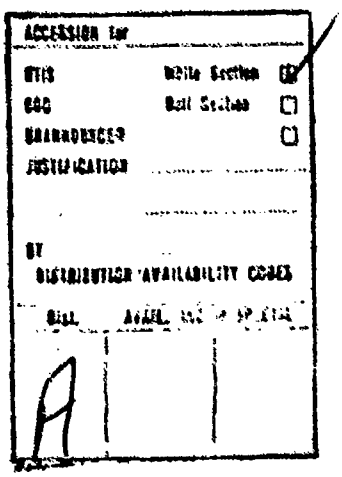
Acute rodent toxicities of hydrogen chloride, hydrogen fluoride and their mixtures with and without alumina dust were determined. The acute effects of tetranitromethane, the isomeric nitrotoluenes and methyl nitrate by various routes of administration were examined. Oral, cutaneous and inhalation toxicity determinations and skin corrosion studies were made on a number of transportable chemical agents.



PREFACE

This is the thirteenth 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 No. F33615-76-C-5005. This document constitutes the first report under the current contract and describes the accomplishments of the THRU from June 1975 through May 1976.

The current contract for operation of the Laboratory was initiated in 1975 under Project 6302 "Toxic Hazards of Propellants and Materials," Task 01 "Toxicology" Work Unit No. 63020115. K. C. Back, Ph.D., Chief of the Toxicology Branch was the technical contract monitor for the Aerospace Medical Research Laboratory.

J. D. MacEwen, Ph.D., served as co-principal investigator and Laboratory Director for the THRU of the University of California, Irvine. Acknowledgement is made to C. E. Johnson, C. C. Haun and G. L. Fogle for their significant contributions and assistance in the preparation of this report. Partial support for this program was provided by the National Institute of Occupational Safety and Health, the U. S. Army Medical Research and Development Command and the Department of Transportation.

TABLE OF CONTENTS

TABLE OF CONTENTS (CONT'D)

LIST OF FIGURES

LIST OF FIGURES (CONT'D)

LIST OF TABLES

LIST OF TABLES (CONT'D)

LIST OF TABLES (CONT'D)

LIST OF TABLES (CONT'D)

LIST OF TABLES (CONT'D)

SECTION I

INTRODUCTION

This document constitutes the 13th annual report of the Toxic Hazards Research Unit, (THRU), a research team which operates a dedicated inhalation toxicology laboratory to investigate potentially hazardous chemicals and materials of interest to the Air Force and other governmental agencies. The THRU research team is an interdisciplinary group of University of California, Irvine, toxicologists, chemists, statisticians, and engineers supported by Air Force pathologists, veterinarians, and medical technologists.

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

During the first six years of operation, the primary research efforts of the THRU were directed to obtaining information on health hazard of spacecraft flight, and the biological data obtained have been used as criteria for setting continuous exposure limits and for engineering design factors. The primary research efforts have in recent years focused more on problems of aircraft environments, chronic occupational health problems and the potential oncogenicity of chemicals used

in military and civilian activities. To this end many of the current research programs serve the mutual interest of the Air Force and other governmental agencies such as the National Institute of Occupational Safety and Health, and the Department of Transportation.

As part of its contract responsibilities, UCI/THRU presents an annual technical conference to disseminate new toxicological information to Air Force, other governmental and industrial scientists. This year's conference chaired by Dr. James E. Sterner presented 27 technical papers covering a broad range of occupational and environmental toxicology problems. Fourteen papers were presented by University of California faculty and staff members. The open forum discussion following each session resulted in significant contributions of additional technical information and scientific exchange. The conference, held 21 October through 23 October, 1975 drew 164 participants including speakers.

The papers presented at the conference were published as the Proceedings of the 6th Annual Conference on Environmental Toxicology, AMRL-TR-75-125, Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio.

Next year's conference, currently in the development stage, will be held in October, 1976 at the Stouffer's Inn, Dayton, Ohio.

SECTION II

RESEARCH PROGRAM

The research activity of the THRU is a continuing program independent of contract years, with several studies in progress at the beginning and end of each report period. Experiments that were initiated and completed during the past year and were of sufficient magnitude to merit separate technical reports are only summarized in this document. This year's research program was conducted on a broad range of chemical materials and includes inhalation studies of rocket fuels, coal tar aerosols, and combinations of solid rocket propellant exhaust products. Acute oral and dermal toxicity studies on transportable materials were also conducted.

A Study of the Oncogenic Capacity of Hydrazine

Hydrazine, unsymmetrical dimethylhydrazine (UDMH) and monomethylhydrazine (MMH) have each been shown to produce carcinomas in experimental animals by various oral modes of administration (IARC, 1974; Clark et al., 1968). Although this information about the hydrazines and many other chemical compounds has scientific interest, it is frequently unrealistic in terms of actual or practical human exposures.

Recently, however, experiments in the Toxic Hazards Research Unit Laboratory of UC, Irvine have confirmed the carcinogenic risk of the Threshold Limit Values (TLV) of 1 ppm hydrazine (MacEwen and Vernot, 1974). In these experiments, mice held 1 year postexposure after 6 hours daily, 5 days per week inhalation exposure to N_2H_4 over a 6-month period were necropsied and found to have a significant increase in alveolargenic carcinomas at the industrial TLV concentration. At a 5-fold higher dose, 11 of 15 exposed mice had alveolargenic carcinoma, 2 had lymphosarcomas and 1 mouse had a malignant hepatoma with metastasis to the spleen. Two of the mice with alveolargenic carcinomas also had metastatic lesions, one in the heart and another in the rib cage.

The results of these studies indicate a strong dose relationship for the production of carcinomas in mice. A dose relationship was seen for other inhalation effects of hydrazine and MMH, either acute or chronic (Haun, 1970; MacEwen and Haun, 1971).

The evidence that N_2H_4 produces pulmonary carcinomas at the TLV concentration, based on small numbers of animals and in only one species, should be confirmed in multiple species and with large numbers of animals.

There is a question as to the likelihood that human exposures of this chronic nature have occurred during the production and use of hydrazine, UDMH and MMH. Moreover, there is no certainty that chemicals that produce tumors in mice will be carcinogenic for man. In spite of these reservations but cognizant of the fact that the hydrazines do cause carcinogenic reaction in animals. it was desired that inhalation studies be performed by THRU which would be definitive of a suitable carcinogenic risk or no effect level for these highly important industrial and military chemicals.

The experimental protocol designed for the study of hydrazine oncogenic potential was modified from that used for the study of oncogenic capacity of UDMH. The modification was based on several considerations among which were a tentative change in the TLV from 1.0 ppm to 0.1 ppm and a request from an international consortium of hydrazine manufacturers. The tentative change in the TLV indicated a need for testing N_2H_4 concentrations spanning both current and proposed values with at least one animal species.

The request from the hydrazine manufacturers group asked that we include both sexes of rats in the experiment. extend the exposures from 6 months to one year and that we increase the numbers of rat

controls to permit intermittent necropsies for spontaneous increases in deaths of individual exposure groups. They also wished to increase the number of concentrations tested to span the current and proposed TLV's. Since their request for modification of the protocol consisted of changes which would be beneficial to our goal of determining the oncogenic capacity of hydrazine and the added costs were indirectly covered, by the provision of pathologic evaluation services from an independent consulting research laboratory for 2 species of animals, the request was acted upon in a favorable manner.

The animal exposures were initiated during the summer of 1975 and will be continued into the next reporting period. Not all experimental groups are in phase with respect to the starting dates for their exposure. Even though all animals for the study were acquired in time for simultaneous insertion into the experimental groups the hamsters received were not suitable for use. A second group of hamsters was also found to be diseased upon arrival and consequently a 4-month delay was encountered in the insertion of hamsters into the experiment. Due to chamber equipment malfunction, the exposure of the original group of mice to 1 ppm hydrazine was stopped. The mice were replaced and a separate set of controls were set aside for comparison. These mice have been in the study for 5-1/2 months.

The animals used in this study consist of C57 black/6 mice obtained from Jackson Laboratories, CDF (Fisher 344 derived) albino rats from Charles River, Engle Golden Syrian hamsters, and beagle dogs. The numbers of animals of each sex and species are listed in Table 1 which also shows the chambers used and exposure concentrations.

TABLE 1. EXPERIMENTAL DESIGN FOR HYDRAZINE INHALATION EXPOSURE CONCENTRATIONS

Hydrazine Concentration, ppm	Animals Numbers, Sex and Species	Chamber Number
0.05	100♂, 100♀ rats; 400♀ mice	7
0.25	200♂ hamsters; 400♀ mice	5
0.25	100♂, 100♀ rats; 4♂, 4♀ dogs	6
1.0	200♂ hamsters; 400♀ mice	1
1.0	100♂, 100♀ rats; 4♂, 4♀ dogs	4
5.0	100♂, 100♀ rats; 200♂ hamsters	8
Control	150♂, 150♀ rats, 400♀ mice; 200♂ hamsters; 4♂, 4♀ dogs.	Vivarium

Exposures are conducted on a 6 hour/day, 5 day/week schedule without exposures on weekends and holidays. The Thomas Dome Chambers are being operated with nominal airflows of 35 CFM at a slightly reduced pressure (725 mm Hg) to prevent hydrazine leakage into the laboratory atmosphere.

The safety protocol and laboratory contaminant monitoring protocols are the same as those used during the UDMH study as described in the last annual report (MacEwen,and Vernot, 1975).

As shown in Table 1, four inhalation experiments are currently underway to determine lifetime response of different animal species to selected low level concentrations of hydrazine such as 0.05 ppm, 0.25 ppm, 1 ppm and 5 ppm. The exposures are to continue for one year with 6 hours a day, 5 days a week exposure. No exposures are made on holidays and weekends.

Hydrazine is introduced into the dome by using a Sage Pump Model 355 kept inside a plexiglas hood. One pump is used for each exposure concentration experiment. The contaminants are carried to the dome through a 1/4" teflon line in which vaporization takes place. The contaminant generation system takes advantage of the negative air pressure inside the dome which permits a constant air flow through the contaminant line of about 2.5 liters per minute. Slight heat is applied to the contaminant line to increase the volatilization of the hydrazine for the highest concentration levels.

The continuous monitoring of the hydrazine concentrations in the chambers is accomplished with a Technicon AutoAnalyzer. The exposure chamber atmosphere is pulled through a 1/4" polyethylene line into a scrubber tower loaded with glass beads. A buffered iodine solution is added at the top of the tower and it runs down through the beads. The hydrazine from the dome reacts with the iodine solution and reduces it, causing a loss in color. This color change is measured as absorbance. The absorbance is proportional and linear when compared to concentration and a calibration curve can be prepared daily. To use the curve it is necessary to keep the chamber sampling air flow through the tower at the same rate as was used to make the standard curve. The iodine solution flow rate must also be maintained at the same rate as used for the standard curve.

The sampling solution buffer contains 40 grams/L KI, 20 grams/L Na_2HPO_4, and 6 grams/L KH_2PO_4. An iodine solution of 0.1 M is used to add iodine to the buffer. Because each experiment has different hydrazine concentrations, the quantity of iodine added to its buffer is different. This is done to improve each analysis. To each liter of buffer, 15 ml of 0.1 M iodine is added to analyze the 5.0 ppm N_2H_4 experiment; 3.5 ml/L for the analysis of the 1.0 ppm hydrazine concentration, and 1 ml/L is used

for the 0.25 and 0.05 ppm concentrations. Different filters are used to either increase or decrease the colorimeter sensitivity as needed. For 0.25 ppm hydrazine, a 400 mμ filter is used while the 0.05 ppm concentration requires a 352 mμ filter. The 1.0 ppm N_2H_4 concentration takes a 420 mμ filter and 5.0 ppm requires a 466 mμ filter.

The effects of hydrazine exposure thus far have been limited to dose related depression of growth rates for male rats as shown in Figure 1 and hamsters receiving 5, 1 or 0.25 ppm hydrazine as shown in Figure 2. Weights of female rats taken on the same biweekly schedule are somewhat erratic. Subnormal weight gain patterns appear clear only for those in the 5 ppm and 1 ppm exposure groups.

Blood samples are drawn biweekly from all control dogs and the test dogs which are exposed to 1 ppm and 0.25 ppm N_2H_4 concentrations. After 9 months of exposure, most results collected for the entire battery of tests which include as liver function tests, SGPT determination and BSP retention time, are completely normal when compared with preexposure biweekly results or control values. Total protein and serum albumin values appear to be significantly elevated in the 1 ppm N_2H_4 exposure group and there may be a trend

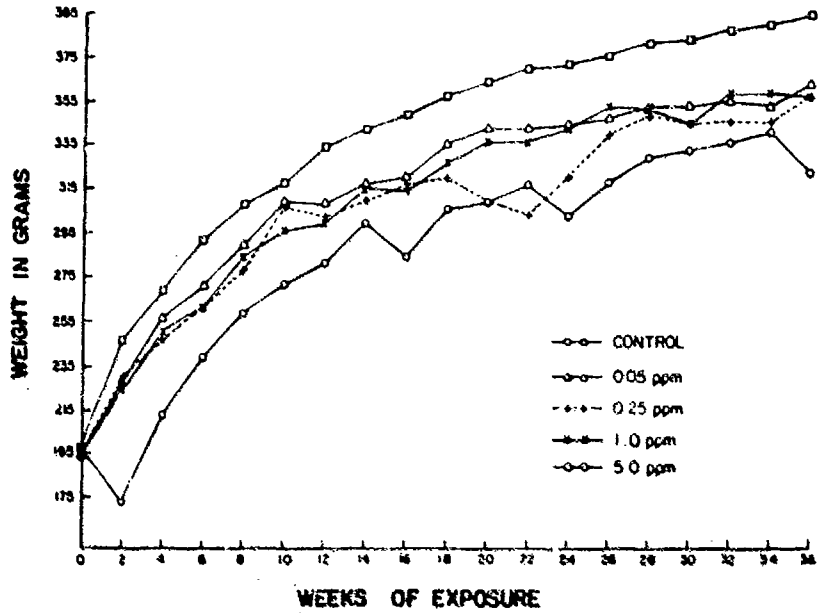


Figure 1. The effect of chronic exposure to inhaled hydrazine on the growth rate of male rats.

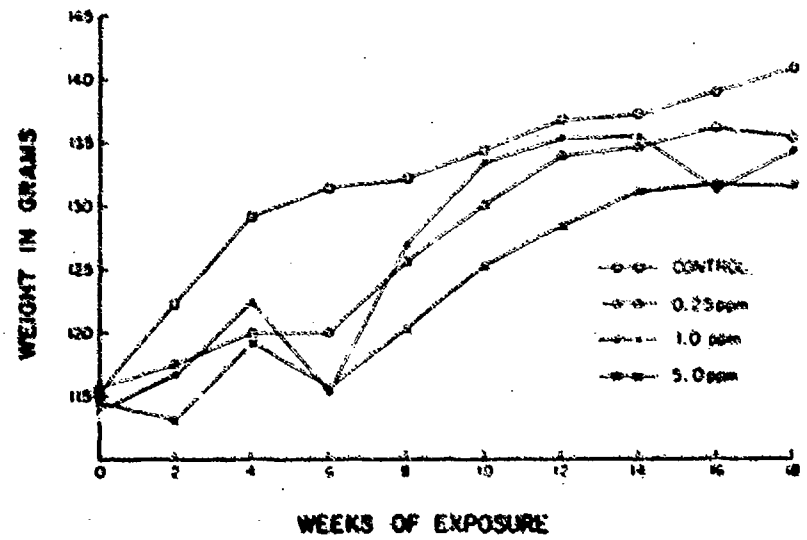


Figure 2. The effect of chronic exposure to inhaled hydrazine on the growth rate of Golden Syrian hamsters.

of higher values for these determinations in the 0.25 ppm N_2H_4 exposure group. Evaluation of these findings is complicated by one elevated value during the preexposure period and a change in clinical chemistry methodology by the laboratory group providing this service.

Mortality in exposed and control groups has been very sparse. There have been deaths in all groups of exposed and control hamsters and mice but no dose response relationship is in evidence. Ten percent mortality is the highest in any hamster group, while no more than 4% have died in any group of mice. Very few rats have died and at the levels tested, dogs are showing no signs of ill health.

Histopathology information is not available at this time although all dead animals receive preliminary examination inhouse to determine cause of death. Paraffin embedded tissues from mice and rats will be sent to Huntingdon Research Center, England for final processing and definitive examination while hamster tissues will be sent to USAF School of Aerospace Medicine/VSP, Brooks AFB, Texas. The study is continuing and no conclusion or comment on the oncogenic potential of N_2H_4 can be made at this time.

90-Day Continuous Coal Tar Aerosol Inhalation Studies

Two 90-day continuous coal tar aerosol studies (2.0 and 0.2 mg/m^3 coal tar) were concluded during the past year. The 10 mg/m^3 coal tar aerosol study was concluded last year as reported in the 1975 Annual Technical Report (MacEwen and Vernot, 1975). The descriptions of the coal tar generation system, methods of analyses and experimental protocols are in a previous annual report (MacEwen and Vernot, 1974). During the postexposure holding period, the animals were examined biweekly for development of skin lesions.

A summary of the mouse skin tumors found after 90-day continuous exposure to 2.0 mg/m^3 coal tar aerosol and lifetime observation is shown in Tables 2 and 3. Of the 14 skin tumors found in the ICR/CF-1 mice exposed to 2.0 mg/m^3 coal tar, 9 or 64% of these were found during the first 22 weeks of postexposure observation.

Only one skin tumor was found during the 98-week postexposure observation period for the animals exposed to 0.2 mg/m^3 coal tar aerosol in each mouse strain tested, the ICR/CF-1 and JAX/CAF-1, both observed after 81 weeks postexposure. In addition, one of the JAX/CAF-1 control mice developed a skin tumor at 84 weeks postexposure.

The following tabulation, showing a cumulative number of skin tumors that developed after a comparable postexposure period for all three aerosol studies, demonstrates a definite dose-response effect. This effect is strongly apparent in the ICR/CF-1 mice but less obvious in the JAX/CAF-1 mice where smaller numbers of skin tumors occurred. However, in a comparison of all data on JAX/CAF-1 mice (MacEwen and Vernot, 1975), it can be seen that the number of tumors increased during the later stages of the postexposure holding period. It appears that the JAX/CAF-1 mice have a much longer period of onset than the ICR/CF-1 strain for development of skin tumors.

Exposure Concentration mg/m^3	Week of Observation	Cumulative Numbers of Tumors			
		ICR/CF-1*		JAX/CAF-1*	
		Exposed	Control	Exposed	Control
10	100	44	3	18	1
2	103	14	0	3	0
0.2	101	1	0	1	1

*N = 75 female mice in each group.

All 90-day coal tar aerosol studies have been terminated. The surviving animals were sacrificed and representative tissues were sampled for histopathological examination. Histopathology of all animals in these studies has been contracted to another source and the results, when available, will be reported by that group.

TABLE 2. SUMMARY OF SKIN TUMORS FOUND IN ICR/CF-1 MICE EXPOSED TO 2 MG/M^3 COAL TAR AEROSOL

Number of Weeks Postexposure	Total Weeks on Experiment	New Tumors	Cum. No. of Tumors	Total Number Examined
6	19	3	3	75
8	21	1	4	75
10	23	0	4	75
12	25	0	4(4)	75
14	27	0	4(4)	74
16	29	0	4(4)	59
18	31	1	5(5)	69
20	33	0	5(4)	66
22	35	4	9(8)	66
24	37	0	9(7)	61
26	39	0	9(7)	60
28	41	0	9(7)	58
30	43	1	10(7)	58
32	45	0	10(7)	48
34	47	0	10(7)	45
36	49	0	10(7)	45
38	51	0	10(7)	44
40	53	0	10(7)	44
42	55	2	12(9)	44
44	57	0	12(9)	44
46	59	0	12(7)	44
48	61	0	12(7)	43
50	63	0	12(7)	43
52	65	0	12(7)	43
54	67	0	12(7)	43
56	69	0	12(7)	43
58	71	0	12(6)	40
60	73	0	12(6)	40
62	75	1	13(6)	37
64	77	0	13(6)	35
66	78	0	13(5)	33
68	81	0	13(5)	33
70	83	0	13(5)	32
72	85	0	13(5)	31
74	87	0	13(4)	29
76	89	0	13(4)	29
78	91	0	13(4)	27
80	93	0	13(2)	24
82	95	0	13(2)	22
84	97	0	13(2)	22
86	99	0	14(3)	21
88	101	0	14(3)	19
90	103	0	14(3)	18

( ) = Number of mice with tumors which are alive on this date.

TABLE 3. SUMMARY OF SKIN TUMORS FOUND IN JAX/CAF-1 MICE EXPOSED TO 2 MG/M^3 COAL TAR AEROSOL

Number of Weeks Postexposure	New Tumors	Cumulative Number of Tumors	Total Examined
6	0	0	65
8	0	0	65
10	0	0	65
12	0	0	64
14	0	0	62
16	1	1(1)	59
18	0	1(1)	59
20	0	1(1)	54
22	2	3(3)	54
24	0	3(3)	51
26	0	3(2)	50
28	0	3(2)	47
30	0	3(2)	47
32	0	3(2)	44
34	0	3(2)	44
36	0	3(1)	40
38	0	3(1)	39
40	0	3(1)	36
42	0	3(1)	36
44	0	3(1)	36
46	0	3(1)	31
48	0	3(1)	31
50	0	3(1)	31
52	0	3(1)	31
54	0	3(1)	31
56	0	3(1)	31
58	0	3(0)	30
60	0	3(0)	30
62	0	3(0)	30
64	0	3(0)	30
66	0	3(0)	30
68	0	3(0)	30
70	0	3(0)	29
72	0	3(0)	28
74	0	3(0)	27
76	0	3(0)	24
78	0	3(0)	24
80	0	3(0)	23
82	0	3(0)	22
84	0	3(0)	22
86	0	3(0)	20
88	0	3(0)	20
90	0	3(0)	17

( ) = Number of mice with tumors which are alive on this date.

Chronic Inhalation Toxicity of JP-4 Jet Fuel

An 8-month intermittent exposure study of the toxicity of inhaled JP-4 jet fuel vapors to various animal species was detailed in a previous annual report (MacEwen and Vernot, 1974). The study was terminated after 33 weeks of exposure and, with the exception of 20 rats and 20 mice to be held for one year postexposure, all animals were sacrificed.

Gross examinations of the animals that died during the exposure period or at the 33 week sacrifice date revealed no lesions which could be attributed to exposure. The organ and body weight ratios of rats were analyzed statistically following sacrifice with significant differences from control values being found in organ weights and organ to body weight ratios only in the rats exposed at the 5.0 mg/liter JP-4 concentration. An increase in weight was found in lung, liver, spleen and kidney. Micropathological examination of these tissues failed to reveal any dose related effects which could be attributed to this increase in organ weights.

The only other significant pathological finding after the 8-months of repeated daily exposures to JP-4 was an increase in the incidence of chronic murine bronchitis in the rats exposed to either concentration of the jet fuel. Table 4 shows the incidence of this manifestation in the various exposure groups.

TABLE 4. INCIDENCE OF CHRONIC MURINE BRONCHITIS IN RATS EXPOSED TO JP-4 VAPORS

	Controls	Benzene Controls	5 mg/liter JP-4	2.5 mg/liter JP-4
Number Examined	24	25	30	29
Number with Bronchitis	0	2	8	8

After 12 months, approximately 80% of the rats and 46% of the mice held for postexposure observation died. Mortalities were equally distributed between all exposure and control groups with chronic respiratory disease being the principal cause of death. There were no gross lesions in any of the animals which could be attributed to exposure.

Histopathological examination of the animals held for postexposure observation revealed an increase in hemosiderin deposits in the spleens of the exposed rats as well as the benzene exposed controls. The tumorigenic response of these animals is listed in Table 5. The tumor response in either species was slight and not dose-related, therefore not considered physiologically significant.

In all, no significant pathological lesions were found in any of the animals held one year postexposure which would warrant changing the conclusions stated in the last annual report in which we recommended that an exposure standard for workmen should not exceed 2.5 mg/liter JP-4 vapors (12.5 ppm benzene equivalent) for extended periods of time.

TABLE 5. TUMOR INCIDENCE IN ANIMALS EXPOSED TO JP-4 VAPORS FOR 6-MONTHS AND HELD ONE YEAR POSTEXPOSURE

	Controls	25 ppm Benzene*	5.0 mg/liter JP-4	2.5 mg/liter JP-4
Mouse Tumors:				
Alveolargenic Adenoma	3/19**	6/17	4/16	7/21
Lymphosarcoma	0/19	1/17	1/16	2/21
Mammary Carcinoma	0/19	1/17	0/16	0/21
Hepatoma	1/19	0/17	0/16	0/21
Hematopoietic Tumors	6/19	1/17	4/16	3/21
Thyroid Carcinoma	0/19	0/17	1/16	0/21
Rat Tumors:				
Mammary	0/15	0/16	1/20	0/18
Thyroid Adenoma	0/15	1/16	0/20	0/18
Pancreatic Islet Cell Adenoma	0/15	1/16	0/20	0/18

* Positive control for maximum benzene concentrations.
**Number of tumors found/number of animals examined microscopically.

Carcinogenic Effects of Chronic Inhalation Exposure of Animals to Coal Tar Aerosol

Long-term daily repeated inhalation exposures of four animal species to the complete complex mixture of coal tar volatiles was initiated for comparison with 90-day continuous exposure studies to the same material for validation of the experimental approach of time compression with continuous animal exposures. This study was also designed to examine the effects of inhaled coal tar aerosols on Macaca mulatta, a species more closely related to man.

The description of the coal tar generation system, method of analysis, and experimental protocols are given in a previous annual report (MacEwen and Vernot, 1975). Some groups of animals were placed into exposure after the experiment had begun because initial shipments did not meet quality control standards. Since exposure for all groups ended on 7 January 1976, the rabbits were exposed 16 months, JAX mice 17 months, and all other groups 18 months. During the post-exposure holding period, the rats were weighed on a biweekly schedule. All rodents were also examined biweekly for the development of skin lesions.

The chamber coal tar aerosol concentrations were analyzed using gravimetric sampling to trap the aerosol droplets on a millipore filter. The fluorescent materials were then dissolved from the filter with toluene and the fluorescence measured by a Turner fluorometer. The mean concentration of coal tar after 18 months was 9.97 mg/m^3 in one exposure chamber and 9.98 mg/m^3 in the other. These mean concentrations were calculated over 371 exposure days (542 calendar days).

Each exposure chamber was also sampled daily during the first month of exposure and monthly thereafter for benzene vapor concentration. The benzene analysis, measured by gas chromatography, averaged 9.1 mg/m^3 in Dome 2 and 10.2 mg/m^3 in Dome 3. The concentration range was 4.0 to 25.8 and 4.8 to 38.5 in Domes 2 and 3, respectively. A benzene hazard did not exist as these concentrations were well below the Threshold Limit Value concentration of 80 mg/m^3.

Aerosol particle size determinations were made monthly during the study following the procedure of Vooren and Meyer (1971). A minimum of 99% of the total droplets in both chambers were five microns or less in diameter. Therefore, most aerosol droplets within the chambers were of a respirable size.

At the conclusion of the aerosol exposure portion of the study, four male and four female rats from both the exposed and the control group were sacrificed for gross and histopathologic examination. Gross examination showed all of the males and three of the four female test rats' lungs were filled with irregular to confluent white masses. These were flat, umbilicated and fungiform on the pleural surface. In most cases, the lungs of the exposed rats did not collapse at necropsy.

Histopathological examination of the sacrificed rats and those that died postexposure is now complete. A high incidence of squamous cell carcinomas of the lungs was seen in the exposed rats (Table 6) while no lung carcinomas were found in any of the control rats. All of the male rats examined had lung carcinomas while 82% of the females were similarly affected. Of the seven female rats which did not develop squamous cell carcinomas, three died during the first eight months of exposure, possibly before the lesions developed in any of the rats. None of the deaths in the male rat group occurred prior to eleven months of exposure.

TABLE 6. COAL TAR TUMORIGENESIS IN RATS

	Controls		Exposed	
	Males	Females	Males	Females
Number Examined Histologically*	36	37	38	38
Tumors found:				
Squamous cell carcinoma, Lung	0	0	38	31
Sebaceous cell carcinoma	0	1	0	0
Intraabdominal carcinoma	0	1	0	0
Mammary fibroadenoma	0	1	0	3
Mammary adenocarcinoma	0	1	0	0
Other tumors	0	1	8	2
Overall Tumor Incidence (%)	0	13	100	82

* The original number of rats per group was 40. However, because of autolysis and/or cannibalization, a few animals were unsuited for histopathological examinations.

Some histopathology results have been received for mice that died during the course of the study. Enough data has been received on the CF-1 mice to show definite lung tumor effects (Table 7). Twenty-one of 107 CF-1 test mice developed alveolargenic carcinomas while only two of 126 control mice showed this lesion. Most other tumors found histologically were equally distributed between test and control mice and not considered coal tar related although there may be a reduction in total numbers of sarcomas and hemopoietic tumors in the exposed mice. There still remain some surviving mice that are being held postexposure for skin and other tumor examination.

TABLE 7. COAL TAR TUMORIGENESIS IN MICE

	Controls		Exposed	
	ICR/-CF-1	JAX/-CAF-1	ICR/-CF-1	JAX/-CAF-1
Number Examined Histologically	126	12	107	15
Tumors found:				
Alveolargenic carcinoma	2	0	21	4
Alveolargenic adenoma	5	1	5	0
Bronchiogenic carcinoma	0	0	1	0
Squamous cell carcinoma	0	0	1	0
Lymphosarcoma	13	1	3	2
Reticulum cell sarcoma	10	0	6	2
Hemangiosarcoma	3	1	3	0
Hemopoietic tumors	20	1	11	0
Subcutaneous sarcoma	1	0	1	0
Other tumors	4	2	7	0

A summary of the fluorescence values found in the serially sacrificed ICR/CF-1 mice is shown in Table 8. The values for hide deposition of coal tar appear to reach an early equilibrium between the amount being deposited on the fur and the amount being removed by grooming. The lung fluorescence data shows a rapid increase during the first 25 days followed by a gradual increase for the next 423 days. After that time the amount of fluorescent compounds found in the lungs of the mice increases rapidly. This latter increase could be a factor of age with resultant breakdown of clearance mechanisms in the lungs. The age of the mice at the 448th exposure day was approximately 16 months.

TABLE 8. COAL TAR FLUORESCENCE RETAINED IN MOUSE LUNG AND SKIN TISSUES (N=4)

Days of Exposure	Calendar Days	Tissue Fluorescence*	
		Lung (μg/g)	Hide (μg/cm^2)
1	1	6	1.4
7	9	19	3.2
20	25	173	4.9
60	88	180	3.8
108	147	210	3.7
149	210	236	2.7
185	266	332	3.5
233	329	315	3.2
270	385	296	2.1
309	448	383	6.7
352	511	567	3.8
371	542	584**	6.3**

* Values are test animal values minus control animal values.
** N = 8.

A summary of the mouse skin tumors is shown in Table 9. Beginning at 30 weeks of exposure, the animals were examined biweekly. A skin tumor was found on one control ICR/CF-1 mouse during the first skin inspection at 30 weeks and on two more controls during the 96th week. The first skin tumor found on a test animal was at 50 weeks. In all, skin tumors were found in only 5 test and 3 control ICR/CF-1 mice to date. In the JAX/CAF-1 strain mouse, total tumor incidence to date is 2 in the exposed group and 1 in a control.

TABLE 9. SUMMARY OF SKIN TUMORS FOUND IN ICR/CF-1 MICE INTERMITTENTLY EXPOSED TO 10 MG/M^3 COAL TAR AEROSOL FOR 18 MONTHS

Number of Weeks Exposure	New Tumors		Cumulative Number of Tumors		Total Number Examined	
	ICR/CF-1	JAX/CAF-1	ICR/CF-1	JAX/CAF-1	ICR/CF-1*	JAX/CAF-1**
30	0	0	0	0	73	49
32	0	0	0	0	64	48
37	0	0	0	0	64	48
41	0	0	0	0	62	48
46	0	0	0	0	58	47
50	0	0	0	0	56	41
54	0	0	0	0	53	41
56	0	0	0	0	52	41
58	0	0	0	0	52	41
60	0	0	0	0	52	40
62	1	0	1(1)	0	52	40
64	0	0	1(0)	0	52	40
66	0	0	1(0)	0	48	39
68	0	0	1(0)	0	47	39
70	0	0	1(0)	0	46	38
72	0	0	1(0)	0	45	38
74	0	0	1(0)	0	41	38
76	0	0	1(0)	0	40	38
78	0	0	1(0)	0	38	38
80	1	0	2(1)	0	38	37
82	0	1	2(1)	1(1)	34	37
84	1	0	3(1)	1(1)	32	37
86	0	0	3(1)	1(1)	32	37
88	0	0	3(1)	1(1)	31	37
90	0	1	3(1)	2(2)	31	36
92	1	0	4(2)	2(2)	29	35
94	0	0	4(2)	2(2)	28	35
96	0	0	4(2)	2(2)	26	35
98	1	0	5(3)	2(2)	23	32
100	0	0	5(3)	2(1)	23	30

( ) = Number of mice with tumors which were alive at examination period.
*Original N = 75, ** Original N = 50.

These results differ greatly from the skin tumor results of the 10 mg/m^3 coal tar exposure 90-day continuous study in which 102 test and 5 control mice developed lesions. Further comparison of these groups appears later in the text.

During the course of the study, rat, monkey and rabbit weights were monitored on a regularly scheduled basis. Figures 3 and 4 show the mean body weight relationship of the test animals with their respective controls.

Except for an early six-week period and at the 12-month weighing period where the weights of the male control rats dropped for some unknown reason, the exposed rat group showed a statistically significant depression in mean body weight. This was apparent from the first weighing after the start of the study, at two weeks, and on through 18 months of exposure. The rabbits show a similar pattern with the exposed rabbits having a statistically significant weight depression. Rabbit body weight comparisons were discontinued after nine months due to the small number of surviving test rabbits.

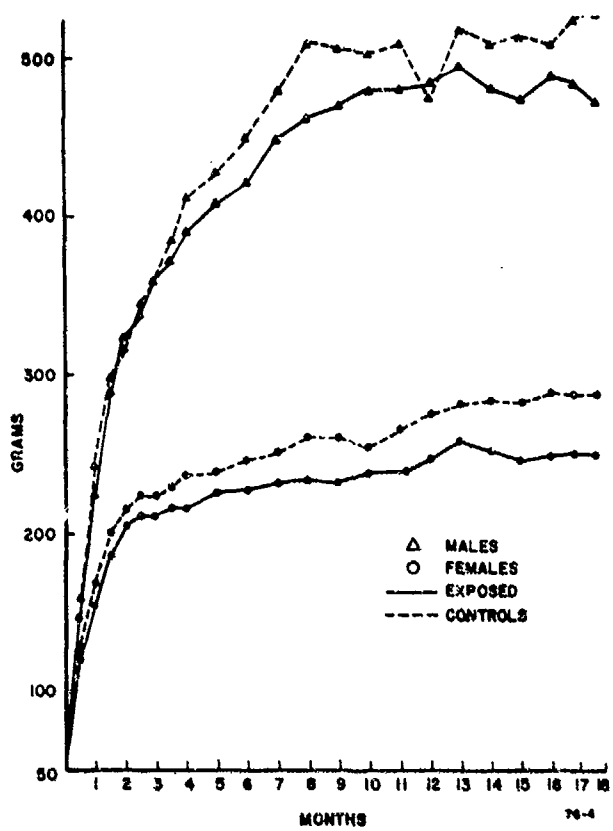


Figure 3. The effect of repeated exposure to 10 mg/m^3 coal tar aerosol on growth of rats.

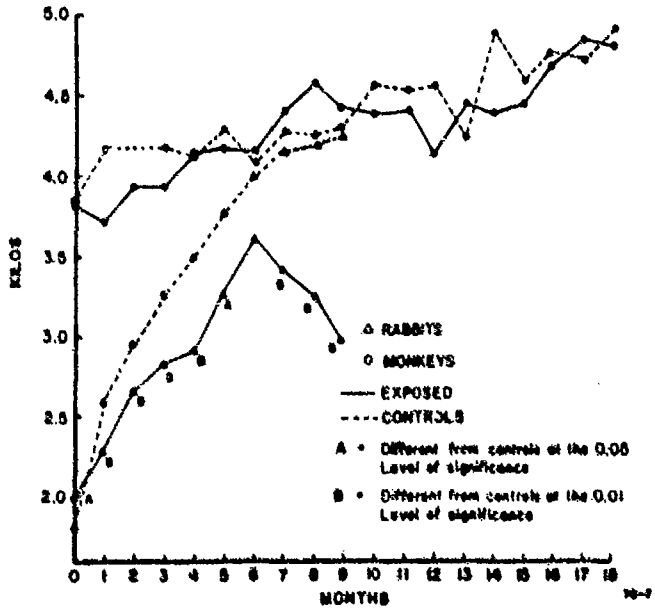


Figure 4. The effect of repeated exposure to 10 mg/m^3 coal tar aerosol on growth of rabbits and monkeys.

Sixteen test and 6 control rabbits died during the exposure period. This mortality has been attributed to a chronic respiratory infection which caused severe debilitation and dehydration. At the conclusion of the study surviving rabbits were removed to the NIOSH laboratories in Cincinnati, Ohio for postexposure observations.

One of the purposes of this industrial exposure type of study was to validate the experimental approach of time compression in the 90-day continuous studies. Incidence of skin tumors in the ICR/CF-1 mice provides one basis for comparison of the two studies. A summary of hide fluorescence and cumulative numbers of skin tumors is found in Table 10.

The animals in the two 10 mg/m^3 studies were exposed to the coal tar aerosol for a comparable number of total exposure hours. In the continuous 90-day study, the hours of exposure were compressed while the similar number of hours of the intermittent study extended over an 18-month time period. The amount of fluorescent compounds found on the hides of the mice of the continuous study is much greater than what was found on the hides of the intermittently exposed mice at any of the examination points. This is due, of course, to the amount of exposure-free time the intermittent group had for grooming each exposure day and on weekends.

TABLE 10. SUMMARY OF HIDE FLUORESCENCE ($\mu g/cm^2$) OF CF-1 MICE DURING AND AFTER EXPOSURE TO COAL TAR AEROSOLS

Aerosol Conc.*	Hours of Exposure												ICR/CF-1 Mouse Skin Tumors at 100 Weeks
	6	42	120	360	648	894	1110	1398	1620	1854	2112	2226	
10 mg/m^3 (Intermittent)	1.4	3.2	4.9	3.8	3.7	2.7	3.5	3.2	2.1	6.7	3.8	6.3	5/75**

	Hours of Exposure					
	24	168	720	1440	2160	
10 mg/m^3 (Continuous)	26.9	34.9	19.2	23.8	21.6	102/225
2 mg/m^3 (Continuous)	2.4	9.3	7.6	4.4	9.9	14/75
0.2 mg/m^3 (Continuous)	0.4	1.6	4.9	1.0	3.8	1/75

*Intermittent study ran 5 days per week, 6 hours pre day, for 18 months. Continuous studies ran 7 days per week, 24 hours per day for 90 days.

**Number of tumors observed/number of animals exposed.

Not unexpectedly, therefore, the skin tumor incidence in the intermittent group is very low, actually falling between the mice continuously exposed to 2 mg/m^3 and 0.2 mg/m^3 at comparable total exposure periods. The amount of hide fluorescence of the intermittently exposed group also lies between the amount found on the hides of the two low level groups, thus reinforcing the dose-effect relationship between hide exposure and tumor incidence. By this criterion, it appears as though continuous exposure to coal tar was a much greater insult to the skin of animals than intermittent exposure.

A similar comparison of the amount of fluorescent compounds found in the lungs of the CF-1 mice from the two studies is shown in Figure 5. Although the amount of retained fluorescent compounds in the mouse lung is considerably higher during the continuous exposure period, the total lung burden during the entire experimental period is similar. When the postexposure holding period is complete, the final analysis may show that the integrated lung burden of coal tar may be slightly higher in the intermittent exposure group. A comparison of the incidence of lung tumors cannot be made until histopathology data is received from the NIOSH pathology contractor.

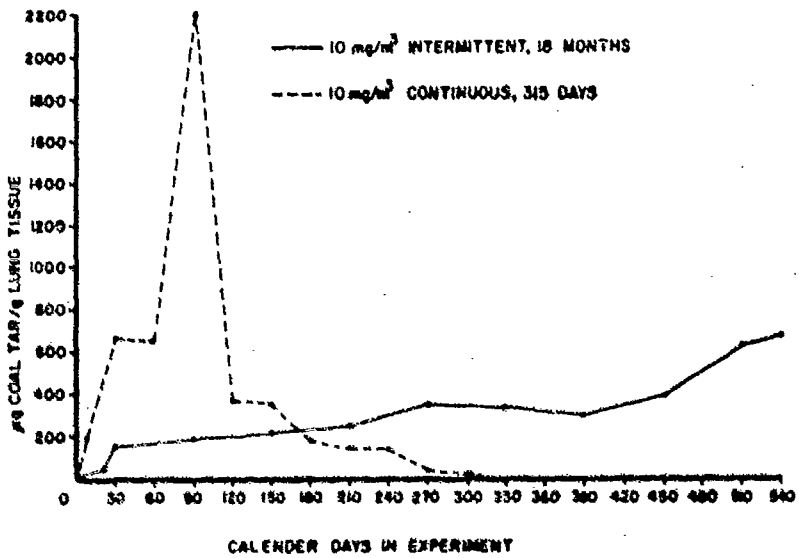


Figure 5. A comparison of total lung burden of fluorescent coal tar compounds between 90-day continuous and 18-month intermittent exposure.

Incomplete results prevent drawing conclusions on the validity of compressed time exposures at this point except in relation to the skin tumor incidence which was increased by continuous exposure.

The most significant finding of this experiment, however, is the evidence that coal tar aerosol mixed with the BTX or light oil fraction collected above coke ovens is carcinogenic in two rodent species. This evidence complements the epidemiologic findings of Lloyd (1971) and of Redmond (1973) concerning the increased incidence of cancer in coke oven workers engaged in activities on the topside of the ovens.

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

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

The details of the experimental rationale and protocol were presented in the last annual report (MacEwen and Vernot, 1975) along with data obtained during the 6-month exposure period of animals to 5 and 0.5 ppm UDMH and through 4 of 6 months planned exposures to 0.05 ppm UDMH. This report covers data accumulated since that time. Currently, all study groups are in the postexposure phase of the experiment. At this writing, animals exposed to 5 ppm or 0.5 ppm are 14-1/2 months postexposure. Animals that received 0.05 ppm are 11-1/2 months postexposure with the exception of the hamsters. Respiratory disease disallowed use of the first group received from the vendor, but the second group was healthy and began exposure 2 months later.

Significant exposure effects of 0.5 and 5 ppm UDMH were limited to slight to moderate but transitory hepatotoxicity in dogs exposed to the 5 ppm concentration. Dogs exposed to 5 ppm UDMH on a 6 hour/day, 5 day/week schedule developed significantly elevated serum glutamic pyruvic transaminase (SGPT) levels by the fourth week of exposure. At 6 weeks, the mean SGPT value for the exposed dogs was 3 times the control level. Throughout the remaining 20 weeks of exposure, SGPT values for the exposed dogs (Table 11) were stable at levels 3-4 times those of the control group. A trend to recovery, approximately 50% reduction, was seen in measurements made 2 weeks postexposure. Subsequent values

TABLE 11. EFFECT OF 6-MONTH INHALATION EXPOSURE TO 5 PPM UDMH ON SERUM GLUTAMIC PYRUVIC TRANSAMINASE LEVELS IN DOGS
[Group Mean Values (N = 8)]

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

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

at 4, 8 and 11 weeks postexposure showed no further reductions. However, when the dogs were sampled again (at Brooks AFB where they are being maintained) at 27 and 47 weeks postexposure, SGPT values were completely normal when compared with control animal values.

Liver function tests were performed on dogs at exposure termination and a 4, 8, 11 and 38 weeks postexposure. Bromsulphalein (BSP) measured in the blood of the 5 ppm exposed dogs 10 minutes following a 10 mg/kg injection showed significant retention at exposure termination, 4 and 8 weeks postexposure. Although the mean BSP retention values for the exposed dogs at 4 weeks postexposure indicated no trend to recovery, an examination of individual values revealed 10-25% reduction in values for 6 of 8 dogs. As seen in Table 12, recovery occurred at 11 weeks postexposure. BSP measurements made at Brooks AFB 38 weeks postexposure show no abnormal values for the exposed dogs. Their values for control and exposed dogs are noticeably less than ours, and probably represent differences in the BSP test method.

TABLE 12. MEAN BROMSULPHALEIN RETENTION VALUES*
IN CONTROL AND 5 PPM UDMH EXPOSED DOGS

Time	Control	5 ppm
Exposure Termination		
26 Weeks	18.1	30.3**
Weeks Postexposure		
4 Weeks	20.7	29.5**
8 Weeks	13.8	30.0**
11 Weeks	18.0	21.8
38[1] Weeks	11.4	12.3

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

The numbers of animals that died during the 6 months of exposure to 5 ppm and 0.5 ppm UDMH are shown in Table 13. This table was shown in the 1975 annual report and cause of death in mouse and hamster groups was discussed. In no case was death attributed to UDMH exposure. Mortality ratios at 12 months postexposure are presented in Table 14. The numbers include animals that died during the exposure phase of the study.

TABLE 13. MORTALITY RATIOS IN CONTROL AND UDMH
EXPOSED ANIMALS AT EXPOSURE TERMINATION

Experimental Group	Dogs	Rats	Mice	Hamsters
Control	0/8	0/200	7/400	13/200
0.5 ppm	0/8	0/200	6/400	24/200
5 ppm	0/8	1/200	8/400	22/200

TABLE 14. MORTALITY RATIOS IN CONTROL AND UDMH EXPOSED ANIMALS AT 12 MONTHS POSTEXPOSURE

Experimental Group	Dogs	Rats	Mice	Hamsters
Control	0/8	54/200	50/400	190/200
0.5 ppm	0/8	14/200	58/400	181/200
5 ppm	0/8	9/200	58/400	183/200

The mortality figures for all hamster groups are quite high in comparison to those for the rats and mice. This is due to their shorter life span. The aging process was reflected in the pathology findings. Fluid filled hepatic cysts were found in almost every animal, but the common cause of death in hamsters from all groups in this study was renal insufficiency due to severe amyloidosis. The small number of surviving hamsters prompted their sacrifice at 12-1/2 months post-exposure. Gross pathology results were the same as seen in hamsters that died postexposure.

Table 14 also shows a disproportionately large number of control rat deaths relative to the exposed groups. Approximately 40 control rats died from an epizootic respiratory infection which occurred in an animal holding facility 3 months after the exposure phase of the study. Fortunately all other experimental animals were maintained in a different location. Deaths in groups of exposed and control mice are nearly the same; therefore, no toxicological significance is attributed to mortality in exposed

mice. At 12 months postexposure the mice were approximately 20 months of age. Mortality ranged from 12.5% in the control group to 14.5% in both exposed groups which probably represents natural attrition. Complete histopathology on all rodents that died postexposure is not available at this time.

As mentioned previously, the 6-month exposure of animals to 0.05 ppm UDMH was completed during 1975 and the dogs, rats and mice are 11-1/2 months postexposure, while the hamsters are 9-1/2 months postexposure.

Hepatotoxicity as measured by SGPT levels was not demonstrated in the 0.05 ppm UDMH dog exposure group. All clinical blood measurements used in the 5 ppm and 0.5 ppm study on the same time schedule were normal and showed no trends to adverse effect. Exposed and control dogs are being maintained at Brooks AFB. Hematology, growth and clinical chemistry values remained normal when determinations were made at 21 and 34 weeks postexposure.

Mean body weights of dogs and mice were comparable to their controls throughout the exposure phase of the study and into the postexposure phase thus far. Weights of exposed hamsters and rats are statistically

lower than controls throughout the 6-month exposure and have continued to lag behind control values during the postexposure observation period.

Very few deaths occurred in rats, mice and hamsters, and none in dogs during the 6 months of exposure to 0.05 ppm UDMH. Deaths that did occur were unrelated to the UDMH exposure. Table 15 shows current mortality ratios.

TABLE 15. POSTEXPOSURE* MORTALITY RATIOS IN CONTROL AND UDMH EXPOSED ANIMALS

Experimental Group	Dogs	Rats	Mice	Hamsters
Control	0/8	8/200	66/400	31/200
0.05 ppm	0/8	7/200	31/400	69/200

* Dogs, rats and mice 9-1/2 months postexposure.
Hamsters 7-1/2 months postexposure.

Cause of death in exposed and control hamsters was largely due to manifestation of aging in this species as discussed in the results of exposure to 5 ppm and 0.5 ppm UDMH. The malfunction of a new automatic watering system caused the drowning of 25 of the exposed hamster group and 29 control mice housed at the Vivarium.

Significant exposure effects of the UDMH concentration levels used in this study were limited to reversible hepatotoxicity in dogs exposed to the 5 ppm level. BSP retention values were normal at 11 weeks postexposure.

Elevated SGPT values showed 50% reductions by 2 weeks postexposure and complete recovery by 27 weeks. On the basis of the tests and measurements used in this study, the current TLV of 0.5 ppm UDMH appears to be well chosen without consideration of the oncogenic capacity of this compound. Tumor incidence will be assessed during the lifetime observation and testing of the rodents and dogs.

The rats and mice from this study will undergo definitive histopathologic examinations in our laboratory while hamsters will only receive preliminary examination for cause of death and presence of neoplasia. The definitive workup of tissue from the hamsters will be done by the USAF School of Aerospace Medicine/VSP, Brooks AFB, Texas. Thirty separate tissues will be regularly examined, along with any tumor masses in other tissues, following the suggested standard protocol for cancer screening studies of the National Cancer Institute.

Histopathology data collected thus far reveals some tumorigenesis in UDMH exposed rats, mice and hamsters. However, tumors have also been seen in the control animals. Insufficient data at this time precludes firm comparisons of tumor incidence in the exposed and control animals. Nevertheless, a few general comments can be made. The presence of hematopoietic tumors, including lymphopoietic and reticulum cell sarcomas,

is common in aged mice, and these types appear to dominate in this study. Tumors seen thus far in the exposed rats are the common high incidence neoplasms of aged rats. The presence of a high percentage of hematopoietic and reticuloendothelial tumors in both control and exposed hamsters was unexpected since these tumors are thought to be uncommon in this species, unlike adrenal tumors which are commonplace in aged hamsters. This study is being continued.

Feroral Toxicity of Dimethylnitrosamine

Dimethylnitrosamine (DMNA), a well-known carcinogen, was found to be a trace impurity in commercial UDMH, and was found to be present at a 0.12% concentration in the supply of UDMH used in the oncogenic studies of that chemical conducted at the THRU. At the time the UDMH experiments were initiated, we were not concerned with this trace amount of DMNA as a contaminant since it was ubiquitous in any sample of UDMH having been an intermediary in the manufacturing process. We had calculated that this amount of contamination of the UDMH would, at worst, result in a 6 ppb concentration of DMNA in the highest UDMH exposure concentration. We did not think that this level of DMNA could cause any effects.

We became concerned with the possible effect of DMNA on the experimental results of the UDMH exposures when the dogs showed evidence of mild hepatotoxicity, a finding that had not been previously reported from acute or subchronic exposures of animals to UDMH.

There was adequate evidence of the hepatotoxicity of DMNA but all published data were from exposures to grossly higher concentrations (or doses several orders of magnitude greater) and no dose response was available for extrapolation to the probable DMNA contamination levels experienced in our studies. In order to obtain some information on the possible hepatotoxicity of DMNA at very low doses, a study of its oral toxicity to mice was performed.

Male mice (CF-1, 15 to 20 grams, Harlan Industries, Inc.) received repeated oral doses of various concentrations of DMNA each working day over a period of 28 calendar days. Mice at the two lowest concentrations and controls continued to be dosed through 60 calendar days. The doses given were the oral equivalents of calculated inhalation concentrations expected to result from 6-hour exposures to vapors of DMNA. The oral doses used in this study along with the equivalent inhalation concentrations are listed in Table 16.

TABLE 16. COMPARISON OF DAILY ORAL DOSES OF DMNA GIVEN TO MICE WITH THE CALCULATED THEORETICAL EQUIVALENT 6-HOUR INHALATION CONCENTRATION

Oral Dose	Equivalent	Mice per Group
5 μg/kg	5 ppb	55
10 μg/kg	10 ppb	55
50 μg/kg	50 ppb	35
500 μg/kg	500 ppb	35
1 mg/kg	1000 ppb	35
5 mg/kg	5000 ppb	35
controls	0 ppb	55

The compound was administered as a solution in distilled water. Glass syringes with special oral dosing needles were used to administer the compound to the mice. The nonfasted mice were weighed daily, prior to dosing, to determine the proper injection volume. Control mice received a daily peroral injection of distilled water.

Five mice from each group (including controls) were sacrificed after 7, 9, 14, 21 and 28 calendar days. At the four highest dose levels, all survivors were sacrificed after 28 days. The two lowest dose levels and controls continued to receive daily doses through 60 calendar days at which time all survivors were sacrificed.

The livers were removed from all mice that died or were sacrificed. The liver tissue was processed and prepared for histopathologic examination. The remaining mouse carcass was labeled and stored in formalin for possible future evaluation.

Very few deaths occurred during the dosing regimen. Most deaths were caused by extraneous infections and were unrelated to the toxic action of the contaminant or trauma induced by the dosing procedure.

Histopathological examination of the mice that were sacrificed at the prescribed time periods revealed some fatty change in the livers of mice receiving doses of 50 micrograms/kg or higher. Table 17 gives a summary of the histological results. The mice from the two low dose levels show varying degrees of hepatocyte vacuolation in 73 to 79% of the mice examined with increased fat deposition. However, the control group also shows hepatocyte vacuolation in 64% with minimal fatty changes in about 30-40% of the mice which indicates that the small increase over control levels in these two low dose groups may be insignificant. The incidence of hepatocyte vacuolation in all other dose groups is 90% or greater, is dosage related, and most likely biologically significant. The livers of the mice at the highest dose level also had a high incidence (94%) of hepatocellular necrosis, a definite indication of liver tissue damage. There also appeared to be a dose related increase in fat accumulation of the treated animals.

Liver sections of these animals are in the preparation stage for electron microscopy analyses. This examination should be able to determine

the subcellular effects of DMNA on the livers of all the animals but, more particularly, determine whether or not the livers of the mice at the two lower dose levels were affected.

TABLE 17. LIVER CHANGES IN MICE AFTER REPEATED ORAL EXPOSURE TO DIMETHYLNITROSAMINE

Contaminant Concentration	Effects Observed
Controls	Varying degrees of hepatocyte vacuolation in 66% of mice.
5 micrograms/kg	Varying degrees of hepatocyte vacuolation in 79% of mice.
10 micrograms/kg	Varying degrees of hepatocyte vacuolation in 90% of mice.
50 micrograms/kg	Varying degrees of hepatocyte vacuolation in 100% of mice.
500 micrograms/kg	Varying degrees of hepatocyte vacuolation in 92% of mice.
1 mg/kg	Varying degrees of hepatocyte vacuolation in 97% of mice.
5 mg/kg	Varying degrees of hepatocyte vacuolation in 97% of mice. Hepatocellular necrosis in 94% of mice.

Hepatotoxic Response in Dogs to the Inhalation of Trace Amounts of Dimethylnitrosamine (DMNA) in 1, 1-Dimethylhydrazine (UDMH)

The finding of significantly elevated serum glutamic pyruvic transaminase (SGPT) levels in dogs exposed to 5 ppm UDMH on a 6 hour/day, 5 day/week regimen, as previously reported, was surprising since UDMH had not been shown to be hepatotoxic by earlier investigators conducting acute and subchronic toxicity studies. The corroborative finding of moderately but significantly elevated bromsulphalein (BSP) retention values in the dogs was also strong evidence of hepatotoxicity.

The effect was shown to be reversible when the dogs were removed from the exposure and placed in clean air. BSP values returned to control levels within 3 months and the enzyme measurement of SGPT was within normal limits at 27 weeks postexposure.

Since the UDMH used in the oncogenic studies contained approximately 0.12% DMNA, a known liver toxin, we initiated a series of short studies to clarify this matter. The first of these was the peroral dosing of mice with graded doses of DMNA previously described and the second study consisted of exposing four dogs (2 male and 2 female) 6 hours daily 5 days/week to a 5 ppm concentration of UDMH which

had been redistilled and which was, by the best analytical techniques available, free from any DMNA. A similar group of 2 male and 2 female dogs served as controls for comparative blood tests and were sham exposed in an adjacent Rochester inhalation exposure chamber. Both chambers were operated with nominal airflows of 30 cfm at a slightly reduced pressure to prevent leakage of the test chemical into the laboratory atmosphere. The analysis of chamber contaminant concentration was continuous, utilizing an AutoAnalyzer colorimetric technique which was the same method used for monitoring UDMH in the 6-month inhalation study mentioned previously. Blood samples were drawn from all dogs at biweekly intervals or more often and clinical determinations made for the following battery of tests:

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

Bromsulphalein (BSP) retention times were determined at the beginning and end of the study. Baseline measurements were available for all dogs for at least 2 months prior to this study, thus insuring selection of healthy animals with stable blood parameters.

During the course of the experiments, liver wedge biopsies were taken from all dogs for pathologic examination. At the conclusion, all dogs were sacrificed and major organs submitted for gross and histopathology.

In the first and second phases of this study, the test dogs were exposed to 5 ppm UDMH determined to be free of DMNA by mass spectrometric analysis. In the final phase, 2 dogs that served as controls in the first phases were exposed to a mixture of 0.12% DMNA in UDMH which gave an air concentration of an estimated 6 ppb DMNA and essentially 5 ppm measured UDMH.

The first exposure phase was conducted for 8-1/2 weeks on a 6 hour/day schedule omitting weekends and holidays. This was a duplication of the exposure regimen used in the 6-month study. As seen in Table 18, there were no significant changes in mean SGPT values for the exposed dogs and all other clinical chemistry determinations were normal

TABLE 18. MEAN SGPT VALUES OF DOGS EXPOSED TO 5 PPM UDMH[1] AND CONTROLS

Sample Period	Mean SGPT Value (International Units)	
	Exposed[2]	Control[2]
Preexposure		
2 Months	36.8	30.0
1 Month	25.8	24.0
1 Week	31.5	30.0
Intermittent Exposure		
2 Weeks	37.8	32.8
4 Weeks	37.8	34.0
6 Weeks	32.0	29.0
8-1/2 Weeks	36.8	32.0
Before Continuous Exposure	48.9	45.1
7 Days Continuous Exposure	41.5	37.5
13 Days Continuous Exposure	44.8	30.1

[1] Free of DMNA by mass spectrometric analysis.

[2] N = 4

when compared to control values. To examine the possibility that 5 ppm UDMH (free of DMNA) may have caused liver changes not revealed by SGPT measurements, liver wedge biopsies were taken from all exposed and control dogs for pathologic examination at the conclusion of 8-1/2 weeks of exposure. Histopathologic differences between exposed and control tissues were marginal, but it appeared that cytoplasmic degenerative changes in liver cord cells might be slightly greater and more frequent in the UDMH exposed animals. There was also a modest increase in the amount of yellow-brown granular material seen accumulated in Kupffer's cells of exposed dog tissue.

The dogs were rested 5 days following surgery after which continuous exposure to 5 ppm UDMH (free of DMNA) was begun to see whether this would cause SGPT changes in exposed animals. Results of SGPT determinations made after 7 days of exposure and at the conclusion of the second phase, 13 days, are shown in Table 18. The data collected in this study clearly illustrate that neither intermittent exposure for 8-1/2 weeks nor continuous exposure for 13 days to 5 ppm UDMH (free of DMNA) has any hepatotoxic effect in dogs.

In the final test phase, the female control dogs were placed in the exposure chamber and the female exposed dogs were placed in the control chamber. This interchange was made to eliminate any possibility of sensitization of the dogs by exposure to UDMH before this final experiment began. The experiment was started within a few hours after the cessation of the previous one and the dogs were exposed for 16 days continuously to the mixture of UDMH and DMNA. As seen in Table 19, there were significant SGPT elevations in blood samples taken from exposed dogs after 10 and 16 days. Noticeable is the 25% increase in mean SGPT values from 10 through 16 days of exposure. BSP determinations made on all dogs at exposure termination showed no significant differences between exposed and control mean values, nor any trend toward elevations in individual values of the 2 exposed dogs. This study was concluded and all dogs were sacrificed and submitted for gross and histopathologic examination. Histopathology results were the same for exposed and control dogs. Hepatocytes were relatively uniformly pale and swollen although this alteration was slightly more prominent in the periacinar regions. The cytoplasm contained many eosinophilic granules as well as some yellow-brown pigment granule. The latter were also noted in Kupffer cells. Several nonspecific eosinophilic intranuclear inclusions were also seen. The exposure to DMNA was sufficient to produce enzyme changes but apparently not quite severe enough to cause visible cellular injury.

It appears from the results of these experiments that DMNA was the active agent producing increased SGPT levels but that the level tested was insufficient to cause discernible hepatocellular changes or alterations in liver function as measured by BSP retention.

Since DMNA is a known potent hepatotoxin and proven carcinogen in animals, the results of the 6-month UDMH (containing DMNA) chronic inhalation exposure and the subsequent assessment of oncogenicity during the animals lifespan should be considered carefully to avoid unwarranted incrimination of UDMH.

TABLE 19. MEAN SGPT VALUES OF DOGS CONTINUOUSLY EXPOSED TO 5 PPM UDMH WITH 0.12% DMNA[1] AND CONTROLS

Sample Period	Mean SGPT Values (International Units)	
Days of Exposure	Exposed[2]	Control[2]
10 days	68.8**	42.3
16 days	85.8**	37.5

(1) Determined by mass spectrometric analysis.

(2) N = 4.

** Higher than control mean value at the 0.05 level of significance.

Toxicity of Solid Rocket Motor Exhaust - Effects of HCl, HF and Alumina on Rodents

The firing of certain solid fuel rocket engines has been shown to result in the emission of large quantities of hydrogen chloride (HCl), hydrogen fluoride (HF) and submicron sized particles of aluminum oxide (alumina). The possible exposure of Air Force personnel and local residents to these combustion products prompted a rodent lethality study to determine the degree of hazard posed by such exposures. Although much acute toxicity data on HCl and HF has been generated in this laboratory, no information existed on effects resulting from simultaneous exposure to these two compounds plus alumina particles.

Three series of 60-minute inhalation exposures were performed in an attempt to classify the toxicity to rats and mice of combinations of the three materials. First, single contaminant exposures were undertaken to determine the dose-response relationship of HCl and that of HF. Second, concurrent exposures to both HCl and HF were performed to discover if they act in an additive, less than additive, or more than additive manner as indicated by rodent mortality. The third series of experiments comprised the duplication of exposure concentrations and conditions of selected concurrent HF and HCl exposures with the addition of high concentrations of alumina dust. Any interaction of the alumina to cause an alteration in toxicity would be indicated by greater or lesser animal mortality.

Test animals consisted of groups of ten male CFE (Sprague-Dawley derived) rats weighing between 250 and 325 grams and groups of 10 CF-1 (ICR derived) mice with weights ranging from 25 to 32 grams.

Animals were observed for toxic signs and mortality during the exposure and for a period of 14 days postexposure. A representative number of test animals that died following treatment or were sacrificed after the 14-day observation period were submitted for gross and histopathologic examination.

The exposure chamber was a modified Longley type utilizing a sliding cage drawer to permit rapid insertion and withdrawal of test animals from the contaminant equilibrated chamber. Partially dried air with a relative humidity of approximately 23% was metered at a rate of 11 cfm to one quadrant of chamber having a volume of 22.1 cubic feet.

Hydrogen fluoride vapor was supplied to the exposure chamber from steel tanks of the pure liquid material (Matheson Gas Products) or from gas cylinders of a 1% concentration HF in dried nitrogen. Metering of the contaminant was accomplished with a Teflon® flowmeter (Mace 16032) or a Hastings Raydist Mass Flowmeter (Model LF-20K).

A glass flowmeter was used to meter HCl vapors from a steel cylinder of the pure liquid material (Matheson Gas Products).

Anhydrous aluminum oxide powder of the gamma form with an upper particle size limit of 1.4 micron was acquired from a commercial source (Research Organic/Inorganic Chemical Corporation). Delivery of the alumina particles was accomplished with a modified fluidizing dust generator (Figure 6) of the type described by Drew and Laskin (1971). Modifications consisted of a more powerful fan motor and the pressurization of the fluidizing chamber. This enabled the generation of much higher dust concentrations for longer periods than possible with the unmodified device.

Continuous analyses of HF and HCl concentrations in the exposure chamber were provided by utilizing specific ion electrodes. Known volumes of chamber atmosphere were mixed in a gas scrubber column with known amounts of an aqueous reagent absorber. The solution was then passed through a flow cell containing the ion and reference electrodes. Calibration of the electrodes was done prior to every exposure.

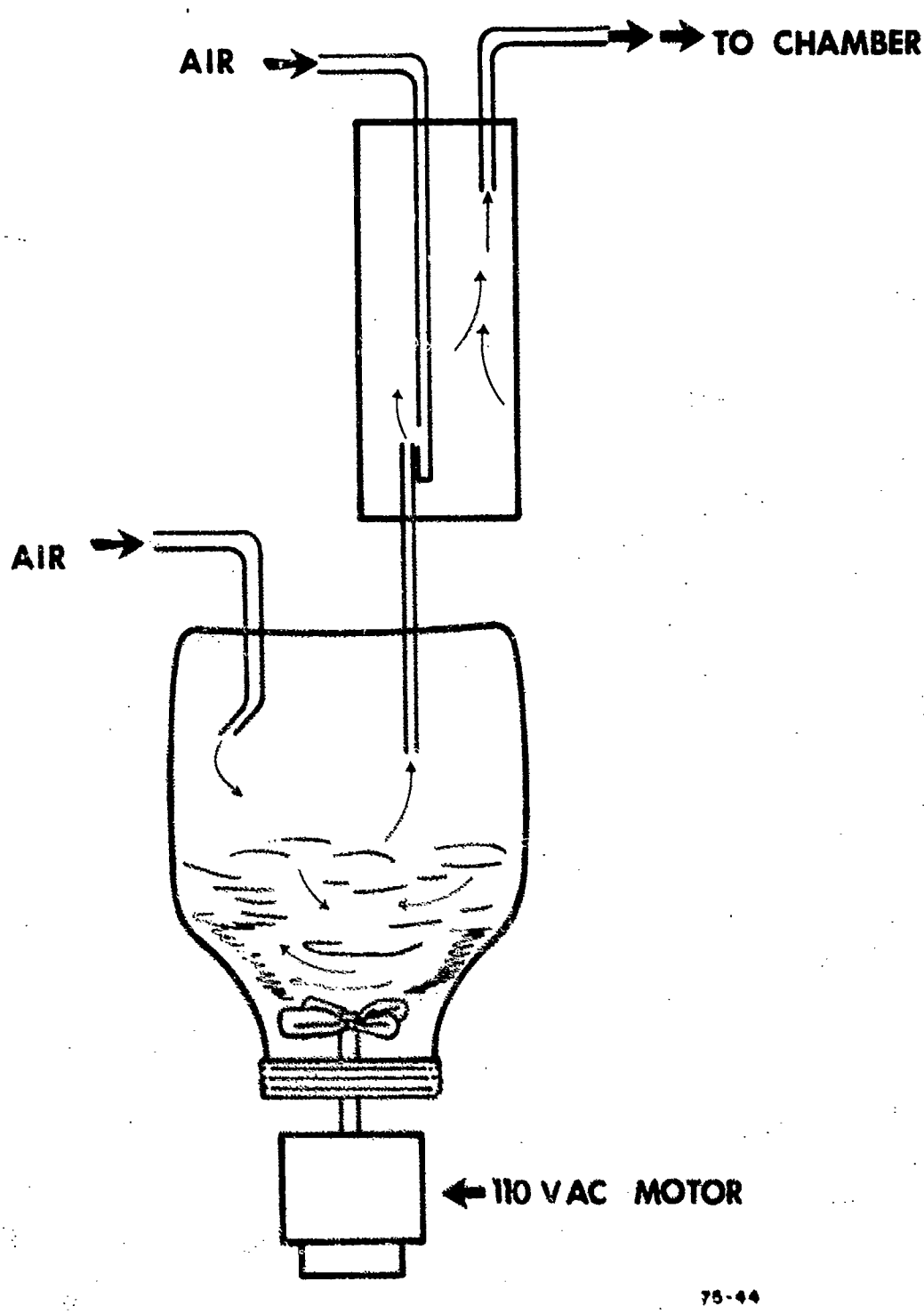


Figure 6. Fluidizing duct generator used for aerosolization of alumina particles.

The analysis of alumina exposure concentrations was performed by a gravimetric method. Samples of the chamber atmosphere were drawn at a rate of 10 liters per minute through a sample head (Gelman Model 1220) containing two membrane filters having a pore size of 0.45 and 0.2 micron (Gelman, 47 mm, type GA-6 and type GA-8). Alumina concentrations, expressed in milligrams per cubic meter, were determined 12 times for each one hour exposure.

Statistical analysis of mortality data was accomplished using the BMD03S Biomedical Computer Program, Biological Assay - Probit Analysis Method.

The 60-minute LC_{50} values for rats and mice exposed to HCl vapors alone are 3124 ppm and 1108 ppm, respectively. Mortality response data for individual exposures are shown in Table 20.

Toxic signs noted during exposure to HCl included increased grooming, irritation of eyes, mucous membranes and exposed skin. A rapid, shallow breathing pattern and fur discoloration to a yellow-green were noted by the end of 60 minutes.

TABLE 20. MORTALITY RESPONSE OF RATS AND MICE EXPOSED TO HCl VAPORS FOR 60 MINUTES

Rats		Mice	
Concentration (ppm)	Mortality Ratio	Concentration (ppm)	Mortality Ratio
1813	0/10	557	2/10
2585	2/10	985	3/10
3274	6/10	1387	6/10
3941	8/10	1902	8/10
4455	10/10	2476	10/10
LC_{50} and 95% C. L. = 3124 ppm (2829-3450)		LC_{50} and 95% C. L. = 1108 ppm (874-1404)	

Necropsy of animals dying during or after exposure revealed pulmonary congestion and intestinal hemorrhages in both rats and mice, with rats also exhibiting thymic hemorrhages.

Mortality data for rats and mice exposed to various concentrations of HF vapors alone are shown in Table 21. LC_{50} concentrations were 1395 ppm for rats and 342 ppm for mice.

TABLE 21. MORTALITY RESPONSE OF RATS AND MICE EXPOSED TO HF VAPORS FOR 60 MINUTES

Rats		Mice	
Concentration (ppm)	Mortality Ratio	Concentration (ppm)	Mortality Ratio
1087	0/10	263	0/10
1108	2/10	278	1/10
1405	3/10	324	7/10
1565	8/10	381	6/10
1765	10/10	458	9/10
LC_{50} and 95% C. L. = 1395 ppm (1302-1495)		LC_{50} and 95% C. L. = 342 ppm (315-372)	

Symptoms of rats and mice during exposure included eye and mucous membrane irritation, respiratory distress, corneal opacity and erythema of exposed skin.

Pathologic examination of rats dying during or after exposure showed pulmonary congestion, intraalveolar edema and some cases of thymic hemorrhage. Mice exhibited pulmonary congestion and hemorrhage.

The purpose of the next series of experiments was to determine if any toxic interactions exist from simultaneous exposures to HCl and HF. By defining a dose response relationship, it can be shown whether the combination results in additive, less than additive, or more than additive effects. The mortality response of rats obtained from simultaneous exposure to various concentrations of HCl and HF is shown in Table 22.

TABLE 22. MORTALITY RESPONSE OF RATS EXPOSED FOR 60 MINUTES TO COMBINATIONS OF HCl AND HF VAPORS

HCl, ppm	HF, ppm	Mortality
1292	493	80%
1366	580	50%
1421	610	30%
1564	570	40%
1505	640	10%
1685	786	60%
1845	823	80%

Figure 7 is a graphic representation of the mortality response of rats obtained from exposure to HCl and HF singly and in combination. The line marked HCl is the probit regression line obtained from plotting percent mortality (converted to probits) versus the log of the average concentration during the exposures to HCl alone. The line marked HF is the probit regression line for the HF only exposures. The equations of the probit regression lines were obtained from the computer printouts during statistical analysis and are as follows for rats:

$$\text{Probit response} = 4.9\ (\ln \text{ of HCl conc.}) - 34.426 \quad (1)$$

$$\text{Probit response} = 6.481\ (\ln \text{ of HF conc.}) - 41.928 \quad (2)$$

Analysis of the simultaneous exposure data was performed by the use of these equations to convert HF concentrations to the equivalent HCl concentration necessary to produce the same mortality response. The addition of the actual HCl concentration plus the concentration of HF expressed as its equivalent HCl concentration equals a total effective HCl concentration. For example, a concentration of 786 ppm HF and 1685 ppm HCl was achieved during a simultaneous exposure and produced 60% mortality in rats. The probit response for an exposure of rats to HF alone was calculated by inserting the log of 786 into equation 2. The obtained probit response (1.2803) was then substituted into the HCl equation and the equivalent concentration of HCl was calculated to be 1460 ppm.

The point plotted from the total 3145 ppm (1685 ppm + 1460 ppm) giving a 60% mortality plus the other points derived from the above method are represented by circles in Figure 7.

Note that the points are distributed along and slightly below the probit regression line for HCl. This indicates that the effects of HCl and HF are almost entirely additive or more than additive effects respectively. Additivity is not surprising considering the similar chemical nature and target organs of the two compounds.

Table 23 shows the mortality response of mice exposed to HCl and HF concurrently.

TABLE 23. MORTALITY RESPONSE OF MICE EXPOSED FOR 60 MINUTES TO COMBINATIONS OF HCl AND HF VAPORS

HCl, ppm	HF, ppm	Mortality
555	169	20%
816	197	20%
816	197	20%
547	230	60%
808	267	40%
875	284	20%
911	290	70%
1090	332	80%

Figure 8 depicts graphically the dose response data for mice exposed to HCl and HF singly and in combination.

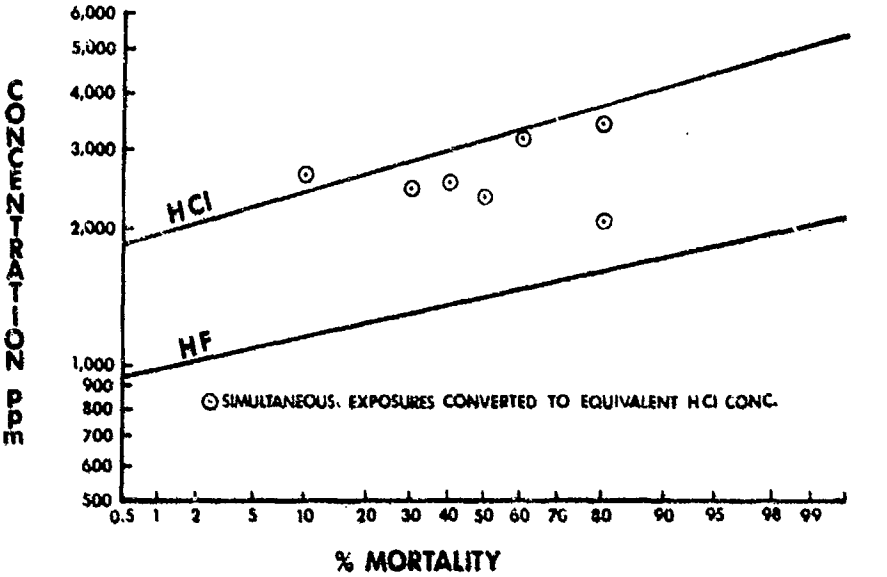


Figure 7. HCl and HF probit regression lines for rats.

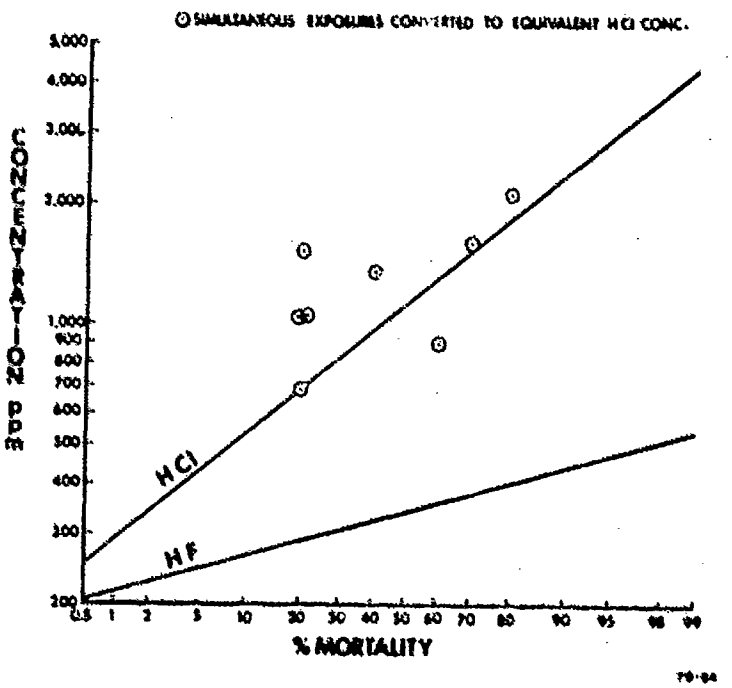


Figure 8. HCl and HF probit regression lines for mice.

The equations of the probit regression lines for mice are as follows:

$$\text{Probit response} = 1.737\ (\ln \text{ of HCl conc.}) - 7.177 \quad (3)$$

$$\text{Probit response} = 5.075\ (\ln \text{ of HF conc.}) - 24.618 \quad (4)$$

As in the rat data, the circles in Figure 8 are plots of the total equivalent HCl concentration versus mortality during simultaneous exposures. Again, the points are distributed along the HCl probit regression line. Therefore, additivity of effect of HCl and HF for mice is also indicated.

Gross and histopathologic examination of rats and mice exposed to combinations of HCl and HF revealed no additional sites of damage from those seen of single exposures.

At this stage of investigation, an exposure of 10 rats and 10 mice to an average 478 mg/m^3 of alumina dust for 60 minutes was performed. Symptomatology included vigorous grooming during the first quarter of the exposure, followed by signs of mechanical irritation to the eyes and nasal passages as indicated by half closed eyes and sneezing-like activity. No toxic effects were apparent immediately postexposure, nor in the 14-days following. All animals survived and had normal weight gains. Gross and histopathologic examination at the end of the postexposure observation period showed normal tissue with no changes attributable to the exposure.

Spectrographic analysis of lung tissue from control animals and from rats and mice sacrificed immediately after the exposure to alumina dust revealed that a significant amount of alumina had been deposited in the lungs of exposed animals.

The last series of exposures investigated the change in toxicity, if any, produced by the addition of high concentrations of alumina dust to an atmosphere containing HCl and HF gases. Although HCl and HF act primarily as pulmonary irritants and mainly affect upper respiratory tissue, it is possible that the gases could be adsorbed onto the alumina particles and carried into the more inaccessible parts of the lung.

An apparent positive or negative effect of alumina to produce rat mortality greater or lesser than seen in the duplicate exposure without alumina is represented by a plus or minus sign in Table 24. The same information for mice is shown in Table 25.

Each exposure pair was conducted on the same day using animals from the same lot, with contaminant and analysis chemicals from the same batch.

TABLE 24. MORTALITY RESPONSE OF RATS EXPOSED TO HF, HCl AND ALUMINA FOR 60 MINUTES

HCl Conc. ppm	HF Conc. ppm	Al_2O_3 Conc. mg/m^3	Mortality	Apparent Alumina Effect
875	284	-	0	
911	290	232	0	
1421	610	-	30%	
1366	580	505	50%	+
1564	570	-	40%	
1505	640	538	10%	-
1845	823	-	80%	
1685	786	610	60%	-

TABLE 25. MORTALITY RESPONSE OF MICE EXPOSED TO HF, HCl, AND ALUMINA FOR 60 MINUTES

HCl Conc. ppm	HF Conc. ppm	Al_2O_3 Conc. mg/m^3	Mortality	Apparent Alumina Effect
375	151	-	0	
428	147	121	0	
555	169	-	20%	
541	175	128	0	-
816	197	-	20%	
816	197	156	20%	0
875	284	-	20%	
911	290	232	70%	+
1564	570	-	100%	
1505	640	538	100%	

Gross and histopathologic examination of animals exposed to all three materials showed the same type of damage as exposure to HCl and HF combinations.

Our results indicate that the addition of alumina dust to HCl and HF combination exposures had no net effect on rodent mortality.

In summary, the purpose of this investigation was to determine the degree of hazard posed by simultaneous acute inhalation of HCl, HF and alumina dust. LC_{50} concentrations for rats and mice were determined for 60-minute exposures to HCl alone and to HF alone. Combination exposures to various concentrations of HCl and HF vapors provided information that could be analyzed by probit analysis techniques. It was shown in exposures of rodents to both corrosive gases simultaneously that the mortalities produced were due to a physiologically additive effect. That is, one compound did not potentiate or antagonize the effects of the other. The addition of alumina dust to atmospheres containing HCl and HF vapors did not increase or decrease rodent mortality.

Toxicity of High Density Jet Fuel Components

A new aircraft fuel has been developed for extending the flight range before refueling. The fuel designated JP-9 is a mixture of three primary ingredients, namely RJ-4, RJ-5, and methylcyclohexane. RJ-4 and RJ-5 are high density hydrocarbons yielding a greater BTU output per unit volume than conventional jet aircraft fuels. They also have a higher viscosity which causes pumping or flow problems at low temperatures which is the reason for the addition of methylcyclohexane to the mixture. The precise composition of the JP-9 fuel is not fixed but will be tailored for use in specific aircraft systems. Although no toxicity data are available for JP-9 fuel, it is not meaningful to evaluate the entire mixture for two reasons: first, the actual mixture has not been set, and second, methylcyclohexane is extremely volatile in comparison with the other constituents and would dominate the vapor exposure mixture, thus masking the effects of RJ-4 and RJ-5.

The acute and chronic toxicity studies on methylcyclohexane have been reported by Treon et al. (1943). Acute exposures of rabbits to inhaled concentrations of methylcyclohexane above 10,000 ppm (≈ 40 mg/liter) caused significant weight loss, narcosis and convulsions while a concentration of 15,227 ppm was fatal in slightly over one hour.

Repeated daily, 5-hour exposures of rabbits to concentrations of 1162 ppm or lower for periods up to 10 weeks produced no measurable or observable signs of toxicity.

Some of the physical chemical properties of RJ-4, RJ-5 and methylcyclohexane are shown in Table 26. RJ-4 is a mixture of isomers of perhydromethylcyclopentadiene. RJ-5, also known as "Shelldyne H", is a mixture of reduced dimers of bicycloheptadiene. Noticeable in Table 26 are the very low vapor pressures of RJ-4 and RJ-5 relative to that for methylcyclohexane. The vapor pressures of RJ-4 and RJ-5 are approximately 100 and 1500 times less, respectively, than methylcyclohexane. This information is basic to the consideration of the hazard (the probability of injury in use) of these fuels.

To examine the acute inhalation hazard and to obtain experimentally determined saturation concentrations as an aid in the selection of vapor levels for the chronic study, groups of six rats each were exposed for 6 hours to essentially saturated vapors of each compound. No adverse effects were seen during exposure. Pathologic examination after 14-day postexposure observation showed no abnormalities. Peroral doses of 4 g/kg RJ-5 in corn oil were not lethal to a group of 3 rats; however, 2 of 3 mice succumbed to a 250 mg/kg dose.

The toxicity of RJ-4 and RJ-5 has not been reported previously and it was, therefore, necessary to conduct chronic inhalation studies with these materials to evaluate their potential health hazard.

Accordingly then, concentrations of 0.15 mg/liter RJ-5 and 2 mg/liter RJ-4 were selected for the 6-month chronic exposure of 4 animal species. The levels chosen are slightly below saturation vapor pressures so that condensation on chamber surfaces would not occur.

Each experimental group and the unexposed chamber controls consisted initially of 4 female and 4 male beagle dogs, 50 male CFE rats, 40 female CF-1 mice, and an uneven mixture of male and female Macaca mulatta monkeys, 4 per chamber.

TABLE 26. PHYSICAL CHEMICAL PROPERTIES OF RJ-4, RJ-5 AND METHYLCYCLOHEXANE

	RJ-4	RJ-5	MCH
Empirical Formula	$C_{12}H_{20}$	$C_{14}H_{20}$	C_7H_{14}
Molecular Weight	164	188	98
Boiling Point (°F)	431	522	213
Vapor Pressure (70 F)	0.354 mm Hg	0.025 mm Hg	42 mm Hg
Density (70 F)	0.925 g/ml	1.0813 g/ml	0.7660 g/ml

Each group of animals was housed in separate Thomas Domes operated with nominal airflows of 40 cfm at a slightly reduced pressure, 725 mm Hg, to avoid leakage of the hydrocarbons. Temperatures were controlled at 72 $\pm$ 2 F and relative humidity at 50 $\pm$ 10%. Exposures were conducted on a 6 hour/day, 5 day/week schedule. No exposures were made on weekends and holidays. Upon completion of the daily exposures, the chambers containing RJ-4 and RJ-5 were purged with air for 30 minutes before lifting the dome tops. Cleaning of the chambers was done and residual food replaced with fresh supplies at this time.

Although expected to be low, the toxicities of the chemicals under study are unknown except for the minimal acute animal information mentioned earlier. Personnel working with these materials avoided skin contact and inhalation. The vapor generation apparatus and chemical supplies were in ventilated hoods and the areas were no smoking zones.

The chamber concentrations of RJ-4 and RJ-5 were continuously monitored using flame ionization hydrocarbon analyzers. The generation and monitoring techniques were identical to those used during the JP-4 toxicity study.

To measure the chronic toxicity of RJ-4 and RJ-5, a limited number of parameters were selected, with the view toward increasing the variety of tests should the basic battery reveal trends, or deleterious effects during the course of the study.

All exposed animals were observed for signs of toxic stress as well as mortality. Gross and histopathologic examinations were made on all dead animals. Body weights of dogs, monkeys and rats were measured on a biweekly schedule. Table 27 shows the reduced battery of clinical hematology and chemistry tests performed on blood samples taken from dogs and monkeys on a biweekly basis. A complete battery of clinical laboratory tests were made at the start and at the completion of the exposures. These tests include, in addition to those shown in Table 27, creatinine, chlorides, cholesterol, BUN, total inorganic phosphorus, bilirubin and serum triglycerides. At final blood sampling or sacrifice of the large animals, additional blood samples were drawn for identification and refrigerated for storage of serum. These "banked" serum samples were stored until histopathology reports were received and reviewed. Twenty rats and mice from each of the study groups were retained for one-year observation following exposure termination to evaluate any postexposure effects from RJ-4 and/or RJ-5

inhalation. All remaining animals were sacrificed at exposure termination and submitted for gross and histopathologic examination. Major organs were taken from 20 rats from each group and weighed to allow for comparison of mean organ weights and organ to body weight ratios.

TABLE 27. CLINICAL BLOOD TESTS PERFORMED ON RJ-4, RJ-5 EXPOSED AND CONTROL DOGS AND MONKEYS

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

A curious effect occurred in the rats exposed to RJ-4. Diarrhea was evident in the majority of the rats at 10 weeks of exposure and continued throughout the duration of the exposure portion of the study. Frequent postexposure observation of surviving rats revealed gradual alleviation of this condition. At 14 weeks postexposure, there were no signs of diarrhea.

There were six deaths during the 6 months of exposure. One male monkey died during the seventh week of exposure to RJ-5. Pathology revealed death was due to gastric dilatation of unknown etiology, but believed to be unrelated to exposure. One mouse in each of the exposure groups was sacrificed at 4 and 16 weeks of exposure due to accidental injuries. Remaining mortality was limited to control rodents. One mouse and one rat died of pneumonia at 1 and 24 weeks, respectively. One rat was sacrificed at 10 weeks because of abnormal behavior indicative of middle ear infection.

Mean body weights of exposed monkeys obtained on a biweekly schedule were normal when compared with control weights taken on the same time schedule. However, weight depressions were noted for rats and dogs exposed to RJ-4 and RJ-5.

The growth rate of rats is shown in Figure 9. Noticeable is the apparently subnormal gain from 2 weeks forward for both exposed groups The mean weights of the RJ-4 exposed animals are statistically different from control values at all time periods. However, the weights are only about 5% less than the controls. This is certainly an indication of stress, but of no great importance biologically. Although at several time periods the mean weights of the RJ-5 exposed rats were

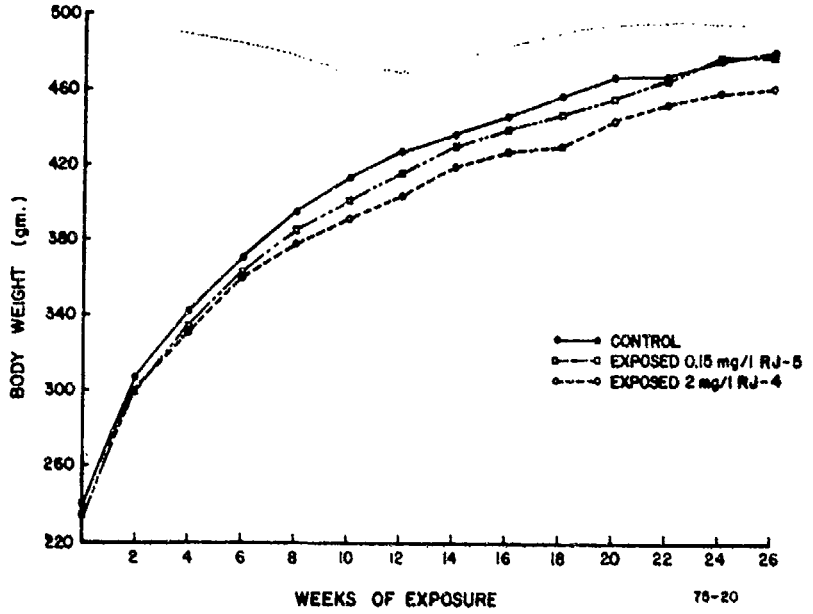


Figure 9. The effect of repeated daily exposures to RJ-4 or RJ-5 on rat growth.

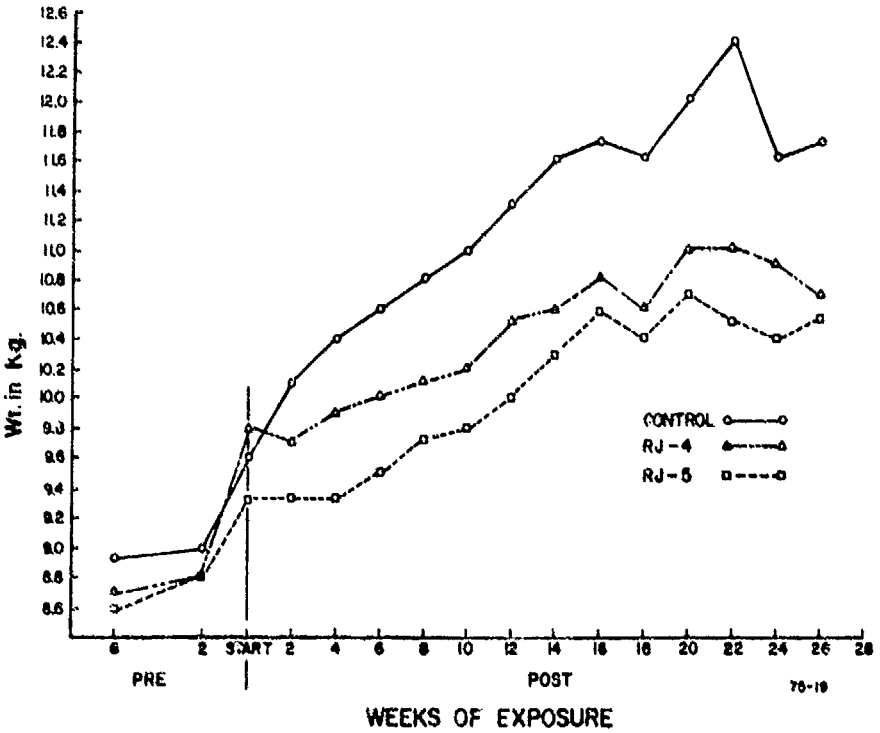


Figure 10. The effect of repeated daily exposures to RJ-4 or RJ-5 on dog growth.

10-12 grams less than control, statistical calculations revealed no significant differences from control weights. The odors of RJ-4 and RJ-5 were very noticeable and objectionable even after purging of the chambers after each exposure period and were thought possible to cause appetite suppression in rats resulting in growth suppression. To test this theory, food consumption measurements were made over a 3-day period during the 10th week of exposure. The daily results were variable, but overall information suggested there was no real difference in food consumption between control and exposed rats.

Dog mean body weights are shown in Figure 10. Both exposed groups of dogs gained less weight than controls throughout the course of the study. At first glance, it would appear that weight depression was greater for the RJ-5 exposed dogs. This is not the case in that the RJ-5 group weighed 0.5 kilograms less than the RJ-4 group at the beginning of the study. An examination of initial and final group mean body weights revealed that the controls, the RJ-5 and the RJ-4 groups gained 2.10, 1.22 and 0.98 kilograms respectively. Therefore, comparable subnormal weight gains occurred for dogs exposed to RJ-4 and RJ-5.

Biweekly clinical blood test results collected on dogs and monkeys showed no abnormalities or trends to adverse hematological effect.

There was no abnormal change in organ weights in rats exposed to RJ-5. Mean organ weights and organ to body weight ratios are shown in Table 28 for rats exposed to RJ-4 and controls. No toxicologic significance is attached to the lung weight difference between RJ-4 exposed and control rodents in that body weights of RJ-4 rodents were also significantly lower than controls. However, mean liver and kidney weights as well as the ratios for the RJ-4 exposed rats are statistically higher than control values.

TABLE 28. THE EFFECT OF 6-MONTH CHRONIC INHALATION EXPOSURE TO RJ-4 ON RAT ORGANS[1]

Organ	Mean Organ Weight, Grams		Organ/Body Weight Ratio	
	RJ-4	Control	RJ-4	Control
Liver	15.61**[2]	12.96	3.536**[2]	2.820
Kidney	3.50**[2]	3.20	0.792**[2]	0.674
Lung	1.92**[3]	2.16	0.434**[3]	0.471
Spleen	0.84	0.91	0.188	0.198
Heart	1.47	1.55	0.333	0.339

** Statistically different from control at 0.01 level.

[1] N = 20

[2] Significantly higher than control.

[3] Significantly lower than control.

Gross pathology results for animals sacrificed at exposure conclusion revealed no changes attributed to RJ-4 or RJ-5 exposure. There were no significant histopathology findings in monkeys and mice. However, acute inflammation was noted in the lungs of dogs and rats exposed to RJ-4 and RJ-5. This information is shown in Table 29. It can be seen that lung lesions were restricted to male RJ-4 and female RJ-5 exposed dogs while 8 of 20 RJ-4 exposed rats and 6 of 20 RJ-5 exposed rats showed bronchopneumonia.

TABLE 29. LUNG HISTOPATHOLOGY IN DOGS AND RATS EXPOSED TO JP-9 CONSTITUENTS (RJ-4 AND RJ-5)

	RJ-4 Exposed			RJ-5 Exposed			Controls		
	Dogs		Rats	Dogs		Rats	Dogs		Rats
	♂	♀	♂	♂	♀	♂	♂	♀	♂
Broncho-pneumonia	3/4	0/4	8/20	0/4	2/4	6/20	0/4	0/4	2/20
Bronchitis	1/4	0/4	1/20	0/4	3/4	0/20	0/4	0/4	1/20
Abscess	0/4	0/4	0/20	1/4	0/4	0/20	0/4	0/4	0/20

Cause of death in groups of 20 rats and mice during the 12-month postexposure observation period, almost without exception, was pneumonia. Table 30 shows the number of rodent survivors at sacrifice, one-year postexposure. Gross pathology results for the few remaining rats, 2 RJ-5 exposed and 3 controls, revealed chronic respiratory

infection in all cases. Nodules, tumor like lesions,were seen on the lungs of exposed and control mice. One control mouse, 3 RJ-4 and 5 RJ-5 exposed mice showed these lesions.

TABLE 30. JP-9 STUDY SURVIVORS AT ONE-YEAR POSTEXPOSURE

Group	Rats	Mice
RJ-4 Exposed	0/20	12/20
RJ-5 Exposed	2/20	14/20
Control	3/20	12/20

Histologic examination of rats that died or were killed during the 52nd week postexposure observation period were remarkably similar with consistent findings of chronic respiratory disease and glomerulonephrosis. One rat exposed to RJ-5 that survived the entire postexposure period had thyroid carcinoma.

Mice that died during the postexposure period had a high incidence of pathologic change in all three groups as shown in Table 31.

TABLE 31. INCIDENCE OF TUMORS IN MICE THAT DIED DURING THE POSTEXPOSURE PERIOD

	Control	RJ-4 Exposed	RJ-5 Exposed
Sarcomas	2/5	3/6	4/6
Alveolargenic Carcinomas	1/5	0/6	0/6
Other Tumors	0/5	1/6	0/6

Most of the sarcomatous lesions observed were undifferentiated but were thought to be of hemopoietic origin. These lesions were commonly seen in multiple organs including the lung, spleen, liver and kidney. Table 32 shows total incidence of the significant lesions found in all mice during the 52-week postexposure observation period.

TABLE 32. INCIDENCE OF SIGNIFICANT LESIONS IN MICE EXPOSED TO JP-9 CONSTITUENTS

	Controls	RJ-4 Exposed	RJ-5 Exposed
Lymphosarcoma	0/17	0/18	2/20
Alveolargenic Carcinoma	1/17	0/18	5/20
Alveolargenic Adenoma	0/17	2/18	0/20
Bronchogenic Carcinoma	0/17	0/18	1/20
Hemopoietic Sarcoma	2/17	2/18	3/20
Myelosarcoma	1/17	1/18	1/20
Total Tumors	4/17	5/18	12/20

No lethality occurred in four animal species during 6-month inhalation exposures to near saturation concentrations of RJ-4 and RJ-5. Dogs and rats in both exposure groups experienced body weight depressions relative to their controls. Weight depressions proved to be statistically lower than controls except for rats exposed to RJ-5 vapor.

Mean liver and kidney weights as well as their ratios were significantly elevated in RJ-4 exposed rats when compared with control data. Histopathology which included Oil-Red-O staining failed to reveal any fat deposition or abnormal alterations in liver and kidney tissue which could account for increased organ weights in RJ-4 rats. Histopathologic findings in exposed monkeys and mice showed no abnormalities that were treatment related. However, for dogs and rats, considering acute pulmonary inflammation as a group, i.e., abscess, bronchopneumonia and bronchitis, the frequencies suggest respiratory irritation with the probability of secondary bacterial inflammation. The results of clinical hematology and chemistry tests performed on dogs and monkeys provides evidence that no kidney, liver or hematological toxicity occurred from chronic exposure to RJ-4 or RJ-5 vapors.

The results of this study demonstrate the low order of toxicity of JP-9 constituents exhibited in experimental animals. Kidney and liver hyperplasia in RJ-4 exposed rats and pulmonary irritation in dogs and monkeys exposed to RJ-4 and RJ-5 emerge as the salient results of this study. Although the reasons for organ hyperplasia in rats is not clear, it is of little toxicologic significance in that there was no tissue destruction or alteration. Although there is some indication of increased

tumor incidence in a small number of mice held for one year after exposure to near saturated RJ-5 vapors there is no clear cut evidence that this compound is carcinogenic. The latter finding of respiratory irritation should be considered relative to possible human experience of chronic exposure to RJ-4 or RJ-5. In this regard, certain factors must be considered. Due to their low vapor pressures, the inhalation hazard (the probability of injury in use) is extremely low. The odors of RJ-4 and RJ-5 are extremely objectionable and it is, therefore, doubtful that workers would tolerate concentrations far less than those used in this study for any substantial time period. Furthermore, as constituents of JP-9 fuel, the toxicity of the mixture would be largely that of methylcyclohexane. However, considering RJ-4 and RJ-5 as separate entities as they were tested, both fuels show a relatively low order of toxicity in experimental animals and are judged to be of a low inhalation hazard to man.

Percutaneous, Oral and Inhalation Studies for Classification of Toxicity Ratings for Transportable Chemical Agents

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

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

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

During the current reporting period, a group of compounds was received and were assigned code numbers prior to testing. These compounds, their code numbers and the tests to be done on each are listed in Table 33.

A description of the methods and procedures for oral, dermal, corrosion and inhalation testing were detailed in the last annual report (MacEwen and Vernot, 1975). The only change from the described methods was the fact that some inhalation exposures were done in a 9-liter glass chamber with an air flow of 9 liters/minute. The exposure groups for this chamber remained at 5 rats per contaminant level.

The results of the completed acute toxicity tests and the assigned classification are shown in Tables 34 through 37.

Four compounds, sodium trichloro-s-triazinetrione, fumaric acid, oxalic acid and tris-2-hydroxyethyl isocyanurate caused no deaths when applied to the clipped skin of rabbits at the highest dose tested (20,000 mg/kg) and were thereby designated "below toxic." Two compounds, perchloromethyl mercaptan and methyl chloroformate, caused death at very low inhalation levels and were classified as "extremely toxic."

TABLE 33. LIST OF COMPOUNDS TESTED FOR ACUTE ORAL, INHALATION AND PERCUTANEOUS TOXICITY

Code No.	Compound	Route of Administration: Oral Tox.	Inhal. Tox.	Skin Absorp.	Skin Corrosion
107	Perchloromethyl mercaptan		X	X	X
143	Boron trichloride		X		
144	Boron trifluoride		X		
165	Ethyl chloroformate		X	X	
180	Methyl chloroformate		X	X	
183	Nitric acid				X
192	Pyridine		X		
198	Fuming sulfuric acid		X		
253	Hydrochloric acid				X
254	Sodium hydroxide				X
255	Sulfuric acid				X
256	Hydrofluoric acid				X
258	Cresol (from coal tar) technical			X	
259	Cresol (from petroleum) technical			X	X
260	o-Cresol, practical			X	
261	m-Cresol, practical			X	
262	p-Cresol, practical			X	
263	Sodium trichloro-s-triazinetrione			X	
264	Fumaric acid			X	
265	Maleic anhydride			X	
266	Ammonium hydroxide				X
267	Oxalic acid, 5% concentration			X	
270	3-Methyl butyric acid			X	
271	Tris-2-hydroxyethylisocyanurate			X	
273	p-Cresol (Sherwin-Williams)			X	X
274	Potassium hydroxide				X
275	Acetic acid				X
276	Benzene sulfonic chloride		X		
277	Benzene sulfonic fluoride		X		
278	Sulfur dioxide		X		
279	Chloroacetyl chloride		X		
280	Trichloroethylene		X		
281	Sulfuryl chloride		X		
282	Sulfuryl fluoride		X		
283	Sulfur chloride		X		
284	Sulfur dichloride		X		
286	Commercial carburetor cleaners				
	Gumout (Pennzoil)			X	X
	No. 7 Carburetor cleaner (Dupont)			X	X
	B-12 Chemtool (Berryman)			X	X

TABLE 33 CONTINUED

Code No.	Compound	Route of Administration: Oral Tox.	Inhal. Tox.	Skin Absorp.	Skin Corrosion
287	Phosphotungstic acid	X			
288	Chromic nitrate	X		X	X
289	Calcium chromate	X		X	X
290	Propargyl alcohol	X	X	X	

TABLE 34. CORROSIVE EFFECTS OF VARIOUS COMPOUNDS ON RABBIT SKIN

Compound	Rabbit # 1	2	3	4	5	6	Remarks
Sulfuric acid 4%	o	o	o	o	o	-	Noncorrosive
Hydrofluoric acid 4%	o	o	o	o	o	-	Noncorrosive
Ammonium hydroxide 10%	o	o	+	o	o	o	Noncorrosive
" " 20%	+	+	-	-	-	-	Corrosive
" " 15%	o	+	o	o	o	+	Corrosive
" " 12%	+	+	+	+	o	+	Corrosive
Hydrochloric acid 20%	+	+	-	-	-	-	Corrosive
" " 15%	+	o	o	o	o	o	Noncorrosive
" " 17%	o	+	o	+	-	-	Corrosive
Potassium hydroxide 4%	+	+	-	-	-	-	Corrosive
" " 2%	+	+	-	-	-	-	Corrosive
" " 1%	o	o	o	o	o	-	Noncorrosive
Nitric acid 4%	o	o	o	o	o	-	Noncorrosive
" " 8%	o	+	o	o	o	+	Corrosive
" " 6%	o	o	o	o	o	o	Noncorrosive
Acetic acid 20%	o	o	o	o	o	-	Noncorrosive
" " 40%	o	o	o	o	o	-	Noncorrosive
" " 80%	o	o	o	o	o	-	Noncorrosive
" " 100%	o	o	o	o	o	-	Noncorrosive
Sodium hydroxide 4%	+	+	+	o	-	-	Corrosive
" " 2%	+	o	o	+	+	-	Corrosive
" " 1%	o	o	o	o	o	-	Noncorrosive
para-Cresol (Sherwin-Williams)	+	+	-	-	-	-	Corrosive
Cresol (petroleum)	+	o	+	+	+	-	Corrosive
Perchloromethyl mercaptan	o	o	o	o	o	-	Noncorrosive
Carburetor cleaners							
Gumout (Pennzoil)	o	o	o	o	o	-	Noncorrosive
No. 7 Carburetor Cleaner (Dupont)	o	o	o	o	o	-	Noncorrosive
B-12 Chemtool (Berryman)	o	o	o	o	o	-	Noncorrosive
Chromic nitrate	o	o	o	o	o	-	Noncorrosive
Calcium chromate	o	o	o	o	o	-	Noncorrosive

+ = Caused visible destruction or irreversible alteration in skin tissue after 4 hours contact.

o = Did not cause visible destruction or irreversible alteration in skin tissue after 4 hours contact.

- = Not tested in that a positive of 2/6 or a negative of 0/5 rabbits has already been produced.

TABLE 35. DERMAL TOXICITY OF VARIOUS COMPOUNDS ON FEMALE RABBITS

Compound	LD_{50} (95% C.L.) in mg/kg	Data Used to Calculate LD_{50} in mg/kg (Mortality Response, N=3)	Classification
Cresol (coal tar)	2000 (700-5900)	1000(1), 2000(1), 3000(3)	Toxic
Cresol (petroleum)	2000 (700-5900)	1000(1), 2000(1), 3000(3)	Toxic
o-Cresol	890 (460-1690)	500(0), 1000(2), 2000(3)	Toxic
m-Cresol	2830 (no range)	1000(0), 2000(0), 4000(3)	Toxic
p-Cresol	300 (130-910)	250(1), 500(3), 1000(3)	Toxic
Ethyl chloroformate	7120 (no range)	5040(0), 6350(0), 8000(3)	Toxic
Sodium trichloro-s-triazinetrione	>20,000	20,000(0)	Below toxic
Methyl chloroformate	7120 (no range)	5040(0), 6350(0), 8000(3)	Toxic
Fumaric acid	>20,000	20,000(0)	Below toxic
Perchloromethyl mercaptan	1414 (363-5513)	500(0), 1000(1), 2000(2)	Toxic
Maleic anhydride	2620 (1930-3550)	2000(0), 2520(1), 3170(3), 4000(3)	Toxic
Oxalic acid	>20,000	20,000(0)	Below toxic
3-Methyl butyric acid	3560 (1880-6770)	2000(0), 4000(2), 8000(3)	Toxic
Tris-2-hydroxyethyl isocyanurate	>20,000	20,000(0)	Below toxic

TABLE 36. ONE-HOUR INHALATION TOXICITY OF VARIOUS COMPOUNDS FOR MALE AND FEMALE RATS

Compound	Sex	LC_{50} (95% C.L.) in ppm	Data used to Calculate LC_{50} in ppm (Mortality Response, N=5)	Classification
Ethyl chloroformate	M	145 (138-152)	117(0), 138(1), 152(4)	Highly toxic
Ethyl chloroformate	F	165 (148-184)	118(0), 148(1), 184(4)	Highly toxic
Methyl chloroformate	M	88 (64-123)	42(1), 78(0), 92(2), 101(5)	Extremely toxic
Methyl chloroformate	F	103 (90-118)	92(2), 110(3), 120(4), 128(4)	Extremely toxic
Boron trichloride	M	2541 (2243-2878)	2032(0), 2270(1), 2627(5), 3019(4), 3717(4), 3742(5)	Toxic
Boron trichloride	F	4418 (3921-6149)	2844(0), 3443(1), 3792(2), 4092(0), 4370(3), 5201(4)	Toxic
Boron trifluoride	M	387 (320-467)	317(1), 398(4), 437(3), 513(3), 675(5)	Highly toxic
Boron trifluoride	F	371 (293-469)	266(2), 290(0), 312(3), 399(3), 468(4), 557(3), 650(3), 723(5), 864(5)	Highly toxic
Perchloromethyl mercaptan	F	16 (13-22)	10(1), 16(3), 20(2), 28(4), 31(5), 43(5)	Extremely toxic
Perchloromethyl mercaptan	M	11 (10-13)	7.0(0), 9.0(1), 10.8(2), 11.2(4), 14.0(3), 17.0(5)	Extremely toxic

TABLE 37. ORAL TOXICITY OF VARIOUS COMPOUNDS TO MALE AND FEMALE RATS

Compound	Sex	LD_{50} (95% C.L.) in mg/kg	Data Used to Calculate LD_{50} in mg/kg (Mortality Response, N=5)	Classification
Phosphotungstic acid[a]	M	3297 (2558-4249)	2520(1), 3175(2), 4000(4)	Toxic
Phosphotungstic acid[a]	F	4925 (3577-6780)	2000(0), 4000(1), 8000(5)	Toxic
Calcium chromate[b]	M	746 (627-887)	504(0), 635(2), 800(2), 1008(5)	Toxic
Calcium chromate[b]	F	327 (260-410)	252(1), 318(2), 400(4), 504(5)	Toxic
Chromic nitrate[a]	M	1543 (1266-3011)	500(1), 1000(2), 2000(2), 4000(5)	Toxic
Chromic nitrate[a]	F	1414 (923-2168)	500(0), 1000(1), 2000(4), 4000(5)	Toxic
Propargyl alcohol[a]	M	93 (58-151)	25(0), 50(1), 100(2), 200(5)	Toxic
Propargyl alcohol[a]	F	54 (37-78)	25(0), 50(2), 100(5), 200(5)	Toxic

(a) diluted in H_2O.
(b) diluted in Agar (0.6%).

One compound on which inhalation exposures were conducted presented several practical as well as theoretical problems. Boron trifluoride (BF_3) is a very unstable gas which immediately forms an aerosol when released from the gas cylinder. The aerosol formed is a reaction product with water vapor in the air and is a mixture of hydrates of BF_3. Their precise composition is unknown although currently the subject of study in other laboratories. To further complicate matters BF_3 reacts readily with liquid water to form fluoroboric acid and it is probably in the fluoroborate form when it contacts lung surfaces in man or in experimental animals.

The series of inhalation exposures presented to male and female rats were chemically monitored to determine exposure concentration by collecting the air samples in an absorbing tower in water and measuring tetrafluoroborate ion using an ion specific electrode. The chamber concentrations were calculated as if they were BF_3 by use of the theoretical equation:

$$4BF_3 + 3H_2O \rightarrow 3HBF_4 + B(OH)3$$

Standards made with $NaBF_4$ were compared with bag standards made with measured amounts of BF_3 and calibration curves were made for the measuring electrode. Thus the LC_{50} value is reported for BF_3

which cannot be directly seen or measured in ambient air and probably does not exist in the lung of man or animal. The toxicity data obtained for BF_3 is however adequate to identify the hazard of exposure.

The acute toxicity tests for several compounds are still in progress. Primarily, the remaining tests are for inhalation LC_{50} determinations which require chemical monitoring and are, therefore, more time consuming. These tests are progressing as scheduled and the remaining results will be reported as available.

Toxic Hazard Evaluation of Five Atmospheric Pollutant Effluents from Ammunition Plants

Acute toxicity studies were conducted on five atmospheric pollutants resulting from the manufacture of munitions to evaluate the community and environmental health hazard associated with their emission. These studies were undertaken at the request of the U. S. Army as a preliminary step in the establishment of criteria for setting environmental or emission standards.

The group of compounds and the route of administration for each are shown below:

Compound	Route of Administration				
	Oral	Intravenous	Skin Absorption	Skin Irritation	Inhalation
Methyl Nitrate	x				x
Tetranitro-methane	x	x			x
o-Nitrotoluene			x	x	x
m-Nitrotoluene			x	x	x
p-Nitrotoluene			x	x	x

The experimental animals were fasted for a minimum of 16 hours prior to administration of the oral dose. This allows for more uniform absorption in all animals of the same species since the amount of food in the stomach varies greatly from animal to animal in the unfasted condition. Both rats and mice were weighed individually at the time of dosing to determine the proper injection volume. Glass syringes with special oral dosing needles were used to administer the compounds to the rats and mice.

Rangefinding doses were given for each compound. These consisted of dosing five rats and five mice at three levels determined from available evidence in the literature. After finding the proper range, geometrically spaced doses were administered to determine the LD_{50}. Ten male Sprague-Dawley albino rats and ten male CF-1 albino mice were dosed at each level and the LD_{50} with its 95% confidence limits were

determined by the probit analysis method of Finney (1952). Deaths which occurred during the 14 days immediately following the administration of the single dose were included in the final mortality tally. Any animal which survived the 14-day postexposure observation period was sacrificed at that time.

The patch-test method was used to measure the degree of primary irritation of intact and abraded skin of female New Zealand albino rabbits. The rabbits were clipped of all possible hair on the backs and flanks 24 hours prior to exposure to allow for recovery of the skin from any abrasion resulting from the clipping. Six areas on the back, three on each side, were designated as patch areas. This allowed for the simultaneous testing of the three compounds on each rabbit, with each compound being tested on both the intact and abraded skin. Three areas on the right side of each rabbit were abraded by making minor incisions through the stratum corneum, but not sufficiently deep to disturb the derma or to produce bleeding. These were made in a square pattern with a syringe needle to make the incisions. Six rabbits were tested for each compound.

The test material was applied in its native state in the amount of 0.5 gram for solids and 0.5 ml for liquids. The compound was applied to the designated areas and then 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 then covered with a rubber dental dam strip and secured with more elastoplast tape. The patches remained on the rabbits for 24 hours. During that time, the rabbits were fitted with leather restraining collars to prevent disturbance of the patch area, but allowing the rabbits freedom of movement and access to food and water.

After 24 hours, the wrap and patches were carefully removed and the test areas evaluated for irritation using the Draize table as a reference standard. Readings were again made at 72 hours (48 hours after the first reading). The scores for each category in the table are the average score of the six rabbits tested. The higher the score, the more severe the irritation caused by the compound.

Skin absorption toxicity was determined using female New Zealand albino rabbits. All rabbits were clipped as closely as possible with an Oster clipper fitted with surgical blades. The back of the rabbits and the sides down to about half-way to the stomach area were clipped from the saddle area of the shoulders to the top of the rear leg area.

The rabbits were weighed prior to dosing to determine the proper dose volume. The compound was applied undiluted to the back of the rabbit, divided equally between the two sides of the rabbit. The compound was kept in place with 8-ply gauze patches. Latex rubber dental dam was then applied over the entire back area where clipped. Elastoplast tape was then used to keep the dose in place. Restraining harnesses, described earlier, were fitted to each rabbit during the entire dosing period.

All doses were kept in contact with the rabbits skin for 24 hours. After this period of time had elapsed, the tape, latex and gauze were removed and the animal was observed for 14 days. Three rabbits were dosed per level.

The inhalation exposures for 4-hour LC_{50} determinations were done using male, Sprague-Dawley, CFE rats and male CF-1 mice, ten per exposure level. All animals were observed for signs of toxicity and mortality during exposure and for the 14 days immediately following exposure. Animals were weighed immediately prior to exposure and survivors at 14 days postexposure.

The three nitrotoluene isomers, having a low order of toxicity, were tested at vapor saturation levels. Production of essentially saturated vapors of the two liquid isomers was accomplished by bubbling dry air through a fritted disc immersed in the sample. The resultant vapors were then passed through a 9-liter glass chamber containing the experimental animals. The one solid isomer was tested by a static technique whereby an excess of the compound was sealed into a 120-liter plexiglass chamber for a period of 24 hours. The experimental animals were then rapidly introduced into the chamber by means of a sliding cage drawer designed to minimize vapor loss. All exposures were continuously analyzed using a Beckman Model 400 total hydrocarbon analyzer.

Analysis of tetranitromethane (TNM) was done using a colorimetric method to determine contaminant concentration. In this method, Lyshkow reagent (modified Saltzman reagent) was allowed to mix and react with the sampled air in a glass delay coil. The resultant color developed was related to sample concentration and read using a Technicon Auto-Analyzer I system.

Standardization was based on a gravimetric technique using diffusion tubes. These were constructed by sealing the narrow end of disposable Pasteur pipettes resulting in straight wall tubes 110 mm long by 5 mm I.D., which when less than half full would diffuse approximately 3.5 µl of TNM vapor per minute. Two tubes showed a combined mean diffusion rate of 7.06 (s.d. $\pm$ 0.37) ppm/minute at 30 C.

For TNM generation, a modification of the diffusion tube system using two short tubes (30 mm length by 15 mm I.D.) gave a stable source of TNM from 70 µl/minute with one tube at 30 C to 680 µl/minute for two tubes at 60 C.

A Miran® infrared analyzer was used for the analysis of methyl nitrate concentrations. Calibration was achieved by use of standard bags in the 0-1000 ppm range. The conditions for the Miran IR analyzer were as follows:

Pathlength	0.5 M	Absorbance	1.0 A
Slitwidth	1.0 mm	Time Constant	1 0
Wavelength	5.98 µ	Sample Flow	500 cc/min.
Gain	10 X		

An ethanolic solution of TNM was used for an intravenous LD_{50} determination in groups of rats and mice. Rats were injected using the lateral saphenous vein of the hind leg while mice were injected in the right lateral tail vein. The experimental animals were weighed prior to dosing to determine the proper injection volume. Ten animals were dosed at each level and the LD_{50} with its 95% confidence limits was determined by the probit analysis method previously mentioned.

Deaths which occurred during the 14 days immediately following the administration of the single dose were included in the final mortality. Any animal that survived the 14-day postexposure observation period was sacrificed at that time.

Tetranitromethane (TNM)

The rat 4-hour LC_{50} of TNM vapor was determined to be 17.5 ppm or 0.14 mg/liter. The mouse 4-hour LC_{50} for TNM is 54.4 ppm or 0.44 mg/liter (Table 38). Responses of the animals, within each species, were consistently dose-related and followed a general pattern of lethargy and inactivity with some eye and nose irritation at the toxic levels. All animals remained inactive during exposure with noticeable decrease in rate and depth of respiratory movements.

Deaths which occurred following exposure generally occurred within 12 hours. If the animals survived this time period, they usually lived to the 14-day sacrifice period. Rats at the nonlethal exposure level lost weight through the first four days postexposure but recovered and had normal weight gains by the end of the 14 days. Rats exposed at the partial mortality levels showed a definite depression in growth rate during the 14 days following exposure. Scattered weight losses were found in all mice that survived the 14-day observation period.

TABLE 38. 4-HOUR INHALATION LC_{50} VALUES FOR RATS AND MICE EXPOSED TO TETRANITROMETHANE VAPOR (N=10)

Rats		Mice	
Conc., ppm	Mortality Ratio	Conc., ppm	Mortality Ratio
23	10/10	76	10/10
21	10/10	63	5/10
19	6/10	55	4/10
18	3/10	47	3/10
15	3/10	42	3/10
10	0/10	32	1/10
		17	0/10
		14	0/10
LC_{50} =	17.5 ppm	54.4 ppm	
95% C.L. =	16.4 to 18.7 ppm	48.0 to 61.7 ppm	

Gross pathologic examination of the animals that died showed multifocal areas of moderate to severe lung congestion throughout all lobes with many of these areas appearing hemorrhagic. Animals examined after exposure to nonlethal levels of TNM vapors showed mild congestion of lungs upon gross examination.

Intravenous administration of TNM to rats and mice resulted in a LD_{50} of 12.6 mg/kg and 63.1 mg/kg, respectively (Table 39). As was seen in the results of inhalation exposures, rats are much more susceptible to TNM than mice. Mouse deaths occurred within minutes after dosing. Gasping with a foamy nasal discharge and tonic convulsions at the highest level preceded death in the rats. Rat deaths occurred within two hours following administration of the compound. Control rats and mice given intravenous injections of equivalent volumes of ethanol alone had normal weight gains and survived the 14-day observation period.

Intragastric administration of undiluted tetranitromethane to fasted male rats produced an LD_{50} value of 130 mg/kg (Table 40). Similar administration of TNM to male mice produced an LD_{50} of 375 mg/kg. All animals remained inactive and lethargic for several hours after administration of the compound. Most deaths occurred during the 12-hour period immediately following dosing and delayed deaths were an uncommon occurrence.

TABLE 39. INTRAVENOUS TOXICITY OF TETRANITROMETHANE TO RATS AND MICE

Rats		Mice	
Conc., mg/kg*	Mortality Ratio	Conc., mg/kg*	Mortality Ratio
31.3	10/10	125	8/10
15.6	7/10	62.5	6/10
7.8	1/10	31.3	1/10

*Diluted in ethanol

LD_{50} =	12.6 mg/kg	63.1 mg/kg
95% C.L. =	10.0 to 15.9 mg/kg	45.0 to 88.7 mg/kg

TABLE 40. ACUTE ORAL TOXICITY OF TETRANITROMETHANE IN RATS AND MICE

Rats		Mice	
Conc., mg/kg	Mortality Ratio	Conc., mg/kg	Mortality Ratio
500	10/10	1000	10/10
250	8/10	500	7/10
125	5/10	250	2/10

LD_{50} =	130 mg/kg	375 mg/kg
95% C.L. =	83 to 205 mg/kg	262 to 511 mg/kg

The difference between LD_{50} values determined for the oral and intravenous routes was most striking and suggestive of different mechanisms of toxicity dependent upon the route of administration. Additional studies were conducted to explore this unusual difference.

Groups of 2 rats each were given single oral doses of TNM and blood samples were taken 90 minutes after treatment. The blood samples were analyzed for methemoglobin content and a dose dependent response was measured as shown in Figure 11. In the rats dosed at 62.5 mg/kg and 125 mg/kg a second set of methemoglobin samples were collected three hours after exposure and the measured values had only decreased slightly below those found at 90 minutes as shown in Table 41.

TABLE 41. METHEMOGLOBIN RESPONSE TO ORAL DOSING OF RATS WITH TETRANITROMETHANE

Oral Dose mg/kg	Methemoglobin 90 Minutes g/100 ml	%	180 Minutes g/100 ml	%
62.5	3.5	28	3.3	26
125	5.9	47	5.3	42

The toxic signs observed in rats and mice after oral administration of TNM and the measured methemoglobin values are consistent with acute methemoglobinemia which is believed to be the toxic mechanism involved in lethality following this route of entry.

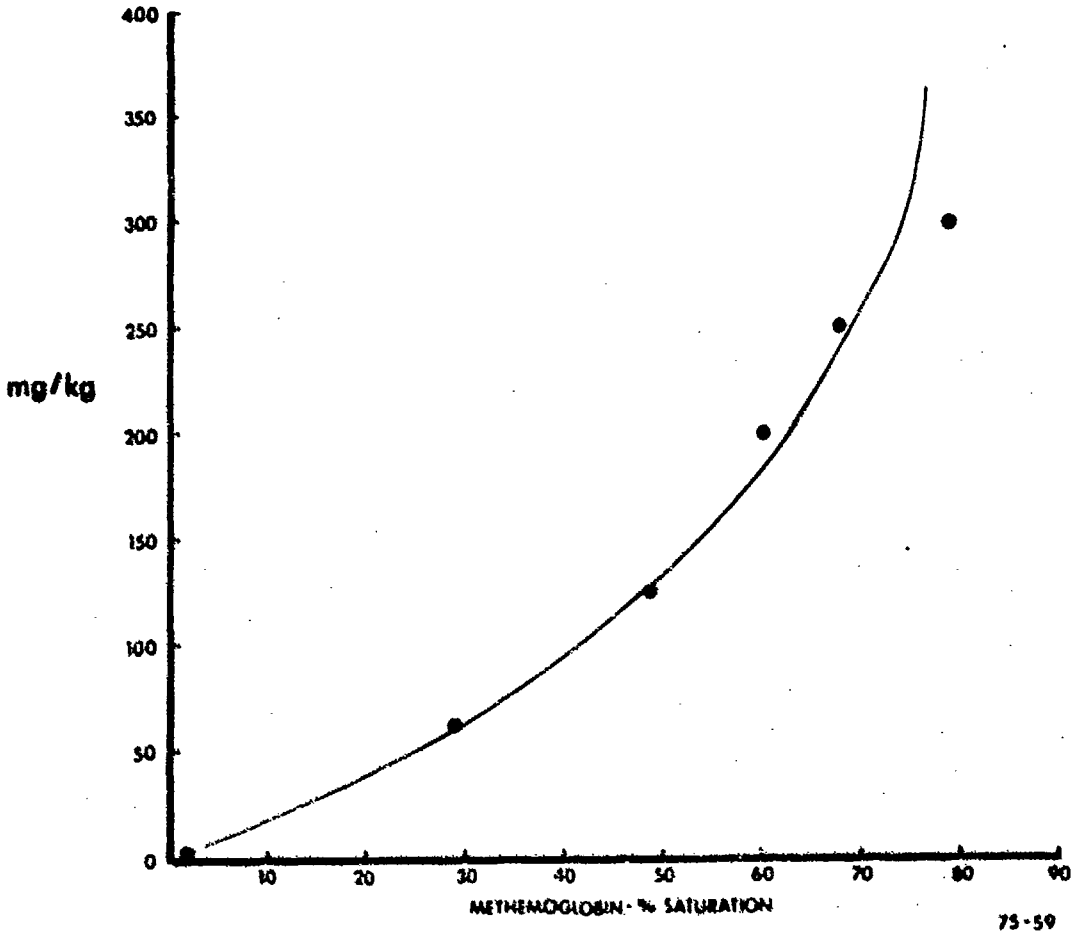


Figure 11. Methemoglobin response in rats given a single oral dose of tetranitromethane.

In order for TNM to form methemoglobin the compound would have to be metabolized to nitrite ion. If NO_2 were liberated from the molecule it could be converted to nitrate and nitrate ions. The nitrate ion would then be converted to nitrite ion by bacterial action in the intestine. Since the mechanism of toxicity of ingested TNM appeared to be nitrite induced methemoglobinemia, the rat oral LD_{50} was compared with the rat oral LD_{50} reported for sodium nitrate by Smyth et al. (1969) of 180 mg/kg. Calculation of the NO_2^- content of each compound gives an adjusted LD_{50} for NO_2^- of 120 mg/kg for TNM and 122 mg/kg for sodium nitrite.

The signs of toxicity observed in the inhalation and intravenous exposures of rats and mice were consistent with acute pulmonary irritation and respiratory deaths. At necropsy animals exposed by these routes had congested and hemorrhagic lungs. Two rats were given an IV injection dose of 15 mg/kg TNM, slightly above the LD_{50} dose, and sampled for methemoglobinemia at 90 minutes and had the same methemoglobin values as untreated controls (0-3% saturation).

Animals exposed to TNM by the inhalation and intravenous routes reacted as if they had been exposed to nitrogen dioxide gas (NO_2). If one assumed that NO_2 was liberated from TNM in the lung on an equimolar

basis (4 volumes of NO_2 for each volume of TNM) the measured TNM 4-hour LC_{50} value of 17.5 ppm would yield 70 ppm NO_2 which is comparable with the 4-hour LC_{50} for NO_2 of 88 ppm reported by Gray et al. (1954). Furthermore, a calculation of the dose of TNM inhaled by a 200 gram rat at the 4-hour LC_{50} concentration of 17.5 ppm assuming 100% absorption and a minute volume of 75 ml/minute yields 12.6 mg/kg, a value identical to the IV LD_{50}.

A second study was designed to test the hypothesis that the effects obtained by exposure of rats to a given concentration of tetranitromethane are equal to those obtained by exposure to 4 times the molar concentration of nitrogen dioxide at levels lower than the LC_{50} values.

Tandem, 2-week continuous exposures were conducted on theoretically equivalent concentrations of TNM and NO_2. Exposure groups consisted of 100 male rats, housed 10 per cage, in Longley chambers. Two smaller Rochester chambers housed the control rats which were exposed to air alone.

The concentrations used in the tandem experiments are as follows:

Experiment No.	TNM Conc., ppm	NO_2 Conc., ppm
1	7.5	30
2	5.0	20
3	3.5	14
4	7.5	40

All rats were examined daily for general appearance, behavior, signs of toxic stress and lethality with body weights recorded immediately prior to the start of exposure and at the conclusion, 14 days later.

Methemoglobin determinations were made on 10 rats per group, including controls, at the conclusion of the 14-day exposure period. In addition, groups of rats (consisting of 10 per group from the first experiment and 20 per group for all other experiments) had lungs precisely removed for wet weight determinations. The wet lung weights of each group were statistically analyzed for determination of edematous effects.

Gross and histopathologic examinations were made on all animals that died during exposure or were sacrificed at the conclusion of the 14-day study. Organ weights of lung, liver and kidneys were recorded for all animals examined at sacrifice. Statistical comparisons were performed on the mean organ weights and the organ to body weight ratios.

The contaminant concentrations were continuously monitored using a colorimetric method whereby a modified Saltzman reagent was allowed to mix and react with the sampled air in a glass delay coil. The resultant color developed was then related to the sample concentration and read using a Technicon AutoAnalyzer system.

In addition, a Wilkes Miran IA infrared analyzer was also used to monitor the TNM chamber. The absorbance measurement of the Miran was continuously recorded on a strip chart recorder.

These two methods were calibrated using pure TNM and were used concurrently during the first two days of the first experiment. The purpose of dual analysis was to determine if any dissociation of tetranitromethane into nitrogen dioxide takes place in the exposure chamber which would result in a decrease in infrared absorbance without affecting the AutoAnalyzer result, since the AutoAnalyzer measures NO_2 as efficiently as TNM. There proved to be no evidence of spontaneous dissociation during the two days; therefore, use of the AutoAnalyzer system for monitoring TNM concentrations was discontinued.

The rats in both contaminant chambers showed lethargy, dyspnea, kyphosis and general poor health with the TNM exposed rats showing yellowing of the fur during the first exposure series. The TNM exposed rats showed these symptoms of toxic stress to a greater degree than the NO_2 exposed rats. The rats exposed to 5 ppm TNM or 20 ppm NO_2 also showed similar signs of toxic stress but to a lesser degree. Although the toxic signs decreased with the decrease in concentrations, they were still visible at the lowest concentrations tested.

Mortality occurred in 75% of the TNM exposed rats and 31% of the NO_2 exposed rats in Experiment No. 1 (Table 12 and Figure 12). Deaths were minimal until after the fifth exposure day at which time mortalities began to accelerate. This was the lowest concentration of NO_2 which resulted in lethality. Deaths did occur in the 5 ppm TNM exposure, starting after the seventh exposure day.

Several problems occurred during the third and fourth day of the first experiment which resulted in concentration excursions in the TNM chamber. Two excursions, although for short duration, exceeded the 4-hour LC_{50} of 1[illegible] ppm. The following day four TNM rats died. As the rats had appeared to show signs of toxic stress prior to the concentration increase, it is difficult to determine just what effect the excursions had. The problems were eliminated and concentration control was satisfactory thereafter.

Gross pathology of the animals that died from either contaminant during exposure revealed a red-tinged exudate around the external nares. The lungs which failed to collapse upon opening of the pleural cavity had a few areas of focal consolidation and hemorrhage scattered throughout all lobes. Multifocal areas of emphysema was a common finding in most of the rats. The livers of the exposed animals appeared to be moderately congested.

TABLE 42. CUMULATIVE MORTALITY OF RATS CONTINUOUSLY EXPOSED TO TNM OR NO_2 (% Mortality)

Days of Exposure	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Compound and Conc., ppm							(Experiment 1)							
TNM - 7.5	0	0	1	1	5	8	12	17	21	24	31	44	58	75
NO_2 - 30.0	0	3	3	3	3	3	7	7	9	9	14	18	21	31
							(Experiment 2)							
TNM - 5.0	0	0	0	0	0	0	0	2	2	5	6	9	11	16
NO_2 - 20.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
							(Experiment 3)							
TNM - 3.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NO_2 - 14.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
							(Experiment 4)							
TNM - 7.5	0	0	1	1	1	1	3	7	14	23	30	42	54	65
NO_2 - 40	0	21	26	27	27	27	27	27	28	29	35	41	46	50

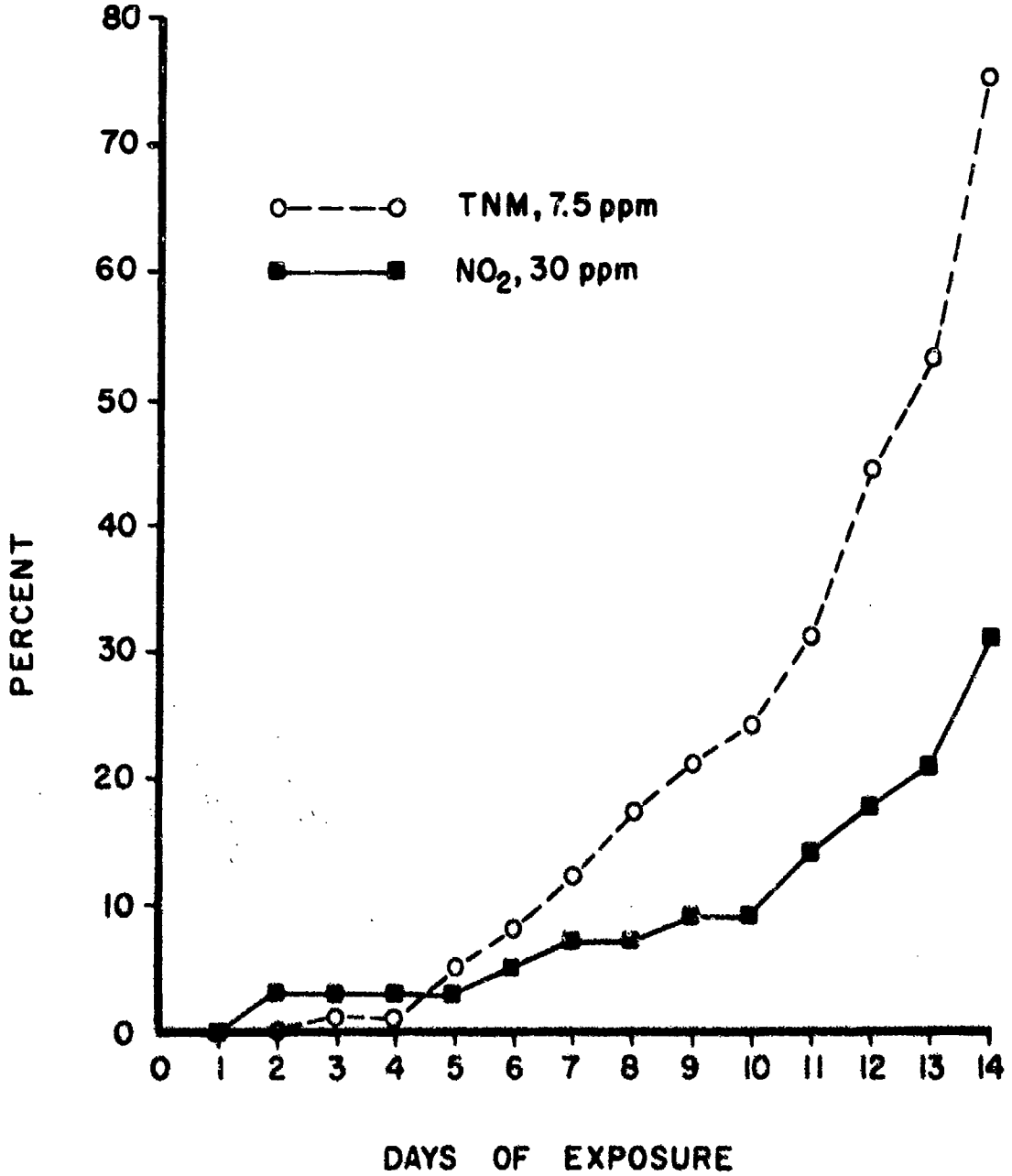


Figure 12. Cumulative mortality resulting from continuous exposure to 7.5 ppm TNM or 30 ppm NO_2.

The lungs of the animals sacrificed at the conclusion of the 14-day exposure period showed similar lesions except that rarely were any hemorrhagic areas seen. These same lesions were noted at each of the other levels examined but the severity of the lesions decreased as the contaminant concentration decreased.

A statistically significant increase in methemoglobin was found in both test groups at all exposure levels when compared to their respective controls. However, although the exposed groups showed an increase, it was minimal and well below a level which could have a biological effect.

The results of the wet lung examinations are shown in Tables 43 through 46. At the 7.5 ppm TNM or 30 ppm NO_2 level, the test rats differed statistically from the controls at the 0.01 level of significance in body weight, wet lung weight and lung to body weight ratios. Additionally, a statistically significant difference is also noted when comparing the two test groups. The TNM exposed group showed a greater toxic response than the group exposed to NO_2.

A similar effect was found in the 2nd and 3rd experiments, although to a lesser degree. Again, the TNM exposed group differed statistically from the NO_2 exposed group in most parameters examined. Although

TABLE 43. SUMMARY OF EFFECTS OF 14-DAY CONTINUOUS EXPOSURE TO 7.5 PPM TNM OR 30 PPM NO_2 ON RATS

	Controls	TNM	NO_2
$\bar{x}$ Body Weight at Start, g (N=100)	187.8	184.5	186.8

Summary of Wet Lung Examination, N=10

	Controls	TNM	NO_2
$\bar{x}$ Body Weight, g	267.8	143.1**B	215.6**
$\bar{x}$ Lung Weight, g	1.290	2.274**A	1.933**
Lung/Body Weight Ratio	0.483	1.606**B	0.902**

Summary of Body and Organ Weights at Necropsy

	Controls, N=70	NO_2, N=48
$\bar{x}$ Body Weight, g	275.7	209.8**
$\bar{x}$ Lung Weight, g	1.696	2.394**
$\bar{x}$ Liver Weight, g	10.987	7.683**
$\bar{x}$ Kidney Weight, g	2.371	1.935**
Lung/Body Weight Ratio	0.616	1.166**
Liver/Body Weight Ratio	2.983	2.657**
Kidney/Body Weight Ratio	0.862	0.928**

** = Different from controls at the 0.01 level of significance.
A = Different from NO_2 group at the 0.05 level of significance.
B = Different from NO_2 group at the 0.01 level of significance.

TABLE 44. SUMMARY OF EFFECTS OF 14-DAY CONTINUOUS EXPOSURE TO 5 PPM TNM OR 20 PPM NO_2 ON RATS

	Controls	TNM	NO_2
$\bar{x}$ Body Weight at Start, g (N=100)	199.0	199.7	199.1

Summary of Wet Lung Examinations, N=20

	Controls	TNM	NO_2
$\bar{x}$ Body Weight, g	262.3	194.5**B	250.1
$\bar{x}$ Lung Weight, g	1.401	1.922**A	1.784**
Lung/Body Weight Ratio	0.536	1.012**B	0.716**

Summary of Body to Organ Weights at Necropsy

	Controls, N=60	TNM, N=49	NO_2, N=63
$\bar{x}$ Body Weight, g	263.4	198.3**B	254.9**
$\bar{x}$ Lung Weight, g	1.672	2.094**	2.171**
$\bar{x}$ Liver Weight, g	8.405	6.833**B	9.063**
$\bar{x}$ Kidney Weight, g	2.012	1.551**B	1.995
Lung/Body Weight Ratio	0.636	1.071**B	0.853**
Liver/Body Weight Ratio	3.188	3.461**	3.557**
Kidney/Body Weight Ratio	0.763	0.787	0.785

** = Different from controls at the 0.01 level of significance.
A = Different from NO_2 group at the 0.05 level of significance.
B = Different from NO_2 group at the 0.01 level of significance.

TABLE 45. SUMMARY OF EFFECTS OF 14-DAY CONTINUOUS EXPOSURE TO 3.5 PPM TNM OR 14 PPM NO_2 ON RATS

	Controls	TNM	NO_2
$\bar{x}$ Body Weight at Start, g (N=100)	247.3	246.2	243.4

Summary of Wet Lung Examination, N=20

	Controls	TNM	NO_2
$\bar{x}$ Body Weight, g	319.0	264.6**B	310.4
$\bar{x}$ Lung Weight, g	1.511	1.821**	1.841**
Lung/Body Weight Ratio	0.474	0.691**B	0.595**

Summary of Body and Organ Weights at Necropsy

	Controls, N=60	TNM, N=60	NO_2, N=60
$\bar{x}$ Body Weight, g	319.7	258.7**B	305.4**
$\bar{x}$ Lung Weight, g	1.773	2.165**	2.169**
$\bar{x}$ Liver Weight, g	11.982	9.217**B	10.000**
$\bar{x}$ Kidney Weight, g	2.680	2.358**B	2.569*
Lung/Body Weight Ratio	0.556	0.848**B	0.714**
Liver/Body Weight Ratio	3.731	3.557**B	3.276**
Kidney/Body Weight Ratio	0.840	0.913**B	0.843

*=Different from controls at the 0.05 level of significance.
**= Different from controls at the 0.01 level of significance.
B = Different from NO_2 group at the 0.01 level of significance.

TABLE 46. SUMMARY OF EFFECTS OF 14-DAY CONTINUOUS EXPOSURE TO 7.5 PPM TNM OR 40 PPM NO_2 ON RATS

	Controls	TNM	NO_2
$\bar{x}$ Body Weight at Start, g (N=100)	254.7	254.4	256.4

Summary of Wet Lung Examinations, N=20

	Controls	TNM	NO_2
$\bar{x}$ Body weight, g	327.6	171.9**B	199.1**
$\bar{x}$ Lung Weight, g	1.651	2.687**	2.780**
Lung/Body Weight Ratio	0.505	1.572**	1.433**

Summary of Body and Organ Weights at Necropsy

	Controls, N=60	NO_2, N=19
$\bar{x}$ Body Weight, g	315.4	210.5**
$\bar{x}$ Lung Weight, g	1.785	3.183**
$\bar{x}$ Liver Weight, g	10.745	6.789**
$\bar{x}$ Kidney Weight, g	2.573	1.874**
Lung/Body Weight Ratio	0.567	1.556**
Liver/Body Weight Ratio	3.408	3.241*
Kidney/Body Weight Ratio	0.815	0.898**

* = Different from controls at the 0.05 level of significance.
** = Different from controls at the 0.01 level of significance.
B = Different from NO_2 group at the 0.01 level of significance.

the body weights of the rats exposed to NO_2 were not affected (lung water group only) at the lowest concentrations, the lung weights showed an increase in weight indicating an edematous effect. The lung/body weight ratio continues to show a difference between the two contaminants, TNM exposed animals being more severely affected.

Because of the high mortality in the TNM exposed rats in the first experiment, not enough remained at the sacrifice date to statistically compare their organ weights with those from the NO_2 exposure and control groups. Statistical comparisons were made on the NO_2 exposed animals and (Table 43) every parameter examined differed from the controls at the 0.01 level of significance.

Evaluation of the data accrued from the first three experiments indicated that the toxic effect of TNM vapors was greater than four times the NO_2 effects. The edematous reaction in the lungs and the resultant effects on body and organ weights indicated that the actual ratio was more like 1 to 5 or 6.

Based on this premise, an additional set of exposures was performed at concentration levels of 7.5 ppm TNM and 40 ppm NO_2, a ratio of 1 to 5.3. The 7.5 ppm TNM level was repeated to determine whether the concentration excursions mentioned previously of the first 7.5 ppm TNM experiment had an adverse effect on the experimental results.

The mortality results of this study (Figure 13) indicate that the TNM concentration excursions in the initial experiment at 7.5 ppm possibly accelerated the onset of death by two days. However, the slope of the curve in the second 7.5 ppm TNM study is much steeper and the end result in 14 days is similar to the original 7.5 ppm TNM study.

The initial effects of 40 ppm NO_2 resulted in deaths to one-fourth of the rats. The rats that died during this early period all had a clear exudate flowing from the mouth and nares and congested lungs, indicating irritation and edematous reaction to the NO_2 vapors. The remaining animals were able to compensate or adjust to the NO_2 vapors and few mortalities occurred during the succeeding seven exposure days. However, at eleven days mortalities began to occur again. By the fourteenth exposure day, deaths were comparable for both compounds.

The lethal effects of TNM and NO_2 appear to be comparable at a ratio of 4:1 TNM/NO_2 as seen in Table 12 but growth inhibition is not comparable at any exposure combination tested. On the other hand, lung weights are similarly affected at various ratios of TNM to NO_2 and increase with concentration indicating increased edemagenesis.

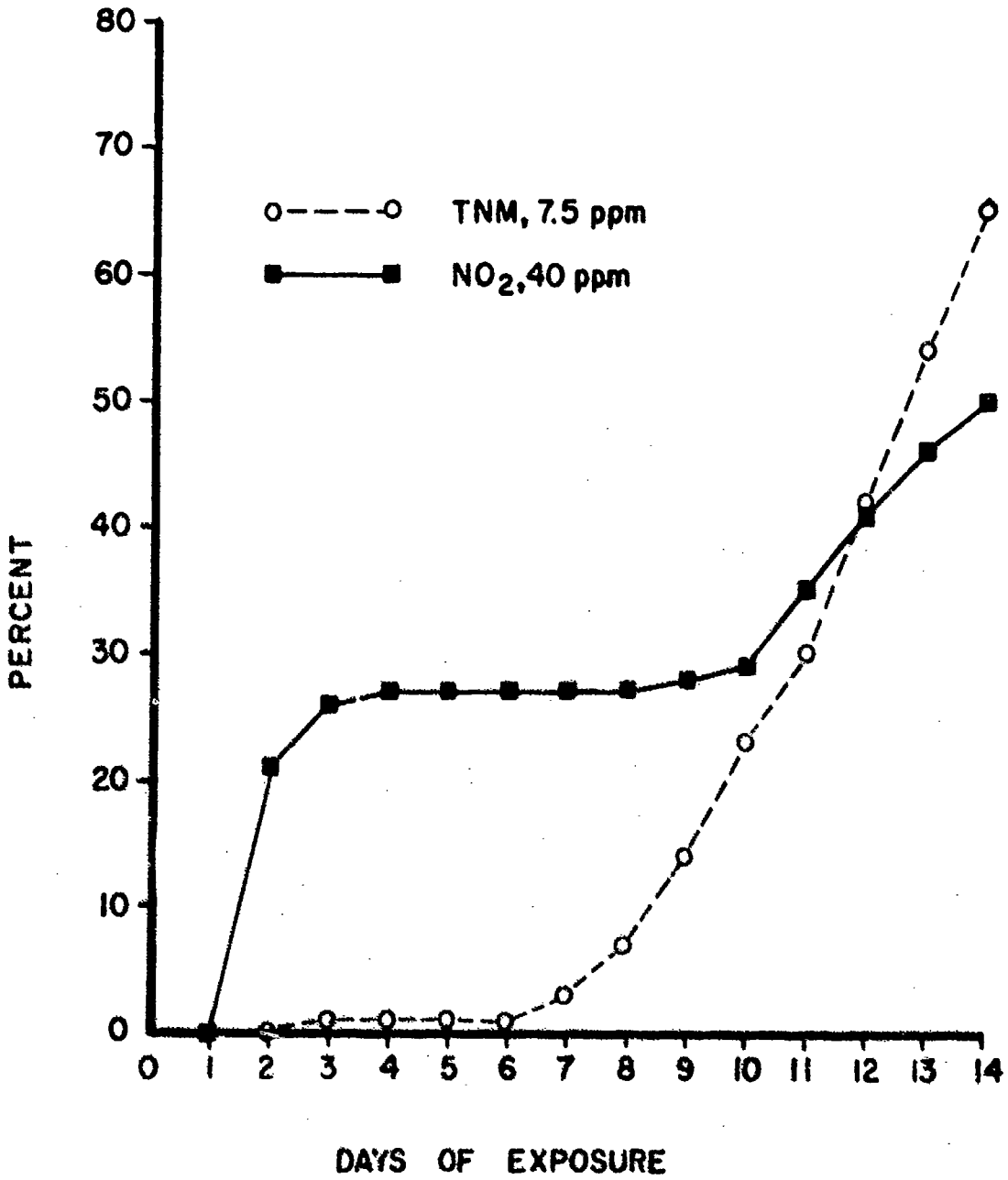


Figure 13. Cumulative mortality resulting from continuous exposure to 7.5 ppm TNM or 40 ppm NO_2.

The results of the pathologic examinations of the contaminant exposed animals indicate that the physiological responses of the rats to either compound are very similar. Both are severe respiratory irritants causing edema and resultant lung congestion with emphysema.

The present Threshold Limit Value (TLV) published by the American Conference of Governmental Industrial Hygienists (1975) for NO_2 is 5 ppm C. On the basis of the results presented in this report, it seems reasonable to suggest that limits for TNM be based on 1/5 the limits already set for NO_2, i.e., a limit of 1 ppm for tetranitromethane vapors should be safe for workmen for normal working periods. A ceiling (C) limit was attached to the 5 ppm NO_2 limit because of the possible lung-tumor accelerating capacity of free radical compounds. Since TNM is not a free radical compound a ceiling limit, if necessary, must be based on other criteria.

Methyl Nitrate

The rat 4-hour LC_{50} of methyl nitrate vapor was experimentally determined to be 1275 ppm or 4 mg/liter and for mice the 4-hour LC_{50} for methyl nitrate was 5942 ppm or 18.7 mg/liter (Table 47). Responses of the animals were dose-related and followed a general pattern of lethargy, decreased respiratory rate and cyanosis. All animals were inactive throughout the 4-hour exposure period.

TABLE 47. 4-HOUR INHALATION LC_{50} VALUES FOR RATS AND MICE EXPOSED TO METHYL NITRATE VAPOR

Rats		Mice	
Conc., ppm	Mortality Ratio	Conc., ppm	Mortality Ratio
1608	10/10	7560	10/10
1285	5/10	6530	10/10
1121	1/10	6020	6/10
642	0/10	5800	3/10
		5500	0/10
		4990	0/10
LC_{50} =	1275 ppm	5942 ppm	
95% C.L. =	1200 to 1355 ppm	5827 to 6509 ppm	

TABLE 48. ACUTE ORAL TOXICITY OF METHYL NITRATE TO RATS AND MICE

Rats		Mice	
Conc., mg/kg	Mortality Ratio	Conc., mg/kg	Mortality Ratio
1000	10/10	2000	8/10
500	10/10	1800	6/10
397	6/10	1590	0/10
315	4/10	1260	0/10
250	1/10		
LC_{50} =	344 mg/kg	1820 mg/kg	
95% C.L. =	308 to 384 mg/kg	1738 to 1906 mg/kg	

Rats that died as a result of vapor exposure died either during exposure or in the following 12 hours. Mouse deaths were often delayed, ranging from 3 to 11 days postexposure. With few exceptions, rats and mice that survived the 14-day postexposure period showed normal body weight gains.

Mild to moderate pulmonary congestion with focal areas of hemorrhage was seen in both rats and mice. Nonlethal doses showed similar gross changes.

Single peroral doses of methyl nitrate to fasted male rats produced an LD_{50} value of 344 mg/kg as shown in Table 48. Administration of methyl nitrate to fasted male mice by the peroral route resulted in an LD_{50} of 1820 mg/kg. Both rats and mice were inactive and lethargic immediately following the intragastric dosing. Rats also exhibited labored breathing and gasping at the highest dose level. Deaths were seldom delayed with most occurring during the 12-hour period immediately following dosing. Control animals given ethanol alone survived the 14-day observation period and showed normal body weight gains.

Since the toxicity of methyl nitrate is much greater in rats than in mice by either the oral or inhalation routes of exposure, the possibility existed that this difference of approximately 5-fold could be a function of body size and if so it could be significantly more toxic in man. To eliminate this possibility a third species, guinea pigs, was examined for comparison of oral toxicity to the preceding data. It was suspected that the metabolism of the methyl nitrate was different in the rat than in the mouse and that the toxicity difference would not necessarily be related to body size or surface area.

Ten guinea pigs were dosed at each concentration level and the LD_{50} calculated using the probit analysis method of Finney (1952). Deaths which occurred during the 14 days immediately following the administration of the single oral dose were included in the final mortality (Table 49). All that survived the 14-day postexposure observation period were sacrificed at that time.

Gross pathologic examinations were done on all guinea pig that died following the administration of the oral dose. Also, representative animals from groups sacrificed at the conclusion of the 14-day holding period had gross examinations.

TABLE 49. ACUTE ORAL TOXICITY OF METHYL NITRATE TO GUINEA PIGS

Dose Level, mg/kg	Mortality Ratio*
1000	10/10
800	7/10
600	7/10
500	3/10
400	1/10
200	1/10

LD_{50} and 95% C. L. = 548 (456-658) mg/kg

*Number that died over the number dosed.

All guinea pigs that died as a result of the peroral administration of the compound did so within 24 hours of dosing with the exception of the guinea pigs that died at the 200 and 400 mg/kg levels. The one animal that died at 4 days after dosing had an extraneous infection with abscess in the trachea and it is, therefore, doubtful that death could be attributed to the methyl nitrate toxicity. The other guinea pig had a slight respiratory infection which may have attributed to its demise.

Gross examination of the guinea pigs that died following the single oral dose revealed chocolate-brown discoloration of the blood and lungs. Except for the livers appearing slightly pale, no other lesions were observed. Gross examinations of the animals surviving the 14-day observation period revealed no treatment related lesions.

The guinea pig oral LD_{50} of methyl nitrate (548 mg/kg) would place it in the toxic category of most classification systems. These results showed that the methyl nitrate toxicity in this species was not higher than that found earlier in rats (344 mg/kg). Therefore, the hypothesis of a relationship between methyl nitrate toxicity and body surface area or size was rejected.

Nitrotoluenes

Exposures to essentially saturated vapors of each of the nitrotoluene isomers, ortho, meta and para, resulted in no deaths of either rodent species. The saturation concentrations at 22 C (chamber temperature) of the nitrotoluenes were calculated from the Antoine Equation (Lange, 1956).

$$\text{Log } P = \frac{-52.23B}{T} + C_1$$

where T is temperature in Kelvin and B and C are the constants below:

Isomer	B	C	Temperature Limits (C)
ortho	48.114	7.9728	50-225
meta	50.128	8.0655	55-235
para	49.95	7.9815	80-240

Although the lower temperature limits of this equation are higher than the exposure chamber temperature, the values were used to check saturation concentrations obtained in standard bags. The following table compares the experimental and theoretical values.

	Saturation Concentration, ppm	
Compound	Theoretical*	Experimental
ortho	392	416
meta	200	203
para	174	228

*Obtained from Antoine Equation.

It can be seen that the theoretical and experimental values for the ortho and meta isomers are in good agreement. The greater deviation of the theoretical value for para-nitrotoluene from experimental is probably due to the fact that the lower temperature limit of the Antoine Equation for this compound is 80 C, much higher than for the other two. Extrapolation to chamber temperature might, therefore, lead to greater error for para-nitrotoluene.

Rats and mice were exposed for 4 hours to the highest concentrations attainable for these compounds. The concentrations obtained are listed below:

Compound	Species	Conc., ppm	Percent of Saturation
ortho	Rats	320	77
	Mice	354	85
meta	Rats	157	77
	Mice	151	74
para	Rats	152	67
	Mice	228	100

No deaths occurred in any of the exposures or in the subsequent 14-day observation period. The experimental results show that 4-hour exposures to essentially saturated vapors of the nitrotoluenes do not present a toxicity hazard to either rats or mice.

All animals gained weight normally during the 14-day observation period. Gross pathological examination of animals sacrificed after 14 days revealed no lesions which could be attributed to exposure.

When held in covered contact with the clipped trunks of female albino rabbits for 24 hours, the undiluted nitrotoluene isomers, ortho, meta and para, proved to be nontoxic. The dose level of 20 g/kg was not absorbed during the 24-hour period and increasing the dose beyond this level would be uninformative. All rabbits were symptom free and gained weight normally during the subsequent 14-day observation period.

Primary irritation tests on intact and abraded skin were negative for all three nitrotoluene isomers. Readings taken at 24 and 72 hours averaged at less than one per rabbit using the Draize method of scoring. These scores indicate a complete lack of skin irritating potential for these three compounds.

The isomers of nitrotoluene, ortho, meta and para, were essentially nontoxic by the inhalation and transdermal routes of administration. None of the isomers was irritating to the skin of rabbits after 24 hours. These findings are not surprising since the end products of the metabolism of p-nitrotoluene are reported by Williams (1959) to be p-nitrobenzoic acid and p-nitrohippuric acid. Williams further states that the primary products of o-nitrotoluene are o-nitrobenzoic acid and a glucuronide of o-nitrobenzyl alcohol. All of the metabolites are readily excreted and are considered essentially nontoxic. The metabolic fate of m-nitrotoluene has not been identified but it probably follows similar pathways since its low order of toxicity is comparable to the other isomeric forms. These three compounds should not present a hazard to humans by the routes examined.

SECTION III

FACILITIES

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

Analytical Chemistry Programs

During the past year, the chemistry department of the THRU has continued to exercise its function of developing and operating continuous procedures for the analysis of contaminants being tested in the toxicology program. In addition to this primary responsibility, efforts have been directed toward estimation of the concentration of contaminants or metabolic products of contaminants in the urine and blood of experimental animals. In cases where the chemical and physical properties of the contaminant were such as to require nonroutine methods of introduction, the chemistry department has been assigned the task of designing, testing and operating the contaminant introduction procedures.

Results of initial studies on gas and liquid chromatography of physiological fluids, with most work being done on urine, were reported in last year's annual report. The benefits of developing precise techniques for chromatographic analysis of body fluids were given as:

1. Identification of compounds whose concentrations change upon intoxication with a particular compound. This would lead to inferences concerning metabolic effects of the toxicant.

2. Use of the chromatographic technique to identify individuals who have been exposed before overt toxic signs appear.

This report will outline the progress which has been made in both areas of chromatography, liquid and gas.

Liquid Chromatography

Since the last annual report, considerable progress has been made in developing procedures for liquid chromatographic separation of urine constituents. Initial experiments with anion and cation exchange columns gave poor separations as did normal elution chromatography on silica columns. The only columns which appeared promising in the preliminary work were reverse phase columns. In reverse phase

chromatography, materials are eluted from the column by a liquid more polar than the column stationary phase. The most polar materials are eluted first contrasted to the situation in regular liquid chromatography where the less-polar eluant removes materials with lower polarity first. Because the great majority of urine components are very polar, it was extremely difficult to obtain any elution at all using a polar column and a non-polar elution liquid. Since reverse phase uses a non-polar stationary phase and polar eluant, the urine components are much more easily eluted. The reverse phase material showing most promise was Bondapak C_{18}® in which fatty acid groups containing 18 carbons in the chain are chemically bonded to the polymer matrix of the beads making up the column packing.

Many early experiments demonstrated that even polar organic solvents such as methanol, or their mixtures with water, failed to elute urine constituents satisfactorily from reverse phase columns. Pure water did appear to be capable of giving separation. However, until a smaller particle size packing was tried, 10 μm diameter instead of 45 μm, separations were poor. It was found that the smaller particle size material gave efficiences of up to 10,000 theoretical plates/foot compared to 100 for the 45 μm diameter material. Using the small particle size material, direct injections of filtered rat urine into the chromatograph followed by water elution gave 28 peaks in 3 hours at a water carrier

flow rate of 0.2 ml/min and 30 peaks in 6 hours at 0.1 ml/min. There was no advantage gained in separating 2 more peaks at the expense of doubling analysis time, so 0.2 ml/min was chosen as optimum flow rate.

Since high solvent polarity was necessary for good elution, the usefulness of increasing the polarity of the aqueous eluant was considered. Solutions of sodium acetate trihydrate at concentrations of 2.5, 5.0 and 10 g/liter were tried, and the 2.5 g/liter solution yielded the greatest number of peaks, 38. The effect of pH was tested by adding acetic acid to give solutions of pH from 4.0 to 7.0. Neutral solutions were found to perform best with optimum results obtained by dissolving 2.5 g/liter sodium acetate trihydrate in distilled water which had sat for several days and adjusting the pH to 7.0 with acetic acid. Using this solvent system with a 1/4" x 1' micro Bondapak C_{18}® column at a flow rate of 0.2 ml/min up to 56 peaks could be separated. Figure 14 is a typical chromatogram of filtered urine under these conditions.

In order to determine whether liquid chromatographic techniques were capable of distinguishing between intoxicated and normal animals, pooled 24 hour urine samples from a group of 3 rats were taken 5 successive times and chromatographed. Urine from the same group of rats was sampled on 4 separate occasions during which the animals were

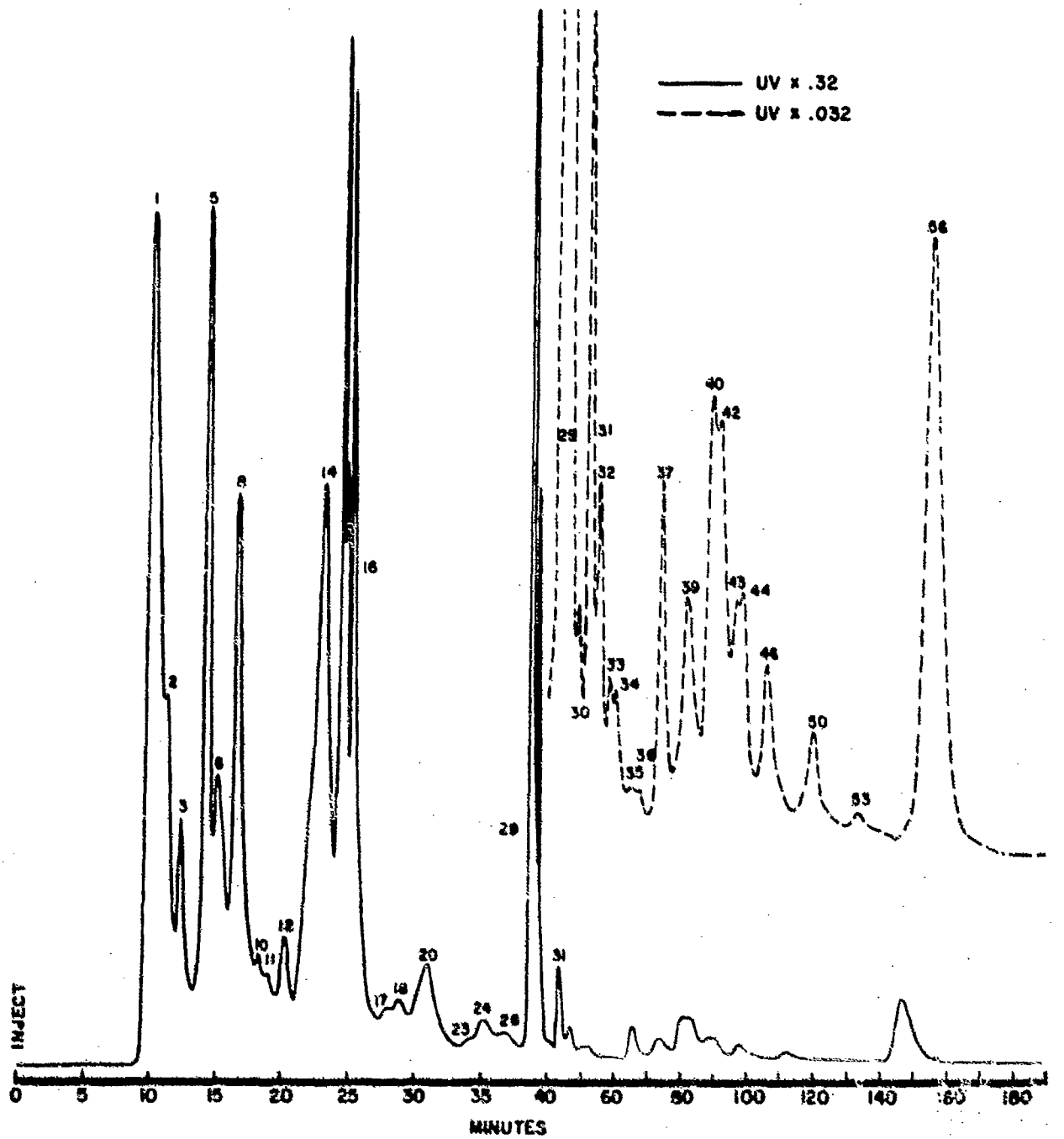


Figure 14. Liquid chromatogram of filtered normal rat urine.

fasted. This was done because the animals became anorexic after intoxication, and we wished to make certain that any differences noted were due to intoxication rather than fasting. A second group of rats was sampled once with no removal of food and 4 times during fasting. Animals from each group were given i. p. injections of 0. 2 ml carbon tetrachloride and 24 hour urine samples were collected after food removal. Tables 50 and 51 present data from each group. Reference to the tables reveals that there are significant differences between peak heights obtained from the urine of fasting animals and those allowed to eat normally. The fasting peak height ranges marked with asterisks are completely outside of the normal peak height ranges indicating that significant changes have taken place. The underlined peaks in both tables have peak heights after intoxication which lie outside the ranges of normal and fasting peak heights. If the peak after administration of carbon tetrachloride is outside of ranges of the fasted baseline group but not of the normal group, it is coded 'FF." All asterisked, underlined and coded peaks are the same in both tables since the criteria of difference had to be met in both groups to be judged significant.

TABLE 50. EFFECT OF FASTING AND INTOXICATION WITH CARBON TETRACHLORIDE ON LIQUID CHROMATOGRAM OF POOLED RAT URINE, GROUP A[1]

Peak No.	Normal Baseline[2] Avg.	Normal Baseline[2] Range	Fasting Baseline[3] Avg.	Fasting Baseline[3] Range	Fasting 0.2 ml CCl_4, i.p.	2 Weeks Post-injection Normal
1	1894	1656-2150	344	*314-388[4]	217	1641
2	152	63-225	348	*326-375	288	81
3	383	289-475	378	351-401	312 FF[5]	444
4	0	0-0	14	0-37	0	0
5	1689	1380-1805	879	*733-1003	634	1668
6	321	287-437	67	*34-85	20[6]	215
7	3.4	0-17	50	0-82	0	0
8	960	812-1091	691	*522-804	2983	1114
9	0	0-0	3.0	0-12	0	11
10	68	36-134	23	7.9-47	0	108
11	48	15-90	4.1	1.8-7.9	21	16
12	181	80-325	40	*20-56	5.8	196
13	51	0-219	35	8.5-5.2	10	40
14	530	148-1101	303	223-367	441	1291
15	0	0-0	0	0-0	0	0
16	2843	2525-3081	2430	2295-2560	2373	2582
17	26	1.2-50	2.3	0-9.3	0	22
18	50	5.5-118	9.0	0-21	1.0	0
19	0.6	0-3.0	9.5	0-28	0	0
20	90	0-161	40	5.6-76	5.1 FF	102
21	64	0-122	33	0-56	0	129
22	44	0-118	3.2	0-9.2	0	9.4
23	25	0-116	6.7	0-17	0	15
24	15	0-64	7.1	0-19	15	0
25	7.4	0-19	10	0-18	0	32
26	53	26-75	16	*7.6-21	1.7	0
27	20	0-47	9.2	5.2-13	14	43
28	2.2	0-6.1	8.6	4.3-17	6.1	2.7
29	2403	2012-2918	966	*835-1048	400	2905
30	12.8	8.6-17	5.6	2.0-8.5	21	5.4
31	191	153-197	143	111-180	53	215
32	34	16-51	64	*54-76	15	54
33	21	17-25	15	9.9-21	53	20
34	6.9	2.1-17	1.6	0-3.9	9.5 FF	5.4
35	1.7	0-8.5	0	0-0	1.4 FF	0
36	3.8	0-10	1.0	0-2.6	1.7	4.0

TABLE 50 CONTINUED.

Peak No.	Normal Baseline(2) Avg.	Range	Fasting Baseline(3) Avg.	Range	Fasting 0.2 ml CCl_4, i.p.	2 Weeks Post-injection Normal
37	70	59-77	50	*45-56	78 FF	89
38	0	0-0	0	0-0	4.1	0
39	19	11-38	16	11-19	9.2	27
40	78	61-87	56	52-63	61	86
41	19	0-31	0	0-0	0	0
42	3.4	0-17	0	0-0	0	0
43	24	10-39	14	5.6-22	0	59
44	27	13-38	16	8.?-20	4.1	0
45	1.2	0-6	2.5	0-10	0	0
46	18	14-31	11	6.8-20	15	32
47	0	0-0	0	0-0	0	3.2
48	1.0	0-3.8	4.4	0-17	0	0.8
49	0	0-0	1.3	0-2.2	0	2.7
50	18	15-21	6.6	*5.6-7.8	1.4	20
51	0.2	0-0.8	0	0-0	0	0
52	0	0-0	0	0-0	0	4.0
53	1.5	0-3.8	0.5	0-1.1	1.0	0
54	1.6	0-3.6	0.5	0-1.1	0	1.3
55	1.6	1.1-2.1	1.8	1.0-2.5	2.8	0
56	102	69-145	46	38-52	34	207

(1)Three rats in the group.

(2)Five samples taken at different times.

(3)Four samples taken at different times.

(4)Asterisked values outside of normal baseline range.

(5)FF coded values - outside of fasting range.

(6)Underlined values - outside of fasting and normal range.

TABLE 51. EFFECT OF FASTING AND INTOXICATION WITH CARBON TETRACHLORIDE ON LIQUID CHROMATOGRAM OF POOLED RAT URINE, GROUP B[1]

Peak No.	Normal Baseline[2]	Fasting Baseline[3] Avg.	Range	Fasting 0.2 ml CCl_4, i.p.
1	1380	133	*86-165[4]	272
2	161	375	*351-403	336
3	299	327	302-349	275 FF[5]
4	0	0	0-0	48
5	1621	779	*467-906	608
6	276	62	*48-72	9.6[6]
7	0	14	0-56	0
8	994	686	*656-734	3328
9	0	0	0-0	0
10	0	14	0-32	0
11	28	14	0-37	7.4
12	108	27	*0-57	102
13	0	23	0-51	12
14	805	492	437-582	672
15	0	0	0-0	0
16	2553	2295	2051-2660	2416
17	46	2.0	0-8.1	0
18	23	14	11-16	16
19	0	0	0-0	0
20	140	78	66-96	32 FF
21	78	22	0-43	32
22	0	16	0-41	0
23	0	0	0-0	0
24	0	5.1	0-12	16
25	35	11	0-32	0
26	21	2.9	*0-9.6	0
27	13	11	6.4-13	21
28	27	5.6	0-13	7
29	2139	1029	*866-1152	576
30	4.6	2.1	0-6.0	24
31	144	183	163-238	64
32	38	60	*43-77	13
33	29	16	11-23	83
34	3.5	2.5	0-4.3	21 FF
35	0	1.3	0-2.9	3.2 FF
36	0	0.8	0-3.2	0

TABLE 51. CONTINUED

Peak No.	Normal Baseline(2)	Fasting Baseline(3) Avg.	Range	Fasting 0.2 ml CCl_4, i.p.
37	99	62	*58-64	90 FF
38	0	1.2	0-4.8	0
39	16	17	15-22	11
40	214	147	144-150	187
41	0	0	0-0	0
42	0	0	0-0	0
43	51	16	0-32	6.4
44	0	15	0-36	11
45	0	1.6	0-6.3	0
46	23	12	2.9-20	14
47	0	0	0-0	0
48	1.6	0	0-0	0
49	0	1.6	0-2.8	0
50	12	7.0	*6.0-8.7	3.8
51	0.5	0	0-0	0
52	0	0	0-0	0
53	1.6	1.5	0.8-2.3	1.6
54	0.9	0.4	0-1.1	2.6
55	2.3	3.3	2.2-4.8	0
56	78	59	39-78	62

(1)3 rats in group

(2)One sample taken.

(3)Four samples taken at different times.

(4)Asterisked values - range does not include normal baseline value.

(5)FF coded values - outside of fasting range.

(6)Underlined values - outside of fasting and normal range.

On intoxication, a large increase in height occurs at peak 8 which is 3-6 times higher than in normal or fasted baseline animals. Less striking increases occur in peaks 30, 33, 34, 35 and 37. Most other peaks decrease after administration of carbon tetrachloride with the greatest relative differences from baselines occurring at peaks 6, 29, 31 and 32. A normal urine chromatogram taken 2 weeks after intoxication demonstrated that most peaks had returned to expected values.

Future work will be done on liquid chromatographic changes induced by monomethylhydrazine intoxication and on the reproducibility of alterations in the chromatograms caused by the 2 model toxic chemicals, carbon tetrachloride and monomethylhydrazine.

Gas Chromatography

The gas chromatographic method of measuring urine components reported last year was altered significantly to improve volatilization of vapors, eliminate the need of an internal standard, concentrate the sample to a greater degree and improve peak resolution. Instead of condensing the vapors in a liquid distillate, nitrogen is bubbled through 5 ml of urine for 20 minutes. The urine to which 25 μl of concentrated sulfuric acid has been added is heated in a boiling water bath. The

exhaust vapors are cooled to room temperature at which point water condenses. The remaining volatile substances are trapped on a 1/8" x 4" SS column containing Chromosorb 101 cooled to 0 C. Very little water is trapped on the column, and the trap weight gain is less than 5 mg. The trap is capped and stored at 0 C or used immediately. For chromatography, the trap is uncapped and quickly inserted through a ball valve into the injection port which has been heated to 200 C. The column temperature is then programmed at the rate of 1 degree/min to 203 C and held at that temperature to termination of the chromatogram. Other chromatographic conditions are as follows:

Gas Chromatograph:	Hewlett-Packard Model 5750 with dual Columns, flame ionization detection.
Column:	1/8" O. D. SS containing Tenax® 60/80 mesh.
Temperatures:	Injector - initially 200 C, slowly drops to column temperature during programming.
	Column - After injection, 2 minutes at ambient temperature then programmed 50-203 C at 1 degree/min.
	Detector - 250 C
Gas Flows:	Helium carrier - 25 ml/min Hydrogen - 30 ml/min Air - 300 ml/min.

Using these conditions, 47 measurable peaks are separated in 3 hours. Figure 15 is a chromatogram of pooled urine volatiles from a group of 3 untreated rats.

The same rat groups sampled for liquid chromatographic analysis were also sampled for gas chromatographic analysis before and after i. p. administration of carbon tetrachloride with the results given in Tables 52 and 53.

As in the case of the liquid chromatograms, fasting of the rats produced significant changes in some of the peaks. Administration of carbon tetrachloride caused peak alterations which could be easily distinguished from those caused by fasting alone.

Engineering Programs

Noise Reduction Program

A study of the sources of noise in the altitude laboratory areas was reported in the last annual report along with proposed remedial actions. This year, vibration isolators were installed in the dome exhaust lines leading to the vacuum pumps. Sound absorbing tape was applied to dome input and exhaust lines, and the exhaust duct in Facility A dome room was completely covered with foam absorber. The basement pump room in Facility A was also completely insulated with absorber material.

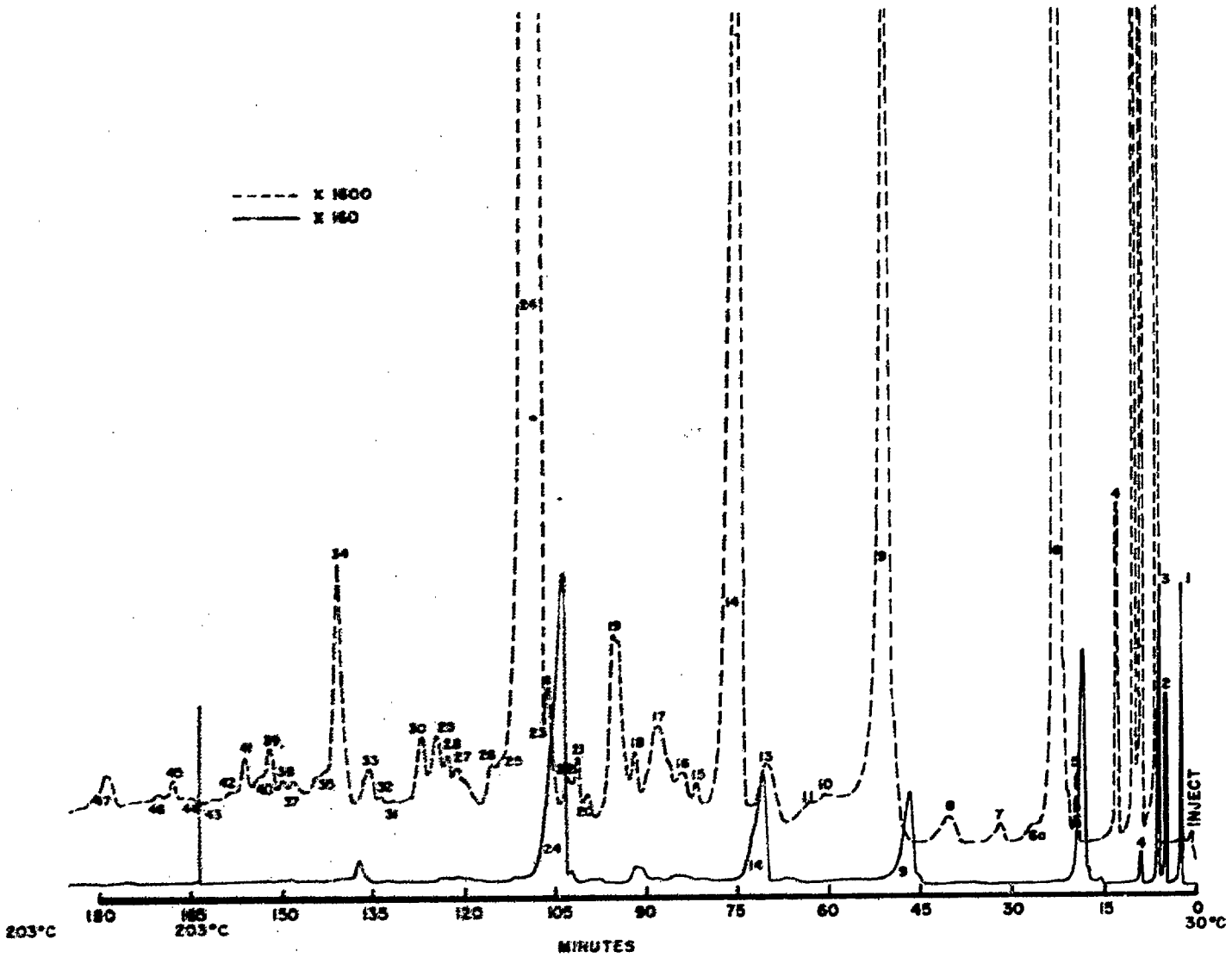


Figure 15. Gas chromatogram of normal rat urine volatiles.

TABLE 52. EFFECT OF FASTING AND INTOXICATION WITH CARBON TETRACHLORIDE ON GAS CHROMATOGRAM OF POOLED RAT URINE, GROUP A[1]

Peak No.	Normal Baseline[2] Avg.	Range	Fasting Baseline[3] Avg.	Range	Fasting 0.2 ml CCl_4, i.p.	2 Weeks Post-injection Normal
1	3228	445-6800	932	238-1276	1153	2268
2	1626	600-2880	1718	877-3213	3481[5]	2520
3	2894	655-5280	3095	1082-5220	3481	4872
4	491	202-828	1968	572-3788	10300	546
5	50	28-74	32	21-33	108	66
6	1949	1462-2333	4970	*3321-7424[4]	7972	2058
7	27	14-72	18	7-27	270	19
8	36	27-77	9.6	8.2-12	15	32
9	1026	928-1344	1126	656-1624	937	1041
10	9.4	6.7-13	2.1	*0-4.1	5.3 FF[6]	11
11	7.6	5.6-11	7.2	3.5-8.8	11 FF	11
12	0	0	0	0	0	0
13	97	74-127	100	84-136	244	84
14	778	640-952	2146	*1722-2807	1617	924
15	37	24-58	16	7.0-25	21	29
16	30	13-45	32	21-46	5.3	35
17	103	74-131	73	66-81	113	134
18	37	21-48	18	1.6-29	18	42
19	181	110-274	207	150-226	294	235
20	22	6.7-51	17	16-19	3.1	25
21	82	22-146	77	52-94	261	76
22	17	12-30	11	8.2-16	5.3	24
23	84	54-125	79	70-87	69	119
24	1904	1427-2800	1462	1172-2053	2204 FF	3041
25	7.5	0-48	0	0	0	4.2
26	58	25-115	38	20-48	146	56
27	18	10-36	89	*40-211	21 FF	25
28	27	5.6-52	9.7	1.8-16	21 FF	21
29	30	19-39	29	14-44	74	48
30	80	62-103	70	60-81	18	83
31	3.1	1.1-6.2	3.0	0-8.2	2.1	2.5
32	13	1.7-36	0.6	0-2.5	2.1	8.4
33	26	11-39	80	12-170	52	33
34	292	121-408	110	82-134	19	389
35	11	8-16	3.3	*0-6.6	11 FF	17
36	2.6	0-7.7	4.4	0.8-12	2.1	1.7

TABLE 52. CONTINUED

Peak No.	Normal Baseline[2] Avg.	Range	Fasting Baseline[3] Avg.	Range	Fasting 0.2 ml CCl_4, i.p.	2 Weeks Post-injection Normal
37	3.6	0.8-8.4	1.2	0-2.3	0	8.4
38	41	1.8-224	145	2.3-362	11	105
39	56	28-94	21	16-29	11 FF	50
40	4.4	1.1-10	4.6	2.6-8.2	0	2.5
41	23	14-46	7.4	*1.6-9.8	44	38
42	5.0	2.5-10	4.1	1.6-8.2	11	4.2
43	2.1	1.1-4.8	5.4	0-18	0	4.2
44	2.6	0-4.8	5.2	2.3-8.2	2.1	4.2
45	6.7	2.5-16	2.7	0-8.2	0	25
46	3.6	2.1-6.8	2.7	1.6-4.1	2.1	4.2
47	49	11-141	25	12-49	11	34

(1)Three rats in group.

(2)13 Samples taken at different times.

(3)Four samples taken at different times.

(4)Asterisked values - outside of normal baseline range.

(5)Underlined values - outside of fasting and normal baseline ranges.

(6)FF coded values outside of fasting baseline range.

TABLE 53. EFFECT OF FASTING AND INTOXICATION WITH CARBON TETRACHLORIDE ON GAS CHROMATOGRAM OF POOLED RAT URINE, GROUP B[1]

Peak No.	Normal Baseline[2]	Fasting Baseline[3] Avg.	Fasting Baseline[3] Range	Fasting 0.2 ml CCl_4, i.p.
1	1008	1491	530-3672	2290
2	1404	1727	912-2124	2740[5]
3	1692	2966	1520-4032	5200
4	850	2377	479-7224	640
5	29	13	5.3-28	28
6	1944	3866	*2970-4505[4]	7080
7	18	27	11-48	17
8	29	8.7	7.6-9.5	10
9	684	811	757-920	700
10	8.6	2.8	*1.5-4.5	8.0 FF[6]
11	4.3	4.6	1.7-6.9	8.0 FF
12	101	1.7	0-6.9	0
13	97	87	63-107	285
14	972	2091	*1982-2318	2003
15	27	12	7.6-15	16
16	32	38	12-58	20
17	85	61	46-87	80
18	36	17	13-22	20
19	217	210	153-271	138
20	11	19	17-21	10
21	68	42	32-54	310
22	18	12	7.2-24	10
23	144	78	64-89	68
24	1498	1216	1084-1323	1870 FF
25	0	0	0-0	0
26	58	32	15-59	12
27	18	29	*27-30	5.0 FF
28	3.6	4.1	1.1-6.9	12 FF
29	35	34	26-46	40
30	58	44	33-56	82
31	3.6	3.7	2.1-6.3	10
32	9.4	1.8	1.5-2.1	2.0
33	22	21	8-36	6.0
34	565	189	67-315	53
35	7.2	3.4	*2.3-4.5	13 FF
36	3.6	6.3	3.8-8.6	0
37	5.8	1.1	0-2.7	0

TABLE 39. CONTINUED

Peak No.	Normal Baseline(2)	Fasting Baseline(3) Avg.	Range	Fasting 0.2 ml CCl_4, i.p.
38	20	39	1.6-101	15
39	40	17	8.0-22	5.0 FF
40	3.6	2.0	1.1-2.7	0
41	22	8.5	*4.3-17	40
42	3.6	2.7	1.6-4.3	5.0
43	3.6	0.3	0-1.1	2.0
44	3.6	5.4	0-9.0	2.0
45	11	3.6	1.1-9.1	5.0
46	3.6	2.4	1.1-4.5	3.0
47	31	12.9	6.4-22	5.0

(1)Three rats in group.

(2)One sample taken.

(3)Four samples taken at different times.

(4)Asterisked values - range does not include normal baseline value.

(5)Underlined values - outside of fasting and normal baseline ranges.

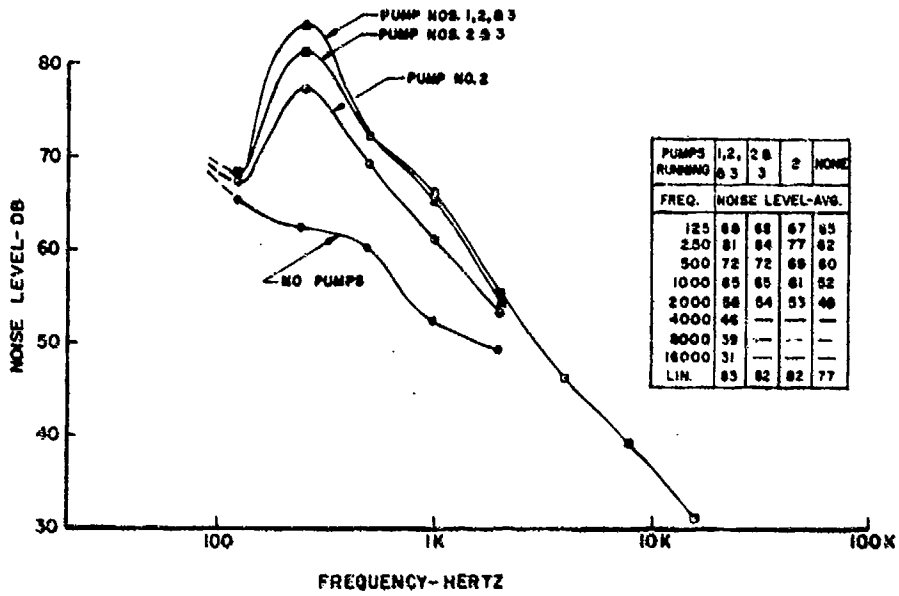
(6)FF coded values - outside of fasting baseline range.

Sound levels were measured at different points in the Facility A dome room, the noisiest area in the THRU laboratory, before and after remedial measures were taken. Figure 16 shows the average noise levels at various sound frequencies measured before any attempts were made to reduce them. Maximum intensity occurred at about 250 Hertz, and running 2 or 3 pumps increased sound levels over a single pump as expected, approximately doubling the intensity for each pump added. (Increasing the decibel value by 3 doubles the intensity.)

Sound levels were measured again after acoustical insulation had been installed, with the results plotted in Figure 17. Here, the level with all 3 pumps running had been reduced more than half. However, there was now no difference between levels produced by Pump #2 alone or in combination with one or both other pumps, and Pump #2 now caused higher acoustic levels than previously. This was attributed to bearing deterioration which had developed after the first measurement. The pump bearings will be replaced and sound levels measured again.

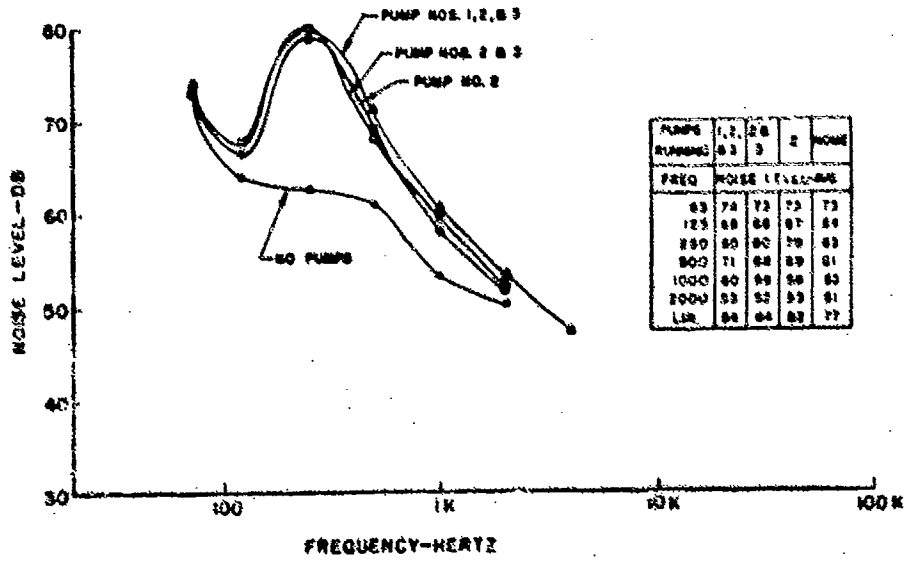
Plethysmograph Chamber

It has been demonstrated by Alarie, (1973) and Freeman et al., (1968), among others, that lower respiratory tract irritants increase the respiration rates in exposed mammals. Equipment which could



PUMPS RUNNING	1,2, & 3	2 & 3	2	NONE
FREQ.	NOISE LEVEL-AVG.			
125	68	68	67	65
250	81	84	77	62
500	72	72	69	60
1000	65	65	61	52
2000	58	54	53	48
4000	46	—	—	—
8000	39	—	—	—
16000	31	—	—	—
LIN.	83	82	82	77

Figure 16. Facility "A" dome room noise levels with and without vacuum pumps in operation before treatment.



PUMPS RUNNING	1,2, & 3	2 & 3	2	NONE
FREQ	NOISE LEVEL-AVG.			
63	74	73	73	73
125	68	68	67	64
250	80	80	78	63
500	71	68	69	61
1000	60	59	58	53
2000	53	52	53	51
LIN.	84	84	83	77

Figure 17. Facility "A" dome room noise levels after minimal acoustic treatment.

measure these rates quickly and precisely in rodents was considered to be a necessary addition to the experimental capability of the THRU. Therefore, a project was instituted during the past year to develop instrumentation which would meet the following design criteria.

1. Simultaneous exposure and measurement of respiration rates of 10 mice.

2. Easy insertion of the mice into and removal from the exposure chambers.

3. Minimization of physical stress in immobilizing the mice.

In most plethysmographic chambers, respiratory parameters are determined by measurement of slight pressure changes caused by breathing. This requires, in addition to a sensitive and precise manometer, a leak-free system since any movement of gas into or out of the chamber would lead to erroneous pressure readings. In order to obviate this drawback, it was decided to measure breathing rate by detecting the flow of air in an open tube leading from the chamber. Since the system is open and at atmospheric pressure, leakage of air in other parts of the system should not be as likely as in a closed system. Thermistors were chosen as the most sensitive detectors of air flow. In the system as presently designed,

it is necessary only to trigger a counter once the air flow has reached a critical value. In this way a single breath inhalation and exhalation will trigger the counter twice. As experience is gained in the use of the equipment, it is possible that breath volumes can be measured by calibration of the thermistors using known flow rates.

In order to test the system before final construction a unit holding one mouse was built. This was found to operate well in the measurement of respiratory rate without imposing undue stress on the test animal. Following this, construction of the final manifold system was begun and is now almost complete. This system is similar to ones which have been used in the past for simultaneous cephalic exposure of a number of mice. Modifications have been made in the general design to facilitate insertion of the animals and to keep them in position as comfortably as possible.

As shown in Figure 18, the main portion of the chamber consists of a transparent, cylindrical, plastic tube with an inside diameter of 3-3/4" and 17" in length. A cap of flat transparent plastic was secured to each end with appropriate gasketing to achieve an airtight condition. A port and baffle was placed in one end for introduction of the exposure atmosphere. The opposite end contains a port for exhausting the chamber.

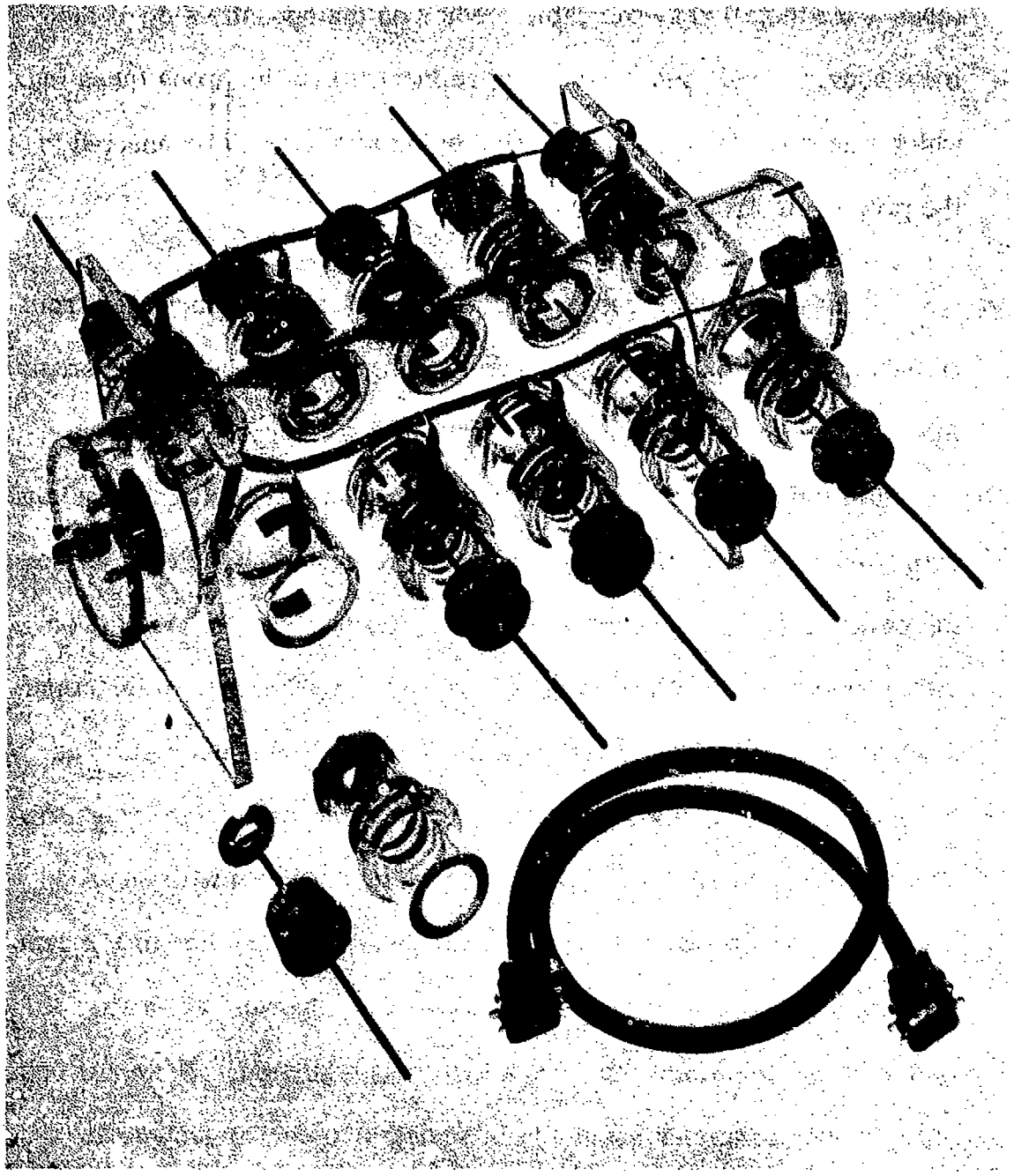


Figure 18. Plethysmograph chamber for mice.

Five intersecting transparent cylindrical tubes were installed on one side of the main body of the chamber by solvent bonding. Five other tubes were installed by the same method on the opposite side of the main body. Small sensor tubes were mounted on the cross tubes into which was threaded a connector containing a thermistor connected to the proper electronic instrumentation.

The assemblies used to contain the mice were fabricated from transparent, cylindrical plastic tubing designed to fit inside the cross tubes on the main body chamber. Three annular grooves were machined in the outer surfaces of these components. A collar around the outer surface locates the center groove to correspond with the sensor tube when inserted into the cross tube. The two annular grooves on either side of the manifold groove are used for "O" rings to maintain airtight integrity. The front of these assemblies consists of two additional collars locking in a rubber dam between two Mylar® washers. The discs and collars are then assembled and secured with threaded fasteners. The heads of the mice are inserted through the rubber dam, forming a seal around the neck. This entire subassembly is attached to the mouse container units and secured with screws. The outer end of the mouse container assembly is sealed by a rubber stopper through which a piston rod is passed to prevent the mice from backing up in the container.

Statistics Programs

In the evaluation of experimental toxicological data one of the most frequently utilized statistical techniques is testing the hypothesis that mean values of some parameters are equal in 2 groups of animals or that the variances of the mean values are equal. These techniques are standard in determining whether exposure to a particular material has had a measurable toxic effect. An exposure is judged to have had an effect when the probability of the means and/or variances being equal is below a certain level of significance, say 0.05 or 0.01. This probability is determined from distribution curves, T-distributions for means, and F-distributions for variances.

Previously, computer programs had been developed for calculation of the probabilities from F and T statistics obtained from the data. These utilized a numerical analysis technique known as Simpson's Rule. Investigation of the literature indicated that there were alternative methods available which were less mathematically ambiguous and which took up less computer time. After comparing several methods it was determined that best results could be obtained from use of the Incomplete Beta Function to generate both T and F-distributions. By changing the parameters of the function in a simple manner, the codes for the two

separate routines could be reduced to one program. This significantly decreased the number of program cards requiring storage and reduced code length and computer time. The code for use of the Incomplete Beta Function for this purpose was obtained from the International Mathematical Statistical Library. A large scale test of the new method was conducted comparing calculations with those obtained from the Simpson's Rule technique and an IBM library program. The new procedure gave results essentially identical with the others in faster time.

During this year, many computer subroutines were transferred to the EDITLIB library which is a computer machine language storage area for programs indexed according to user. A parallel library of these programs in Fortran computer cards called UPDATE was built up to back up the EDITLIB library in case it is wiped out accidentally in some way. The advantage of the EDITLIB library is that it permits commonly used subprograms to be called up automatically for use in a program through use of simple code identification. Different programs can share a simple subprogram, and the user may add elements to the library or delete them. The size of most programs is decreased considerably through use of EDITLIB and a great deal of flexibility is contributed to programming.

Two new large programs, MODPLOT and AUWEIGH, were written. Program AUWEIGH was written to process the data available from the load cell weighing console. This program converts binary code into decimal form, then processes the data and records the results on standard hard copy. Program MODPLOT was written to replace an earlier Calcomp data plotting program. The earlier verson had not been designed for the extended time periods which current experiments cover. MODPLOT is capable of plotting data from both body weight and clinical chemical measurements. Previously, each kind of data required a different program.

Training Programs

Chamber Technicians

Since last year's annual report, one technician has been hired. Phase I and Phase II formal training cycles were scheduled for the new technician. Both phases were completed and all experienced technicians participated in the on-the-job training of this new technician.

Written, deliberate and simulated monthly Emergency Training Procedures were given to all chamber technicians during the year. These procedures provide refresher training as well as insuring that the technician will react properly in the event of an actual emergency. The technicians involved in these training procedures are monitored by their supervisor to insure that the SOP is adhered to.

The following list details the emergency training procedures covered during the past 12 months:

Date	Procedure	Personnel Participation*
June 1975	Unscheduled Complete Power Failure	A
July 1975	Complete Power Failure	A
August 1975	Air Compressor Failure	A
September 1975	Vacuum Pump Failure	All
October 1975	Complete Power Failure	A
November 1975	Rescue of Incapacitated Dome Entrant	All
December 1975	Fire in Airlock During Entry	All
January 1976	Air Supply Fan Failure	A
February 1976	Fire in Airlock During Entry	A, B, C
March 1976	Fire in Exposure Laboratory Area During Dome Entry	A, B, C

Date	Procedure	Personnel Participation*
April 1976	Operation of the Scott Air Pak	A
May 1976	Fire in Dome - No Entrant	A

*A - Shift Operator
B - Safety Observer B
C - Safety Observer C
D - Dome Entrant
All - All Chamber Technicians

The Animal Care Training Program described in the 1974 and 1975 annual reports was continued this past reporting period as programmed. This program has been most successful and now most chamber technicians can successfully draw blood samples from several species of laboratory animals. The chamber technicians have all had considerable practice in the handling of many species of laboratory animals.

All chamber technicians have been trained in the operation of the weighing console for weighing animals in the exposure chambers. A new addition to the weighing system, badge ID cards, also required a new training program to be given to the technicians. A Standard Operating Procedure (SOP) was written to accommodate this instruction.

Animal Technicians

Since last year's annual report, several technicians have become certified in the AALAS program. One became certified at the second level (Animal Technician), while six were certified at the first level (Assistant Animal Technician). Several technicians plan to become certified during the ensuing months as time and experience requirements are fulfilled. UCI animal care personnel certification in the AALAS program is as follows:

2 - Laboratory Animal Technologists

2 - Laboratory Animal Technicians

6 - Assistant Animal Technicians.

Upon completion of the first level AALAS examination, six Animal Caretakers were promoted to Animal Technician positions.

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

The need for a formal course in laboratory animal science was evident after conducting many carcinogen experiments over the last several years. All animals in these experiments are held for their lifetime after the exposure phase of the experiment. The THRU technicians must now be able to identify various animal diseases as well as

skin tumors in our colony of approximately 7700 animals currently being housed at the THRU. Current trends in the conduct of chronic inhalation studies suggest the need for long-term postexposure observation and testing of experimental animals. Therefore, education of the entire group of technicians in the field of laboratory animal science is of great concern particularly as it relates to animal care and maintenance.

Videotapes of the animal courses described in last year's technical report (MacEwen and Vernot, 1975) were utilized in the training of four animal caretakers and two chamber technicians. The following list details the training procedures covered by the course.

Subject	Number of hours
Introduction of Anatomy and Physiology	1
Skeletal System	2
Muscular System	3
Central Nervous System	4
Respiratory System	5
Cardiovascular System	6
Cardiovascular System and Urinary System	7
Reproductive System	8
Digestive System	9-10
Endocrine System	11
Skin and Appendages	12
Fundamentals of Disease	13-15
Metric System	16
Fundamentals of Disease	17-18
Animal Welfare Act	19
Primatology	20
Pharmacology	21-24

Subject	Number of hours
Clinical Laboratory	25
Parasitology	26-27
Procurement and Quarantine	28
Standardization	29
Records, Identification and Gnotobiology	30
Nutrition	31
Primate Diseases	32-33
Dog Diseases	34
Cat Diseases	35
Sanitary Standards	36
Rabbit and Rodent Diseases	37-39
Zoonoses	40-42

Prosectors

A new category of staff positions was established in the Toxic Hazards Research Unit during the past year to handle the larger numbers of animals, particularly rodents, used in oncogenic studies. The greater emphasis placed on oncogenic studies in the THRU during the past few years has overwhelmed our limited pathology capability and a group of light prosectors have been hired and placed in training to alleviate this problem. Each of the prosectors is trained to act as part of a team accomplishing four functions. The senior prosector performs necropsies to expose and remove important organs and has the ability to identify all organs and tissues and to recognize abnormalities while the assistant prosector prepares the tissue for fixation, dissecting if necessary for proper fixation of larger organs or abnormal sections of organs. The other 2 functions are those of tissue cutting and tissue embedding.

The new prosectors were given a 24-week training program that consisted of both classroom lectures and laboratory exercises as shown in the following outline:

Training and Qualification of Parapathologists

A. 1st Level (3 weeks)

1. Lectures followed by laboratory demonstration

Subject	Hours Lecture	Hours Lab. Demonstration
a. General Gross Anatomy		
1) Terminology	1	0
2) Systems	1	1
3) Organology	1	1
b. Specific Anatomy by Species		
1) Mouse	1	1
2) Rat	1	1
3) Hamster	1	1
4) Other Species	1	1
c. Gross Pathology		
1) Basic Tissue Changes	1	1
2) Autolytic Changes	1	1
3) Systemic Pathology	1	1
4) Recognition of Lesions	1	1
d. Histopathology Techniques		
1) Fixation of Tissue	1	0
2) Tissue Trimming	1	1
3) Tissue Processing (Operation of Technicon)	1	2
4) Embedding Techniques (Operation of Embedding Center)	1	3

2. One lecture hour followed by laboratory demonstrations was given each day. The remainder of each day was devoted to:

   a. Observation of necropsies.

   b. Preparation of instruments, solutions and supplies for necropsies.

   c. Indoctrination on documentation for necropsy and tissue embedding.

   d. Fundamentals in performance of necropsies.

B. 2nd Level (9 weeks)

1. Practice in Necropsies - 4 hours/day

   a. Mice
   b. Rats
   c. Hamsters

2. Observation of necropsies performed on diseased animals - 2 hours/day.

3. Practice in tissue embedding - 2 hours/day.

4. At the conclusion of the 2nd level, a written examination was given over all subjects covered during lectures, and a practical examination was given covering ability to recognize the various tissues and organs of the body, dexterity in removal of tissues, ability to trim fixed tissues and properly embed them.

C. 3rd Level (12 weeks)

1. Team exercise in necropsies stressing cooperation between prosector, assistant prosector, tissue trimmer and tissue embedding technicians - 3 hours/day.

2. Performance of necropsies on diseased animals, stressing identification of lesions and description of lesions with respect to color, texture, size, consistency, location, plus alterations in anatomy stressing abnormal location, absence and/or abnormal growths - 2 hours/day.

3. Practice in necropsy preparation, documentation and tissue handling techniques - 3 hours/day.

After completion of the 24-week program the parapathology trainee was expected to be able to recognize and describe any pathological change in tissue that could be seen with the unaided eye, be able to recognize autolytic changes in tissue, have demonstrated capability in the teamwork approach to high volume necropsy procedures, and be accomplished in the care and precision needed in necropsy and necessary histotechnology techniques for small rodent tissues.

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