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TOXIC HAZARDS RESEARCH UNIT ANNUAL TECHNICAL REPORT: 1977

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TECHNICAL REVIEW AND APPROVAL

AMRL-TR-77-46

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
Toxic Hazards Division
Aerospace Medical Research Laboratory

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The research programs of the Toxic Hazards Research Unit (THRU for the period of June 1976 through May 1977 are reviewed in this report. Acute and subchronic inhalation toxicity studies were conducted on decalin. Studies were carried out on the oncogenicity of inhaled hydrazine, 1,1-dimethylhydrazine and monomethylhydrazine. Acute oral, dermal and inhalation toxicity studies were conducted on a large variety of chemical agents used by the Air Force and Navy on transported in interstate commerce. Sensitization, eye and skin irritation studies were also made on these chemicals.

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PREFACE

This is the fourteenth annual report of the Toxic Hazards Research Unit (THRU) and concerns work performed by the Department of Community and Environmental Medicine of the University of California, Irvine on behalf of the Air Force under Contract Number F33615-76-C-5005. This document constitutes the first report under the current contract and describes the accomplishments of the THRU from June 1976 through May 1977.

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

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

TABLE OF CONTENTS

TABLE OF CONTENTS (CONT'D)

LIST OF FIGURES

LIST OF FIGURES (CONT'D)

LIST OF TABLES

LIST OF TABLES (CONT'D)

SECTION I

INTRODUCTION

This document constitutes the 14th annual report of the Toxic Hazards Research Unit (THRU), a research team which operates a dedicated inhalation toxicology laboratory to investigate potentially hazardous chemicals and materials of interest to the U. S. Air Force, U. S. Navy and other governmental agencies. The THRU research team is an interdisciplinary group of University of California, Irvine, toxicologists, chemists, statisticians, and engineers supported by Air Force pathologists, veterinarians, and medical technologists.

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

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

During this past year a Toxicology Detachment of the Naval Medical Research Institute was transferred to Wright-Patterson Air Force Base and has joined the Toxic Hazards Division of AMRL in sponsoring the research activities of THRU.

As part of its contract responsibilities, UCI/THRU presents an annual technical conference to disseminate new toxicological information to the U. S. Air Force, and other governmental and industrial scientists. This year's conference, chaired by Dr. Seymour L. Friess, presented 23 technical papers covering a broad range of occupational and environmental toxicology problems. Eight 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 13 October through 15 October, 1976 drew 146 participants including speakers.

The papers presented at the conference were published as the Proceedings of the 7th Annual Conference on Environmental Toxicology, AMRL-TR-76-125, Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio.

Next year's conference, currently in the development stage, will be held in October, 1977 at the Biltmore Towers Hotel, 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 and fluomine aerosols. Acute oral and dermal toxicity studies on transportable materials were also conducted.

Chronic Inhalation Effects of Low Level Exposures to Fluomine Particulates

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

Information regarding the chronic toxicity to man or animals of this compound was not available. Acute toxic effects were reported by MacEwen and Vernot (1974) and by Kinkead et al., (1975). They reported single peroral doses of fluomine as toxic to both rats and mice at relatively low dose levels (LD_{50}'s of 187 and 123 mg/kg, respectively). The dust particles are highly irritating to the eyes of rabbits causing severe irritation to the conjunctivae resulting in chemosis and marked swelling. Single inhalation LC_{50} values were reported for rats and mice for six- and one-hour exposures. The six-hour LC_{50}'s were 112 mg/m^3 for rats and 416 mg/m^3 for mice. The one-hour LC_{50} for rats was 712 mg/m^3 but it was not possible to cause deaths in mice at 890 mg/m^3 for this time period. A single two-hour inhalation exposure to 30 mg/m^3 caused deaths in guinea pigs.

Kinkead et al. (1975) also reported that the dust of this compound was a potent sensitizer by the intradermal route to guinea pigs. Although an anaphylactic-type response was not elicited by the guinea pigs given an inhalation sensitizing regimen, intradermal injections showed that it was possible for the animals to produce antibodies if exposed to repeated inhalations of low concentrations of fluomine.

This study was designed to determine the chronic effects of low level, nonirritating concentrations of fluomine particulates to rodents and dogs for use in recommending safe exposure limits. It was designed to examine effects immediately following long-term exposure as well as effects up to one year postexposure. The sensitizing potential of low level exposure was also examined.

Prior to initiating the long-term study, two-week preliminary exposures were conducted to determine nonirritating concentrations which could be used for the six-month exposure. The animal groups, consisting of 20 male rats and 20 male guinea pigs, were exposed to fluomine particulates, 6 hours per day, 5 days per week, in a small Rochester chamber. A similar control group, consisting of the same numbers, was maintained for comparative purposes.

The initial concentration examined was 2 mg/m^3 based on the previous guinea pig studies by Kinkead. At the conclusion of the two-week exposure period, one half of the rats were sacrificed and the lungs precisely excised and weighed after which they were analyzed for fluomine deposition. The wet lung weights were statistically analyzed for determination of edematous effects. The remaining rats and one-half of the guinea pigs were sacrificed at the conclusion of the exposure period and examined pathologically for toxic effects. The remaining guinea pigs (10 test and 10 controls) were held for a three-week period after which time they were given an intradermal injection of fluomine to determine their sensitization response.

None of the rats died during the exposure period nor were any signs of toxic stress noted. All test and control rats gained weight normally during the two-week study.

The guinea pig deaths occurred, one on the second exposure day, the other at four exposure days. Signs of toxic stress prior to death included labored breathing, diarrhea, and a general unthrifty appearance. The surviving test guinea pigs appeared lethargic and several exhibited intermittent labored breathing. The guinea pigs appeared normal when each day's exposure was concluded and the fluomine removed from the chamber atmosphere. The control guinea pigs appeared normal throughout the study.

Gross pathologic findings in the guinea pigs that died during exposure included diffuse, mild congestion in all lung lobes and the kidneys. In addition, the animal that died following four exposure days showed dark red consolidation of several lung lobes with emphysema. No gross findings, which could be considered treatment related, were found in the remaining guinea pigs.

A significant finding in the rats examined grossly at the conclusion of the study was mild congestion and emphysema. The control rats did not show similar signs and it was therefore considered that the congestion and emphysema were treatment related effects.

A tabular summary of the mean body weights and mean wet lung weights of the rats is presented in Table 1. The results of guinea pig growth measurements made during exposure to the fluomine aerosol are shown in Table 2.

TABLE 1. EFFECT OF 2 WEEK INHALATIONEXPOSURE TO 2 MG/M^3 FLUOMINE AEROSOL ON RAT GROWTH AND LUNG WEIGHT

	Body Weight (grams) Preexposure	Final	Lung Weight (grams)	Lung/Body Wt. Ratio
Exposed*	215	268	1.96**	.0073
Unexposed Controls*	211	265	1.35	.0051

* N = 10
** Significant at the 0.01 level.

TABLE 2. EFFECT OF 2 WEEK INHALATION EXPOSURE TO 2 MG/M^3 FLUOMINE AEROSOL ON GUINEA PIG GROWTH

	Body Weight (grams) Preexposure	Final
Exposed*	408	451
Unexposed Controls*	409	482

* N = 20

Mean body weight gains of neither species were adversely affected by the fluomine exposure. However, a significant difference was noted in the mean wet lung weights of the exposed animals which would indicate an edematous effect probably caused by irritation. This, as well as the rat lung pathology, strongly suggests that 2 mg/m^3 is an irritating concentration.

The lungs from six control rats along with lungs from eight exposed rats were wet ashed and analyzed for cobalt by means of atomic absorption spectroscopy. No statistical differences could be found between the values for the two groups. Apparently the deposition of the fluomine dust in the lungs of rats could not be measured by this method.

Guinea pigs given an intradermal injection of 0.05 ml of a 0.1% suspension of fluomine in peanut oil three weeks after the conclusion of exposures failed to show a response that would be indicative of a sensitization reaction.

The 2 mg/m^3 fluomine aerosol concentration was definitely irritating and ruled out as a possible concentration for the 6-month dome study. A second 2-week preliminary study was conducted in the same manner using a 0.2 mg/m^3 concentration of fluomine. At the conclusion of the two weeks, the animals were treated the same as described for the first preliminary study.

No signs of toxic stress occurred in either species and all animal groups showed normal body weight gains at the end of the two-week exposure period. The mean body weights of the animal groups and the results of the rat wet lung examination are shown in Table 3. A statistically significant difference was seen in the mean wet lung weights of the test rats when compared to the controls but the biological significance is negated by the fact that the lung/body weight ratios were not statistically different and that the growth of the exposed rats was slightly greater than control animals.

TABLE 3. EFFECTS OF 2 WEEK INHALATION EXPOSURE TO 0.2 MG/M^3 FLUOMINE AEROSOL ON RAT GROWTH AND LUNG WEIGHT

	Body Weight (grams) Preexposure	Final	Lung Weight (grams)	Lung/Body Wt. Ratio
Exposed*	195	276	1.53	.0055
Unexposed Controls*	193	265	1.43	.0054

* N = 10

From the results of the preliminary studies, two concentrations were chosen for the six-month intermittent study. These concentrations were 0.5 and 0.1 mg/m^3. The animal groups consisted of 100 male rats, 140 female mice, 25 male guinea pigs and 8 dogs. The test groups were housed in Thomas domes and exposed six hours per day, five days per week. The control group was housed in an animal holding facility within the same building.

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

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

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

Particle size and mass distribution of the chamber aerosol was determined using an Aries® cascade impactor. The impactor was used for this purpose since the background in the domes was too high and variable for the Royco® particle counter. The background did not interfere with the impactor measurements because the stages were washed with 1N nitric acid and the fluomine determined colorimetrically. A summary of the impactor samples taken over the 6-month period is shown below.

Chamber	Parameter	N	Mean (μm)	Standard Deviation (μm)	Range (μm)
A	MMD	20	2.093	0.149	1.82 - 2.39
A	σg	20	1.747	0.094	1.64 - 1.99
B	MMD	16	2.031	0.130	1.79 - 2.25
B	σg	16	1.897	0.196	1.59 - 2.42

During the six-month inhalation study none of the animal species showed any outward signs of toxic stress. However, some deaths did occur during the study, primarily in the mice. A significant number of mice died during a twelve day period in the low level exposure dome from an infection identified as type G streptococcus. All mice, including controls, were treated for 5 days with tetracycline to prevent an apparent epizootic. This treatment was successful in that deaths were significantly reduced. Other species deaths (except dogs) were sporadic and scattered through the three groups. In no case did the gross examinations reveal lesions which could be attributed to exposure.

All dogs and 10% of the rats and mice were sacrificed at the conclusion of the 6-month exposure portion of the experiment. Gross examinations of all of these animals also failed to reveal exposure-related lesions. Results of microscopic examinations of the tissues are not yet available.

Exposed rats showed a statistically significant depression in mean body weight gain (Figure 1) throughout the study. Although the exposed rat groups differed from the control group, they rarely differed from each other and a dose-effect relationship was not established. The guinea pig mean weights did not differ significantly from the control group except for two short periods of successive weighings as shown in Figure 2. The differences noted above were transient in nature and not consistent throughout the experiment. Again, a dose response relationship was not seen.

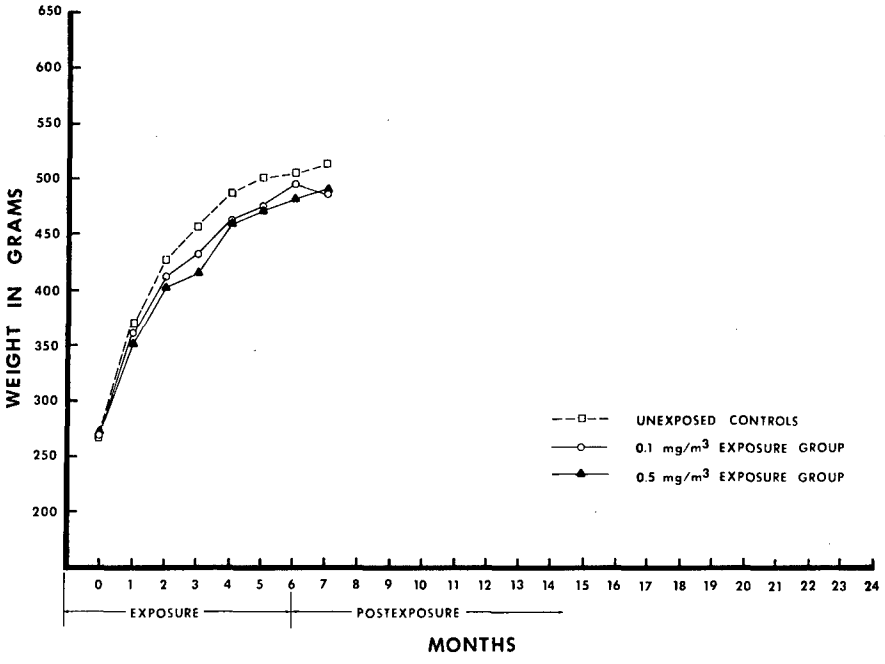


Figure 1. Mean body weights of rats exposed to inhaled fluomine aerosol.

At the conclusion of exposures, the lungs from ten rats from each group were excised and weighed. The wet lung weights were statistically analyzed for determination of edematous effects. The mean wet-lung weights of the exposed animals did not differ significantly from the mean wet-lung weights of the control rats. If the dust particles were irritating to the rats during any portion of the study, they were obviously able to adjust as no edematous effects were noticeable at the conclusion of the study.

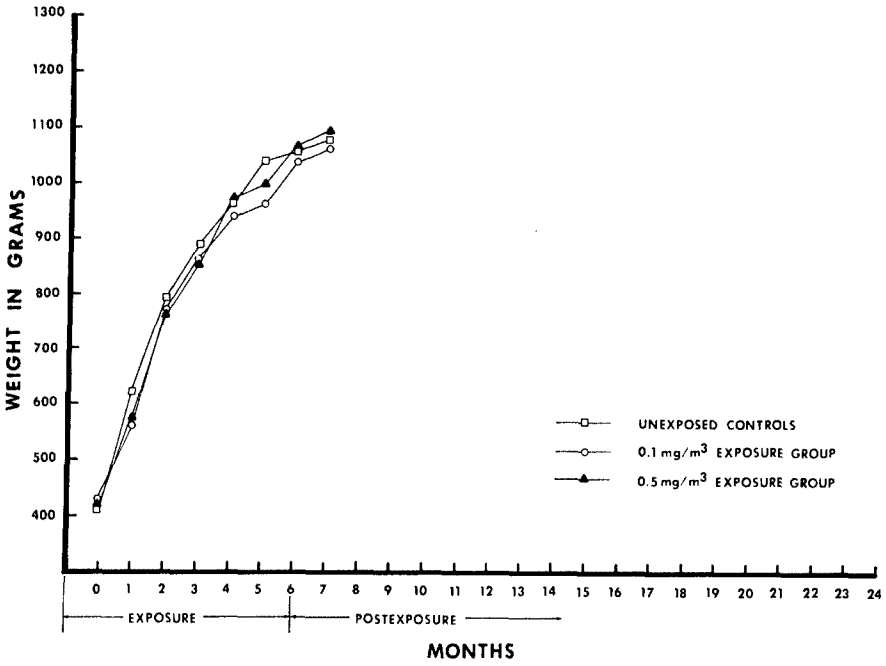


Figure 2. Mean body weights of guinea pigs exposed to inhaled fluomine aerosol.

Guinea pigs given an intradermal injection of fluomine one-month postexposure failed to elicit a skin reaction which would be indicative of a sensitization response.

Recommendations for safe exposure concentrations cannot be made until final reports of histopathologic examination are received. The exposure concentrations evaluated in this study were tolerable to all species tested and failed to elicit the sensitization response seen in guinea pigs at higher aerosol concentrations.

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

In the 1976 annual report (MacEwen and Vernot, 1976), we concluded that significant non-oncogenic effects of 6 months of 6 hour/day, 5 day/week exposures of 4 species of animals to 5, 0.5, and 0.05 ppm UDMH were limited to slight to moderate transitory hepatotoxicity in dogs exposed to the 5 ppm concentration. 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. We also concluded that on the basis of the tests and measurements used in this study, the current TLV of 0.5 ppm UDMH was well chosen without consideration of the oncogenic potential of this compound.

The postexposure phase of the study was terminated with the sacrifice of all surviving animals, except dogs, in 1976 and early 1977. All dogs are maintained at Brooks AFB where they receive quarterly physical examinations and periodic clinical chemistry evaluations. The surviving dogs are in good health and clinical chemistry values are at normal levels. Table 4 shows the experimental design, the number of months postexposure when sacrifices were made, and the number of survivors from each group of animals.

TABLE 4. EXPERIMENTAL DESIGN USED FOR UDMH INHALATION EXPOSURE CONCENTRATIONS, TIME OF POSTEXPOSURE SACRIFICES AND NUMBER OF SURVIVORS

UDMH Conc., (ppm)	Animal Numbers, Sex and Species	Sacrifice (Months Postexposure)	Number of Survivors
5.0	200 Male Hamsters	12-1/2	17
	200 Male Rats	18-19*	142
	400 Female Mice	19-1/2	75
	4 Male, 4 Female Dogs	Not Sacrificed	7
0.5	200 Male Hamsters	12-1/2	19
	200 Male Rats	18-19*	145
	400 Female Mice	19-1/2	103
	4 Male, 4 Female Dogs	Not Sacrificed	8
Control	200 Male Hamsters	12-1/2	10
	200 Male Rats	18-19*	74
	400 Female Mice	19-1/2	56
	4 Male, 4 Female Dogs	Not Sacrificed	8
0.05	200 Male Hamsters	17-18*	46
	200 Male Rats	19-20*	145
	400 Female Mice	19-20*	142
	4 Male, 4 Female Dogs	Not Sacrificed	8
Control	200 Male Hamsters	17-18*	37
	200 Male Rats	19-20*	139
	400 Female Mice	19-20*	88
	4 Male, 4 Female Dogs	Not Sacrificed	8

*Serial sacrifices over a one-month period.

Mean body weights measured biweekly during exposure and monthly postexposure to 5.0 and 0.5 ppm UDMH are shown for rats and hamsters in Figures 3 and 4 respectively.

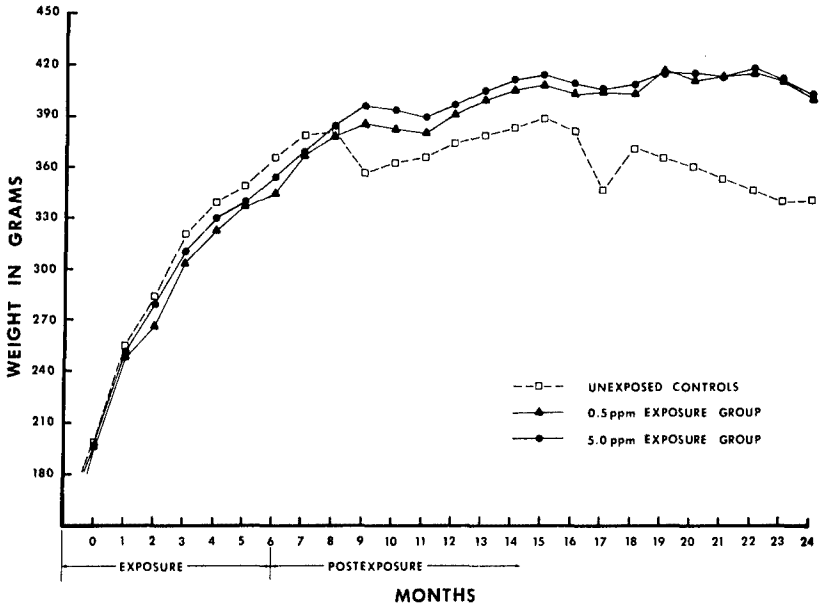
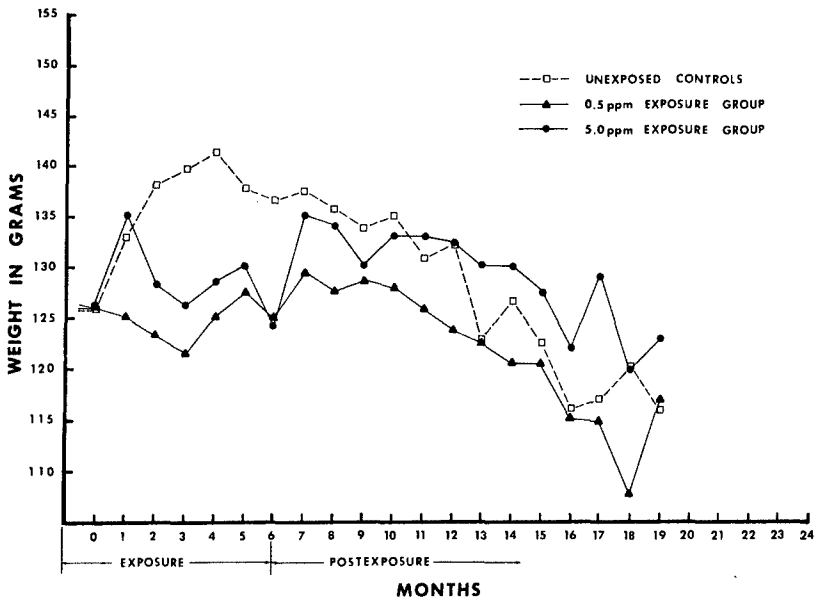


Figure 3. The effect of chronic exposure to inhaled UDMH on the growth rate of male rats.

Figure 4. The effect of chronic exposure to inhaled UDMH on the growth rate of male hamsters.



Body weights of rats and hamsters were also taken during exposure to 0.05 ppm UDMH but were less remarkable than at the higher doses. In general, all groups of exposed rats and hamsters gained weight during exposure at a lower rate than controls. The differences were statistically significant in many cases, but not dose related. The postexposure weight patterns for all exposed and control rat and hamster groups are difficult to interpret relative to exposure effects. They probably reflect the general health condition of the various animal groups and the aging process, especially in hamsters whose life span is much shorter than rats.

The rats and mice from this study are undergoing definitive histopathologic examinations in our laboratory. The results for rats will be collated and reported inhouse. Histopathology information on mice will be sent to the National Cancer Institute for analysis utilizing their Biocollaborative Carcinogenesis Studies Program. The definitive workup of tissue from hamsters is being done at the USAF School of Aerospace Medicine/VSP, Brooks AFB, Texas. Computerization of results will also be performed at Brooks using the OP Scan Program.

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

Histopathology examinations are incomplete for the three rodent species and the partial data obtained have not been evaluated. A separate report will be issued during he next year and a summary report prepared for the ensuing annual technical report.

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

The rationale, purpose, and details of the experimental protocol for conducting inhalation studies to determine the oncogenicity of hydrazine in four species of laboratory animals were presented in the 1975 (AMRL-TR-75-57) and 1976 (AMRL-TR-76-57) annual reports and will not be reiterated here except for a restatement of the experimental design shown in Table 5.

TABLE 5. EXPERIMENTAL DESIGN FOR HYDRAZINE INHALATION EXPOSURE CONCENTRATIONS

Hydrazine Concentration (ppm)	Animal Numbers, Sex and Species	Chamber Number
0.05	100 Male Rats 100 Female Rats 400 Female Mice	7
0.25	200 Male Hamsters 400 Female Mice	5
0.25	100 Male Rats 100 Female Rats 4 Male Dogs 4 Female Dogs	6
1.0	200 Male Hamsters 400 Female Mice	1
1.0	100 Male Rats 100 Female Rats 4 Male Dogs 4 Female Dogs	4
5.0	100 Male Rats 100 Female Rats 200 Male Hamsters	8
Control	150 Male Rats 150 Female Rats 400 Female Mice 200 Male Hamsters 4 Male Dogs 4 Female Dogs	Vivarium

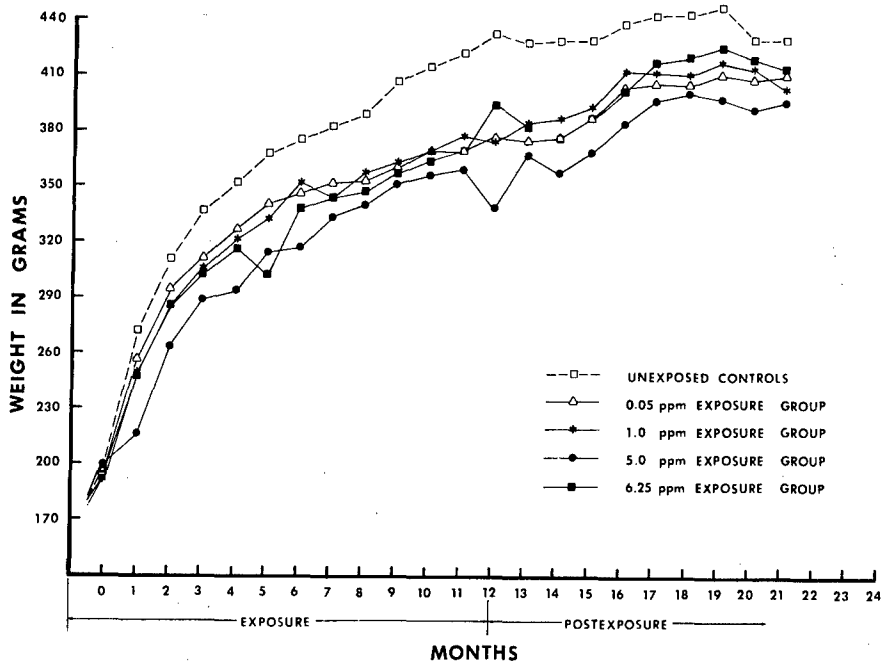


Figure 5. The effect of chronic exposure to inhaled hydrazine on the growth rate of male rats.

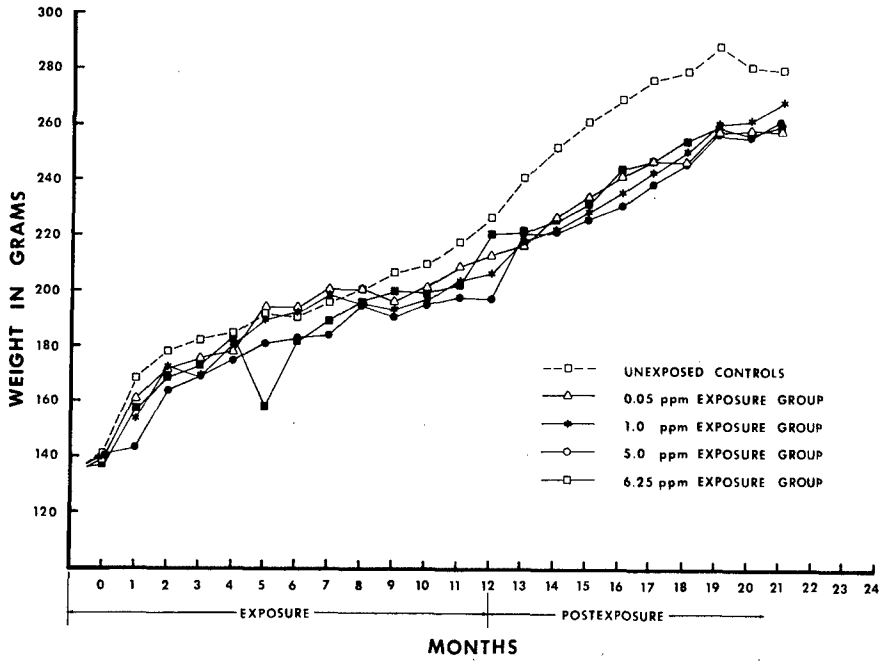


Figure 6. The effect of chronic exposure to inhaled hydrazine on the growth rate of female rats.

The animal exposures were inititated during the summer and winter of 1975 and completed one year later. 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 various 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 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 was set aside for comparison. These mice were placed in the experiment about 2 weeks before the hamsters.

The 1976 Annual Report (AMRL-TR-76-57) provides animal mortality data, body weight information and comments on clinical chemistry results for dogs current at that time.

At the present time, the animals are well into the postexposure phase of the experiment. As of the beginning of June 1977, it was 10-1/2 months since the completion of hydrazine exposure of dogs, rats and mice except for the 1 ppm group and their controls. These mice are 7 months postexposure while the hamsters are 6-1/2 months postexposure. All dogs were sent to Brooks AFB following 15 weeks of postexposure observation at the Vivarium facility of the AMRL-Veterinary Division. Postexposure observation of the dogs at Brooks includes quarterly physical examinations and periodic clinical chemistry determinations. Until recently, all hamsters, rats and mice were maintained at the Vivarium under as close observation as possible with that facility being separated from the THRU laboratory. Due to aging of the animals and the increased mortality in the groups of hamsters and mice, all of the hamsters and 50% of the mice were transferred to the animal quarters at the THRU laboratory where a surveillance schedule every 4 hours is currently maintained. Additionally, the numbers of animals in each cage have been reduced to permit better observation for dead and moribund animals to obtain fresh tissue for pathologic examination for tumorigenesis. Animals are weighed on a monthly schedule.

The effects of 1 year hydrazine exposure on the body weights of male and female rats and male hamsters are shown in Figures 5, 6 and 7, respectively. Generally, subnormal weight gains were seen during exposure for all animal groups compared with controls, although weights of female rats were somewhat erratic. Dose related subnormal weight gain patterns are distinct only for animal groups exposed to 5 ppm hydrazine. Postexposure weights show no weight recovery relative to controls except in the case of the hamster group exposed to 1 ppm hydrazine. Mean body weights of mice are shown in Table 6 along with their survival tally. Body weights of exposed mice were unaffected by hydrazine exposure when compared with their control groups.

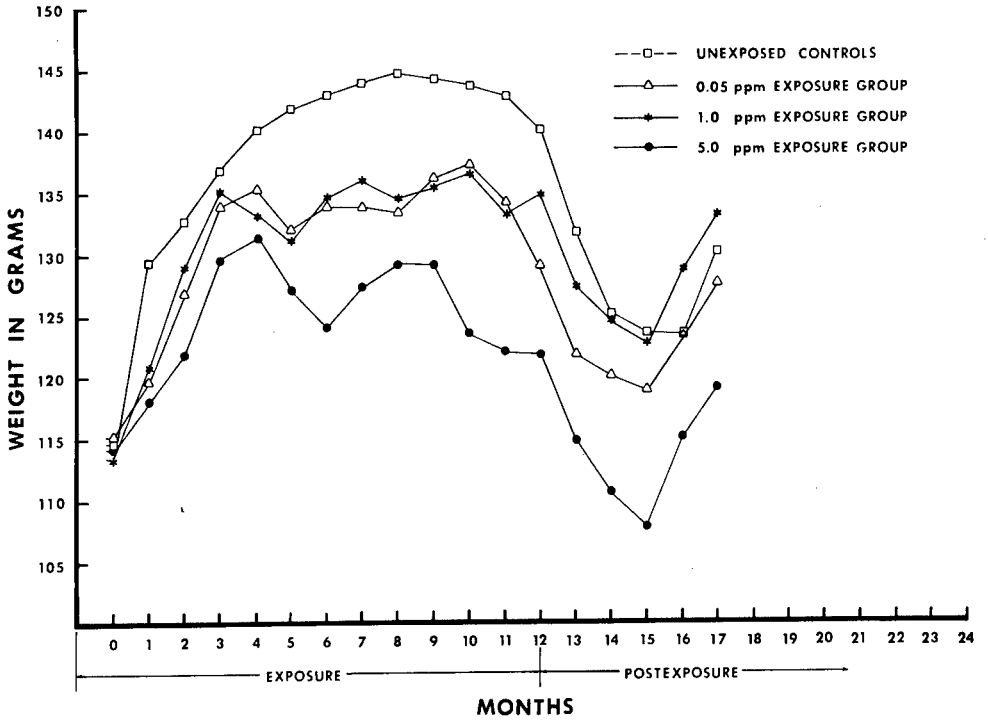


Figure 7. The effect of chronic exposure to inhaled hydrazine on the growth rate of male hamsters.

TABLE 6. MEAN BODY WEIGHTS AND SURVIVAL TALLY FOR MICE EXPOSED TO HYDRAZINE FOR ONE YEAR

Exposure Time, Months	Controls for 0.05,0.25 ppm No.	Weight,g	0.05 ppm No.	Weight,g	0.25 ppm No.	Weight,g	Exposure Time, Months	Controls for 1.0 ppm No.	Weight,g	1.0 ppm No.	Weight,g
Pre	400	16.2	400	18.0	400	16.4					
1	397	20.0	396	21.5	399	20.5					
2	397	21.6	396	22.2	395	22.0					
3	397	22.3	396	23.2	394	22.6					
4	397	22.5	396	23.6	393	23.4	Pre 10/27/75	400	17.0	400	16.9
5	397	22.5	396	24.1	390	24.0	1	400	19.3	395	20.2
6	397	23.1	395	24.2	384	24.5	2	399	20.7	394	22.0
7	397	23.6	393	24.2	384	25.1	3	399	21.1	393	22.7
8	397	24.0	392	25.3	384	25.5	4	399	22.0	391	23.4
9	394	23.8	391	25.3	383	26.5	5	398	22.3	391	24.1
10	392	24.1	390	25.6	383	25.9	6	397	22.5	390	24.5
11	390	24.6	379	26.9	377	27.0	7	396	23.6	382	25.4
12	390	25.1	376	27.0	373	27.6	8	394	25.6	380	26.0
Months Post											
1	387	26.5	372	26.4	373	26.5	9	393	25.3	379	26.1
2	382	27.5	369	26.9	370	27.5	10	391	24.9	376	25.9
3	377	27.4	364	27.0	367	27.2	11	388	25.2	373	27.2
4	374	27.2	344	26.6	364	26.8	12	Not Weighed		371	26.8
5	369	27.0	335	26.2	358	27.0	1 Mo.Post	385	25.2	368	26.0
6	350	27.6	319	26.0	351	26.1	2	375	25.5	350	26.6
7	344	28.7	312	26.7	342	27.1	3	372	26.6	349	27.4
8	337	28.4	295	27.0	330	27.2	4	367	26.4	345	26.8
9	324	29.1	285	27.8	310	27.7	5	358	27.5	342	27.8

Blood samples were drawn biweekly from control dogs and the dogs exposed to 1 ppm and 0.25 ppm hydrazine during the course of the exposure and at 2, 5, 9, 14 and 33 weeks postexposure. After one year 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 results or control values. When compared with controls, total protein values resulting from elevated globulin values appear to be significantly higher in the 1 ppm hydrazine exposure group, both during and following exposure, and to a lesser extent in the 0.25 ppm exposure group during exposure only. However, all values for exposed dogs are within normal range of the globulin determination and are not believed to be of any significance relative to hydrazine exposure.

Cumulative mortality was less than 20% in any group of exposed or control male or female rats at 10 months postexposure. Hamster mortality was approximately 50% at four months postexposure and 60% at six months postexposure (19 months of age).

The survival rate of hydrazine exposed rats and their controls has surpassed our expectations and therefore an interim sacrifice will be made at 12 months postexposure (24 months on study). Twenty rats of each sex from each exposure group and 30 of each sex from the control group will be necropsied during the month of July, 1977. The remaining rats will then be held until the number in any exposure group of males reaches 10% at which time all male rats will be necropsied. Likewise, when any female N_2H_4 exposure group reaches 10% of the original number, all remaining females will be necropsied.

If the mice have not reached a 75% mortality rate by 12 months postexposure, they will continue to be held until one exposure group reaches that point, at which time we shall necropsy all survivors of the same age. If 90% mortality is reached in any group prior to the 12 month postexposure point, we shall necropsy the survivors of that age group.

Approximately 4 months postexposure, rectal bleeding was observed from one dog in the 0.25 ppm exposure group. A biopsy was taken from a 3 x 2 cm cauliflower-like growth on the ventral surface of the rectum extending from the muco-cutaneous border craniad. The histological examination revealed a low grade adenocarcinoma.

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

The Acute and Subchronic Inhalation Toxicity of Decalin in Rodents

Decalin, a commonly used alicyclic hydrocarbon solvent, does not have an established Threshold Limit Value (TLV) and the available experimental data are insufficient for establishing limits. The acute oral LD_{50} for rats is reported to be 4170 mg/kg of body weight indicating a low order of toxicity by this route although it is reported as capable of producing dermatitis. Cardani (1942) reported that repeated daily eight-hour exposures to three guinea pigs at 1.8 mg/liter (320 ppm) of decalin resulted in one death at 2 days, a second death at 21 days, and the third animal died on the 23rd day. Gage (1970) described the exposure of eight rats to 200 ppm decalin for 20 days on a 6 hour/day schedule with no toxic signs and grossly normal visceral organs at necropsy. A 4-hour single exposure to 1000 ppm caused death in three of eight rats. The deaths were preceded by tremors and convulsions.

These data were not sufficient for estimating safe interim exposure limits pending chronic toxicity studies since no controls were evaluated and histologic examinations were not conducted. Therefore, a study was designed to provide more meaningful data on the inhalation toxicity of decalin.

Acute Studies

Prior to initiation of the subchronic portion of these studies, some preliminary acute inhalation exposures were performed on rats, mice, and guinea pigs. Prior to exposing the animals to known concentrations, rats and mice were exposed to essentially saturated vapors for various time periods. From vapor pressure data these exposures should result in calculated concentrations of approximately 1150 ppm.

Exposure of groups of five rats to saturated vapors for 4, 2 and 1-hour periods resulted in deaths of 5, 5 and 2 rats, respectively. The rats were hyperactive early in the exposure but by 40 minutes were exhibiting tonic convulsions, tremors, and prostration. Rats that survived two or more hours of exposure were paralyzed in the posterior half of the body. Even though some of these rats survived as long as 8 days postexposure, they never regained use of this portion of the body. None of the paralyzed rats survived the 14-day postexposure observation period.

Five of 8 mice died after 4 hours exposure to saturated vapors while none of 10 died after 1 hour. Symptoms were similar to the rats with tonic convulsions and tremors. However, none of the survivors showed any paralysis as was seen in the rats.

Following the saturated vapor exposures, rats and mice were exposed to varying concentrations of decalin vapors for 4 hours to determine the LC_{50}. The concentrations and mortality results are shown below.

Rats		Mice	
Conc. (ppm)	Mortality	Conc. (ppm)	Mortality
980	5/5	1085 (saturated vapor)	5/10
820	4/5	993	1/10
785	2/5		
625	2/5		
375	0/5		

LC_{50} = 710 ppm (619-816)

The 375 ppm concentration was chosen for the low level rat exposure to correspond to the chosen 6-hour daily inhalation concentration of 250 ppm. The rats at this concentration were asymptomatic throughout the 4-hour exposure and showed no apparent effects during the subsequent 14-day observation period. Five guinea pigs were exposed to 375 ppm decalin to determine possible effects during the dome study. The guinea pigs were also asymptomatic during the 4-hour exposure period and during the 14-day postexposure observation period.

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

Subchronic Studies

Three groups of 100 male Sprague-Dawley rats (Harlan Industries, initial weight range 183-282 grams), 100 female CF-1 mice (Charles River Breeding Laboratories, not weighed) and 25 male Harlan - derived guinea pigs (Sweetwater Farms, initial weight range 479-665 grams) were exposed to decalin in inhalation exposure chambers at concentrations of 50 or 250 ppm, or served as controls in conventional housing for 30 days. Exposures in the chambers were conducted on a 6-hour per day, 5 day per week basis with continuous monitoring of the decalin concentration. The rats and guinea pigs were weighed initially, at 15 days, and at termination. Wet organ weights (lung, heart, liver and kidney) were determined on 20 rats from each group and organ-to-body weight ratios were calculated. These animals were killed by barbiturate over-dose followed by exsanguination. The body weights used in this calculation were obtained in the fasting state. Terminal body weights for purposes of determining overall weight gain were obtained on the previous day in the nonfasting state.

Representative tissues from the remaining animals (80 rats, 100 mice, and 25 guinea pigs from each group) were preserved in formalin. Histological evaluation was generally confined to the trachea, lungs, liver, kidneys, and urinary bladder. In addition, any lesions noted during the gross necropsy were similarly examined. The animals generally exhibited normal behavior and appearance during the study.

The body weight data for each group of guinea pigs and rats are presented in Table 7. Exposure to decalin produced a statistically significant lower growth rate for the guinea pigs at each interval as compared to the unexposed controls. The exposed groups of rats showed a greater rate of gain than the controls at the two-week interval but at termination, the exposed rats showed depressed body weight gains as compared to the controls.

TABLE 7. THE EFFECT OF 30-DAY INTERMITTENT SUBCHRONIC INHALATION EXPOSURE TO DECALIN ON GROWTH RATES OF GUINEA PIGS AND RATS

(weight in grams)

		Week Number		
Species	Conc.,(ppm)	0	2	4
Guinea Pig	50	698.2 (539-665)	681.2* (567-757)	757.3** (635-846)
	250	598.0 (479-651)	661.7** (568-750)	751.4** ¢682-853)
	0	600.4 (521-665)	725.7 (571-820)	806.9 (652-935)
Rat	50	210.7 (183-240)	298.3** (244-378)	330.4** (247-397)
	250	212.3 (189-239)	299.8** (232-356)	336.5** (288-398)
	0	211.0 (183-282)	287.8 (242-328)	351.1 (275-422)

* Significantly different from controls (p = 0.05).
** Significantly different from controls (p = 0.01).

Exploratory studies on urinary metabolites of decalin were conducted using the physiological fluid "fingerprint" gas and liquid chromatography techniques described in our last annual report (MacEwen and Vernot, 1976).

Urine samples were collected from groups of three rats held overnight in metabolic cages. Three groups of controls were run to determine group to group range. A group of rats from the 50 ppm decalin exposure were held overnight after 16 exposure days and returned the next morning for the start of that day's exposure. Rats from the 250 ppm decalin exposure were run in a similar manner overnight after the seventeenth exposure day. The liquid chromatography results are in Table 8. Peaks numbered 1, 2, 8, 14, 43 and 49 of the exposed rats are low compared to the controls. Peaks 1, 2, 14 and 43 may be dose related. Peaks 4, 16, 41 and 47 are higher than controls with only 41 possibly dose related. Decalin was injected on the column and no peak was seen. Decalin is either not UV absorbent or is retained too strongly on the column. Decalin was therefore, not one of the urine peaks seen in the exposed animals. Decreases in peaks 1 and 2 have also been seen with carbon tetrachloride exposures.

TABLE 8. LIQUID CHROMATOGRAPHIC PEAKS FOUND IN URINE OF RATS EXPOSED TO DECALIN

Peak Number	Control 1	Control 2	Control 3	Exposed 50 ppm	Exposed 250 ppm
1	2059	1428	1851	1232	880
2	506	362	657	268	48
3	0	133	0	0	35
4	133	66	398	3294	741
5	0	166	17.3	0	0
6	1134	822	1125	1720	653
7	58	0	52	12.2	40
8	1139	1016	929	681	768
9	0	46	17.3	0	944
10	0	0	21	0	29
11	104	19.9	31	46	48
12	0	16.6	0	0	0
13	0	199	388	0	208
14	2128	1809	1695	1440	224
15	265	0	185	207	1400
16	8.1	15.3	13.8	24	22
17	25	3.0	0	0	0
18	0	74	135	15.9	117
19	87	0	0	76	0
20	16.1	3.7	12.1	3.7	14.4
21	0	22	8.7	168	11.2
22	2875	2374	2820	2062	2400
23	518	166	329	146	192
24	736	714	727	525	744
25	69	116	104	0	253
26	311	33	40	122	110
27	120	66	213	104	133
28	0	339	112	232	192
29	322	83	230	98	189
30	7.6	3.3	28	15.3	27
31	48	174	55	34	256
32	6.0	39	8.7	6.7	0
33	6.2	12.9	20	7.7	22
34	13.3	4.2	1.7	20	12.8
35	50	48	61	50	99
36	0	4.5	3.8	0	0
37	10.8	0	0	5.5	3.2
38	9.7	83	55	6.7	3.2
39	18.4	5.3	0	7.3	49
40	51	18.3	25	60	6.4
41	7.6	5.3	6.9	10.4	13.8
42	6.9	12.5	2.9	0	15.0
43	18.4	13.3	15.6	11.6	2.6
44	766	365	787	628	504
45	9.7	4.2	3.5	7.3	4.0
46	54	46	43	1.8	50
47	0	0	0	32	12.8
48	19.6	6.6	38	63	24
49	368	191	237	107	120

The gas chromatographic results seen in Table 9 show that peaks numbered 11, 14, 19, 20, 21, 23, 26, 32, 34 and 35 of the decalin exposed rats are low relative to the controls while peaks numbered 24, 29, 33, 36, 38 and 39 increased; three of these peaks (24, 29 and 34) are also altered when rats are exposed to carbon tetrachloride. Unchanged decalin gives a very broad peak by the GC method which would appear only as a baseline rise on the urine chromatogram. The 50 ppm exposed rats show a baseline rise at that point and the 250 ppm exposed rats show a larger baseline rise which may indicate that unaltered decalin is excreted in urine but cannot be unequivocally determined by this technique. The significant findings of the exploratory study are the loss of three peaks (Numbers 30, 34 and 35) and the appearance of two new gas chromatographic peaks numbered 36 and 38 which appear to be metabolities of decalin.

TABLE 9. GAS CHROMATOGRAPHIC PEAKS FOUND IN URINE OF RATS EXPOSED TO DECALIN

Peak Number	Control 1	Control 2	Control 3	Exposed 50 ppm	Exposed 250 ppm
1	6688	6500	7920	4655	10315
2	1245	1300	1447	855	1690
3	4680	4160	4930	3040	4315
4	16200	6370	6345	2850	6440
5	14.4	5.2	66	27	12.5
6	1080	1300	1231	619	1040
7	2894	3926	2241	2850	4415
8	108	103	100	47	97
9	78	91	85	36	71
10	842	712	961	619	800
11	14.4	6.2	7.0	3.9	1.5
12	48	52	68	36	59
13	209	146	189	108	153
14	3168	3432	3294	2090	1740
15	63	55	79	62	75
16	1152	962	1096	657	950
17	218	260	273	139	235
18	266	0	216	149	108
19	8640	4940	9450	3116	3100
20	13536	16640	12690	7980	8550
21	598	377	675	391	55
22	576	380	761	380	19.0
23	238	619	318	1.9	9.5
24	144	0	0	119	1470
25	288	718	540	451	0
26	1080	1040	284	119	0
27	40	0	13.5	0	0
28	585	291	405	285	200
29	9	0	0	361	1405
30	38	81	8.1	0	0
31	2556	1362	1150	760	5125
32	95	55	176	45	28
33	4.5	3.1	0	16.3	146
34	15.3	3.1	9.7	0	0
35	4.5	33	58	0	0
36	0	0	0	390	188
37	4.5	1.6	0	0	0
38	0	0	0	152	280
39	32	12.0	22	48	40
40	4.5	24	0	0	0
41	10.8	13.0	22	16.7	19

The mean terminal body weights, organ weights, and organ-to-body weight ratios for the rats are shown in Table 10. The wet lung weights for the two test groups were significantly less ($p < 0.01$) than the control group. However, when the organ-to-body weight ratios were examined, there were no significant differences. Similarly, the heart weights for the two test groups were significantly ($p < 0.05$) lower than the control group but the ratios showed no significant differences. The liver weights showed a similar trend with the 50 ppm and the 250 ppm groups showing lower values at $p < 0.01$ and $p < 0.05$, respectively. There was no significant difference in the kidney weights; however, the ratios were significantly ($p < 0.01$) higher for the test group when compared with the control group.

TABLE 10. THE EFFECT OF SUBCHRONIC INHALATION EXPOSURE TO DECALIN ON TERMINAL WEIGHTS, ORGAN WEIGHTS AND ORGAN TO BODY WEIGHT RATIOS OF RATS

	Exposure Concentration		
	50 ppm	250 ppm	Unexposed Controls
Body Weight (gm)	297	305	335
Organ Weights (gm)			
Lung	1.647**	1.662**	1.820
Heart	1.006*	1.008*	1.095
Liver	8.862**	9.583*	10.065
Kidney	2.180	2.231	2.204
Organ to Body Weight Ratios (%)			
Lung	0.555	0.546	0.543
Heart	0.339	0.331	0.327
Liver	2.279	3.147	3.002
Kidney	0.733**	0.733**	0.659

* Significantly different from controls (p = 0.05).
** Significantly different from controls (p = 0.01).

Gross pathology findings for all species were miscellaneous in nature and showed no lesions which could be attributed to the decalin exposure.

Histopathologic findings of significance in the rats were as follows:

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

Liver - Hydropic change was observed in the hepatocyte cytoplasm of all groups. However, there appeared to be a definite dose-relationship in the incidence and severity of these changes. The controls showed an incidence of 25% minimal change without any more severe changes. By contrast, the 50 and 250 ppm groups, while showing approximately the same number of minimal changes - 24 to 31% - showed a dose-related response in the severity of changes. The 50 ppm group exhibited, on a scale of 0-4+, 14% 2+ and 8% 3+, whereas the 250 ppm group showed 21% 2+ and 29% 3+. The pathologist believed that these changes were probably the result of an accumulation of water as the result of biochemical injury but that the alterations would be reversible.

Kidney - Hyalin droplet formation within the proximal tubular epithelial cytoplasm was observed in both test groups and the control group. However, there appeared to be a definite dose-response in both incidence and severity. On a scale of 0 to 4+, the controls showed a minimal change (1+) in 46% of the animals with the remainder showing no changes. The rats at the 50 ppm level showed changes of 1+ in 23%, 2+ in 39%, and 3+ in 25%. No grades of 4+ were reported. The rats at the 250 ppm level showed changes of 1+ in 1%, 2+ in 33%, 3+ in 41%, and 4+ in 25%.

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

The remaining findings were miscellaneous in nature and appeared unrelated to decalin exposure.

The histologic findings in mice generally did not appear related to decalin exposure. One exception was the liver which showed hepatocytic vacuolization of the cytoplasm in 7% of the controls, 5% of the 50 ppm mice, and 52% of the 250 ppm mice. The significance of this finding is not known, although apparently dose related.

In summary, the most significant changes in rats attributable to decalin exposure were:

1. Increased organ-to-body weight ratios for the kidneys when compared with the control values.
2. Hyalin droplet formation within the proximal tubular epithelial cytoplasm of the kidneys which showed a definite dose response in terms of incidence and severity.
3. Hydropic change in the hepatocyte cytoplasm which appeared to be definitely dose related.
4. Lowered organ-to-body weight ratios for the liver when compared with the control values.

The only finding in the mice which appeared related to the exposure was hepatocyte vacuolization of the cytoplasm in which the incidence was markedly greater in the high level (250 ppm) animals than in the control or low level (50 ppm) animals.

Histology on the guinea pigs is not yet available.

Although these histologic changes are regarded as reversible, this was a 30-day study. Strong consideration should be given to conducting a chronic inhalation exposure of six to 12 months to determine the severity of the effects seen in this subchronic study.

A Study of the Oncogenic Capacity of Inhaled Monomethylhydrazine

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

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

MacEwen and Vernot (1975) reported the results of a two year drinking water study in which hamsters were given standard and acidified drinking water containing 0.01% MMH. A third group of hamsters was given acidified water (pH 3.0) as unexposed controls.

Neither the incidence, degree of severity, nor age of onset of nonneoplastic pathologic changes was markedly different between animals drinking MMH in water and control animals. The presence of 23% incidence of adrenocortical tumors in control animals versus 4% in Group I (MMH in tap water) and 12% in Group II (MMH + pH 3.5 water) argues against MMH as a cause of these tumors. The remaining neoplasms, one hemangioendothelioma of the liver, two hepatocellular carcinomas, one cutaneous melanoma, occurred only in the experimental groups. They were derived from four different cell types and as such constitute a 4% incidence for each tumor in their respective groups of animals, except for an 8% incidence of hepatocellular carcinoma. The overall tumor incidence for Group I (MMH + tap water) was 16%, Group II (MMH + pH 3.5 water) was 24%, and Group III (control) was 31%. These findings are in contrast to the findings of Toth and Shimizu.

The reported investigations present some evidence that MMH may be carcinogenic and therefore may pose a hazard to man. The case for carcinogenicity of MMH is, however, inconclusive at this point and for this reason the comprehensive inhalation exposure study outlined in this protocol was developed.

This study was designed to determine the oncogenic potential of inhaled MMH at concentrations ranging from maximum tolerated levels to levels lower than occupational Threshold Limit Values (TLV). The objective was to evaluate the safety margin of current TLV values using animal models.

Monomethylhydrazine for use in this experiment was prepared by Olin Corporation. The batch of MMH was purified as much as possible by bubbling nitrogen gas through it to drive off more volatile contaminants. The batch of 5 liters volume was delivered in two packages and was then repackaged at THRU in 100 ml units under nitrogen to prevent oxidative changes. This repackaging was necessary to minimize oxidative degradation during use and also to reduce occupational health and safety hazards.

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

Prior to beginning the long-term, one-year study, a subacute inhalation study was performed on hamsters. Little information on the toxicity of MMH to hamsters under conditions of repeated, intermittent inhalation exposure was available. The preliminary study was an aid to selecting concentration levels for the one-year study.

A group of 20 male hamsters was exposed to 50 ppm of MMH for 6 hours per day on a five day per week basis. However, 19 of 25 hamsters were dead by the fifth exposure day and the study terminated. Early signs of toxic stress in these hamsters were irritation of the eyes and upper respiratory tract followed by anorexia, emaciation, gasping and prostration. The survivors showed a mean weight loss of 32 grams at termination.

The concentration was halved to 25 ppm and a new group of 25 male hamsters exposed on the above schedule for a total of 30 exposure days. This exposure regimen resulted in five deaths. These occurred between the sixth and sixteenth exposure days. Survivors had a mean weight loss of 22 grams at termination. These results indicated that 5 ppm MMH would be a concentration which the hamsters should be able to tolerate for an extended inhalation study.

Rats, mice, hamsters and dogs are being exposed to MMH by the inhalation route in chambers for one year using an industrial work week schedule of 6 hours/day, 5 days/week with holidays and weekends off to simulate a human exposure regimen.

The highest exposure concentrations selected for each of the animal species are based on previous tests that show them to be the maximum tolerated repeated exposure levels. Other exposure levels are order of magnitude decreases in concentration encompassing the TLV value starting from the maximum tolerated concentration for the most sensitive species (mouse).

All rodents will be held postexposure for an additional year of observation or until cumulative mortality exceeds 90% of the groups at which time necropsies will be performed and tissues taken for histopathologic evaluation of tumorigenesis. The dogs will be shipped to the USAF School of Aerospace Medicine, Sciences Division for postexposure observation of 5 years. Data resulting from postexposure observation will be delivered to the Toxic Hazards Research Unit for inclusion in the experimental record file.

Purebred beagle dogs used in this study were previously quarantined and background clinical studies were conducted for several months. Rodents being used are listed below.

Species	Strain	Source
Rats	Fisher 344	Charles River Breeding Labs.
Mice	C57Bl/6	Jackson Laboratory
Hamsters	Golden Syrian	Charles River Breeding Labs.

The animals are fed ad libitum during nonexposure hours and cleaned daily after completion of the 6-hour exposure and minimum 30-minute air purge period. Industrial hygiene sampling is used to verify the adequacy of the purge time.

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

Each pair of inhalation chambers contains as few species as possible to minimize the risk of cross infection. Dogs and rats are housed in one chamber and the mice and hamsters in the companion chamber. The numbers of animals are based on the maximum numbers for each species which can be exposed in the THRU chambers and be compatible with ILAR standards for animal care. The numbers of rodents were selected to permit a statistically valid number of animals of each species to reach the required age for tumor induction with natural and toxicologic attrition. The chamber loadings and MMH concentrations being used are shown in Table 11.

TABLE 11. CHAMBER ASSIGNMENTS AND CONCENTRATIONS FOR INHALATION EXPOSURES TO MONOMETHYLHYDRAZINE

MMH Conc., (ppm)		5.0	2.0	2.0	0.2	0.2	0.02	-0-
Chamber Number		5	1	2	3	4	8	Vivarium
Species								
Rats	Male	100	-	100	100	-	100	150
	Female	100	-	100	100	-	100	150
Mice	Female	-	400	-	-	400	400	400
Hamsters	Male	200	200			200	-	200
Dogs	Male	-	-	4	4	-	-	4
	Female	-	-	4	4	-	-	4

A Sage pump equipped with a suitable glass syringe is used to inject liquid MMH into a 1/4" polyethylene tubing line open to the dome air supply line where it is vaporized and drawn in against a slight negative pressure. The syringe pump is housed in a fume hood for safety. A flow diagram of the generating system is shown in Figure 8.

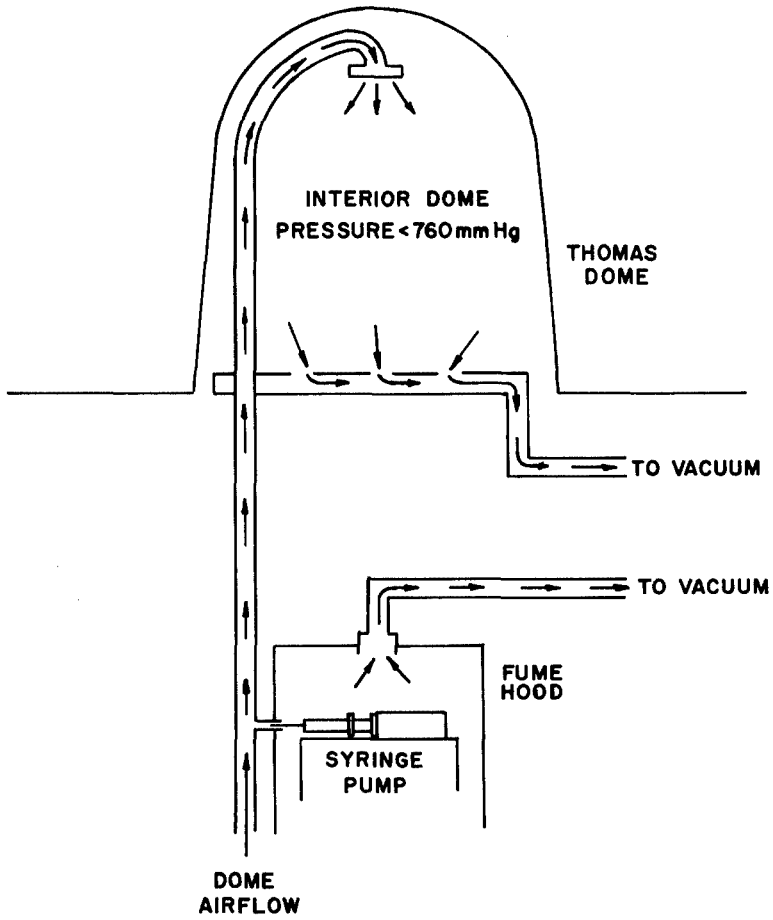
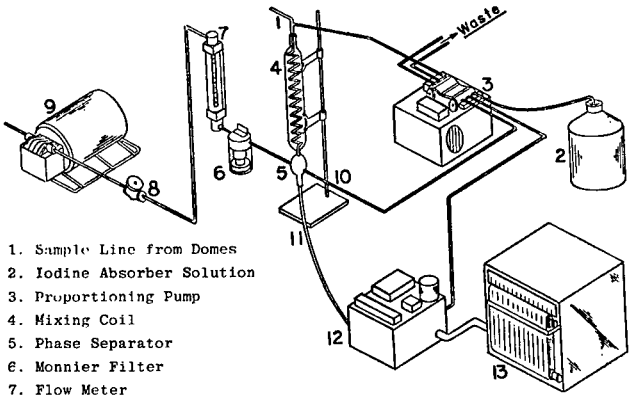


Figure 8. A schematic illustration of the generation system used for MMH chronic inhalation exposures to animals.

The analytical procedure used for monitoring of MMH concentration in the domes is a modification of the method reported by Geiger and Vernot (1967). A 2-liter/minute sample is drawn from the dome using a 1/4" I.D. polyethylene tube. The dome air is then drawn through a glass scrubber column filled with glass beads designed for mixing the MMH vapor with the absorber solution. The absorber solution is prepared by mixing 40 g/liter KI with 20 g/liter Na_2HPO_4 and 6 g/liter KH_2PO_4 in water. A schematic illustration of the analytical system being used is shown in Figure 9.



1. Sample Line from Domes
2. Iodine Absorber Solution
3. Proportioning Pump
4. Mixing Coil
5. Phase Separator
6. Monnier Filter
7. Flow Meter
8. Control Valve
9. Single Stage Dia-Pump
10. Waste Line
11. Analysis Sample Line
12. Colorimeter
13. Strip Chart Recorder

Figure 9. Monomethylhydrazine analytical system.

From 1 to 10 ml/liter of 0.1M iodine is added to the absorbing solution depending on the MMH concentration to be analyzed. The dome air reacts with the iodine in a colorimetric reaction which is proportional to the amount of MMH present according to Beer's Law. The air and liquid are separated and the liquid is pumped to a Technicon AutoAnalyzer® to determine the MMH concentration. A chart recorder is used for continuous monitoring of the concentration. Sequential sampling is conducted on the 2 pairs of chambers in which the exposure concentrations are common.

The analysis is calibrated using a Model 303 Gas Mixing System. The sample holder made from a Pasteur pipet is placed inside a small test tube. The test tube is fitted with wire holders and placed inside a glass chamber which fits inside an oven. At any temperature, MMH is vaporized into the glass chamber at a constant rate. The rate may be varied by changing the oven temperature. A flowing stream of prepurified nitrogen is passed through the chamber to generate low-level gas mixtures continuously.

The weight of the MMH sample holder is noted before and after calibration to obtain the amount of weight loss. This along with the dilution factor can be used to calculate the concentration.

All animals are observed hourly during exposure and will be observed daily until termination of each experiment during the postexposure period.

Rats, hamsters and dogs are weighed individually at biweekly intervals during exposure and will be weighed monthly during the postexposure period. Mice are weighed in groups and group mean weights followed on a monthly basis throughout the experimental period.

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

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

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

All animals that die or are sacrificed in these studies will be necropsied. The necropsy is defined as external examination, including body orifices, and examination and fixation of all of the following tissues for histopathologic examination:

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

Continuous industrial hygiene monitoring of the work environment is carried out by analysis of the room air using MDA Scientific Hydrazine Analyzer Model 7020 and a Technicon AutoAnalyzer II®. The AutoAnalyzer sequentially samples five laboratory locations and takes a baseline sample from the air conditioning systems. The MDA Hydrazine Analyzer samples a baseline from the air supply system and then tests four locations in the room housing the exposure chambers.

Exposures began on 8 March with mice, hamsters and dogs. The first shipment of rats received for this study did not pass quality control examination. A new group has been quarantined and examined and was inserted into the study on 13 April.

An unexpectedly large number of deaths (30/200 exposed to 5.0 ppm MMH, 12/200 exposed to 2.0 ppm) occurred in hamsters during the first 3 weeks of the higher level MMH exposures. In the study preparatory to the current experiment, mortality in hamsters exposed to 25 ppm MMH was only 20% over a 4 week period. The one obvious difference which could be found between these two groups of hamsters was that the mean body weight of the earlier test group was 122 (100-140) grams while the mean body weight of the dome hamsters was 91 (76-107) grams. It is possible that an age-related difference in sensitivity could have resulted in the high mortality incidence now being observed. A new supply of hamsters has been ordered and will be placed in the exposure dome at an older age than were the current animals.

The Effect of Environment on the Growth of Male Fisher 344 CDF Rats

It has been postulated that housing of animals in the Thomas Domes (exposure chambers) might have a deleterious effect on their body weight gains as compared with animals housed in conventional rooms (postexposure) or laminar air flow rooms (postexposure room) or laminar air flow rooms equipped with HEPA filters. The resolution of this potential problem was of importance since the control animals for dome studies are usually housed in either the postexposure room or in a laminar air flow room. This practice has been necessary due to lack of available chamber housing for controls.

Accordingly, a study was designed to attempt to answer the question of impact of housing on rat growth. The foregoing procedures were also carried out to give the pathologists evaluating changes in our animals more background on effects on rats when they were exposed to the dome environment only, i.e., no simultaneous exposure to a contaminant. Three groups, each composed of 50 male Fisher 344 CDF rats (weight range 219-263 grams), were randomly assembled from a group of approximately 200 rats. This rat strain is frequently used in our chronic toxicity studies and these were supplied by Charles River Breeding Laboratory.

One group was housed in a chamber with five rats in each cage. A second group was housed in the postexposure room using plastic solid-bottom cages with three rats per cage. The third group was housed at the Vivarium in a laminar air flow room (Bio-clean) using plastic solid-bottom cages with three rats per cage. This caging scheme is the same as is used in a typical chronic toxicity study. The only deviation from routine was the total animal load in the chamber which was lighter than usual.

At the end of four weeks, 10 rats from each group were sacrificed, a gross necropsy was performed, and tissues were preserved for histologic evaluation. In addition, blood was obtained for CBC's, total protein, and albumin determinations.

At the end of eight weeks, the remaining rats from each group were sacrificed, gross necropsies were performed, and tissues were preserved for histology. Also, blood was obtained from 15 rats from each group for CBC's, total protein, and albumin determination.

The mean body weights and weight ranges for the rats during the study are presented in Table 12 and graphically in Figure 10.

TABLE 12. COMPARATIVE GROWTH OF MALE ALBINO RATS HOUSED IN A POSTEXPOSURE ROOM, A VIVARIUM LAMINAR FLOW ROOM AND AN EXPOSURE CHAMBER
(weight in grams)

Days in Experiment	Chamber	Postexposure Room	Vivarium
0	235	241**	239*
7	254	263**	256
14	270	272	271
21	283	281	279
29	293	301**	294
35	296	301**	297
42	301	312**	300
49	305	321**	307
57	312	328**	314
Net Gain	77	87**	76

* Significantly different from chamber group at the 0.05 level.
** Significantly different from chamber group at the 0.01 level.

In addition, statistically significant differences are indicated in the table. As is evident from this table, the rats in the postexposure room showed a significantly greater weight gain than the animals in the exposure chamber or in the Vivarium at the majority of the weighing intervals. There was no significant difference between the latter two groups except at the initial weighing when the rats at the Vivarium were slightly heavier than the chamber housed rats.

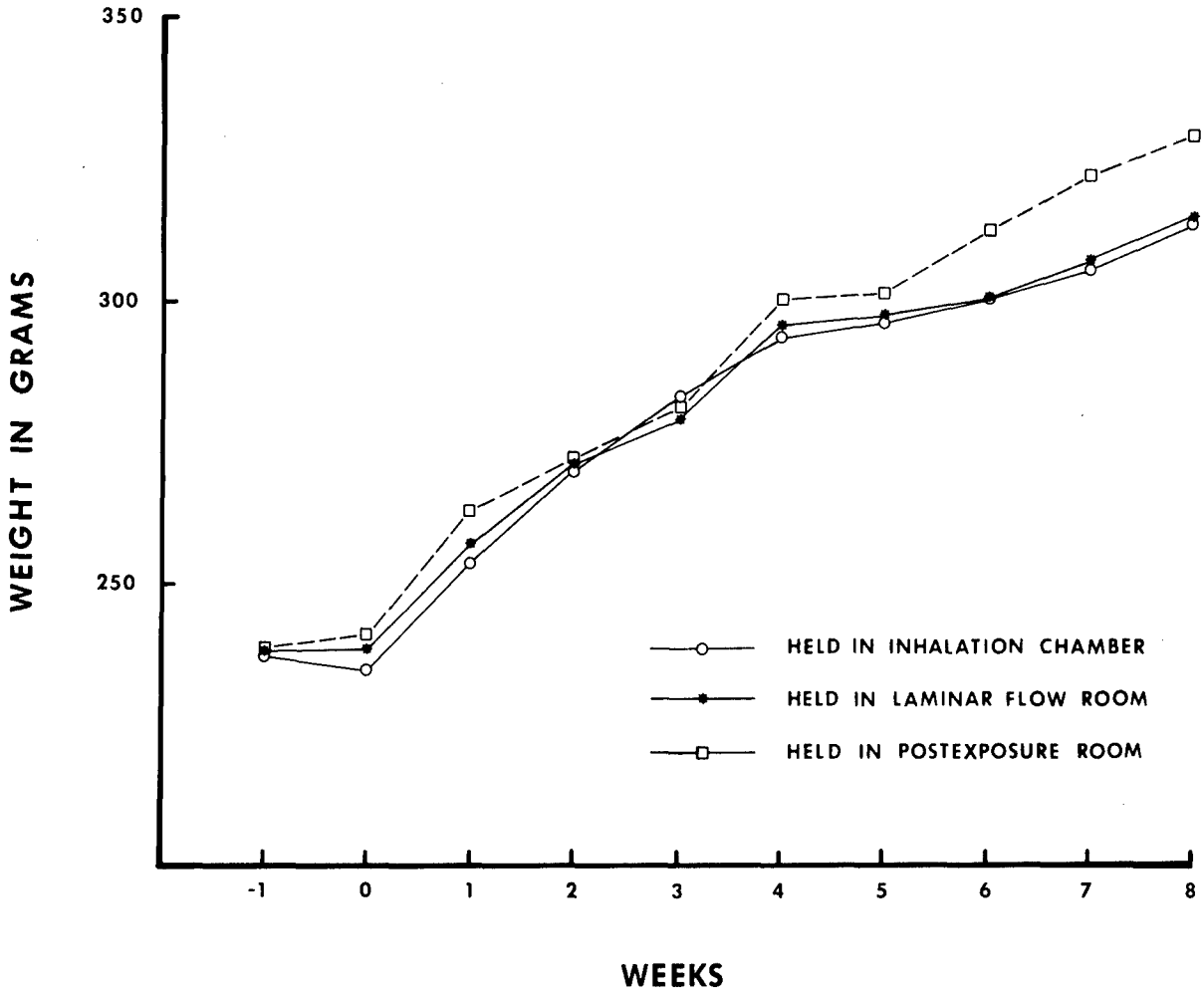


Figure 10. Effect of housing conditions on male Fisher 344 rat growth rates.

Although not part of the basic objective of the study, it is interesting to note that the relative humidity in the chamber is essentially controlled by the animal load. The mean relative humidity in the dome in this study with 50 rats was 28.7% as compared with 57.3% in the postexposure room. With a relatively heavy animal load of 100 rats, 100 mice, and 25 guinea pigs in other ongoing chronic toxicity studies, the mean relative humidity in 6 other chambers was 52.8%. During the same time period, the relative humidity in the postexposure room was 65.2%.

Although no histologic data are available at this date, the numbers of animals with lung, kidney or liver lesions are tabulated below for the 10 rats sacrificed at the end of four weeks.

	Exposure Chamber	Postexposure Room	Vivarium
NGL	6	7	7
Lungs	2	2	3
Liver	0	1	0
Kidneys	3	0	1

As can be seen, there was no significant difference between the groups after four weeks.

A similar comparison of the groups at the end of eight weeks is summarized below.

	Exposure Chamber	Postexposure Room	Vivarium
NGL	20	2	16
Lungs	18	38	24
Liver	4	6	10
Kidneys	3	4	2
Spleen	0	1	0

In both cases, the organ findings were miscellaneous in nature and generally represent mild or moderate changes.

The most obvious difference between the groups at eight weeks is in the higher incidence of lesions observed in the animals housed in the postexposure room as compared with the other two environments. Essentially, all of this is due to lung pathology. The marked increase in the incidence of lesions in this organ with the passage of time is not explainable at this time. The results of the histologic examinations when they are available may clarify this situation.

A general conclusion may be drawn that housing unexposed control rats in the Bio-clean rooms at the Vivarium does not introduce an artifact into comparative body weights.

Intradermal Sensitization Studies on Bis(2,2-Dinitro-2-Fluoroethoxy) Methane and SYFO

The military compounds designated "SYFO" and the compound Bis(2,2-dinitro-2-fluoroethoxy) methane, designated "FEFO", have a high potential for use both as propellant constituents and as explosive materials. Previous acute toxicity studies on FEFO (Barry et al., 1962) determined the mouse intraperitoneal LD_{50} to be 90 mg/kg while the eye and skin irritation to guinea pigs was minimal. MacEwen and Vernot (1975) reported that saturated vapors of FEFO failed to produce any signs of toxic stress after six hours of exposure. Inhalation studies on SYFO were not done because it has extremely low volatility and it would be impossible to achieve a vapor concentration high enough to produce toxic effects at room temperature.

This study was designed to investigate the sensitizing potential of both compounds. The purpose was to determine whether FEFO or SYFO would cause antigen-antibody reaction in male albino guinea pigs.

The experimental groups consisted of 20 male albino guinea pigs. Three additional guinea pigs were used to determine the primary irritation properties of the test substances at the dilution used for the sensitization study.

To determine whether these compounds were irritants, 0.05 ml and 0.10 ml quantities of a 0.1% solution of each chemical in peanut oil was injected into the closely clipped scapular and sacral areas of three guinea pigs. Similar injections of the vehicle alone were also made. The injection sites were examined at 24 and 48 hours after injection. If any response of a value over 25 occurred (see reaction evaluation section), the chemical was considered a primary irritant and more dilute solutions would have been tested for use in the sensitization test. However, neither of these two compounds proved to be irritating at 0.1% and this concentration was therefore used for the sensitization regimen.

The sensitization tests were started on a Monday when the guinea pigs were weighed and closely clipped on the scapular areas. The chemical was injected intradermally (0.05 ml of a 0.1% dilution) into the upper right scapular area of each pig. A similar injection of the vehicle was made concurrently into the upper left scapular area. Readings were made 24 and 48 hours later.

Doses of 0.1 ml of the same dilutions (freshly prepared) were then injected into the clipped dorsal lumbo-sacral areas of guinea pigs the following Wednesday, Friday, Monday, etc. until seven doses had been administered. Care was taken to ensure the repeated doses were not injected into the same site.

The guinea pigs were then rested for 3 weeks (incubation period), weighed and given a challenge dose of 0.05 ml of the 0.1% dilution of the chemical into the lower right scapular area. A control injection of the vehicle alone was also administered into the lower left scapular area at this time. The reactions were read after 24 and 48 hours.

Our grading system (shown below) is designed so that the intensity of the skin reaction is represented by a proportionate numerical value and also that any reaction elicited by the vehicle ("control substance") is subtracted from the reaction elicited by the substance and vehicle combined.

Reaction - Grading System

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

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

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

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

Seventeen of the 20 guinea pigs given intradermal injections of FEFO showed a severe sensitization reaction 24 hours following the challenge injection. The mean reaction score of these 17 animals was 198. At 48 hours, 18 of the guinea pigs showed a severe response to the compound with a mean score of 195.

Seven of the 20 guinea pigs given intradermal injections of SYFO showed a moderate sensitization response with a mean score of 78. At 48 hours, nine of the guinea pigs showed a similar response with an identical mean score of 78.

FEFO and SYFO are both capable of eliciting a sensitization response in guinea pigs; however, FEFO appears to be a stronger sensitizer than SYFO.

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

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

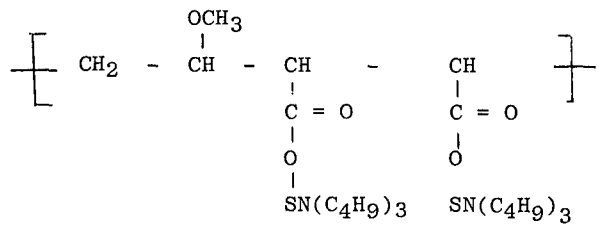
U. S. Navy Materials

Organometallic polymers (OMP) have the potential of being longlasting and effective antifouling agents against organisms such as barnacles, tubeworms, algae, hydroids, sponges and bacteria (Dyckman et al., 1973). The polymers containing trialkyltin have been shown to exhibit some degree of mammalian toxicity. Miller et al. (undated) have found OMP-1 to be irritating to the skin and eyes of rabbits and exhibit an oral LD_{50} in rats of 230 mg/kg. OMP-2 was also irritating to rabbit skin and eyes and was found to have an oral LD_{50} of 280 mg/kg in rats (Bradley, 1976). The present study was undertaken to evaluate the toxic hazards of two additional organometallic polymers, OMP-4 and OMP-5. The acute toxicity tests to be conducted are listed below:

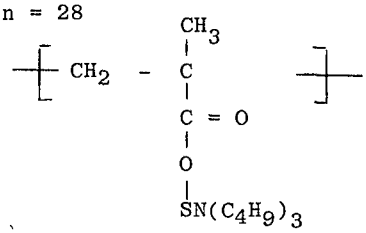
1. Single oral dose LD_{50} in rats and mice.
2. Single intraperitoneal dose LD_{50} in rats and mice.
3. Primary skin irritation in rabbits.
4. Eye irritation in rabbits.
5. Skin sensitization potential in guinea pigs.

The chemical structures of the monomers of the compounds are shown.

OMP-4 -- % Tin = 31.06



OMP-5 -- % Tin = 28



OMP-5 was ground to pass 140 mesh and suspended in corn oil for oral and intraperitoneal administration to rats and mice. Because of the physical nature of OMP-4, suspension in a vehicle was difficult. It was necessary to first prepare a solution of the compound in cyclohexanone, one of the few solvents in which it is soluble, and add this to the propylene glycol vehicle. This mixture was then placed under constant vacuum with stirring to remove the cyclohexanone, which possesses some degree of toxicity. The resulting mixture was an emulsion of OMP-4 in propylene glycol. An additional solution of cyclohexanone and propylene glycol was prepared and treated in a similar manner to be administered to the animals as a control. This insured that any resulting mortality would be attributable to OMP-4 and not cyclohexanone.

The method for oral administration is presented in another section of this report. Single dose intraperitoneal toxicity was estimated by injecting the materials and their respective vehicles into the peritoneal cavity. Injection volumes and procedures for LD_{50} calculations were similar to those used in the oral dose test. Groups consisted of five animals.

The patch test method for primary skin irritation on rabbits can be found in last year's report (MacEwen and Vernot, 1976).

The irritation to the eyes of rabbits was estimated by placing 0.1 gm of the undiluted material in one eye of each of six rabbits. The opposite eye was untreated and served as a control. Examinations for gross signs of eye irritation were made at 24, 48 and 72 hours after application. Scoring of the effects was according to the methods of Draize (1959) in which corneal, iris and conjunctival effects were scored separately.

The skin sensitization potential in guinea pigs along with many of the oral and intraperitoneal tests are still in progress and will be reported later.

The oral and intraperitoneal tests which are complete are summarized in Table 13.

TABLE 13. ACUTE ORAL AND INTRAPERITONEAL TOXICITY OF OMP-4 AND OMP-5

Compound	Route	Species	LD_{50} (95% C.L.) in mg/kg	Data Used to Calculate LD_{50} in mg/kg (Mortality N = 5)
OMP-4	Oral	Rat	268(191-376)	Control (0), 125 (0), 250 (2), 500 (5), 1000 (5)
OMP-5	Oral	Rat	1427(945-2154)	300 (0), 600 (1), 1200 (1), 2400 (5)
	Oral	Mice	406(300-549)	250 (0), 500 (4), 1000 (5), 2000 (5)
	IP	Mice	29(18-47)	7.8 (0), 15.6 (1), 31.2 (2), 62.4 (5), 125 (5)

No signs of skin irritation were seen with OMP-5 application. OMP-4 applied to rabbit skin resulted in skin blanching and edema at the 24-hour examination. The edema had decreased with erythema becoming more pronounced at 72 hours after application. The primary irritation index was 3.0 which classifies it as a moderate irritant. Examination one week after application showed that OMP-4 had produced eschar formation and destruction of the dermal layer down to the fascia, thus classifying it as a corrosive material.

Both OMP-4 and OMP-5 were irritating to the eyes of rabbits. Undiluted OMP-4 produced redness and swelling which decreased in intensity and appeared normal at one week post-treatment. OMP-5 was applied to the eyes in the unground state. Two rabbits showed scattered and diffuse corneal opacity at the 24-hour examination, which cleared by 48 hours post-treatment. The conjunctivae of all the treated eyes were red, swollen and produced a discharge. These findings were moderate at 24 hours and decreased in intensity with time. No irritation was seen at one week after treatment.

Air Force Materials

Acute toxicity tests were performed on a series of miscellaneous materials submitted for testing by the Air Force. These materials are listed in Table 14 along with the physical state and chemical supplier. A preliminary search of the literature and manufacturers information revealed that only one compound, Silicone DC-200, had published documentation of acute toxicity informaion. It was thus necessary to conduct these tests to evaluate potential toxic hazards to the personnel handling the materials.

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

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

   (These tests are currently in progress and the results are not included in this report)

Unfortunately, the supply of some materials was limited, necessitating a prioritization of tests performed.

Animals used for oral dosing were 200-300 gram male Sprague-Dawley rats and 25-40 gram male ICR mice. The animals were fasted at least 16 hours prior to dosing. Solutions of the materials were prepared with corn oil or propylene glycol and administered in geometrically spaced doses using syringes and special oral dosing needles. Animals were individually weighed at the time of dosing to determine the proper dosing volume.

Animals were observed for 14 days after dosing. Any deaths occurring during this period were included in the final mortality results. The moving interpolation method of Weil (1952) was used to determine the LD_{50} and 95% confidence limits. The toxicity classification published by Back et al., (1972) was used to categorize the compounds.

TABLE 14. LIST OF AIR FORCE COMPOUNDS SUBMITTED FOR ACUTE TOXICITY STUDIES

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

As a preliminary screen for the test materials, an oral dose of 5.0 g/kg body weight was administered to two rats. If no mortality occurred, the material was considered to be below toxic and no further oral toxicity tests were conducted. If mortality occurred, an oral LD_{50} was determined.

A patch-test method was used to determine primary skin irritation. A description of this method can be found in the last annual report (MacEwen and Vernot, 1976). The averages of the scores are used to determine a primary irritation index.

Compounds producing an index of 2 or less are mildly irritating while those with indices of 2-5 are moderate irritants and those above 6 are considered severe irritants.

The results of the acute oral toxicity tests and the classifications are shown in Table 15.

TABLE 15. ACUTE ORAL TOXICITY OF AIR FORCE MATERIALS

Compound	Species Male	LD_{50} (95% C.L.) in mg/kg	Data Used to Calculate LD_{50} in mg/kg (Mortality Response N = 2)	Classification
2-amino-1,2,4-triazole	Rat	>5000	5000(0)	Below toxic
Salicyl amino guanidine	Rat	>5000	5000(0)	Below toxic
2,6,di-tert-butyl-di-methylamino-p-cresol	Rat[a]	1189(669-2111)	500(0),1000(3),2000(4)	Toxic
	Mouse[a]	307(190-496)	125(0),250(2),500(4), 1000(5),2000(5)	Toxic
N,N'-disalicylidene-1,2-propane diamine	Rat[a] Mouse[b]	2279(1344-3868)	1140(1).2280(2).4560(5)	Toxic
Silicone DC-200	Guinea Pig	>50 ml/kg	(Rowe et al., 1948)	Below toxic
1,2,3-benzotriazole	b			
Tricresyl phosphate	Rat	>5000	5000(0)	Below toxic
1,4.dihydroxy-anthraquinone	Rat	>5000	5000(0)	Below toxic
Sulfurized 9-Octo-decenoic Acid	Rat	>5000	5000(0)	Below toxic
Azelaic Acid	Rat	>5000	5000(0)	Below toxic
Dimer Acid	Rat	>5000	5000(0)	Below toxic
N-Benzyl-3,7-dioctyl phenothiazine	Rat	>5000	5000(0)	Below toxic
Phenothiazine	Rat[a,b]		5000(3)	
Dioctyl Phenothiazine	Rat	>5000	5000(0)	Below toxic
Sebacic Acid	Rat	>5000	5000(0)	Below toxic
Acryloid HF-866	Rat	>5000	5000(0)	Below toxic
Acryloid HF-844	Rat	>5000	5000(0)	Below toxic
Guanidino Salicyla-mide Salt	Rat[b]		5000(1)	
Nonyl Phenol	Rat[a]	2462(1788-3389)	1000(0).2000(1).4000(5)	Toxic
	Mouse[a]	1231(910-1665)	500(0).1000(1).2000(5). 4000(5)	Toxic
Phosphonate Salt	Rat[b]		5000(2)	
Tris(B-chloroethyl) phosphate	Rat[a]	1131(499-2847)	200(0).400(0).800(0). 1600(3)	Toxic
	Mouse[a]	1866(1289-2701)	500(0).1000(0).2000(3). 4000(5)	Toxic

[a]Five animals per level.

[b]Supply of the compound was limited and used for other tests.

Only two of the compounds produced any irritation at the 24 or 72 hour readings. Salicyl aminoguanidine treatment resulted in slight erythema and edema in three of the rabbits at 24 hours. Examination at 27 hours postexposure showed that the edema had generalized over the patch location and that five of the six rabbits had developed coriaceousness. One week after the exposure the "leathery" patches of skin had fissured and had begun to peel off. The primary irritation index score was determined to be

1.6, characteristic of mild irritation. However, the score is is based upon edema and erythema results with necrotic development not being considered. In view of the tissue damage which resulted, the material should be considered a moderate to severe irritant. Phosphonate salt produced slight erythema and edema at 24 hours. The edema had reduced at 72 hours, but the erythema was still present. The primary irritation index score was determined to be 2.4, thus classifying the material in the mild to moderate irritation range.

All other compounds were found to be non-irritating at 24 and 72 hours postexposue examinations. Many of the liquid compounds apparently reacted with the adhesive of the patches and caused localized reactions. However, the reaction under the gauze patch was nil, thus the classification of nonirritating.

Department of Transportation Materials

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

Certain materials being transported have inadequate toxicology data which is necessary for proper classification by the Department of Transportation. These materials were tested in this laboratory to verify the suitability of proposed transportation health hazards classification criteria. This was done by determining experimentally the 14-day toxicity by skin absorption on rabbits, 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 to determine the category in which each compound was placed.

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

During the current reporting period, a group of compounds was received and assigned code numbers prior to testing. These compounds, their THRU code numbers and the tests to be done on each are listed in Table 16.

TABLE 16. LIST OF COMPOUNDS SUBMITTED BY THE DEPARTMENT OF TRANSPORTATION FOR ACUTE INHALATION AND PERCUTANEOUS TOXICITY STUDIES

Code No.	Compound	Route of Administration: Inhalation Toxicity	Route of Administration: Skin Absorption
192	Pyridine	X	
198	Fuming Sulfuric Acid	X	
273	p-Cresol (Sherwin-Williams)		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
	No. 7 Carburetor Cleaner (DuPont)		X
	B-12 Chemtool (Berryman)		X
288	Chromic Nitrate		X
289	Calcium Chromate		X
290	Propargyl Alcohol	X	X

Methods and procedures for dermal and inhalation testing were described in a previous annual report (MacEwen and Vernot, 1975). The only change from the described methods was the fact that some inhalation exposures were done in a one cubic meter chamber with and air flow of 13-25 cfm. The exposure groups for this chamber remained at 5 rats of each sex per contaminant level.

The results of the completed acute toxicity tests and the assigned classification are shown in Tables 17 and 18.

TABLE 17. 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
Fuming Sulfuric Acid	M	420(397-444)	397(1), 427(3), 606(5)	Highly Toxic
Fuming Sulfuric Acid	F	347(260-464)	229(0), 238(2), 299(2), 531(4)	Highly Toxic
Pyridine	M	9011(8218-9881)	6675(0), 7825(1), 9150(2), 11000(5)	Toxic
Pyridine	F	9023(8164-9972)	5050(0), 7425(1), 9250(1), 9575(4), 11300(5)	Toxic
Benzene Sulfonic Chloride	M	Saturated Vapor (~31 ppm)	31(0)	Less than toxic at room temperature
Benzene Sulfonic Chloride	F	Saturated Vapor (~31 ppm)	31(0)	
Benzene Sulfonic Fluoride	M	Saturated Vapor	315(0)	Less than toxic at room temperature
Benzene Sulfonic Fluoride	F	Saturated Vapor	308(0)	
Propargyl Alcohol	M	1204(1183-1224)	1180(0), 1190(3), 1240(4), 1260(5)	Toxic
Propargyl Alcohol	F	1043(974-1116)	790(0), 970(2), 1050(2), 1130(3), 1180(5)	Toxic
Sulfuryl Fluoride	M	3730(3087-4508)	2700(0), 2950(2), 4000(3), 5000(4)	Toxic
Sulfuryl Fluoride	F	3021(2827-3218)	1210(0), 2950(2), 3250(4), 3600(5)	Toxic
Trichloroethylene	M	26,320(23,714-29,212)	19600(0), 23600(2), 26800(2), 29600(4)	Toxic
Trichloroethylene	F	25,655(22,342-29,460)	29600(1), 23900(1), 26900(3), 29600(4)	Toxic
Sulfur Dioxide	M	2520(2347-2705)	2100(1), 2300(0), 2525(3), 2700(3), 3000(5)	Toxic
Sulfur Dioxide	F	1613(1372-1896)	1225(0), 1300(1), 1400(3), 1910(3), 2900(5)	Toxic
Sulfuryl Chloride	M	131(87-171)	95(0), 120(3), 155(4), 200(4)	Highly Toxic
Sulfuryl Chloride	F	242(183-311)	155(0), 200(2), 300(3), 355(5)	Highly Toxic

TABLE 18. DERMAL TOXICITY OF VARIOUS COMPOUNDS IN FEMALE RABBITS

Compound	LD_{50} (95% C.L.) in ppm	Data Used to Calculate LD_{50} in mg/kg (Mortality Response,N=3)	Classification
Carburetor Cleaners:			
Gumout	5000(3900-6500)	4000(0), 5040(2), 6350(2)	Toxic
DuPont No. 7	9000(4700-17000)	4000(0), 8000(1), 16000(3)	Toxic
B-12 Chemtool	1780(940-3380)	1000(0), 2000(2), 4000(3)	Toxic
Propargyl Alcohol	88.4 (no range)	62.5(0), 125(3), 250(3)	Highly Toxic
Calcium Chromate	>20,000	20000(0)	Less than Toxic
Chromic Nitrate	>20,000	20000(0)	Less than Toxic
p-Cresol (Sherwin-Williams)	180(120-270)	125(1), 250(2), 500(2)	Highly Toxic

SECTION III

FACILITIES

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

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 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.

Analysis of MMH for Purity

The institution of regulations covering non-clinical laboratory studies by the Food and Drug Administration and of guidelines for the conduct of experiments to determine carcinogenesis by the National Cancer Institute have made increasingly important the determination of the degree of purity and stability of any chemical used in long-term toxicity studies. Therefore, at the time that the year-long experiment with MMH was planned, it was decided to develop analytical procedures for measuring degree of purity in the material obtained for use in the study and for identifying and quantifying, if possible, the impurities contained therein. Two complementary approaches were adopted: (1) an iodimetric titration which would measure the total of reducing substances in the sample and (2) a chromatographic procedure which would separate the impurities into individual peaks which could be identified and quantitated.

An iodimetric titration method which had been developed previously for the determination of the stability of aqueous solutions of MMH was adapted to the assay of the material to be used as a contaminant. In this method, 5 µl of MMH is pipetted into 10 ml of an aqueous solution containing 1.269 g/liter

I_2, 40.0 g/liter KI, 20.0 g/liter Na_2HPO_4 and 6.0 g/liter KH_2PO_4. Excess MMH is then titrated to a colorless endpoint with this solution. The technique is very reproducible and MMH degradation can easily be followed in this way.

Additionally, gas chromatography and liquid chromatography were investigated as techniques for the separation of the impurities in commercially available MMH. Attempts to accomplish this using liquid chromatography were not very successful. Several peaks were noted using the UV detector but these were not well separated. Water, water-acetic acid, methanol and acetonitrile were tried as carriers on a C-18 Bondapak column. None of them demonstrated satisfactory separation. Therefore, liquid chromatography was no longer pursued as a separation technique for MMH. Poor gas chromatographic separations were obtained using liquid phases recommended for hydrazine analysis including 1-hydrazinopyridine and Pennwalt 223®. Tenax GC® and Chromosorb 103® were found to provide satisfactory chromatograms when used as column packings. They are porous polymers which have low bleed, long life and are stable in the presence of reactive chemicals. Tenax GC® gave slightly better separation, and it was chosen in the procedure to be used with MMH. The gas chromatographic conditions for purity analysis of MMH were:

Column - 1/8" x 10' stainless steel containing 60/80 mesh Tenax GC®

Carrier - Helium at 25 ml/min

Detectors - flame and thermal conductivity, 1/11 split

Temperature Program - 90 C for 4 minutes, programmed 10 C/minute to 145 C and held for 8 minutes

Injection Temperature - 125 C

Detector Temperature - 170 C.

Under these conditions, 13 peaks were distinguished when MMH was chromatographed.

A quantity of MMH was obtained from Olin Corporation for the year-long exposures of animals. This batch of material was transferred under nitrogen to 100 ml bottles. Two 100 ml bottles were assayed for purity by the gas chromatographic and iodimetric techniques. By iodimetric titration the samples assayed at 100.0 - 100.5% MMH. The same number of impurity peaks were obtained in the samples as had been seen in samples stored for long periods at the THRU, but the peaks were much smaller.

A THRU chemist took samples of pure and contaminanted MMH to the Instrumental Analysis Laboratory at the School of Aerospace Medicine (SAM), Brooks AFB, Texas. The purpose of the visit was to identify the impurities utilizing the gas chromatography/mass spectrometer instrumentation available there. The chemist took with him columns and column packing previously found to be satisfactory for the gas chromatography of MMH. SAM personnel attached the gas chromatographic columns to the mass spectrometer, ran a number of chromatograms and recorded mass spectra under different chromatographic conditions. Tentative identifications of the peak constituents were made using the reference library available with the SAM mass spectrometer. Some of these appeared to be credible considering possible by-products and breakdown products occurring during or after MMH synthesis. These tentatively identified components included methane, nitrogen, ammonia, water, methanol, azomethane and hydrazine. Other peak identifications derived from the computer on the basis of mass spectra did not appear to be compatible with MMH synthesis or breakdown. The mass spectra corresponding to these peaks were analyzed and the largest assigned the structure of formaldehyde monomethylhydrazone, $CH_3NHN=CH_2$. This peak and the water peak rise after a bottle of MMH is opened and used. The mass spectra of two other peaks were examined and tentative structure assigned as $CH_3N=NH$ (or $CH_2=NNH$) and $CH_3N=NOH$. These identifications are uncertain at best since the stability of these compounds is unknown and possibly quite low.

In order to confirm the identification of formaldehyde monomethylhydrazone, formalin (37% formaldehyde, 13% methanol, 50% water) was added to MMH and led to changes in the gas chromatogram. These included increases in the water and methanol peaks and in the peak identified as the hydrazone from its mass spectrum. Since formaldehyde monomethylhydrazone is the expected product of the reaction between formaldehyde and monomethylhydrazine,

$$H_2C=O + CH_3NHNH_2 \longrightarrow CH_3NHN=CH_2 + H_2O,$$

this is strong indication that the assignment is correct. Formaldehyde is probably formed as the first product of oxidation of MMH:

$$2\ CH_3NHNH_2 + 3\ O_2 \longrightarrow 2H_2C=O + 2N_2 + 4H_2O.$$

In liquid MMH, it would react immediately to give the hydrazone. Quantitation of the individual impurities in the MMH being used in the present study has not been accomplished, but the titration procedure demonstrates that the material is quite pure, probably containing no more than 0.5% impurities of which water represents the greatest constituent.

Figure 11 is a gas chromatogram of a sample of this MMH with identifications, some confirmed by injection of pure material, of the peaks included.

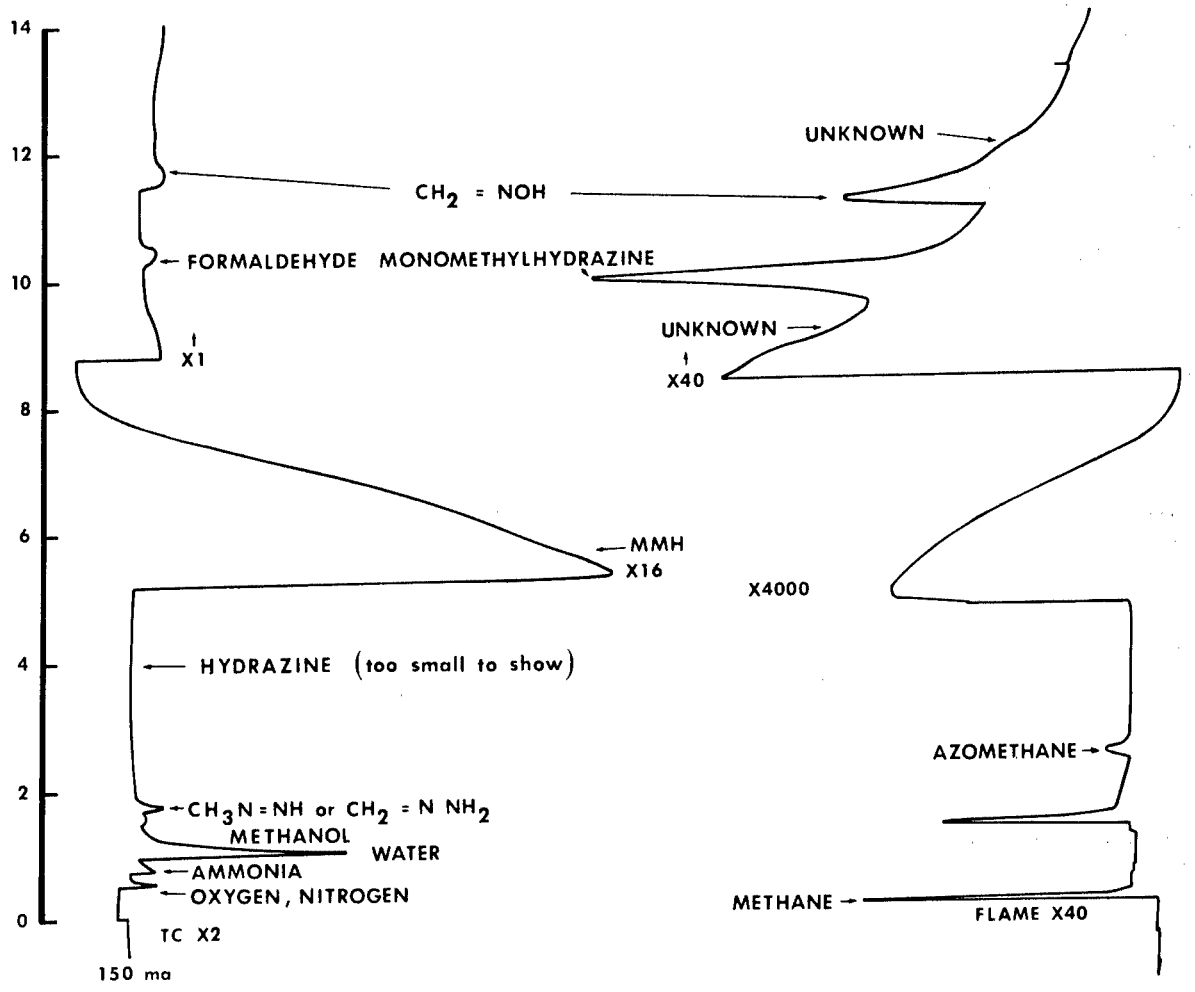


Figure 11. Gas chromatographic separation of MMH impurities.

Generation and Analysis of Sulfur Trioxide

One of the materials for which inhalation toxicity testing was requested by the Department of Transportation was fuming sulfuric acid. Such an exposure would be essentially to sulfur trioxide or to sulfuric acid formed by reaction of SO_3 with moisture in the air. Generation of SO_3 from the fuming acid in a reproducible fashion presented certain problems associated with the fact that the vapor pressure of SO_3 over the fuming acid changed as its concentration changed. Since moisture in the air used to pick up the SO_3 was readily absorbed, leading to dilution of the acid, very dry air had to be supplied for this purpose.

The method found to be satisfactory for generation used 114.63% oleum (fuming sulfuric acid) as a source.

The fumes of SO_3 were generated from a pool of 114.63% oleum contained in a glass flask of approximately 75 cc volume. An air stream measured with a Fischer-Porter 1/16-16 sapphire flowmeter was passed over the surface of the pool. Up to 200 cc/min of dry air (< 4% R.H.) was available for SO_3 pickup. The air/SO_3 from the flask was diluted with 8.0 liter/min dry air (< 10% R.H.) prior to entering the exposure vessel. The exposure volume was 9 liters and the exhaust was passed through a scrubber system prior to venting to the atmosphere. A clean supply of oleum was placed in the flask every fourth exposure. The flask was suspended in a constant temperature bath and allowed to equilibrate before any exposures were attempted.

The analytical method used for measurement utilized the Technicon AutoAnalyzer® I system. Air from the exposure chamber was pumped to a glass scrubber tower at 25-30 cc/min. The scrubber solution was composed of 0.225 M Na_2HPO_4, 0.0015 M NAOH and 0.00009 M phenophthalein and was delivered at a rate of 0.9 ml/min. The analysis was based on the scrubber solution color loss caused by decreasing pH as the acid was scrubbed from the sample stream. Calibration of the analytical system was accomplished using solutions of the scrubber containing known amounts of H_2SO_4. Figure 12 is the calibration curve resulting from ten different sets of standards. The calibration showed a relative error of 6%. The chamber concentration was calculated using the value taken from Figure 12 and the equation.

$$PPM_A = \frac{\text{ml Acid} \times 24.62 \text{ liter/mole} \times \text{Scrubber Soln. Rate} \times 10^3 \text{ ppm}}{\text{Sample Rate}}$$

When animals were placed in the exposure chamber, there was a large increase in the analyzed concentration caused by respiratory CO_2. Therefore, this response was measured before, during and after exposure with the exposure concentration determined as the difference between the exposure response and animal only response.

The analyzed chamber concentration was checked by two independent calculations. One utilized the graph in Figure 13 and the air dilution to calculate the concentrations. The other determined mean concentrations from the difference between the weights of the generation flask at the beginning and end of exposure. Both of these techniques gave values which were consistent with the analyzed concentrations.

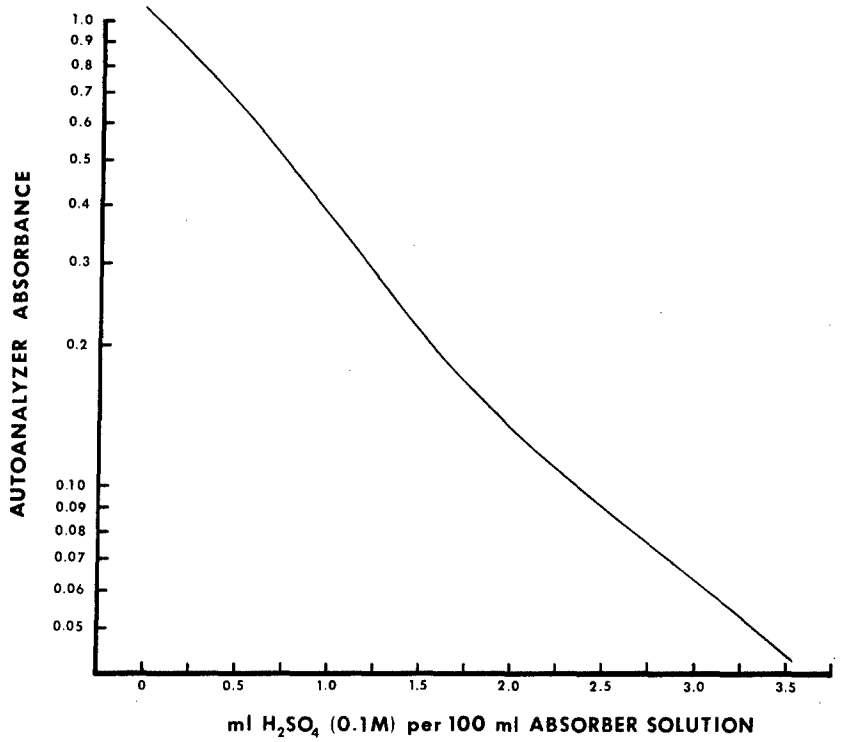


Figure 12. Calibration curve: AutoAnalyzer® absorbance changes with sulfuric acid concentration.

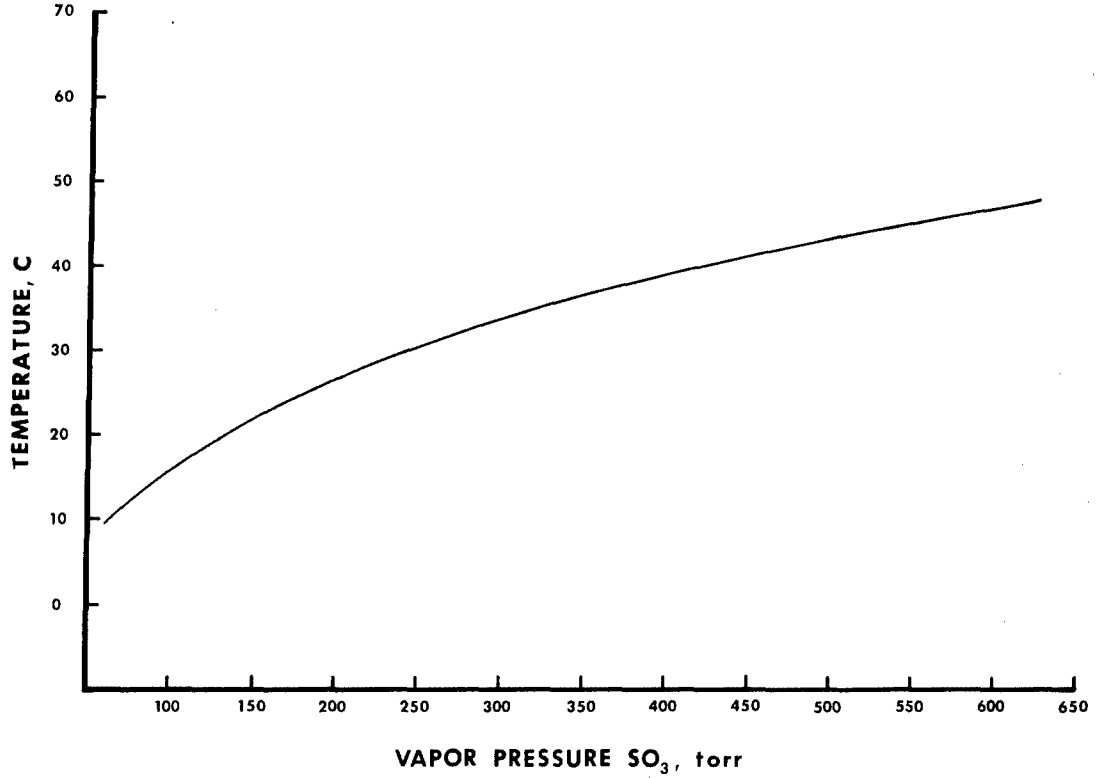


Figure 13. Effect of temperature on SO_3 vapor pressure over oleum.

Physiological Fluid "Fingerprint" Chromatography

Work continued this year on the measurement of changes in urine constituents caused by intoxication with carbon tetrachloride (CCl_4). No changes were made in the technique developed last year for liquid chromatography, but some significant improvements were incorporated into the gas chromatographic procedure, primarily in the area of stripping volatiles from the urine.

In the developed procedure, 5 ml urine is pipetted into a capped midget impinger. One gram of reagent grade lithium chloride is added to reduce the vapor pressure of water and increase the volatilization of nonpolar materials. Reagent grade sulfuric acid, 0.25 ml, is added to reduce foaming and hydrolyze the non-volatile urine components to release more volatiles from the sample. The solution is heated in a boiling water bath and nitrogen bubbled through at 15 ml/min. The effluent is delivered through a room temperature tube to trap the small amount of water given off and prevent clogging of the precolumn. No other volatile is trapped in significant quantities in the water trap. The effluent then passes through a 1/8" x 6" stainless steel 80/100 mesh Chromosorb 101® precolumn cooled to 0 C to reduce the loss of some volatiles particularly those which appeared first on the chromatogram. After 35 minutes the precolumn is then inserted into the GC injector port for chromatographic separation as detailed in last year's annual report (MacEwen and Vernot, 1976).

Groups of 3 rats each were held in individual metabolism cages for periods of 24 hours for urine collection. Twenty-four hour urine samples were collected 4 times with food available to the animals and 4 times while the animals were fasting to provide baselines which would represent extremes of feeding behavior. Then 0.2 ml carbon tetrachloride was injected intraperitoneally into each of the rats and 24 hour urine samples collected while the rats went without food. This procedure was carried out on 2 groups of rats.

The gas chromatograms obtained from intoxicated rat urine differed from those of normal fasted and non-fasted rats specifically in the large variations in 3 peaks caused by injection of carbon tetrachloride. The heights of these peaks with no fasting, during fasting and after intoxication are listed in Table 19. It is apparent that carbon tetrachloride intoxication causes Peak 18 to increase and Peaks 26 and 28 to decrease dramatically. When one group of animals was held 8 weeks postinjection, some recovery of the peaks to normal values was noted. Other gas chromatographic peaks were not affected by carbon tetrachloride or were not consistent in their responses. Carbon tetrachloride itself was not detectable in the urine of injected animals.

TABLE 19. EFFECT OF CARBON TETRACHLORIDE ON RAT URINE GAS CHROMATOGRAPHIC PEAK HEIGHTS

Peak Number	Non-Fasting Baseline[1] Mean	Non-Fasting Baseline[1] Range	Fasting Baseline[1] Mean	Fasting Baseline[1] Range	CCl_4, Immediately Post-Injection	8 Weeks Post-Injection Non-Fasting
			(Group 1 Rats[2])			
18	549	0-2160	810	0-3240	6758	0
26	1036	684-1253	431	84-792	0	152
28	3347	2832-4176	4573	888-4655	0	580
			(Group 2 Rats[2])			
18	0	0	94	0-470	3757	---[3]
26	843	384-1170	123	92-204	0	---[3]
28	2781	1296-3816	1313	912-2091	149	---[3]

[1]Means and Ranges are for 4 determinations on each group.

[2]N = 3, males.

[3]Animals died before long-term post-injection values could be obtained.

ENGINEERING PROGRAMS

Microprocessor - Animal Weighing System Interface

Modification of the Animal Weighing System to output information with a microprocessor was completed during the past report period. Problems with operator interaction with the weighing system had been present since the inception of the system. One problem had been unstable readings due to animal movements on the load cell scales resulting in unsteadiness in the output weight readings. The procedure used originally included a hold button which was activated by the console operator prior to inserting an identification badge in a badge reader to initiate the weighing action. This method depended a great deal on the operator's judgment as to when the reading was stable, and repeatability from operator to operator was very low. Another condition of the initial system was a limitation on the numbers of digits capable of being displayed. The original system could handle a maximum of 20 Binary-Coded-Decimal Digits. These were distributed as follows:

a. 4 Digits - Animal Weight

b. 16 Digits - Animal Identification

This information was available from the load-cell readouts for the animal weight and from punched holes in a plastic badge for the animal identification information.

To encode the information on the plastic badge required punching 4 holes for each digit of information resulting in punching 64 holes in the badge. Punching this number of holes not only required considerable time but the badges were not reusable. The new design continued receiving the 4 digits of animal weight from the load cell readout devices but reduced the badge information to only 1 4-digit animal number. In addition a control column of 4 holes was punched. This resulted in a total of 5 digits or 20 holes to be punched. Not only was the punch-time percard reduced but the cards are reusable resulting in a significant cost-savings. The additional animal information is read from 3 digital thumbwheel switches on the front panel as shown in Figure 14. These are as follows:

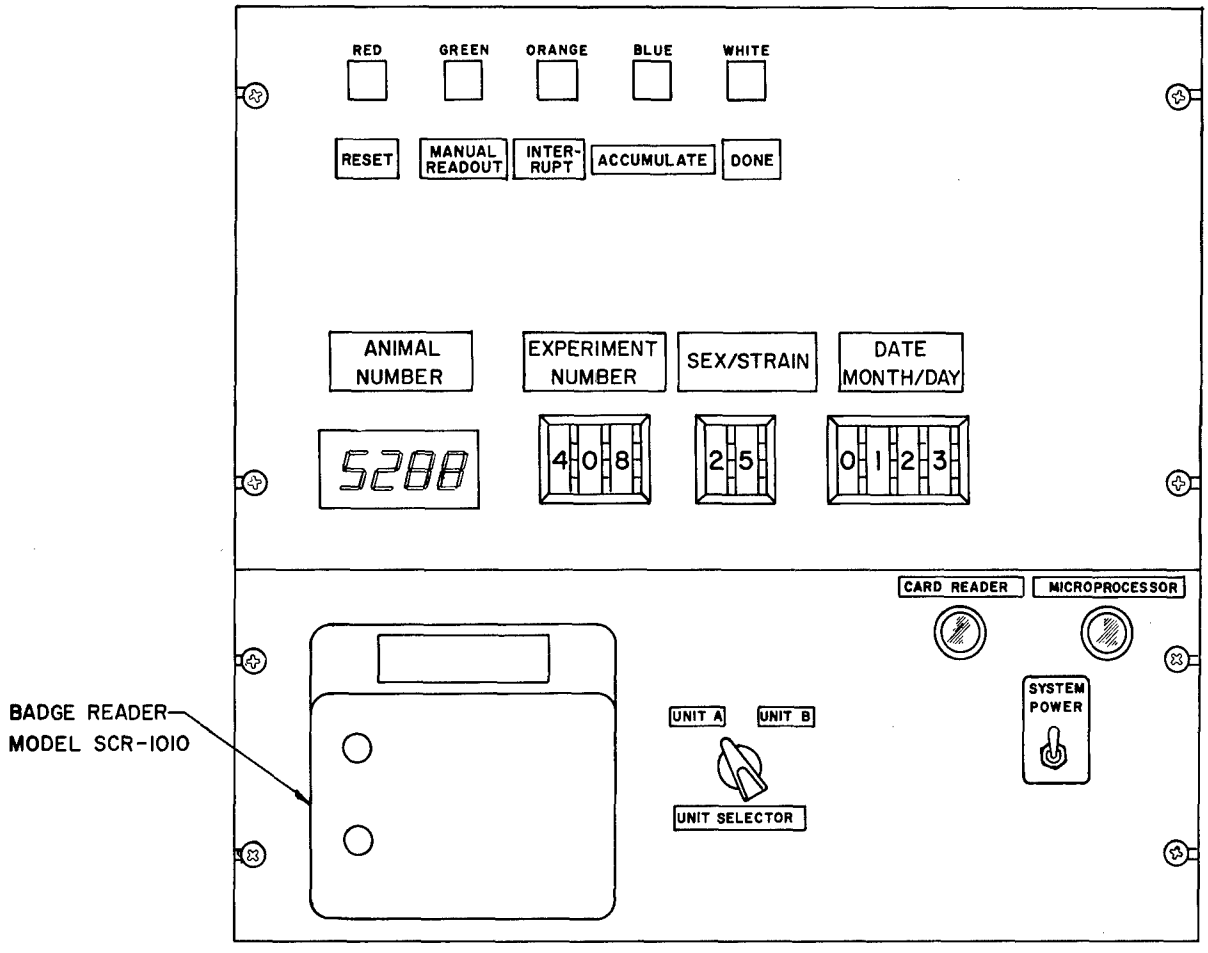


Figure 14. Face panel of animal weighing console.

a. 3-Digit Switch - Experiment
b. 2-Digit Switch - Sex/Strain
c. 4-Digit Switch - Month/Day.

This information may be changed as needed.

The output information is tabulated as shown in Table 20. Each set of animals may be prefaced with the heading as shown.

TABLE 20. AUTOMATED ANIMAL WEIGHT INFORMATION FORMAT

EXPER. NØ.	SEX/ STRAIN	DAY/ MØ.
0413	32	0628

ANIMAL NØ.	ANIMAL WT.
3120	0171
3136	0164
3145	0141
3004	0168
3008	0171
3015	0164
3010	0164
3079	0161
3113	0167
3133	0154
3108	0168

One of the advantages of using microprocessors is the capability of interfacing instruments and control systems to provide accurate and repeatable results. The microprocessor hardware block diagram is shown in Figure 15, and the progress flow chart is shown in Figure 16. As can be seen very effective control is maintained over the complete system

Animal weighing is initiated by insertion of the animal badge. The badge is scanned for the animal number and this is displayed on a large readout on the front panel. The thumbwheel switches are then scanned and the information stored. The badge reader is then deactivated preventing any further badge activations until the weighing of that animal is completed. The weight information is then read from the load cell readouts after the units have been placed in "hold" by the microprocessor. The load cell readouts are then reactivated and after a short delay are placed in hold and read again.

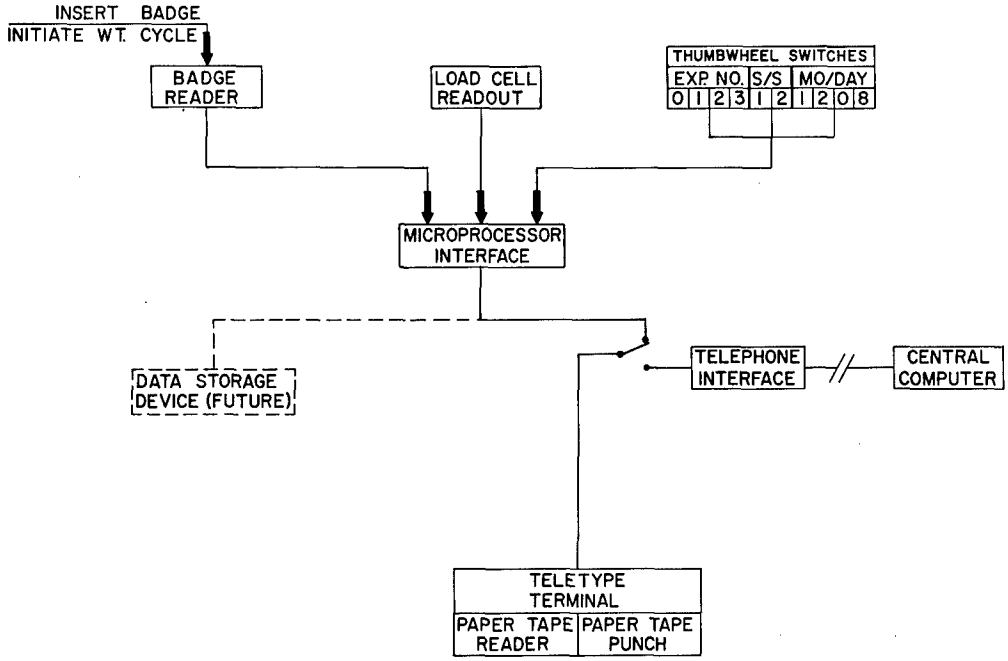


Figure 15. Microprocessor/Animal weighing system interface block diagram.

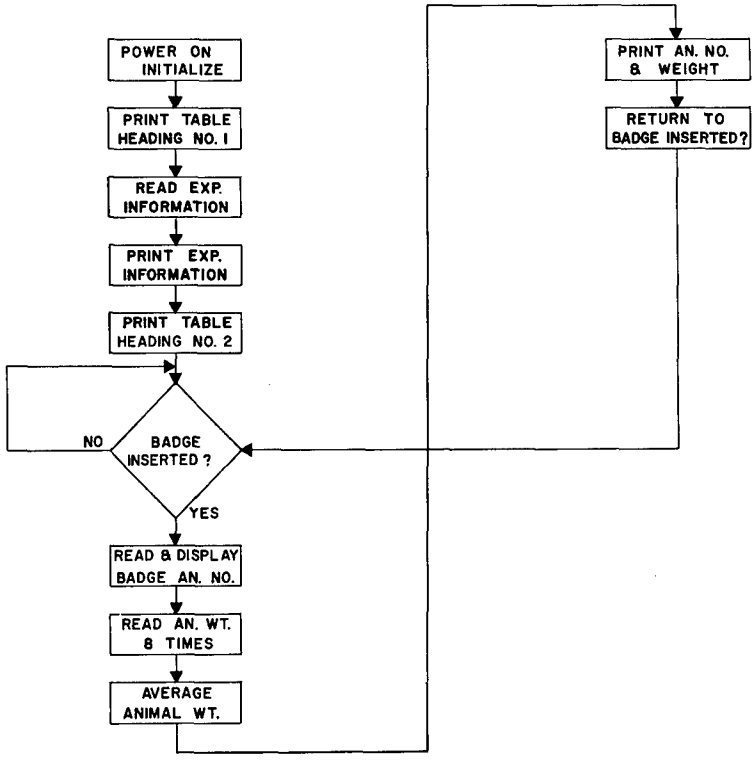


Figure 16. Microprocessor interface program flow chart.

This is repeated 8 times and the readings totaled. The total is then divided by 8 to achieve the average reading. This effectively reduces the effect of animal movement on the read-out device. This completes the load-cell readout cycle and the information is output to the printing device. After printing the Badge-Reader is reactivated and the system is ready for the next animal weighing.

Provisions are incorporated in the system for a manual "read" button to activate the weighing cycle without requiring the insertion of a badge. This function may also be used for calibrating with standard weights as the badge number column appears with all zeros. This can be used to indicate that the weight on this line is a calibration weight. The system increases repeatability and accuracy by removing the decision-making requirements of the operator and simultaneously decreases the time required to weigh animals.

An additional function of the system is to prepare a punched tape of the weight data. This tape is input to a time-shared computer through an interface terminal for data reduction with statistical programs.

Future plans include installation of an integral magnetic cartridge recorder to store data from the weighings which will enable information from a complete experiment, covering one year, to be maintained on site.

Solid State Electronics Development

The utilization of solid-state electronics technology for THRU applications continued during the past report period. Engineering capabilities have been upgraded for the past three years.

The principal effort has been directed towards achieving expertise in the design and use of microprocessors. Many seminars and manufacturers meetings have been attended. Additionally, courses in computer and microcomputer applications have been taken.

Many different types of microprocessors were available with varied operating features. The initial project for development was the Animal Weighing System and extensive surveys of the expanding microprocessor market were conducted. An Intel SBC 80/10 using Intel's 8080 microprocessor was chosen. This is a single-board computer residing in a plug-in card cage. Many manufacturers furnish support devices which are compatible with this equipment.

Design and fabrication of the interface using this equipment provided excellent training for engineering personnel, both in the hardware area and in the program requirements of the device. Techniques used in this project may be easily adapted to other systems.

Future areas for microprocessor utilization are dome atmosphere control systems, comprehensive dome alarm systems with English language diagnostic messages, automatic dome contaminant generating and monitoring systems and various analytical instrument data acquisition systems. A decided advantage of these systems is that they are open-ended for future development. These individual devices may be easily connected to an on-site real time minicomputer thereby providing a comprehensive control and monitoring system for the complete THRU facility.

Repeatability of experimental conditions, increased accuracy, increased reliability, release of technical personnel from routine monitoring duties are just a few of the considerable advantages to be realized from microprocessor technology.

Interactive Program Development - Time-Sharing Computer

A program was initiated at the request of Chemistry Department personnel for aid in daily calculations of inhalation chamber concentration readings. An evaluation of their procedures was conducted to determine their requirements.

Daily concentration readings were taken from each chamber. In some cases 12 readings were taken and in other cases 18 readings. Each of these readings was adjusted for baseline drift and converted from absorbance readings to ppm. The combined readings were then analyzed for the range and the standard deviation. These calculations required approximately two hours to accomplish each day.

A program was developed which accepts the readings from the monitoring system. No further manipulations are required for the operator. These readings are then entered into the computer by the operator. Two programs were developed: one for Baseline Reading and another for Reading Baseline. The appropriate program requires input of the following parameters from the operator:

a. Number of Readings (N)

b. Baseline Correction (B)

c. Conversion Constant (C)

The program performs the following calculations:

a. (N-B) x C = ppm

b. (B-N) x C = ppm

After the readings are entered, the computer provides a readout of the results. After each run the computer inquires again for the parameters of the next run.

After initial training the operation of the program has been entirely conducted by Chemistry Department personnel with a time-shared teletype terminal at the Facility. Minor modifications are made to the program from time-to-time at the request of the operating personnel. Using this program the time required for processing daily information has been reduced from 2 hours to approximately 15 minutes.

An extension of the program is being considered to provide file storage of each day's entries. This will provide for statistical manipulation and/or callback of information on daily concentrations over a selected time period. This procedure currently requires manual manipulations of the information from notebooks.

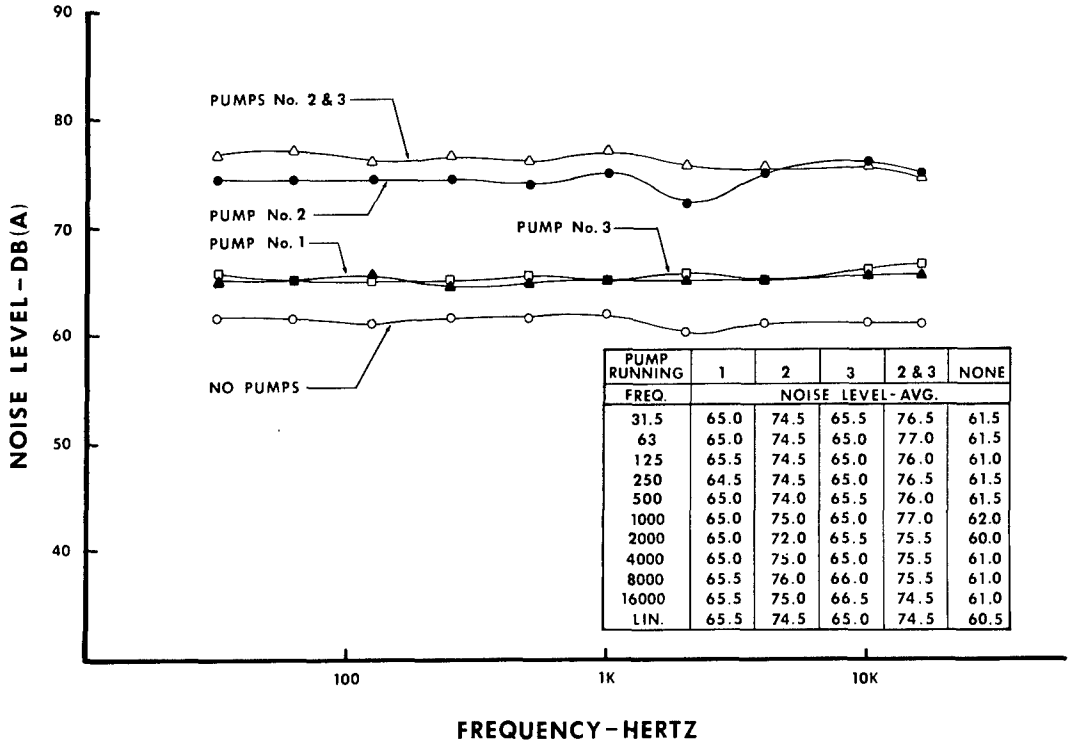
Another possible use of the program is to provide the nucleus of an automatic monitoring system to periodically sample dome concentrations and to provide a printout of the daily concentrations at the end of a run.

Noise Reduction Program

Efforts to reduce unacceptable noise levels in Facility A were continued during the past year. These efforts included vacuum pump overhaul with bearing changes and additional treatment of noise conducting air ducts.

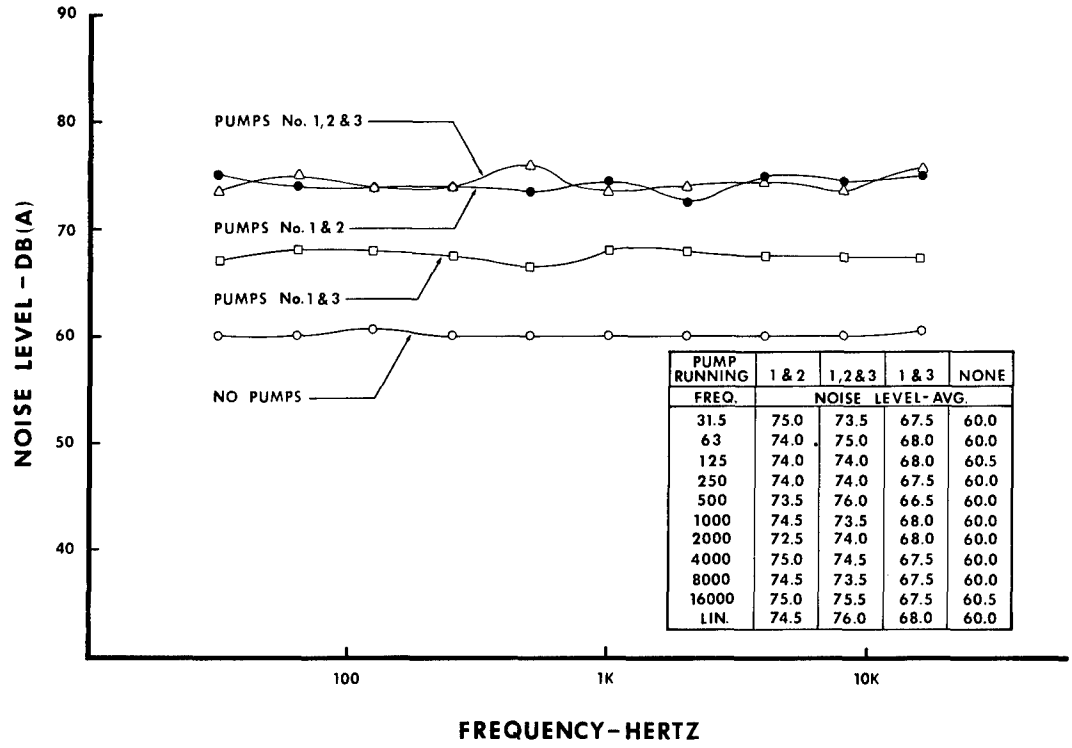
Noise measurements were taken at 9 stations in the room and averaged for each frequency. Figures 17 and 18 show the average noise levels at frequency bands between 31.5 Hertz and 16,000 Hertz. An "A" weighing network was used for these measurements to simulate the sensitivity of the human ear. Previous measurements were taken at the actual sound wave pressures in order to determine the resonant frequency band which required the most reduction in noise level.

The curves in Figures 17 and 18 show a relatively flat response across the entire frequency range. Pumps 1A and 3A when run separately had an average noise level of 65 DB(A). When these pumps were run simultaneously, the noise level was approximately 68 DB(A). Background noise without pumps running was approximately 60 to 62 DB(A).



PUMP RUNNING	1	2	3	2 & 3	NONE
FREQ.	NOISE LEVEL - AVG.				
31.5	65.0	74.5	65.5	76.5	61.5
63	65.0	74.5	65.0	77.0	61.5
125	65.5	74.5	65.0	76.0	61.0
250	64.5	74.5	65.0	76.5	61.5
500	65.0	74.0	65.5	76.0	61.5
1000	65.0	75.0	65.0	77.0	62.0
2000	65.0	72.0	65.5	75.5	60.0
4000	65.0	75.0	65.0	75.5	61.0
8000	65.5	76.0	66.0	75.5	61.0
16000	65.5	75.0	66.5	74.5	61.0
LIN.	65.5	74.5	65.0	74.5	60.5

Figure 17. Effect of noise control efforts on sound level output Facility A vacuum pumps 1,2,3 and 2 & 3.



PUMP RUNNING	1 & 2	1,2 & 3	1 & 3	NONE
FREQ.	NOISE LEVEL - AVG.			
31.5	75.0	73.5	67.5	60.0
63	74.0	75.0	68.0	60.0
125	74.0	74.0	68.0	60.5
250	74.0	74.0	67.5	60.0
500	73.5	76.0	66.5	60.0
1000	74.5	73.5	68.0	60.0
2000	72.5	74.0	68.0	60.0
4000	75.0	74.5	67.5	60.0
8000	74.5	73.5	67.5	60.0
16000	75.0	75.5	67.5	60.5
LIN.	74.5	76.0	68.0	60.0

Figure 18. Effect of noise control efforts on sound level output of various Facility A vacuum pump combinations.

Pump 2A had an average noise level of 74 to 75 DB(A) when run separately and in combination with the other two Facility A pumps. The initial analysis indicated that the cause of the high noise level was due to worn out bearings, but replacing the bearings did not correct the problem. The higher noise level could be an inherent characteristic of this pump, its location on the mounting pad, the alignment of the exhaust duct with respect to the pump or a combination of the above.

All the measurements were well within the OSHA noise limits of 90 DB(A) for an 8-hour day personnel exposure. However, the high reverberation characteristics of the Facility A dome room cause a disturbing effect at 75 DB(A) whereas the 65 DB(A) is tolerable.

Since six pumps are available and only three are necessary as a maximum for operating all Facility A and B domes, pump 2A is now used as a standby system in order to maintain the lower noise level.

Plethysmograph Chamber

In order to measure the respiratory rate of mice, a prototype plethysmograph was designed and constructed for use in conjunction with the 10 mice capacity plethysmograph test chamber reported in the 1976 THRU Annual Report, AMRL-TR-76-57.

The plethysmograph has 10 digital counters to record the breathing of mice simultaneously. The instrument can be operated in a preset automatic time control mode or a manual time control mode. A mode selector switch is provided for that purpose. The automatic time count is set by a 4 digit readout with thumb wheel dials. Counting can be set for 1 to 9999 minutes duration. The manual mode is used for random time counting.

The instrument has a power switch to energize the circuit and a start switch to activate the counting cycle. Lights to indicate counting in progress and completion of count are also on the panel.

A thermistor unit at each of the 10 mouse locations in the plethysmograph chamber transmits each breathing pulse to the plethysmograph for counting. Because of the heat sensitivity of the thermistors, the operation of the plethysmograph is affected by changes in room temperature and room drafts. Methods of eliminating or reducing this interference are being investigated. Meanwhile, the instrument has been turned over to the Toxicology Department for test and evaluation.

Thomas Dome Access Hatch Modification

As a continuing endeavor to improve the operation of the chamber access hatch opening and closure, several component parts of the mechanism have been modified and/or redesigned.

The center shaft of the rotating mechanism had no provision for lubrication. After several years in service, the exposure to various elements affected the shaft to the extent that rotation was difficult and in one case impossible. The shaft was modified by drilling and tapping an end part for installation of a grease fitting. A longitudinal hole was drilled on a vertical plane through the center of the shaft and a cross hole drilled to intersect the longitudinal hole connected to the grease fitting part. Recessed spaces were machined at specified lengths on the shaft and a spiral, annular groove was machined in the outer peripheral surface, intersecting the grease supply holes. As the shaft is rotated lubrication is transferred from the supply holes to the recessed spaces, throughout the annular groove, assuring constant, complete lubrication. Figure 19 depicts the completed machining modification.

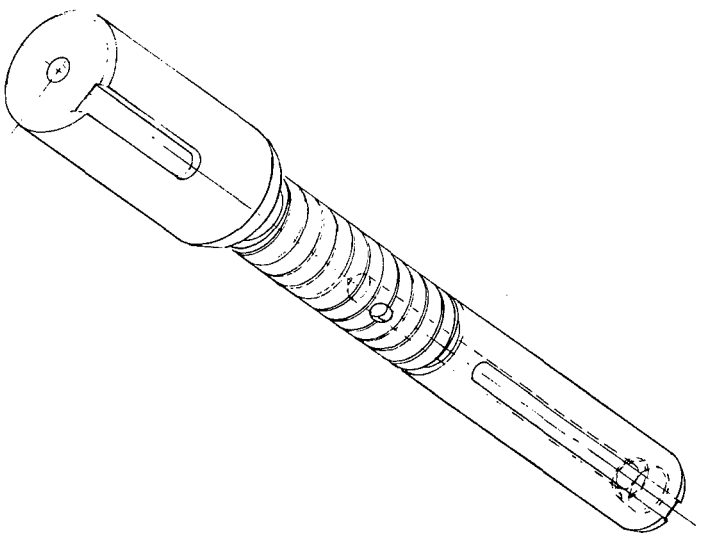
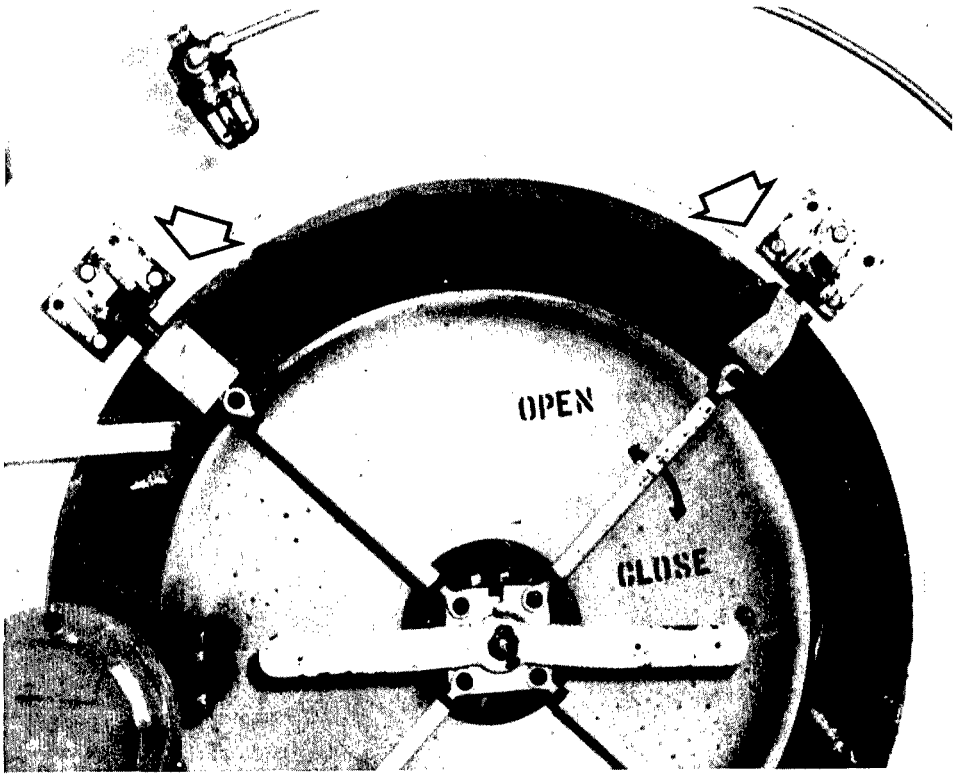


Figure 19. Modified Thomas Dome access hatch shaft.

Bearing pillow blocks used for latching bolt rods were fabricated from solid, aluminum blocks welded to hatch. There was no provision for lubrication of the bearings. The blocks were replaced with modified units bolted in place. The modification consisted of sleeve type bearings fabricated from oil impregnated, sintered bronze material, force fitted into the aluminum blocks.

Figure 20 illustrates both old and new versions of these changes.



Before

After

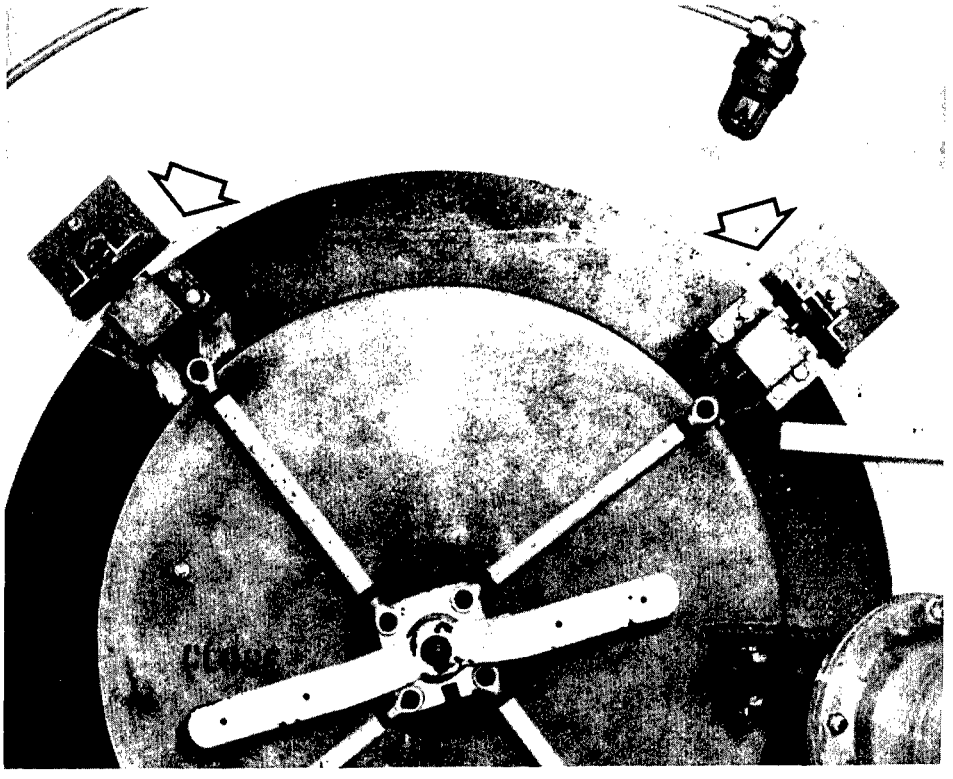


Figure 20. Modifications of Thomas Dome access hatch latching mechanisms.

The latching bolt rods originally contained one level across the mating end. Periodically the rods would rotate causing a malfunction. The problem was corrected by modification of the rod ends to have a tapered bullet nose configuration, permitting the rod to engage the striker bar even if rotated.

To complete the operational improvement, the striker mechanism was also completely redesigned. The original striker assembly was fabricated from carbon steel, which had a tendency to rust. To curtail rust the assemblies were painted. On mating surfaces, the paint would wear off and the rust return. Because of rust and paint accumulations, adjustment of the unit was greatly impaired. The redesigned unit employed a revised method of adjustment and was fabricated from stainless steel.

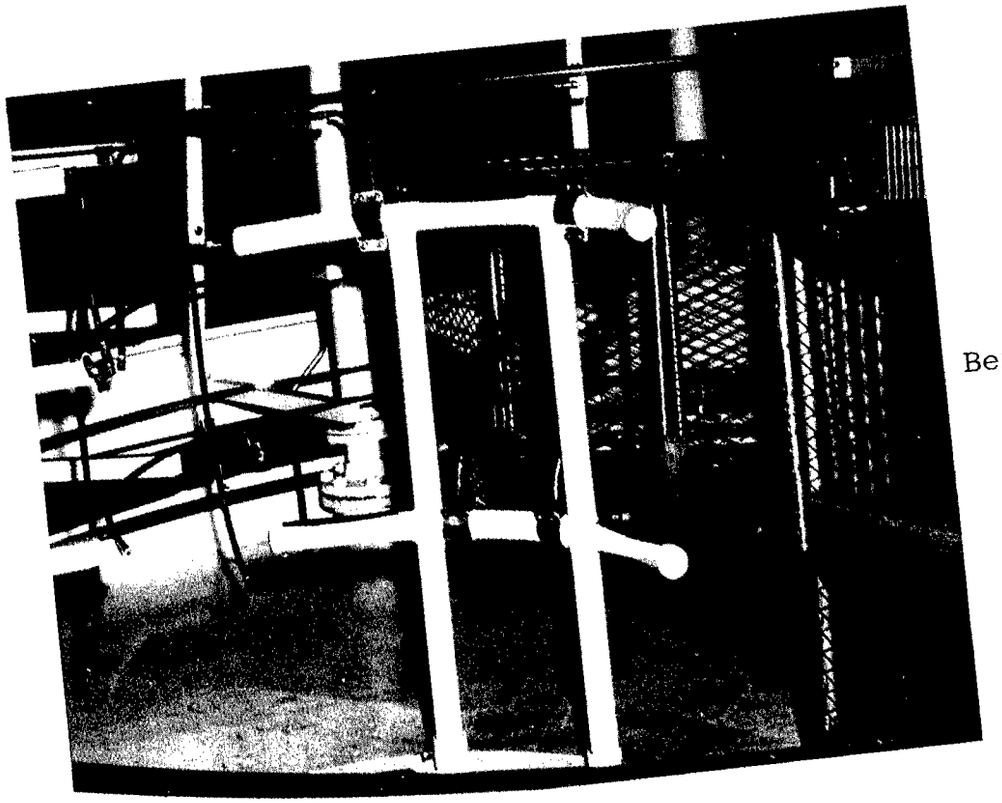
Thomas Dome Access Hatch Safety Railings

New safety railings, located at the top of the access hatch openings were fabricated from a re-designed concept. The railings were constructed of an aluminum material, with shorter top sections and each rung was machine knurled to prevent hand slippage. The old railings were constructed of carbon steel material, painted, and contained a longer top rail that frequently interfered with movement around hatch opening. No provision was included for an abrasive surface to prevent slippage, and glued-on abrasive material frequently came off or was removed and replaced when renovation was completed. Both old and new styles are shown in Figure 21.

Exhaust Filter Boxes

Filter boxes installed in the dome exhaust system were large cumbersome units, fabricated from carbon steel, requiring painting at each renovation. Two access doors were located on a side to accommodate changing of filter elements. The physical size of the units controlled mounting location and often interfered with installation patterns of test animal locations. No provision was contained in the unit for positive clamping of absolute filter elements.

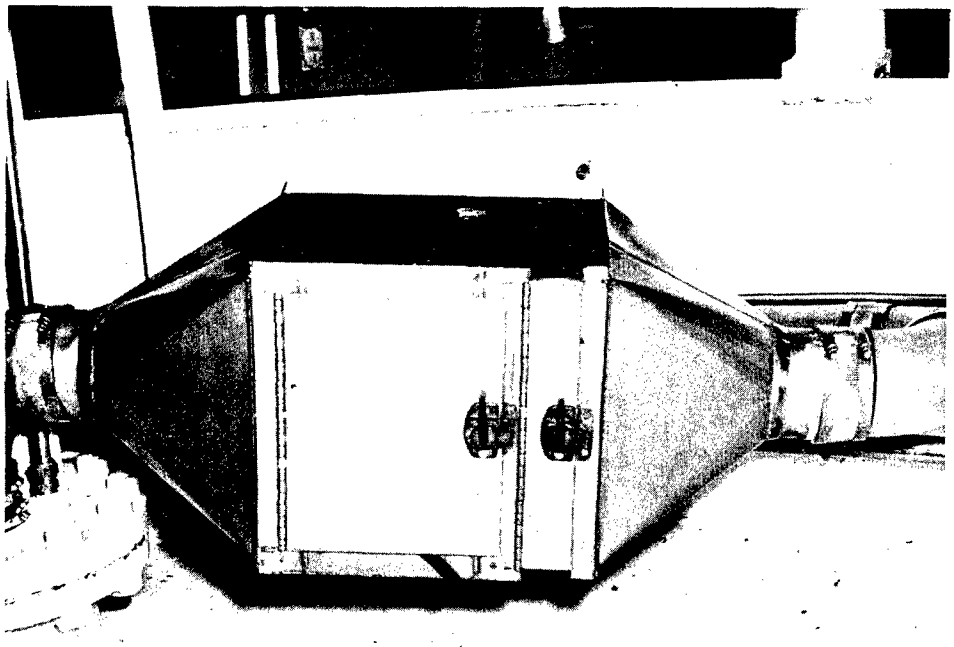
As an effort to alleviate some of the problems, the unit was completely redesigned. The units were made considerably smaller in physical size allowing more selection of mounting location. Material selected for fabrication was stainless steel, removing the required painting operation. Only one access door was employed in the new units, granting access to both element compartments at once, and a positive, clamping element for use of absolute filter elements was installed. Figure 22 shows the contrast between the two units.



Before

After

Figure 21. Modifications to Thomas Dome access safety railings.



Before

After

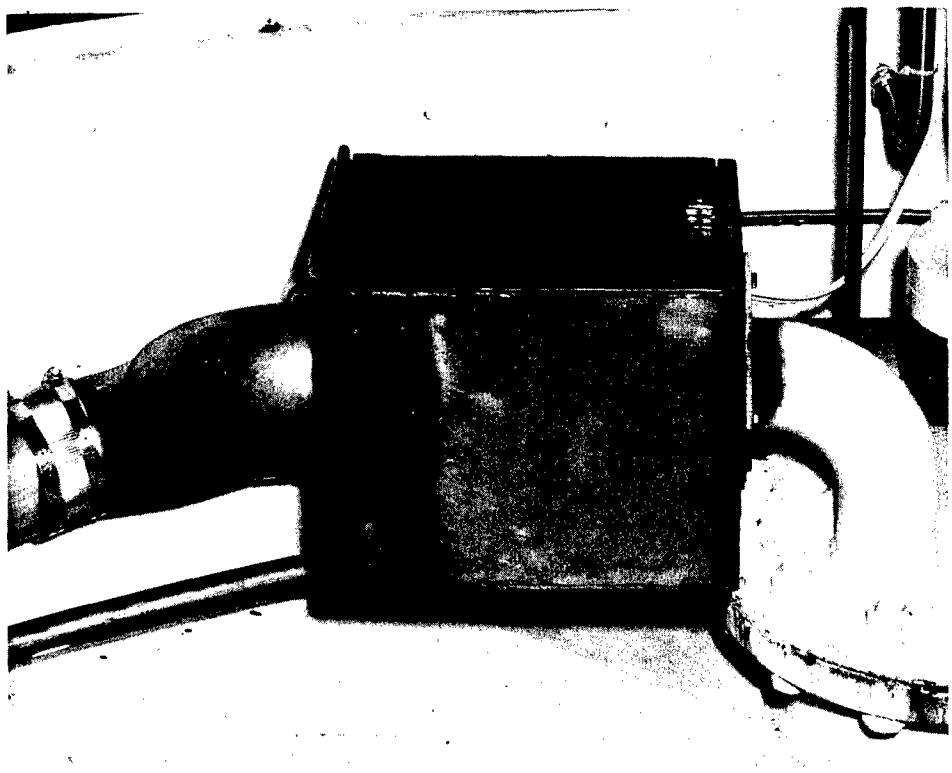


Figure 22. Modifications to Thomas Dome exhaust air filtration system.

The inhalation exposures to MMH conducted in the Thomas Domes required careful attention to insure the safety of scientists and technicians overseeing various aspects of the experiment. To accomplish this, two instruments were set up in Facilities A and B for the detection of very low levels of MMH which may have escaped into the laboratory environment.

An AutoAnalyzer® was installed in Facility A which monitored laboratory air for MMH using the same iodimetric technique employed for chamber MMH analysis. Sample lines were directed to various points in the laboratory where MMH contamination is most likely to occur. In operation, a pump pulls air samples into the analyzer sequentially from each sample point for 10 minutes. A timer opens and closes solenoid valves to control the sequence. The system is diagrammed in Figure 23.

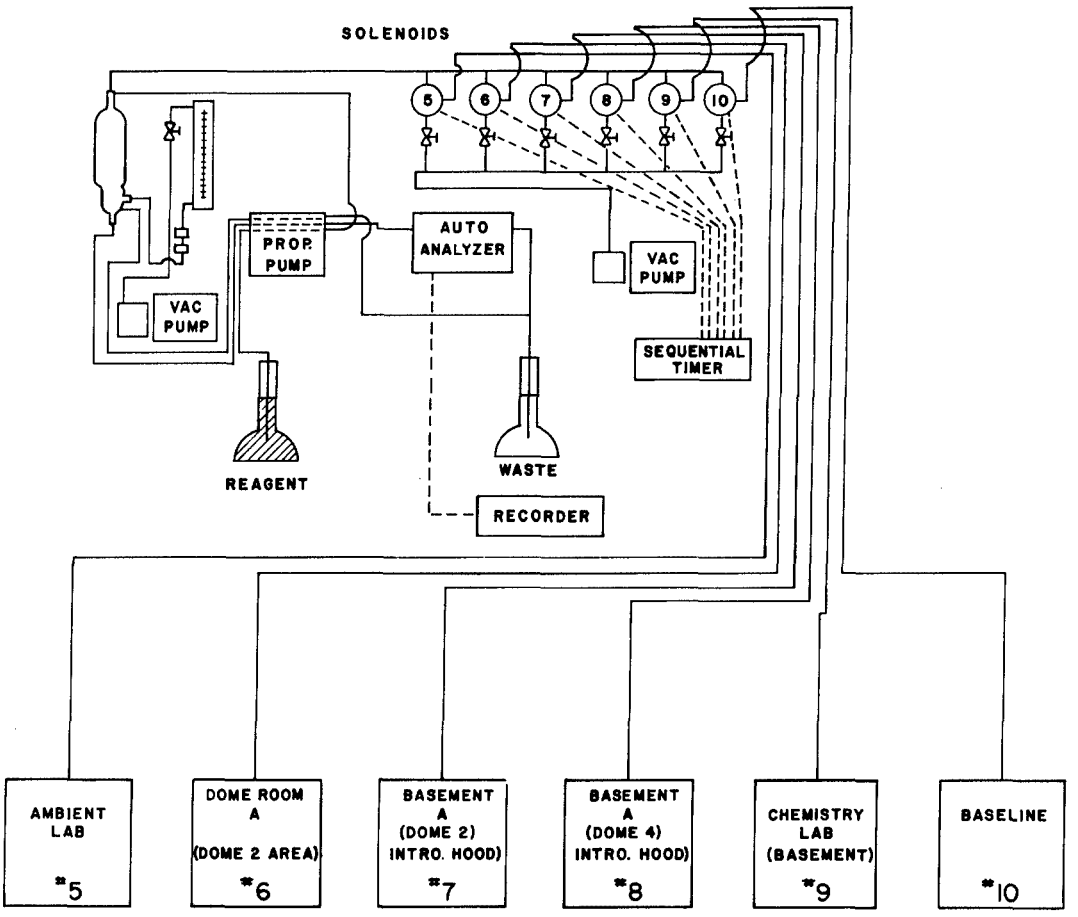


Figure 23. Facility A laboratory air sampling system.

In Facility B, a similar sequence of samples is monitored for MMH using a commercial hydrazine analyzer which measures a color change on continuously moving paper tape. Modifications of the analyzer were made to increase sensitivity. These included discontinuing use of the reference side of the paper tapes which was found to cause rather wide baseline fluctuations and changing instrument tubing from teflon to smaller gauge polyethylene to reduce hydrazine loss and increase sample speed through the tubing. A larger air pump was put on the analyzer to increase sample air flow from 250 ml/min to 1.5 liter/min. The increased air flow decreased response time and increased the amount of sample being pulled through the tape thereby improving sensitivity. Figure 24 illustrates the sampling and analysis utilized for Facility B. Both analyzers are calibrated using a diffusion tube system. The minimum detectable concentration for each system is 10 ppb. If levels of 50 ppb are reached, the laboratory is evacuated and a search instituted for possible leaks. Personnel engaged in this search and in making repairs to the system wear independent breathing equipment.

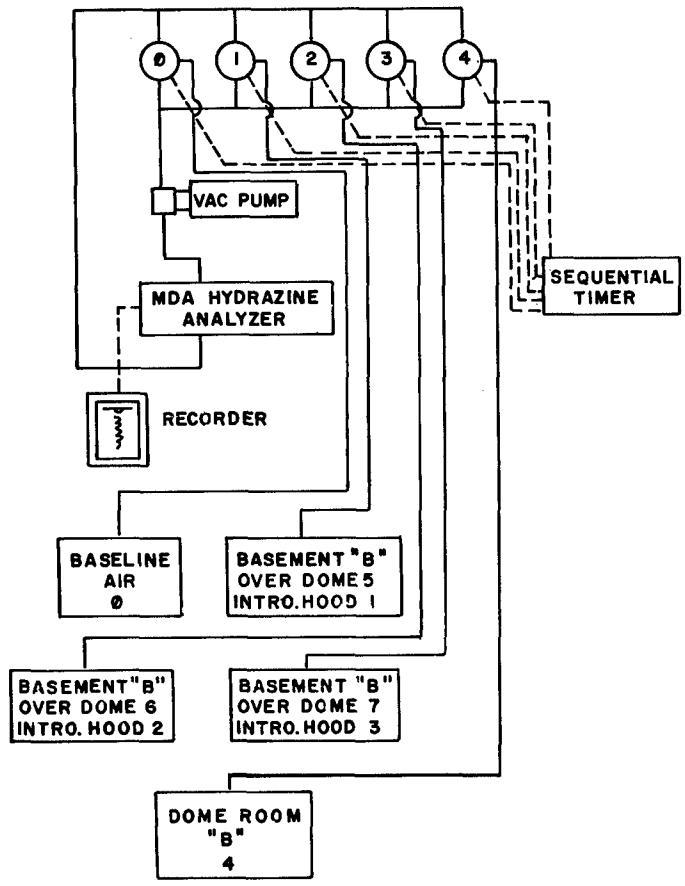


Figure 24. Facility B laboratory air sample system.

TRAINING PROGRAMS

Chamber Technicians

Since last year's annual report, two chamber technicians have been hired. Phase I and Phase II formal training cycles have been scheduled for the new employees and have been initiated. Experienced technicians will participate in the on-the-job training portions of this program. These trainees are also being trained in preparation of taking the junior level of examination for certification by the American Association of Laboratory Animal Science.

Written and practical monthly Emergency Training examinations 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 supervisors to insure that the SOP is properly followed.

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

Date	Procedure	Personnel Participation*
June 1976	Complete Power Failure	A
July 1976	Rescue of Incapacitated Dome Entrant	All
August 1976	Complete Power Failure	A
September 1976	Air Compressor Failure	A
October 1976	Vacuum Pump Failure	All
November 1976	Air Compressor Failure	A
December 1976	Air Supply Fan Failure	A
January 1977	Fire in Exposure Laboratory Area During Dome Entry	A,B,C
February 1977	Fire in the Dome - No Entrant	A
March 1977	Rescue of Incapacitated Dome Entrant	All
April 1977	Air Compressor Failure	A
May 1977	Vacuum Pump Failure	All

*A - Shift Operator
B - Safety Observer B
C - Dome Entrant
All - All Chamber Technicians on Duty.

The animal care program described in previous annual reports was continued. This program has been successful and all experienced technicians are proficient at drawing blood samples from all species, usually in more than one manner. The technicians are capable of handling and restraining all species used in our laboratory. The two new employees are scheduled to receive the entire animal handling training program.

Except for the two new employees, all technicians are capable of operating the weighing system with the computerized output. The new digital readout system has been completed and a new operating procedure will be written by the Development Engineering group.

All incoming technicians are required to complete the Ralston Purina Animal Care Course. This course is primarily a self-study course which lays a foundation for further study in the field of laboratory animal care. The new employees will be receiving this course upon completion of Phases I and II of their formal training. They will proceed through the course at their own study pace under the direction of a supervisor.

Animal Technicians

Animal Care Training Programs described in previous annual reports were continued as programmed.

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 two were certified at the first level, Assistant Animal Technician. There were several technicians who desire to become certified in the AALAS program, but must fulfill the one year experience requirement before taking the examination for the first level of certification. One technician is preparing to take the examination for Laboratory Animal Technologist. UCI animal care personnel certification in the AALAS program is as follows:

1 - Laboratory Animal Technologist

3 - Laboratory Animal Technicians

6 - Assistant Animal Technicians.

Upon completion of the first level AALAS examination, two animal caretakers were promoted to the animal technician position.

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

The need for a formal course in laboratory animal science was evident after conducting many carcinogenicity studies over the last several years. All animals in these studies are held for their lifetimes. The THRU technicians must now be able to identify various animal diseases as well as skin tumors in our colony of approximately 8800 animals currently being housed in our facilities. 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.

Training of Animal Technicians for Chamber Entry Duties

The following is an outline that was followed for the training class in April 1977:

A. General Chamber Concentration

1. Air supply to dome
2. Air outlet from dome
3. Contaminant introduction
4. Contaminant treatment downstream
5. Industrial hygiene apparatus.

B. Safety Systems

1. Alarm systems (visible and audible)
2. Communication system
3. Observers.

C. Responsibility of Observer "B"

1. Prior to chamber entry
2. During chamber entry
3. Following chamber entry.

D. Responsibility of Dome Entrant

1. Clean cage pans
2. Feed animals
3. Remove any sick or dead animals
4. Change water valves (if necessary)
5. Hose down floor.

Advanced Practical Training

The following exercises were made available this reporting period for UCI animal care personnel to complete as time and opportunity became available. Each exercise must be signed by a staff member and he should insure that the technician is able to do each procedure correctly and with confidence. A veterinary officer will give a short practical exam to the technician after all items are signed.

Dog

Restraint and handling ____

IV injection of cephalic, saphenous, jugular, lingual veins ____

Blood collection - clotted and nonclotted ____

I.M., S.Q., I.P. injection ____

Pass endotracheal tube ____

Dog - continued

Knotts technique ____

Place an intercath ____

Be able to give general physical exam - eyes, ears, teeth, mm, skin, nails ____

Be able to place ointment in dogs eyes ____

Be able to pill a dog ____

Be able to trim a dogs nails ____

Be familiar with sanitation and feeding ____

Surgical prep ____

Take temperature, pulse, respiration ____

Clean teeth ____.

Monkey

Restraint and handling ____

Blood collections - femoral, saphenous ____

Injection - S.Q., I.M., I.V. ____

TB tine ____

Pass nasal gastric tube ____

Extract canine teeth of primate ____.

Rabbit

I.M. injections ____

Bleeding via ear vein and artery ____

Ear exam ____

Dental exam ____

Guinea Pig

Venipuncture femoral vein ____

Venipuncture penis ____

Dental exam ____.

Rodent

Venipuncture tail vein ____

Orbital sinus bleeding ____

Heart ____

Mark rodent using toe clip ____.

Miscellaneous Duties

Pass urinary catheter:
Male & female dog ____
Male & female monkey ____

Be able to do a venous cutdown ____

Be able to suture skin ____

Be able to express anal glands of dog ____

Be able to remove brain from dog ____

Be able to lay out necropsy specimen for rapid inspection by veterinary officer ____

Perform tests for CBC, BUN, B. canis ____

Assist surgeon ____.

Necropsy

Do basic necropsy and collect tissue ____

Know major organs ____.

Surgery

Act as circulator ____

Scrub, gown, glove ____

Be able to run autoclave and gas sterilizer ____

Pack and instrument care ____.

X-Ray

Be familiar with protective gear ____

Be able to run the X-ray unit ____.

Be Able To:

Load and operate incinerator ____

Run and maintain cage washers ____

Feed all animals ____

Do health check ____

Tatoo - punch and electric ____

Toe clipping ____

Be familiar with receipt of animals to include Q.C., records, quarantine ____.

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