TOXIC HAZARDS RESEARCH UNIT ANNUAL TECHNICAL REPORT: 1972

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SysteMed Corporation

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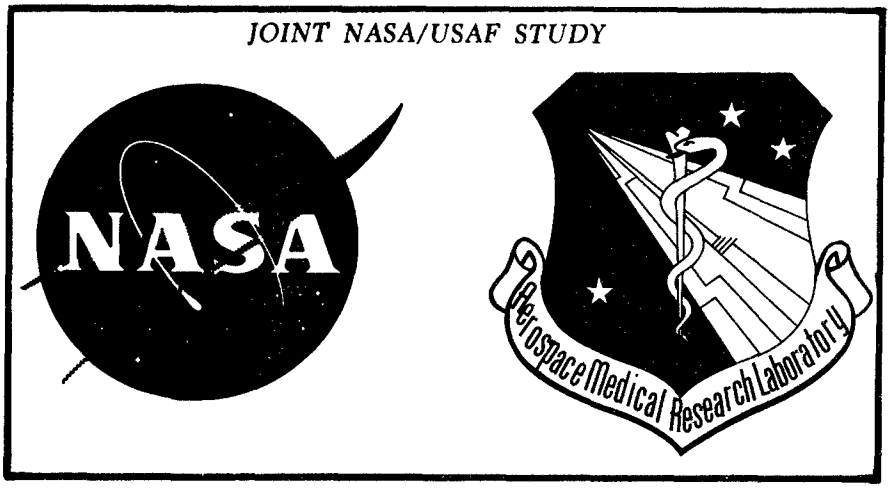

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The experiments reported herein were conducted according to the "Guide for Laboratory Animal Facilities and Care," 1965 prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences—National Research Council.

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13. ABSTRACT

The activities of the Toxic Hazards Research Unit (THRU) for the period of June 1971 through May 1972 are reviewed in this report. Acute inhalation toxicity experiments were conducted on hydrogen chloride (HCl) gas and aerosol, ethyl bromide (C_2H_5Br), hydrogen bromide (HBr), hydrogen sulfide (H_2S), ammonia (NH_3), chlorine (Cl_2), and silane (SiH_4). Subacute toxicity studies were conducted on chlorine pentafluoride (ClF_5), dichloromethane (CH_2Cl_2) and coal tar volatiles. Further toxicity studies of subacute and chronic responses to inhaled monomethylhydrazine (MMH) are also described.

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Oral Toxicity Testing						
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FOREWORD

This is the eighth annual report of the Toxic Hazards Research Unit (THRU) and concerns work performed by SysteMed Corporation on behalf of the Air Force under Contract No. F33615-70-C-1046. This constitutes the third report under the current contract and describes the accomplishments of the THRU from June 1971 through May 1972.

The current contract for operation of the laboratory was initiated in 1969 under Project 6302 "Toxic Hazards of Propellants and Materials," Task 01 "Toxicology" Work Unit No. 63020110. K. C. Back, PhD, Chief of the Toxicology Branch, was the technical contract monitor for the Aerospace Medical Research Laboratory.

J. D. MacEwen, PhD, of SysteMed Corporation, served as principal investigator and Laboratory Director for the THRU. Acknowledgement is made to C. E. Johnson, C. C. Haun, G. L. Fogle and J. H. Archibald for their significant contributions and assistance in the preparation of this report. Partial support for this program was provided by the National Aeronautics and Space Administration, the Department of Transportation and the National Institute of Occupational Safety and Health.

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This technical report has been reviewed and is approved.

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SECTION I

INTRODUCTION

This document constitutes the 8th annual report of the Toxic Hazards Research Unit, (THRU), a research team which operates a dedicated inhalation toxicology laboratory to investigate potentially hazardous chemicals and materials of interest to the Air Force and other governmental agencies. The THRU research team is an interdisciplinary group of 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. The chamber facilities consist of three types, each performing a separate function. Preconditioning chambers are used to prepare and stabilize animals in a controlled environment. Rochester and Longley Chambers are used for exposing animals to airborne contaminants under ambient conditions of pressure and air composition. These ambient chambers are useful for acute inhalation exposures as well as intermittent long-term chronic exposure experiments. Eight unique and extremely versatile altitude chambers (designated herein as Thomas Domes) are used for conducting long term continuous or intermittent subacute and chronic exposure studies. These Thomas Domes are capable of operating at absolute pressures ranging from 260 to 760 torr utilizing gas mixtures ranging from 20 to 100% oxygen and 0-80% of a secondary gas or mixture of gases. Environmental control of relative humidity, temperature, pressure, and gas flow rate is

very stable and precise through continuous monitoring and feedback modulation of regulating valves. The control equipment is provided in replicate and failsafe form so that uninterrupted exposures may be conducted for indefinite periods. More detailed description of the design and operation of the THRU facility is published in references 1 through 7.

With the comprehensive scientific team and exposure resources described above the THRU can conduct realistic simulation of human exposures to contaminants causing adverse health effects. These exposures, provided to multiple animal species, are carefully monitored using continuous analytical techniques. The animals used in the experimental programs are also monitored by continuing visual observation and regularly scheduled biochemical and physiological measurements.

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. This research effort is continuing on a lesser scale while more emphasis has been placed on obtaining data useful for solution of problems of military or civil aircraft emergencies, community emergencies, and chronic industrial exposures. To this end many of the current research programs serve the mutual interest of the Air Force and other governmental agencies such as the National Institute of Occupational Safety and Health, the Department of Transportation and its Federal Aviation Agency.

As part of its contract responsibilities, SysteMed Corporation presents an annual technical conference to disseminate new toxicological information to Air Force, other governmental and industrial scientists. This year's conference presented 23 technical papers and had as a central theme the toxicology of volatile halogenated organic compounds with particular emphasis on the use of fluorocarbons. The open forum discussion following each session resulted in significant contributions of additional technical information and scientific exchange. The conference, held 31 August through 2 September 1971, drew 142 participants including speakers.

SECTION II

RESEARCH PROGRAM

The research function of the THRU is a continuing activity independent of contract years, with several experiments 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 broader range of materials than previously and includes inhalation studies of propellant oxidizers and fuels, organic solvents, coke oven effluents, irritant gases, and space cabin material gas-off products. Oral toxicity investigations on commercial chemicals to which the general public may be exposed through transportation accidents were also conducted.

CONTINUOUS ANIMAL EXPOSURE TO HIGH CONCENTRATIONS OF DICHLOROMETHANE

Dichloromethane, also known as methylene chloride, is used extensively as a solvent in many space cabin construction materials. The provisional space cabin limit has been set at 25 millimoles/25 m^3 (25 ppm) for 90-day flights, and 5 millimoles/25 m^3 (5 ppm) for 1000-day flights (reference 8). To properly assess the inhalation hazard to astronauts, two high levels, 1000 and 5000 ppm, were intentionally selected and four animal species were exposed continuously to these concentrations for periods of not more than 14 weeks. In a previous study Heppel et al. (reference 9) exposed dogs, rabbits, guinea pigs, and rats to

5000 ppm intermittently for 7 hours per day, 5 days per week for periods up to 6 months. They found subnormal weight gains, decreased food intake and death of 3 of the 8 guinea pigs after 35, 90, and 96 exposures, respectively. Examination of the animals that died showed pneumonia and centrilobular fatty degeneration of the liver. However, none of the other species showed any evidence of toxicity during the course of exposure. CNS effects, varying in degree, were produced in the four species previously mentioned, and in monkeys exposed to 10,000 ppm on a 5 day per week, 4 hour per day schedule. Dogs were removed after 6 exposures because of injuries from hyperactivity while all other species finished 36-38 exposures. Lehmann and Schmidt-Kehl (reference 10) observed only drowsiness and slight reduction in body temperature in cats and rabbits exposed 8 hours a day, 6 days a week to concentrations of 1728-2036 ppm for 4 weeks. Little other work has been reported on the chronic toxicity of dichloromethane.

Acute LC_{50} values for mice have been reported as 14,500 ppm for a 2-hour exposure (reference 11) and 16,188 ppm for an 8-hour exposure (reference 12). Human exposure includes the fatality of one of four men accidentally exposed to undetermined concentrations (reference 13) and the nonfatal exposure of 33 workers to levels of approximately 29-5000 ppm (reference 14).

In the study reported here, both exposed groups and the control groups consisted initially of 8 female dogs, 4 female rhesus monkeys, 20 male Sprague-Dawley rats, and 380 female ICR mice. An additional 20 mice were used in the 5000 ppm exposure to measure spontaneous activity.

Each group of animals was housed in a separate Thomas Dome operated at 725 mm Hg pressure to avoid leakage of the gas. Nominal airflows of 40 cfm were used in all cases.

The dichloromethane (CH_2Cl_2) used in this study was technical grade and was analyzed by mass spectrometry to be approximately 99% pure. During exposure, continuous analysis of the concentration level in each dome was performed using a flame ionization hydrocarbon analyzer.

A large number of parameters were selected to measure the chronic toxicity of this compound. Signs of toxic stress were noted as well as numbers that died and times to death. Body weights of rats, dogs, and monkeys were measured on biweekly schedules throughout the duration of the study. Organ weights were taken on rats that were sacrificed after one month of exposure and at the conclusion of the experiment. Gross and histopathologic examinations were made on animals that died or were sacrificed during the study, and on animals that were killed at termination of the study.

Narcotic effects were very noticeable in dogs. The rats appeared normal. By the second day of exposure, dogs and monkeys regained coordination; however, they, as well as the mice, were very lethargic and remained so until death due to exposure or sacrifice. Food consumption was noticeably reduced in all species. Emaciation in dogs and monkeys became progressively worse as exposure continued. In dogs it was so advanced in many cases that blood sampling was difficult and often unsuccessful. Animals exposed to 1000-ppm

dichloromethane showed similar signs of toxicity but to a far lesser degree, except for dogs. Appetite suppression was evident in about 80% of the large animals, while the rats showed little visible evidence of toxicity.

Continuous exposures to 1000- and 5000 ppm dichloromethane were shown to have deleterious effects on the four animal species used in this experiment. The higher concentration was found to be lethal to 50% of the dogs and 30% of the mice after 3-4 weeks of exposure. Although 1000 ppm did not kill significant numbers of mice over 14 weeks, lethality in dogs was still apparent since 75% of these animals died after 5-7 weeks of exposure. Rats and monkeys experienced depressed weight gains as a result of exposure to CH_2Cl_2.

Elevated serum glutamic pyruvic transaminase (SGPT) and isocitric dehydrogenase (ICDH) values were obtained from dogs after 4 weeks of exposure to 1000 ppm. These enzymes are more specific indicators of liver damage than others such as serum glutamic oxaloacetic transaminase (SGOT) and lactic dehydrogenase (LDH) and the high values obtained point to the liver as being a target organ for the toxic action of CH_2Cl_2 on the dog. Monkey serum enzymes, contrastingly, showed little if any effect of exposure.

Striking effects of exposure to CH_2Cl_2 were revealed by histopathologic examination. The dogs in both exposures demonstrated marked fatty liver change which might have been expected to result in cirrhosis if longer exposure times had been possible. It must be recalled that this and other dog liver changes had occurred after only 3 weeks of exposure to 5000 ppm and 6-7 weeks of exposure to 1000 ppm. Mice sacrificed after 5 weeks of exposure to 5000-ppm CH_2Cl_2

had liver changes in nuclei, cytoplasm, and cellular organization. After 14 weeks of exposure to 1000 ppm, these alterations were again present and had progressed to the point where focal collapse and ductal proliferation indicated an early stage of cirrhosis.

Significant histopathological liver changes were noted in all rat groups exposed to CH_2Cl_2, and there was obvious cellular cytoplasmic degeneration in animals exposed to both concentrations for 14 weeks. There was histologic evidence of renal toxicity in the rat with the presence of iron pigmentation in cortical tubular cells. After 14 weeks exposure, cortical tubular cell degeneration was apparent. The pronounced gross and histopathological changes noted in rat livers and kidneys were not accompanied by any significant differences in organ weights from control values. This is an unusual finding, since overt evidence of gross change in an organ is usually accompanied or preceded by organ weight change.

Little pathologic indication of toxicity to monkeys was obtained, except for the one animal that died early in the 5000 ppm exposure. Gross observations of mild to moderate liver changes were made in the animals exposed to 5000 ppm CH_2Cl_2, but no lesions were noted on examination of the animals after 14 weeks of exposure to 1000 ppm. H & E stains of exposed monkey tissue were negative although some fat accumulation in liver was noted in tissues stained with oil red-O in the 14-week, 1000 ppm exposed animals.

Under the conditions of this experiment, CH_2Cl_2 is toxic to all species tested, most to dogs, less so to mice and rhesus monkeys, and least to rats.

METABOLIC CONVERSION OF DICHLOROMETHANE TO CARBON MONOXIDE

As an adjunct to a previous continuous inhalation study and in light of new information (reference 15) showing that the blood of humans exposed to dichloromethane contained significant levesl of carboxyhemoglobin, animals were exposed to 5000 ppm dichloromethane for 24 hours. Although other tests were performed, this experiment was designed primarily to measure carboxyhemoglobin levels, if produced as a result of exposure to the highest concentration level.

Groups of 2 female dogs, 2 female rhesus monkeys, and 10 male albino Sprague-Dawley derived rats were placed in a Thomas Dome by means of the pass-through airlock after the desired 5000-ppm dichloromethane concentration had been established. A flame ionization hydrocarbon analyzer was used for continuous analysis. The dome was operated at 720 mm Hg pressure with a nominal airflow of 40 cfm.

Individual blood samples from dogs and monkeys and a pooled sample from 5 control rats were taken for carboxyhemoglobin, hematocrit, hemoglobin, and red blood cell measurements one day before the initiation of the exposure. In the dome shortly before the conclusion of the exposure, blood samples were taken from dogs and monkeys and pooled samples from 2 groups of 5 rats each. The blood was removed from anesthetized rats by means of cardiac puncture.

Significant increases in COHb were measured in all animals exposed as shown in table I. Preexposure levels were 0. 5% or less for each species. In succeeding tables individual results from dogs and monkeys are given.

Although the mean COHb value for each species probably does not represent equilibrium concentration, note that the 24-hour response was greatest in dogs, followed by rats, then monkeys.

Table I

Carboxyhemoglobin Elevation in Animals after Exposure to Dichloromethane

(% COHb)

Rats	Monkeys	Dogs
Group I - 16.4%	L 19 - 17.1%	N 61 - 21.0%
Group II- 18.7%	L 83 - 12.3%	N 65 - 29.2%
Mean 17.5%	14.7%	25.1%

Hematology measurements made on the test animals showed no change after exposure or in the case of rats from the unexposed control group as shown in table II.

The rats were inactive for the most part during the exposure; however, they did eat and drink and food was found in the stomach and intestines. Both dogs were inactive and slept through most of the exposure time. They were not unconscious; tapping on the chamber window easily aroused both animals. There was no emesis but neither dog attempted to eat during exposure. Monkeys ate, drank, and appeared normal except during the last few hours of exposure. At that time, they seemed to be somewhat lethargic. A measurable weight decrease resulted from the exposure, ranging from 4.6% in monkeys to 7.5% in the rat.

Table II

Hematology Measurements of Animals Exposed to Dichloromethane for 24 Hours

Rats

Test	Units	Controls	Exposed		
			Group A	Group B	Mean (Group A & B)
Hct	Vol. %	44	46	47	46.5
Hgb	Gm. %	13.2	13.6	15.2	14.4
RBC	Millions	6.76	6.18	7.33	6.76

Dogs

Test	Units	Preexposure			Postexposure		
		N-65	N-61	Mean	N-65	N-61	Mean
Hct	Vol. %	51	53	52	52	51	51.5
Hgb	Gm. %	16.6	18.6	17.6	17.6	17.0	17.3
RBC	Millions	6.83	7.53	7.18	7.03	7.14	7.09

Monkeys

Test	Units	Preexposure			Postexposure		
		L-19	L-83	Mean	L-19	L-83	Mean
Hct	Vol. %	38	41	39.5	38	37	37.5
Hgb	Gm. %	12.0	12.8	12.4	12.0	11.4	11.7
RBC	Millions	5.14	5.74	5.44	5.36	5.23	5.30

Formic acid determinations were made on urine samples from both exposed and control dogs and are shown in table III

Table III

Effect of Dichloromethane on Urinary Formic Acid Formation

Dog	ml Urine Collected	Formic Acid μg/ml Urine
Control		
003	34	30.5
009	21	28.3
Exposed		
N-61	35	53.5
N-65	60	60

Formic acid values for the exposed dogs are noticeably higher than those of the controls. However, the findings may not be significant when one considers the wide range of values obtained for exposed and control dogs in the previous 90-day study under similar exposure conditions.

At the conclusion of the exposure, dogs and monkeys were sacrificed and submitted for gross and histopathologic examination. No gross lesions were noted except in one monkey. This animal's liver was mottled and appeared slightly yellow. Fat stains (ORO) revealed mild fatty changes in the livers of both monkeys and minimal accumulation in the livers of both dogs. Vacuolization of hepatocytes was moderate in both dogs, minimal in one monkey, and absent in the other monkey.

Dogs, monkeys, and rats exposed to 5000 ppm dichloromethane for 24 hours showed definite significant increases in COHb blood levels. These elevations, however, were not accompanied by increases in hematocrit, hemoglobin, and red blood cell values which probably did not occur due to the short time of exposure. While formic acid concentrations in the urine of exposed dogs were abnormally high when compared with control measurements, the value of these findings should be interpreted with respect to the large range of results previously reported. Signs of toxicity were minimal in all species tested; mild or moderate CNS depression was a consistent observation. All exposed animals lost body weight. Gross and histopathologic change was absent in rats, but staining for fat in the livers of dogs and monkeys provided evidence of change due to dichloromethane exposure.

CONTINUOUS ANIMAL EXPOSURE TO LOW LEVELS OF DICHLOROMETHANE

Dichloromethane (CH_2Cl_2) is used extensively in formulating materials used in the cabins of Apollo spacecraft and in materials proposed for the Skylab orbiting space station and consequently, NASA desired long-term continuous inhalation toxicity information on this compound. Having found the detrimental effects previously described above in the 1000-ppm and 5000-ppm exposures, it was decided to examine long-term exposures to low levels of CH_2Cl_2. Hence, the present study was undertaken to determine chronic toxicity resulting from continuous exposure to 25 ppm and 100 ppm CH_2Cl_2 for periods up to 14 weeks.

The exposures were conducted in the Thomas Domes at ambient pressure. The airflow pressure, relative humidity, and temperature were all controlled automatically in these domes. Airflow was maintained at 40 cfm, relative humidity at 50 ± 10%, and temperature at 72 ± 5 F. The absolute pressure within the exposure chambers was slightly negative (725 torr) with respect to ambient to insure a tight seal of the chambers and to prevent contamination of the surrounding laboratory air with CH_2Cl_2 vapor.

Reagent grade CH_2Cl_2 was used as the contaminant in these studies. Liquid CH_2Cl_2 was pumped from a drum by a variable-speed peristaltic pump through a flowmeter to a glass evaporating flask to generate the concentrations required for these studies. Concentrations could be adjusted by either changing the pumping speed to deliver more CH_2Cl_2 to the evaporating flask, or by increasing or decreasing the airflow through the chamber. The CH_2Cl_2 concentration in each of the experimental domes was monitored continuously throughout the entire study using a flame ionization hydrocarbon analyzer which was calibrated daily with bag samples of known CH_2Cl_2 concentration. Measurement over the entire exposure period showed that contaminant concentrations never varied more than 10% from the desired values.

Two exposure levels of dichloromethane were tested, 25 ppm and 100 ppm CH_2Cl_2, with one control group, using three domes in all. The control dome was operated under conditions identical to the experimental domes with the exception of contaminant. The experiments were conducted in three phases to accommodate housing of the large numbers of mice used in portions of the

study. In the first phase of the study 320 mice were placed in each dome for evaluation of the fatty liver degenerative changes seen at higher dichloromethane exposure levels. The mice were serially removed from the domes over a 30-day exposure period and their livers examined by oil red-O staining and electron microscopy. Liver triglyceride determinations were also made. In the second phase of the study, 36 mice were placed in the 100 ppm and control domes in groups of six on the appropriate days to give 1, 2, 3, 4, 7, and 14 days of continuous exposure. All these groups of mice were removed at 15-minute intervals on the morning of the last day. All mice were injected intraperitoneally with a dose of tritiated thymidine (1 microcurie/gram body weight) one hour prior to removal from the chamber, and killed immediately upon removal from the chamber. The uptake of the labeled thymidine was then measured by autoradiography. All mice for this portion of the study were individually numbered by toe clipping, and the groups of six were determined by randomization of the entire complement of mice.

The final phase was the actual 100-day exposure of 170 mice, 20 rats, 4 monkeys, and 16 dogs at each dichloromethane level with a comparable number of controls. All mice used in these experiments were ICR derived CF1 strain females. The rats were Sprague-Dawley strain CFE males, monkeys were Macaca mulatta females approximately 1 year old, and the dogs were beagle variety both male and female 8-14 months of age.

Eighty mice were removed from each dome in groups of 20, fasted for 24 hours, and used for determination of hexobarbital induced sleep

time at 30-day intervals from onset of exposure. The hexobarbital induced sleep time test is used as an indicator of liver function or dysfunction. The length of time an animal stays asleep following a given dose of hexobarbital is determined by the rate at which the barbiturate is inactivated by liver enzymes. If sleep time is reduced, it is assumed that whatever the animal had been exposed to has activated the liver microsomal enzymes, and conversely, an increased sleep time indicates inactivation of liver microsomal enzymes, or liver damage. The methods used to determine and interpret sleep time results in mice have been described by Van Stee (reference 16).

Levels of liver microsomal cytochromes P-450, P-420, and b_5 were determined in mice from each dome after 30-, 60-, and 90-day exposure. These mice were fasted for 24 hours prior to removal from the domes. Immediately upon removal, they were sacrificed and the livers were removed, suspended in 0.5M tris buffer pH 7.5, containing 0.25M sucrose, and frozen to the temperature of dry ice. The frozen livers were then packed in dry ice and sent via Air Express to Dr. F. J. Bullock of Arthur D. Little, Inc. for the cytochrome determinations. The determinations were made in the same manner (reference 17) as in the CH_2Cl_2 study discussed earlier.

From each dome, 20 mice were submitted for gross and histopathological examination following 98 days of continuous exposure to CH_2Cl_2.

For activity testing, 10 mice per dome were used. These mice served as their own controls, with daily measurements of their activity over a 2-hour period recorded for 2 weeks preexposure in another dome under the same conditions as in the study, but without CH_2Cl_2. They were housed in specially constructed activity cages in each of the three domes during the study. The cages were painted black to produce contrast with the white mice and render their movement more easily recognized by the television camera mounted on the outside of the dome. The cages were placed directly against a dome window so that the TV camera could view most of the cage. The TV camera was connected to a remote activity monitor and recorder. The activity measuring system has been previously described by Thomas (reference 18).

To determine any change in normal intestinal flora of mice during chronic exposure to CH_2Cl_2, the feces of mice in each of the three domes of this study were monitored. Prior to initiation of the final 100-day exposure test, 15 mice from each group were monitored twice weekly for two weeks for (1) total bacterial count/gm feces, and (2) proportion of lactose fermenters to nonlactose fermenters Fecal samples were taken every Monday and Thursday during exposure. These samples were treated as above for the baseline bacterial determinations. Samples were collected by placing sterile cage trays under the proper cage in each dome during the daily dome entry for animal care. The trays with the fecal samples were removed from the dome through the pass-through airlock. These samples were then accurately weighed and placed in 10 ml of dilution fluid (trypticase soy broth). Appropriate dilutions were made and samples

were plated on trypticase agar for total count. To determine any change in population of various groups of bacteria in relation to each other, a selective medium (MacConkey's) was used to measure the ratio of lactose fermenter to nonfermenter.

The 20 rats in each dome were weighed on a biweekly basis during the study for growth rate determinations. No other testing was made with the rats except for gross and histopathological examination and organ to body weight ratios at the termination of exposure.

The clinical tests used to evaluate chronic toxicity in the exposed dogs and monkeys are shown in the schedule below:

Clinical Test Schedule for 14-Week Exposed Dogs and Monkeys

Week of Exposure	0	2	4	6	8	10	12	13	14
Hematology	X	X	X	X	X	X	X	X	X
Carboxyhemoglobin levels	X	X	X	X	X	X	X	X	X
Blood CH_2Cl_2 levels				X				X	
Body Weight	X	X	X	X	X	X	X	X	X
SMA-12 Battery	X		X		X		X		X
SGPT	X		X		X		X		X
Pathology									X

The serum glutamic pyruvic transaminase (SGPT) levels were measured by the method of Reitman and Frankel (reference 19). Carboxyhemoglobin levels

were determined by use of a CO-Oximeter®. Blood CH_2Cl_2 levels were determined in the manner described in Section III of this report under chemistry.

In addition to the 100-day exposed dogs, three additional groups of 4 dogs each were used for serial sacrifice following differing lengths of exposure to the two levels of CH_2Cl_2. These dogs were placed in and removed from the three domes for exposure periods of 15, 30, and 46 days. The other dogs exposed the full 100 days comprised the final group in the test series.

The following tests were performed upon removal of all dogs from the experiments:

1. Hematology and SMA-12 Battery
2. SGPT
3. Carboxyhemoglobin Levels
4. Liver Triglyceride Levels
5. Electron Microscopy - Liver
6. Liver - Fat Stains and H & E Stains for Pathology
7. Complete Gross and Histopathology

In addition to the scheduled tests described above for all species, gross and histopathological examinations were made on all animals that died for any reason during the course of the study.

At no time during the course of this study did any of the animals exhibit any overt signs of toxic stress. All animals appeared to maintain normal

activity, had normal appetites, and gained weight in a normal manner. No deaths occurred in either the 25 ppm or 100 ppm exposure chambers that were in any way related to the exposure. For all intents and purposes, these exposure levels for the periods of time studied were nontoxic to the experimental animals. However, minor alterations from normal were detected in some of the tests utilized in these studies.

The concentrations of CH_2Cl_2 in the 25-ppm and 100-ppm domes were remarkably constant throughout all phases of this study. The mean concentrations were determined for each 24-hour period, and averages were made over the entire study. The mean concentrations in the exposure domes were 25.2 ppm and 99.5 ppm. No fluctuations in CH_2Cl_2 concentration in either dome exceeded 10% of the predetermined level during the course of the study.

Measurable changes were observed in mouse liver triglyceride levels during the course of exposure. The triglyceride levels increased in each exposure and peaked after approximately 20 days exposure then returned to a level only slightly higher than control values. Complete data for the liver triglyceride levels as well as for the tritiated thymidine uptake studies will be the subject of a separate technical report.

Hexobarbital sleep time determinations were made after 30, 60, and 90 days of exposure for each test group of mice and the controls. There were no significant differences between exposure groups although there were differences

over time within all groups including controls. Thus, there was no measurable effect of continuous exposure on hexobarbital sleep time.

Liver microsomal cytochrome determinations were also made according to schedule. Results received from Dr. Bullock are summarized in table IV. According to these data, mouse liver cytochromes from animals exposed to 100 ppm CH_2Cl_2 for 30, 60, and 90 days were significantly different from controls. The P-450 determinations were lower than control at all time periods. The b_5 and P-420 values were lower than control at 30 days, but higher at 60 and 90 days. The overall significance of these results will await a final report from Dr. Bullock. No significant difference from control was seen in cytochrome values for mice exposed to 25 ppm CH_2Cl_2 for the same periods.

Results of activity measurements in mice exposed to 25- and 100-ppm CH_2Cl_2 for 14 weeks are still incomplete, as are results of the intestinal flora study on mice.

All data from the serially sacrificed dogs are available at this time except for histopathology on the 46-day exposed dogs. In general, few changes from normal were found, with the possible exception of the 15-day exposed dogs. Gross pathological examination of dogs exposed 15 days to CH_2Cl_2 disclosed that minimal changes had occurred. Dogs exposed to both 25 ppm and 100 ppm had slight mottling of the livers (the livers of control dogs were also slightly mottled), and some reddening of the intestinal mucosa. One dog exposed to

Table IV

The Effect of Continuous Exposure to 25 ppm and 100 ppm CH_2Cl_2 on Mouse Liver Microsomal Cytochromes

CH_2Cl_2 Conc. (ppm)	P-450	B_5	P-420
	30 Exposure Days[a]		
Control	0.866	0.860	0.507
25 ppm	0.815	0.780	0.455
100 ppm	0.511**	0.642**	0.227**
	60 Exposure Days[a]		
Control	0.959	0.990	0.504
25 ppm	0.984	0.981	0.460
100 ppm	0.708*	1.173	0.842
	90 Exposure Days[a]		
Control	0.848	0.815	0.506
25 ppm	0.867	0.854	0.419
100 ppm	0.653**	0.944**	0.646*

* Different from controls at 0.05 significance level.
** Different from controls at 0.01 significance level.
a Results expressed as mμ moles cytochrome/mg microsomal protein.

25 ppm had some white areas at the edges of some lung lobes. Three of the four dogs exposed to 100 ppm had some petechial hemorrhage of the thymus as well.

Hematology results from the 15-day exposed dogs showed no significant changes between control and exposed dogs, nor between preexposure and postexposure values of each individual group.

Histopathological examination of the 15-day exposed dogs revealed that some tissue damage was present, and that it probably resulted from the exposures. Two of the four dogs exposed to 25 ppm had diffuse cytoplasmic vacuolization of the liver and sinusoidal congestion. These changes were not deemed to be fatty changes. In the 100 ppm exposed group, one dog had cytoplasmic vacuolization of the liver and sinusoidal congestion, and evidence of a faint staining eosinophilic hyalin in some of the vacuoles which resembled "alcoholic" hyalin. Changes in both the 25 ppm and 100 ppm groups were considered to be "tentatively" exposure-related due to the absence of a dose relationship, and also because only some of the dogs in each group showed evidence of change. The histopathology examinations on serially sacrificed dogs were performed by Dr. R. L. Patrick at the Laboratory for Experimental Biology in St. Louis, Missouri.

Dogs exposed to 25 ppm and 100 ppm CH_2Cl_2 for 30 days appeared normal at time of sacrifice. Gross pathology on these animals disclosed that one dog

in the 25 ppm group had several firm circular black spots present throughout the lung in subpleural areas, and another dog had some thickening of the right atrioventricular valves (diagnosed as endocardiosis), but neither condition was considered to be related to the exposure. In dogs exposed to 100 ppm, one showed several mesenteric lymph nodes which were hemorrhagic, and another had a slightly mottled liver, endocardiosis and minimal fatty change of the liver. One control dog also had thickening of the right atrioventricular valves which was diagnosed as endocardiosis. Whether the changes noted in the exposed dogs were related to their exposure was undeterminable.

Examination of blood test data from these dogs showed no significant change in hematology and SMA-12 values between preexposure tests and after 30 days exposure to either 25- or 100 ppm CH_2Cl_2, or between control and exposed groups at 30 days.

Histopathologic examination of tissues from the 30-day dogs disclosed no lesions which could be related to the exposures to either 25 ppm or 100 ppm CH_2Cl_2.

Gross examination of dogs exposed to 25 ppm and 100 ppm CH_2Cl_2 for 46 days yielded essentially negative results. Blood test data from these animals also showed no significant differences between control and exposed animals. The one parameter measured in which significant changes took place in the serially sacrificed dogs was carboxyhemoglobin levels. These were not done for the 15-day exposed dogs, but data from the 30- and 46-day exposed dogs are presented in table V.

Table V

Carboxyhemoglobin Levels in Dogs Exposed to Dichloromethane at 25 ppm and 100 ppm CH_2Cl_2 for 30 and 46 Days

(COHb %)

	Control	25 ppm	100 ppm
30-Day Exposure	0.6	0.8	2.8
46-Day Exposure	0.5	1.1	2.8

In both 30-day and 46-day exposures to 100 ppm CH_2Cl_2, carboxyhemoglobin levels in dogs were significantly higher than controls at the 0.01 level. This pattern was also seen in the 100-day exposed dogs and monkeys. The carboxyhemoglobin levels in monkeys exposed to 25 and 100 ppm CH_2Cl_2, and dogs exposed to 100 ppm CH_2Cl_2 were significantly higher than controls throughout the entire study (see tables VI and VII). The dogs exposed to 25 ppm CH_2Cl_2 had the least carboxyhemoglobin of the exposed groups, and the monkeys exposed to 100 ppm CH_2Cl_2 had the highest levels. For both the 25 and the 100 ppm exposures, the carboxyhemoglobin levels in monkeys was considerably higher than in dogs (see figure 1).

Table VI

Effect of Continuous Low Level Exposure to Dichloromethane on Mean COHb Levels in Dogs

(COHb %)

Exposure Week	0	4	8	12	14
Controls	<0.5	<0.5	<0.5	<0.5	<0.5
25 ppm Exposed	<0.5	0.7*	0.7	0.5	0.7
100 ppm Exposed	<0.5	2.1**	2.4**	2.2**	2.6**

N=4

* Significant at .05 level
** Significant at .01 level

Table VII

Effect of Continuous Low Level Exposure to Dichloromethane on Mean COHb Levels in Monkeys

(COHb %)

Exposure Week	0	4	8	12	14
Controls	0.8	0.6	0.6	0.8	0.7
25 ppm Exposed	0.6	1.7**	1.8**	1.6*	1.8**
100 ppm Exposed	0.8**	3.3**	3.7**	3.5**	4.0**

N=4

* Significant at .05 level
** Significant at .01 level

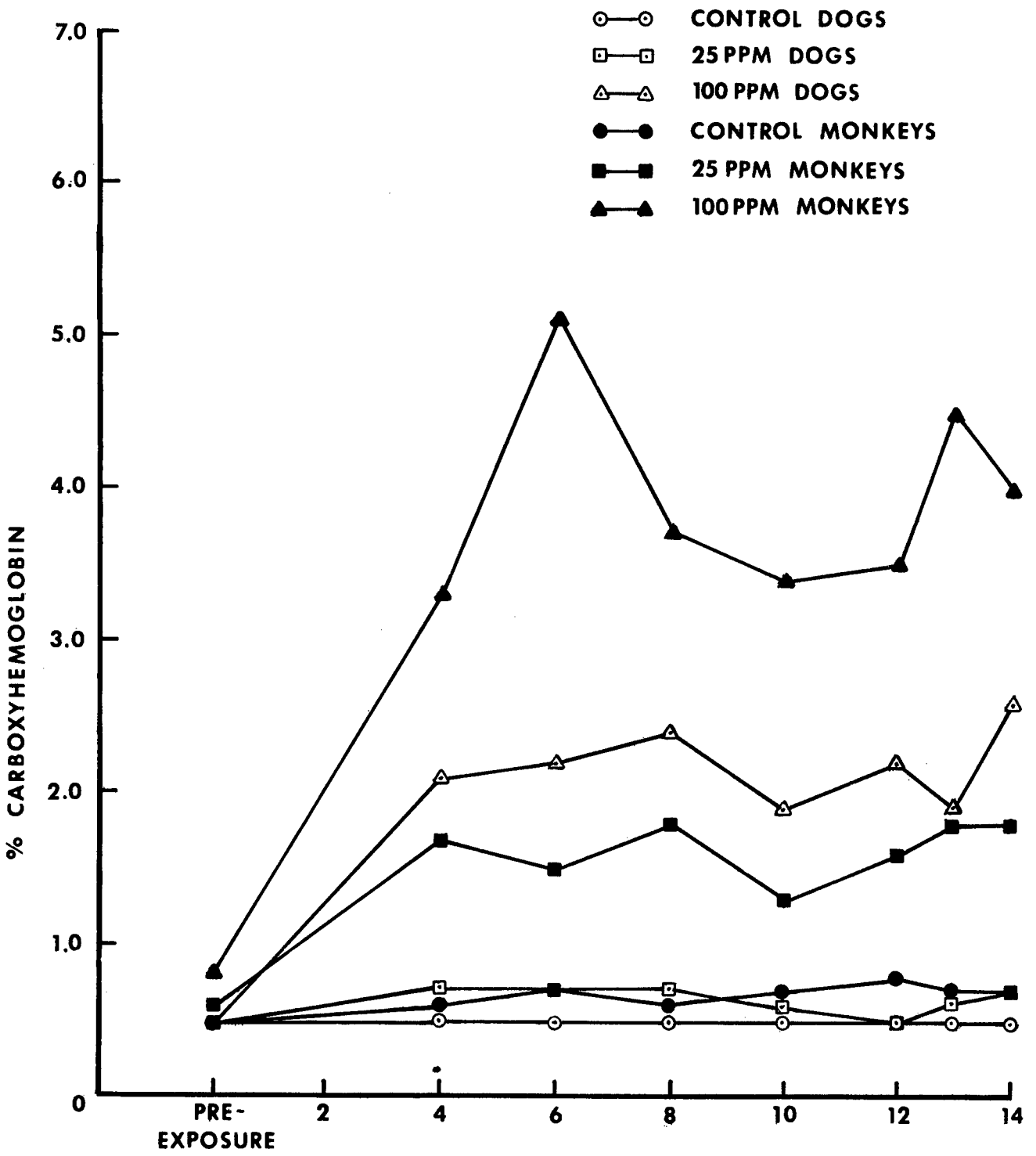


Figure 1

Carboxyhemoglobin Levels in Dogs and Monkeys
Exposed to 25 and 100 ppm CH_2Cl_2

Blood was drawn as scheduled from the exposed dogs and monkeys at 6 and 13 weeks for determination of CH_2Cl_2 levels. Measurable levels were found in both species in both the 25 ppm and the 100 ppm exposure groups. None was found in the blood of either species in the control group. Table VIII shows the data obtained at both 6 and 13 weeks into the study. Dogs had higher CH_2Cl_2 blood levels for both exposure levels.

Table VIII

Dichloromethane in Blood of Dogs and Monkeys
Continuously Exposed at Low Levels

(μg/ml)

Dogs	6 Weeks	13 Weeks
Control	0.0	0.0
25 ppm Exposed	1.1	1.8
100 ppm Exposed	5.1	4.0
Monkeys		
Control	0.0	0.0
25 ppm Exposed	0.6	1.0
100 ppm Exposed	3.1	2.7

The results of hematology measurements made on the exposed dogs and monkeys and their controls are shown as mean values in tables IX and X. There are no significant increases in any determinations resulting from the exposure to dichloromethane. The differences between control and exposed groups, while moderately significant, were present prior to initiation of the experiment and were maintained at essentially a constant level throughout the study.

Table IX

Effect of Continuous Low Level Exposure to Dichloromethane on Dog Hematology Measurements

Exposure Time	Group	HCT (Vol %)	HGB (g%)	RBC (Millions)	WBC (Thousands)
Pre-exposure	Control	43	15	6.1	13
	25 ppm Exposed	47	16	6.7	10
	100 ppm Exposed	50	17	7.0	12
2 Weeks	Control	44	15	6.3	14
	25 ppm Exposed	47	16	6.9	14
	100 ppm Exposed	49	17	7.1	15
4 Weeks	Control	45	15	6.0	14
	25 ppm Exposed	48	16	6.7	13
	100 ppm Exposed	49	17	6.6	12
8 Weeks	Control	44	15	6.5	10
	25 ppm Exposed	46	16	6.8	11
	100 ppm Exposed	48	16	7.3	12
12 Weeks	Control	43	15	6.3	11
	25 ppm Exposed	44	15	6.6	11
	100 ppm Exposed	46	17	6.8	10
14 Weeks	Control	46	16	6.5	10
	25 ppm Exposed	45	15	6.6	11
	100 ppm Exposed	45	16	6.8	12

Mean Values N=4

Table X

Effect of Continuous Low Level Exposure to Dichloromethane on Monkey Hematology Measurements

Exposure Time	Group	HCT (Vol %)	HGB (g %)	RBC (Millions)	WBC (Thousands)
Pre-exposure	Control	41	13	5.6	8
	25 ppm Exposed	43	13	5.9	10
	100 ppm Exposed	46	14	6.1	9
2 Weeks	Control	38	12	5.4	12
	25 ppm Exposed	41	13	5.8	14
	100 ppm Exposed	43	14	6.0	11
4 Weeks	Control	39	13	5.5	10
	25 ppm Exposed	42	14	6.1	10
	100 ppm Exposed	45	14	6.0	11
8 Weeks	Control	38	12	5.4	11
	25 ppm Exposed	40	13	5.8	10
	100 ppm Exposed	43	14	6.0	13
12 Weeks	Control	40	13	5.7	11
	25 ppm Exposed	41	14	5.9	10
	100 ppm Exposed	45	15	6.1	11
14 Weeks	Control	36	11	5.0	12
	25 ppm Exposed	38	12	5.2	10
	100 ppm Exposed	40	13	5.4	13

Mean Values N=4

The results of clinical chemistry determinations for dogs and monkeys after 4, 8, 12, and 14 weeks continuous exposure to 25 ppm or 100 ppm CH_2Cl_2 showed no abnormalities. Only scattered values were found to be satistically different from controls, and none of these differences formed any trends that were suggestive of chronic toxicity in either species.

Mean body weights for exposed dogs and monkeys are shown in table XI. Although the 100 ppm exposed dogs exhibited a slightly different growth rate (see figure 2) than the 25 ppm exposed and control groups, the weight differences were within the normal range in all cases, and no relationship to exposure could be determined. There was no difference between the growth rates of the two exposed groups and control monkeys (figure 3). Growth curves for 100-day exposed rats are shown in figure 3. Again, all groups appeared to experience completely normal growth rates.

Mean organ weights and organ to body weight ratios for exposed rats at time of sacrifice are shown in table XII. Statistical differences from controls were seen in lungs of rats exposed to 25 ppm CH_2Cl_2 (and in the lung to body weight ratio for the same group) and in spleen weights and spleen to body weight ratios in both exposure groups. The significance of these findings cannot be determined until histopathology results become available; however, the lung changes are not a result of exposure or they would have been seen at the higher level.

Table XI

Effect of Continuous Exposure to 25 ppm and 100 ppm Dichloromethane on Dog and Monkey Body Weight

Exposure Time (Weeks)	Mean Body Weight (in kg)					
	Dogs			Monkeys		
	Control	25 ppm	100 ppm	Control	25 ppm	100 ppm
Preexposure	7.87	8.26	9.01	2.65	2.85	2.70
2	7.64	8.96	9.23	2.73	2.96	2.81
4	8.42	8.81	9.38	2.91	3.02	2.89
6	8.78	9.17	9.74	3.14	3.23	3.06
8	8.74	9.09	9.93	3.06	3.23	3.04
10	9.36	9.68	10.38	3.09	3.20	3.00
12	9.08	9.60	9.95	3.13	3.23	3.00
13	9.45	10.08	10.06	3.21	3.48	3.08
14	9.28	9.69	9.97	3.20	3.29	3.16

Mean Values N=4

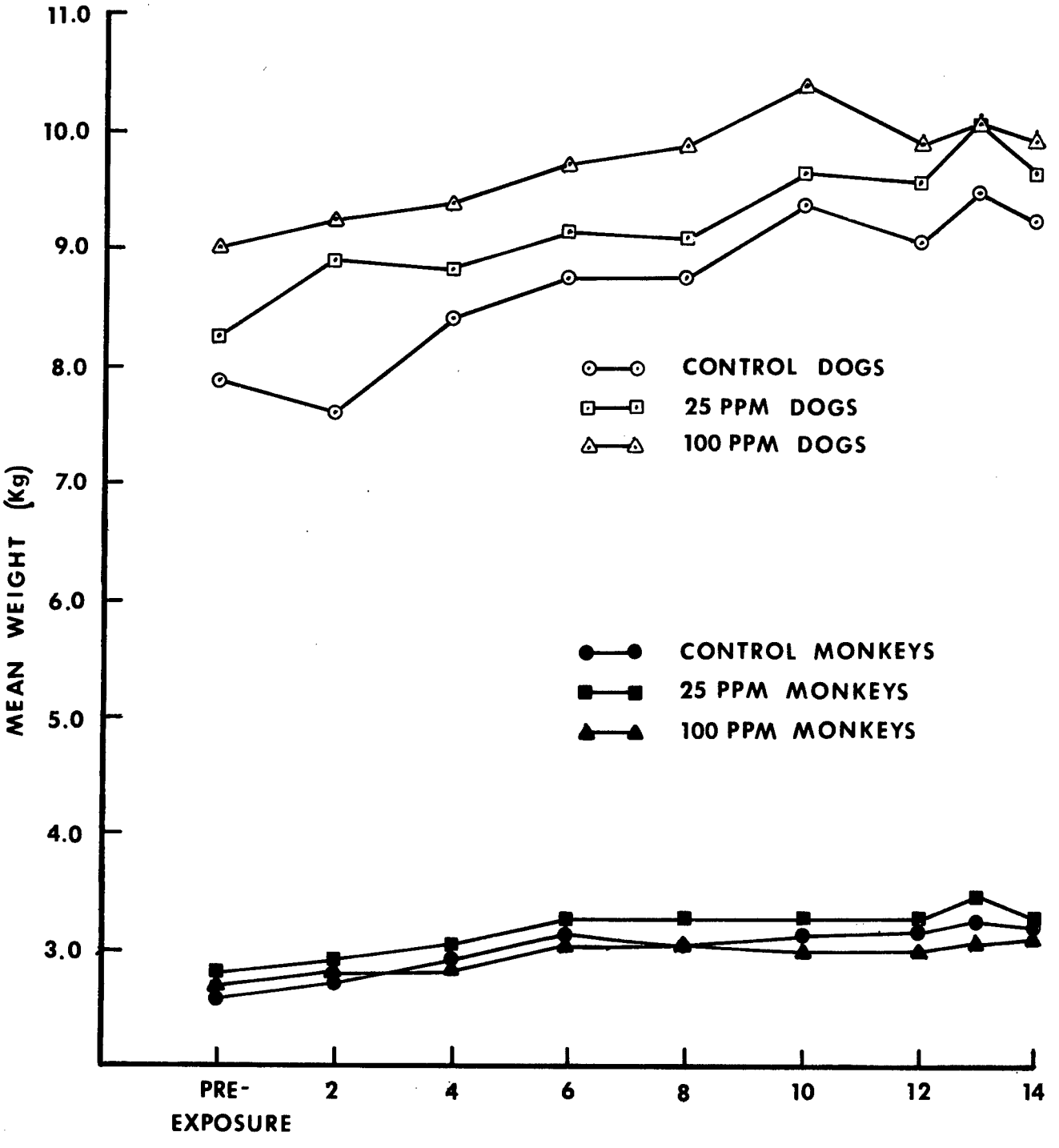


Figure 2

Effect of 14-Week Continuous Exposure to 25 ppm and 100 ppm CH_2Cl_2 on Dog and Monkey Body Weights

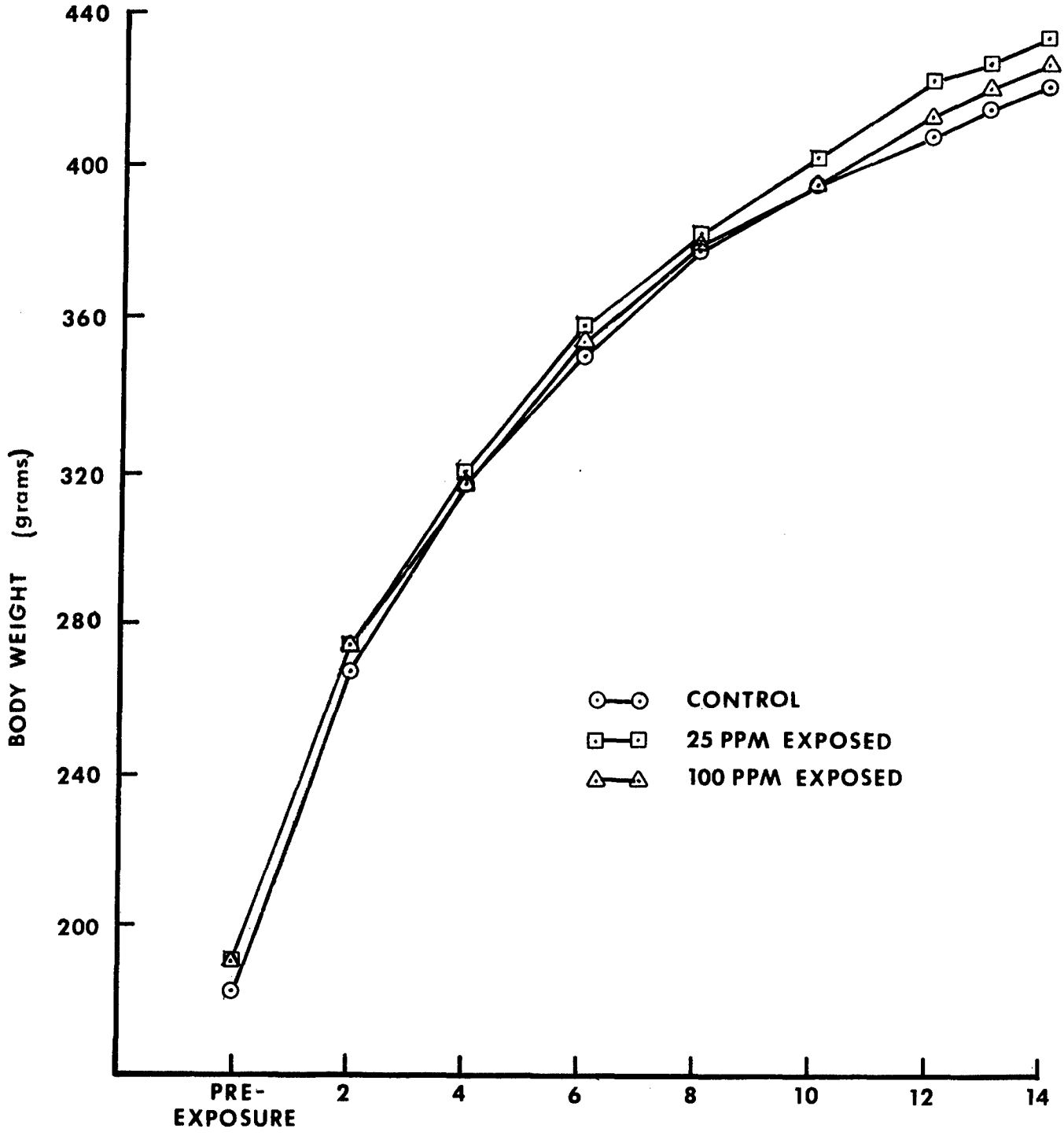


Figure 3

Mean Body Weights for 14-Week Exposed Rats

Table XII

Effect of Continuous Exposure to 25 ppm and 100 ppm Dichloromethane on Organ Weights of Albino Rats

Organ	Mean Organ Weight (grams)			Mean Organ/Body Weight Ratio (grams/100 grams body weight)		
	Control	25 ppm	100 ppm	Control	25 ppm	100 ppm
Heart	1.37	1.44	1.36	.33	.34	.33
Lung	1.94	1.79*	1.83	.47	.43**	.45
Liver	10.68	11.02	11.03	2.59	2.61	2.69
Spleen	.87	.79*	.73**	.21	.18**	.18**
Kidney	2.76	2.74	2.66	.67	.65	.65

N=20

* Different from controls at 0.05 level of significance.
** Different from controls at 0.01 level of significance.

Gross pathology for the full-term exposed rats and mice yielded no differences between the controls and either exposed group. Slight mottling of livers and kidneys was a common finding in all groups.

Gross pathological examination of exposed dogs and monkeys disclosed no evidence of chronic toxicity due to CH_2Cl_2.

Continuous exposures to 25 ppm and 100 ppm CH_2Cl_2 for periods up to 100 days resulted in few, if any, toxic changes in the four species tested. None of the overt signs of toxicity which were so obvious in the previous exposures to 1000 ppm and 5000 ppm CH_2Cl_2 were seen. All animals gained weight in a normal manner, had normal appetites, and appeared to have normal activity throughout the entire study. In the previous exposures to 1000 and 5000 ppm CH_2Cl_2, appetite suppression and resultant weight losses were evident shortly after onset of exposure, with most dramatic effects being seen in dogs and monkeys. Also, activity of the large animals exposed to 5000 ppm was noticeably reduced. Deaths due to exposure occurred in the 1000 ppm and 5000 ppm groups but no exposure related deaths were seen in any of the animals involved in the present study.

In looking at the serial sacrifice portion of the present study, any definite patterns of toxicity due to increased length of exposure to these levels of CH_2Cl_2 were absent. There was a tentative suggestion of liver damage in dogs sacrificed after 15 days continuous exposure to both CH_2Cl_2 levels, but

dogs sacrificed after 30 and 46 days of exposure to the same levels showed no changes from controls. Therefore, either the slight damage present at 15 days was resolved during the course of further exposure, or the tentative designation of damage being related to the exposure at 15 days was not valid. Although the histopathological results from the 46-day exposed dogs are not yet available, all other data (gross pathology and blood determinations) available for these dogs indicate no changes from controls.

In the exposed dogs and monkeys, there was an interesting relationship between carboxyhemoglobin levels and blood CH_2Cl_2 levels. Dogs had higher levels of blood CH_2Cl_2, while monkeys had higher carboxyhemoglobin levels. The monkeys' metabolic processes may be more adept at converting CH_2Cl_2 to carbon monoxide (hence producing carboxyhemoglobin) by some unknown reaction or series of reactions. Assuming that the blood levels of CH_2Cl_2 would be approximately equal in both species exposed to the same concentration if no conversion took place, the conversion of blood CH_2Cl_2 to carboxyhemoglobin at a greater rate in the monkeys would account for the decreased blood CH_2Cl_2 as compared to dogs. The data obtained in this study seem to indicate that this occurred.

The continuous 25 ppm and 100 ppm CH_2Cl_2 exposures presented here were apparently low enough to avoid any major toxic alterations in the normal function of the animals exposed. However, some alterations did occur at these

levels. Mouse liver cytochrome enzymes were significantly altered from controls at 30, 60, and 90 days exposure to 100 ppm CH_2Cl_2, while mice exposed to 25 ppm CH_2Cl_2 for the same periods showed no differences from controls. Carboxyhemoglobin levels were significantly higher in test animals compared to controls. Other minor changes were seen in several of the blood parameters from time to time during the course of the exposures, but these changes formed no pattern, and were not suggestive of any intrinsic damage. The level at which major toxic alterations will occur during continuous exposure lies somewhere between 100- and 1000 ppm CH_2Cl_2. At 100-ppm CH_2Cl_2, the animals were able to compensate for any changes that did occur, and it is probable that more serious alterations would be seen at CH_2Cl_2 levels not too much above 100 ppm. The space cabin TLV of 20 ppm for dichloromethane appears to be a satisfactory level for 100-day missions with a safety factor of at least 5.

PRELIMINARY EXPERIMENTS FOR CHLORINE PENTAFLUORIDE EEL STUDIES

The investigation of the acute toxicity and the establishment of 15-, 30-, and 60-minute LC_{50}'s for chlorine pentafluoride (ClF_5) has been done with rats, mice, dogs, and monkeys, part of which was described in the last annual report (reference 20). This follow-up study was designed to determine histopathological changes which may take place after an acute exposure to very low levels of ClF_5 and to aid in the establishment of emergency exposure limits (EEL) for man. The concentrations and exposure times selected were based on the LC_{50} data reported by Darmer (reference 21).

The current EEL's for ClF_5 are 3 ppm for 10 minutes, 1.5 ppm for 30 minutes, and 0.5 ppm for 60 minutes or CT's (concentration x time) of 30 to 45 ppm minutes. The concentrations and times selected for this preliminary study were 30 ppm for 10 minutes, 20 ppm for 30 minutes, and 10 ppm for 60 minutes.

Groups consisting of 30 male Sprague-Dawley rats, 30 male ICR mice, and 6 rhesus monkeys (4 male and 2 female) were exposed to the concentrations and times mentioned above. A control group of each species was maintained for comparison to the test group. All exposures were made in a Rochester Chamber. The experimental animals were observed for visible signs of toxic stress and mortality both during exposure and for 28 days following the exposure. All animals were weighed weekly. A routine battery of clinical laboratory tests and fluoride analyses was made on blood samples taken from the monkeys at 14 and 28 days postexposure. At sacrifice, a femur was taken from each monkey for analysis of the fluoride content. Gross examination of the animals was performed and histopathology examination of these tissues is presently being done by Dr. Kroe of the Laboratory of Experimental Biology in St. Louis, Missouri.

Contaminant concentrations within the chamber were monitored continuously during all exposures. The analytical determinations were made with a fluoride ion specific electrode.

Toxic signs noted during exposure included lacrimation in all species, salivation by rats, and nausea by the monkeys. These signs of irritation appeared almost immediately after onset of exposure and disappeared within 30 minutes postexposure. No differences were noted among exposure groups in either the onset or disappearance of symptoms.

Of the 30-minute group one mouse was found dead four days postexposure and a monkey six days postexposure. Gross pathology of the monkey revealed some purulent material in the upper respiratory tract and focal areas of severe active subacute bronchopneumonia in the lungs. The pathologist was unable to determine the cause of death in the mouse due to tissue autolysis.

Mean body weight gains in the 60-minute and the 30-minute groups of mice were depressed during the first week postexposure but recovered thereafter. Although no statistically significant differences were found in the mean body weights of the monkeys during the 28-day observation period, all test groups appear depressed when compared to the controls on a weight-gain basis (table XIII). Some scattered statistically significant differences were noted in the monkey blood parameters but no pattern or dose-response relationship can be seen. There appears to be no difference between the exposed monkeys and the controls in the amount of bone fluoride detected in the femurs removed at necropsy (table XIV). The method of bone fluoride analysis is described in detail elsewhere in this report.

Table XIII

Mean Body Weight Gains of Animals Exposed to ClF_5 Vapors

(Cumulative Weight Gain - Grams)

Concentration Level, ppm	Weight 0 Days	Week Postexposure 1	2	3	4
		Rats			
10.5	175.1	42.2	80.4	104.1	127.1
23.0	183.9	44.6	73.8	111.7	131.6
30.0	183.2	50.6	80.9	105.5	126.4
0.0	181.7	43.3	82.5	108.8	129.7
		Mice			
10.5	22.5	2.1	6.1	9.5	9.9
23.0	23.0	1.8	6.0	7.5	9.9
30.0	23.6	3.0	4.7	6.8	9.2
0.0	22.8	4.1	6.6	8.0	10.3
		Monkeys			
10.5	2620	230	0	160	310
23.0	2630	50	-100	250	380
30.0	2830	50	-130	220	370
0.0	2750	350	30	460	600

Table XIV

Bone Fluoride Determinations in Monkeys
Exposed to ClF_5 Vapors

ClF_5 ppm	Time (minutes)	Animal Number	$\mu gF^-/mg$ Actual	Group Mean $\mu gF^-/mg$
30	10	L-11	0.45	
		R-36	0.50	
		L-13	0.31	
		R-44	0.34	
		468	0.48	
		628	0.38	0.41 ± 0.08
20	30	R-42	0.43	
		R-78	0.31	
		R-66	0.38	
		Q-90	0.97	
		L-01	0.36	
		L-17		0.37 ± 0.05*
10	60	R-56	0.27	
		R-70	0.36	
		R-52	0.50	
		R-48	0.45	
		K-91	0.36	
		K-89	0.38	0.39 ± 0.08
Control	--	R-50	0.38	
		R-76	0.27	
		R-68	0.50	
		K-95	0.52	
		K-87	0.34	
		400	0.52	0.42 ± 0.11

*L-17 omitted.

Gross examination of all animals was performed at necropsy. No gross lesions were observed in any of the 10-minute group or in the controls. One monkey of the 30-minute group showed some multifocal, white, caseous material in a cut section of the lung. Significant pathology was observed in the animals exposed for 60 minutes including pale livers and kidneys in the rats and congested lungs in all of the monkeys.

Because of the deaths from the 30-minute exposure and the significant pathology noted one month following the 60-minute exposure, it was concluded that the preliminary test concentrations of ClF_5 were too high. A definitive study is planned using lower CT values and additional animals including dogs. This is expected to be completed in the next report period.

RECLASSIFICATION OF TOXICITY RATINGS OF TRANSPORTABLE CHEMICAL AGENTS

During the past year a study was undertaken to reclassify approximately 200 chemical compounds listed as poisons in Department of Transportation (DOT) and United Nations regulations for transportable materials. An extensive literature search was conducted to determine toxicity status from existing data. Although only oral and inhalation toxicity information was used to determine toxicity classification for this study, other supplemental toxicity information on the compounds was also included on data sheets prepared for each compound. The following criteria were used to determine the category into which each compound would be placed:

	Extremely Toxic	Highly Toxic	Toxic
Oral, 14-Day Single Dose LC_{50}	5 mg/kg	>5-50 mg/kg	>50-5000 mg/kg
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)

Of the approximately 200 chemicals for which the literature was searched to obtain toxicity data, little or no useful information was available for 86 compounds. The information that was available on some of the latter chemicals gave lethal levels which could not be related to LC_{50} values since there was no indication how much greater the doses given were than the minimum lethal dose. The results of this study were submitted to the Department of Transportation and are currently in preparation for a technical report to be issued by that organization.

ACUTE ORAL TOXICITY TESTING PROGRAM

At the request of the Department of Transportation, studies were undertaken to determine the 14-day oral LD_{50} of single doses of a representative group of chemical compounds that could not be given toxicity classifications as a result of the literature search previously described.

For oral toxicity determinations, the following procedures were used. Water soluble compounds were administered in distilled water. All compounds not water soluble were given as a finely ground suspension in corn oil. All of the latter compounds were pulverized using a mortar and pestle to

assure minimum particle size, and hence a better suspension. All suspensions were kept in a turbulent state while in use by agitating on a magnetic stirring platform.

The animals used in this study were 200-300 gram male CFE (Sprague-Dawley derived) rats and 20-30 gram male CF1 (ICR derived) mice obtained from Carworth Farms, Incorporated. All animals were subjected to quality control examinations prior to use to insure that they were in good health.

Glass syringes with special needles were used to intubate all doses. The experimental animals were fasted for at least 16 hours prior to administration of the oral dose and were not given food for at least 2 hours after injection. This was to allow for more uniform absorption in all animals of the same species, since the amount of food in the stomach varies from animal to animal in the unfasted condition.

The intubation volume for both rats and mice was approximately 0.01 ml/gm body weight which meant that the average mouse received a volume of 0.25 ml, and the rat 2.5 ml. The concentrations of the solutions or suspensions were adjusted to give the proper dose in the desired volume. Since the rodents do not possess a vomit reflex, the compounds had to be absorbed or passed on through the gastrointestinal tract. Both mice and rats were weighed individually at the time of intubation to determine actual volume of material required.

Initial rangefinding tests were conducted on each compound at the following dose levels: 5 mg/kg, 50 mg/kg, and 500 mg/kg. After the proper range had been found, subsequent doses were administered according to either a multiple of 2 or 1.26 of the starting dose. This allowed rapid determination of the LD_{50} according to the method described by Weil (reference 22). Five rats and 5 mice were tested at each level chosen.

The animals were observed for toxic signs and mortality for 14 days following the single dose of a compound. Animals that survived the 14-day period were sacrificed at that time.

The oral toxicity data and classification categories are shown for rats and mice in tables XV and XVI, respectively. The tables also show the code numbers for each of the DOT requested compounds tested.

Table XV

Oral Toxicity Results for Department of Transportation Study - Rats

Code No.	Compound	LD_{50} (95% C. L.) in mg/kg	Data Used to Calculate LD_{50} in mg/kg (Mortality Response, N = 5)	Classification
024	Calcium arsenate	812 (712- 924)	630 (0), 790 (2), 1000 (5)	Toxic
056	Mercuric acetate	76 (55- 105)	50 (1), 63 (2), 79 (2), 100 (4)	Toxic
060	Mercuric cyanide	26 (15- 46)	16 (1), 32 (3), 64 (5)	Highly Toxic
064	Mercuric oxide	18 (7- 44)	5 (0), 10 (1), 20 (4)	Highly Toxic
070	Mercuric sulfate	57 (27- 120)	40 (0), 50 (4), 63 (1)	Highly Toxic
076	Mercurous nitrate	297 (248- 362)	252 (1), 320 (3), 400 (5)	Toxic
093	Nicotine sulfate	75 (44- 127)	40 (1), 80 (2), 160 (5)	Highly Toxic
103	o-Nitroaniline	3564 (2590-4910)	1260 (0), 2520 (0), 5040 (5)	Toxic
202	Aniline hydrochloride	1072 (725-1585)	500 (0), 1000 (2), 2000 (5)	Toxic
203	Benzidine	566 (169-5424)	200 (4), 400 (0), 800 (5)	Toxic
204	Benzyl chloride	1231 (1145-1656)	500 (0), 1000 (1), 2000 (5)	Toxic
205	Benzylidine chloride	3249 (2360-4473)	2000 (0), 4000 (4), 8000 (5)	Toxic
206	o-Chloronitrobenzene	268 (181- 396)	125 (0), 250 (2) 500 (5)	Toxic
208	p-Chloronitrobenzene	812 (590-1118)	500 (0), 1000 (4), 2000 (5)	Toxic
214	4, 6-Dinitro-orthocresol	33 (22- 49)	16 (0), 32 (2), 64 (5)	Highly Toxic
216	2, 3-Dinitrotoluene	1122 (501-2516)	500 (0), 1000 (3), 2000 (3)	Toxic
217	2, 4-Dinitrotoluene	268 (181- 396)	125 (0), 250 (2), 500 (5)	Toxic
218	2, 5-Dinitrotoluene	707 (513- 974)	500 (0), 1000 (5), 2000 (5)	Toxic
219	2, 6-Dinitrotoluene	177 (128- 243)	125 (0), 250 (5), 500 (5)	Toxic
220	3, 4-Dinitrotoluene	1072 (725-1585)	500 (0), 1000 (2), 2000 (5)	Toxic
227	2, 2'Dithiobisbenzo-thiazole	>12, 000	2000 (0), 4000 (0), 8000 (1), 12, 000 (0)	Below Toxic
228	m-Nitroaniline	535 (362- 793)	250 (0), 500 (2), 1000 (5)	Toxic
229	p-Nitroaniline	3249 (1984-5702)	2000 (1), 5000 (3), 8000 (5)	Toxic
230	o-Nitrophenol	2828 (2054-3894)	1000 (0), 2000 (0), 4000 (5)	Toxic

Table XV (continued)

Oral Toxicity Results for Department of Transportation Study - Rats

Code No.	Compound	LD_{50} (95% C. L.) in mg/kg	Data Used to Calculate LD_{50} in mg/kg (Mortality Response, N = 5)	Classification
231	m-Nitrophenol	933 (645-1351)	500 (0), 1000 (3), 2000 (5)	Toxic
232	p-Nitrophenol	616 (447- 848)	250 (0), 500 (1), 1000 (5)	Toxic
233	o-Nitrotoluene	891 (500-1584)	500 (0), 1000 (4), 2000 (3)	Toxic
234	m-Nitrotoluene	1072 (725-1585)	500 (0), 1000 (2), 2000 (5)	Toxic
235	p-Nitrotoluene	2144 (1449-3171)	1000 (0), 2000 (2), 4000 (5)	Toxic
238	2, 3-Xylidine	933 (631-1380)	500 (0), 1000 (3), 2000 (5)	Toxic
239	2, 4-Xylidine	467 (315- 690)	250 (0), 500 (3), 1000 (5)	Toxic
240	2, 5-Xylidine	1297 (937-2135)	500 (1), 1000 (0), 2000 (5)	Toxic
241	2, 6-Xylidine	1231 (894-1695)	500 (0), 1000 (1), 2000 (5)	Toxic
242	3, 4-Xylidine	812 (590-1118)	500 (0), 1000 (4), 2000 (5)	Toxic
243	3, 5-Xulidine	707 (469-1068)	250 (1), 500 (0), 1000 (5)	Toxic
244	1-Chloronaphthalene	1540 (1306-1717)	1260 (0), 1588 (4), 2000 (4)	Toxic
245	2-Chloronaphthalene	2078 (1611-2673)	1588 (1), 2000 (2), 2520 (4)	Toxic
246	Mixed Cresols	1454 (563-3550)	1000 (0), 1260 (3), 1588 (2), 2000 (4)	Toxic
247	2, 4-Dichlorophenol	2830 (2054-3885)	2000 (0), 4000 (5), 8000 (5)	Toxic
248	Diethyl sulfate	1412 (1102-1552)	1000 (2), 1260 (0), 1588 (5), 2000 (5)	Toxic

Table XVI

Oral Toxicity Results for Department of Transportation Study - Mice

Code No.	Compound	LD_{50} (95% C. L.) in mg/kg	Data Used to Calculate LD_{50} in mg/kg (Mortality Response, N = 5)	Classification
024	Calcium arsenate	794 (665- 946)	630 (0), 790 (3), 1000 (4)	Toxic
056	Mercuric acetate	62 (41- 92)	20 (1), 40 (0), 80 (4), 160 (5)	Toxic
060	Mercuric cyanide	33 (22- 49)	16 (0), 32 (2), 64 (5)	Highly Toxic
064	Mercuric oxide	22 (10- 48)	10 (1), 10 (1), 40 (4)	Highly Toxic
070	Mercuric sulfate	40 (30- 50)	32 (0), 40 (4), 50 (2)	Highly Toxic
076	Mercurous nitrate	388 (290- 530)	400 (2), 500 (3), 630 (4)	Toxic
093	Nicotine sulfate	16 (12- 21)	12.8 (1), 16 (3), 20 (4)	Highly Toxic
103	o-Nitroaniline	1288 (1131-1467)	1000 (0), 1269 (2), 1588 (5)	Toxic
202	Aniline hydrochloride	841 (474-1493)	500 (1), 1000 (3), 2000 (5)	Toxic
203	Benzidine	214 (144- 317)	200 (2), 400 (5), 800 (5)	Toxic
204	Benzyl chloride	1624 (1153-2185)	1000 (0), 2000 (4), 4000 (5)	Toxic
205	Benzylidine chloride	2462 (1788-3389)	1000 (0), 2000 (1), 4000 (5)	Toxic
206	o-Chloronitrobenzene	135 (110- 200)	62.5 (0), 125 (2), 250 (5)	Toxic
208	p-Chloronitrobenzene	1414 (1070-2044)	1000 (0), 2000 (5), 4000 (5)	Toxic
214	4, 6-Dinitro-orthocresol	21 (12- 37)	16 (1), 32 (4), 64 (5)	Highly Toxic
216	2, 3-Dinitrotoluene	1072 (725-1585)	500 (0), 1000 (2), 2000 (5)	Toxic
217	2, 4-Dinitrotoluene	1625 (1180-2236)	1000 (0), 2000 (4), 4000 (5)	Toxic
218	2, 5-Dinitrotoluene	1231 (730-2077)	500 (0), 1000 (2), 2000 (3), 4,000 (5)	Toxic
219	2, 6-Dinitrotoluene	1000 (589-1697)	500 (0), 1000 (3), 2000 (4)	Toxic
220	3, 4-Dinitrotoluene	1414 (457-4379)	500 (2), 1000 (1), 2000 (4)	Toxic
227	2, 2'-Dithiobisbenzo-thiazole	>12,000	2000 (0), 4000 (0), 8000 (0), 12,000 (0)	Below Toxic
228	m-Nitroaniline	308 (228- 416)	125 (0), 250 (1), 500 (5), 1,000 (5)	Toxic
229	p-Nitroaniline	812 (590-1118)	500 (0), 1000 (4), 2000 (5)	Toxic
230	o-Nitrophenol	1297 (894-1695)	500 (0), 1000 (1), 2000 (5)	Toxic

Table XVI (continued)

Oral Toxicity Results for Department of Transportation Study - Mice

Code No.	Compound	LD_{50} (95% C. L.) in mg/kg	Data Used to Calculate LD_{50} in mg/kg (Mortality Response, N = 5)	Classification
231	m-Nitrophenol	1414 (195-10,270)	500 (0), 1000 (0), 2000 (5)	Toxic
232	p-Nitrophenol	467 (315- 690)	250 (0), 500 (3), 1000 (5)	Toxic
233	o-Nitrotoluene	2462 (1789-3390)	1000 (0), 2000 (1), 4000 (5)	Toxic
234	m-Nitrotoluene	1231 (894-1695)	500 (0), 1000 (1), 2000 (5)	Toxic
235	p-Nitrotoluene	1231 (894-1695)	500 (0), 1000 (1), 2000 (5)	Toxic
238	2, 3-Xylidine	1072 (725-1586)	500 (0), 1000 (2), 2000 (5)	Toxic
239	2, 4-Xylidine	250 (147- 424)	125 (0), 250 (3), 500 (4)	Toxic
240	2, 5-Xylidine	841 (474-1493)	500 (1), 1000 (3), 2000 (5)	Toxic
241	2, 6-Xylidine	707 (522- 957)	250 (0), 500 (0), 1000 (5), 2000 (5)	Toxic
242	3, 4-Xylidine	707 (522- 957)	250 (0), 500 (0), 1000 (5), 2000 (5)	Toxic
243	3, 5-Xylidine	421 (279- 635)	250 (0), 500 (4), 1000 (4)	Toxic
244	1-Chloronaphthalene	1091 (964-1178)	790 (0), 1000 (1), 1250 (5), 1588 (5)	Toxic
245	2-Chloronaphthalene	886 (734-1070)	790 (1), 1000 (4), 1260 (5)	Toxic
246	Mixed Cresols	561 (465- 677)	500 (1), 630 (4), 790 (5)	Toxic
247	2, 4-Dichlorophenol	1625 (1007-2619)	500 (0), 1000 (1), 2000 (3), 4000 (5)	Toxic
248	Diethyl sulfate	647 (507- 827)	500 (1), 630 (3), 790 (3), 1000 (5)	Toxic

There was considerable disagreement between the rat LD_{50} found in this study and that reported elsewhere (reference 23) for the compound calcium arsenate (024). The value obtained in this laboratory was 812 mg/kg (see table XV) while the value previously reported was 298 mg/kg. The major difference in techniques used to obtain these values lies in the vehicle used to administer the dosage. The low value was obtained using a water suspension, as opposed to a corn oil suspension. In order to determine whether or not this difference in vehicle was responsible for the variation in LD_{50} values, a group of five rats was dosed with 500 mg/kg calcium arsenate suspended in water. If the other test was correct, this dose should have killed all or nearly all of the animals. The results of this experiment are shown below:

Species	Vehicle	Dose (mg/kg)	Mortality Ratio*
Rat	Water	500	1/5
Rat	Corn Oil	500	0/5
Rat	CMC**	100	0/5
Rat	CMC	200	0/5
Rat	CMC	400	0/5
Rat	CMC	800	0/5
Rat	CMC	1600	4/5

* Number dead/number dosed
** 1% carboxymethyl cellulose

These results are of a dual interest; they show that the technique used in the present study was reproducible, and that there was little difference in

toxicity between water and corn oil as a vehicle. To further substantiate that the 812 mg/kg value obtained was correct, a series of oral doses of calcium arsenate suspended in 1% carboxymethyl cellulose was administered to rats. The results from these doses are shown in the lower portion of the table above. Calcium arsenate suspended in carboxymethyl cellulose appears to be somewhat less readily absorbed than that in either water or corn oil, as reflected in the lower mortality at the 800 mg/kg level.

The compound 2, 2'-Dithiobisbenzothiazole (227) was classified to be "Below Toxic" with an LD_{50} of >12, 000 mg/kg in both rats and mice. No deaths occurred with any doses of this compound in either rats or mice. A dose level of 12, 000 mg/kg was near the maximum that could be made into a suspension and still be administered with a syringe; in fact, it was extruded as a paste.

Benzyl chloride and benzylidene chloride were tested for both oral and inhalation toxicity. Using the criteria for either route of administration, both these compounds fell in the "Toxic" category.

Benzyl chloride and benzylidene chloride were screened at the upper limits of the "Highly Toxic" range (2000 mg/m^3) and the "Extremely Toxic" range (500 mg/m^3) since the industrial TLV values for these compounds are

based on irritancy to eyes and mucous membranes in workers. Both of these compounds were generated by using a calibrated syringe pump and forcing the liquid into a heated coil through which the chamber input air was passed to pick up the volatilized compound. Nominal concentrations were used for these screening tests. No toxic effects or deaths were noted in either species.

ACUTE INHALATION EXPOSURES OF RATS AND MICE TO SILANE

An investigation of the acute inhalation toxicity response to silane (SiH_4) exposures in rats and mice was conducted at the request of the Department of Transportation.

For the silane exposures, a cylinder of 4.84% silane in nitrogen was purchased, which proved to ignite spontaneously when exhausted into the air. For safety reasons, it was decided not to exceed one percent or 10,000 ppm in the exposure chamber. The silane concentration in the exposure chamber was analyzed using a Beckman infrared 5-A spectrophotometer, and showed a mean recovery of 97% of the nominal.

Male CFE rats and CF1 mice were exposed to the silane vapors in a 30-liter glass bell jar with an air flow of 30 liters per minute under conditions listed in table XVII. The animals exposed for either two or four hours were weighed prior to exposure and at approximately 3, 7, and 14 days postexposure.

Table XVII

Lethal Effect of Silane Vapor Inhalation Exposures on Male Rats and Mice

Species	Nominal Conc. (ppm)	Measured Conc. (ppm)	Measured Conc. (mg/L)	Time (hr)	Mortality Ratio	Time to Death
Rats	1,000	-	-	1.25	0/50	-
Rats	4,000	-	-	1.0	0/5	-
Rats	10,000	9,600	12.6	4.0	0/5	-
Mice	6,000	-	-	1.0	0/5	-
Mice	10,000	9,600	12.6	4.0	4/10	31-45 hrs.
Mice	10,000	9,800	12.8	2.0	0/10	-

Gross pathology was performed on representative animals following the 14-day observation period. Control groups of each species were treated similarly.

The mean weight gain of the 9600 ppm group appears to have been slightly inhibited during the first postexposure week, but fully recovered at 14 days (table XVIII). No gross lesions due to treatment were observed at sacrifice.

Four of 10 mice died following the inhalation of 9600 ppm silane vapor for four hours. All mice appeared normal during and after exposure; however, four were found dead between 31 and 45 hours postexposure. Unfortunately, we were unable to obtain gross pathological examinations of these mice. Another group of 10 mice was exposed to a similar concentration (9800 ppm) for two hours with no visible effects.

The results of these tests indicate that the major hazard associated with acute silane exposures is fire, and concentrations lower than those which ignite spontaneously in air would not constitute a hazard to man provided the insult did not continue for two hours or longer.

ACUTE INHALATION TOXICITY OF ETHYL BROMIDE

Inhalation exposure studies on the acute toxicity of ethyl bromide were conducted on rats and mice to determine the one-hour LC_{50} concentration. These exposures were performed to assist in the toxicity classification of this solvent for the Department of Transportation.

Table XVIII

Effect of Acute Inhalation Exposures to Silane on Growth of Rats and Mice

Species	Exposure Time (hr)	Conc. ppm (mg/m^3)	Preexposure Mean Weights (grams)	Postexposure Mean Weight Change (grams)		
				3 days	7 days	14 days
Rats	4.0	9,600 (12,000)	181	*	+28	+71
Rats	Controls	0	203	*	+39	+76
Mice	4.0	9,600 (12,600)	24.4	-0.3	+ 4.3	+ 4.3
Mice	2.0	9,800 (12,800)	28.1	-2.2	- 0.5	+ 1.9
Mice	Controls	0	28.4	0.0	+ 2.8	+ 3.4

*Not available

Animal exposures were conducted in a 30-liter glass bell jar in groups of ten rats or mice. The exposure concentrations were conducted on a nominal basis since ethyl bromide is relatively nonreactive in air under the experimental conditions used. Confirmatory analysis showed the nominal concentrations to be accurate. The exposures lasted one hour and the animals were held for a 14-day postexposure observation period for visible signs of toxicity and mortality.

The primary response of both rats and mice to ethyl bromide exposure was CNS depression. Diarrhea was noted in rats at the highest level and in mice in all but the lowest concentration, and was probably indicative of gastrointestinal irritation resulting from ingestion of the contaminant when the animals licked their fur. The weight gains of rats exposed to 25, 200 ppm were considerably inhibited during the 14-day observation period (see table XIX). Weight gains of the other rats were either not available due to total mortality, or appeared to be within normal range. Mice exposed to 15, 875 ppm also had a subnormal weight gain at 14 days, and mice exposed to 10, 000 ppm demonstrated a weight suppression as well.

The one-hour LC_{50} values for rats and mice exposed to ethyl bromide were calculated to be 12, 030 mg/m^3 (26, 980 ppm) and 7, 240 mg/m^3 (16, 230 ppm), respectively, using the method of Weil (reference 22), which would place this compound in the "Toxic" category.

Table XIX

Results of One-Hour Exposures of Rats and Mice to Ethyl Bromide Vapors

Nominal Concentration (ppm)	Species	Mortality Ratio*	Mean Weight Gain of Survivors at 14-Days (grams)
20,000	Rats	0/10	60.0
25,200	Rats	2/10	39.6
31,752	Rats	10/10	**
40,000	Rats	10/10	**
Controls	Rats	-	91.6
10,000	Mice	0/10	0.7
12,600	Mice	0/10	4.4
15,875	Mice	4/10	1.7
20,000	Mice	10/10	**
Controls	Mice	-	3.4

* Number dead/Number exposed.
** No survivors.

LC_{50} values (95% confidence limits):

Rats - 26,980 (25,350-28,710) ppm
- 12,030 mg/m^3

Mice - 16,230 (15,360-18,620) ppm
- 7,240 mg/m^3

COMPARISON OF THE ACUTE TOXICITY RESPONSE IN RATS AND MICE RESULTING FROM EXPOSURES TO HCl GAS AND HCl AEROSOL

Hydrogen chloride (HCl) is one of the combustion products formed during the test firing of certain rocket and missile engines. Since the vicinity in which these firings take place is occasionally engulfed with a heavy fog, it was of interest to the Air Force to determine the exposure hazard to Air Force personnel conducting these tests as well as inhabitants of nearby towns who may be placed at risk during either clear or foggy days under prevailing weather conditions.

The present study was undertaken to determine LC_{50} values for exposure to HCl gas for 5 and 30 minutes, and to an HCl aerosol for the same exposure periods. This would define short-exposure toxicity levels for HCl in either state to determine whether the aerosol form is more or less dangerous than the vapor itself.

Since complete details of this study have been published as AMRL-TR-72-21, only summary results will be described herein.

Toxic signs during exposure to HCl vapor and HCl aerosol were essentially identical. The HCl was extremely irritating to the eyes, mucous membranes, and exposed areas of skin. Ulceration of the scrotum was a common finding in both rats and mice. The animals were observed to groom and preen excessively, and usually exhibited a rapid, shallow breathing pattern by the end of the exposure. The fur had a "singed" appearance and texture which was more

noticeable with the aerosol than with the vapor alone. There was evidence of corneal erosion and clouding in both species when exposed to either the vapor or the aerosol.

Gross examination of the animals that died during or shortly following exposure showed that the respiratory tract was the primary target for the HCl damage. Moderate to severe alveolar emphysema, atelectasis, and edema of the lung were observed, and occasional "spotting" of lung tissue was also found. The upper respiratory tract was severely irritated, and the epithelial tissue of nasal and trachael passages was badly damaged in both species. Animals surviving up to 14 days postexposure showed at necropsy that recovery from the exposure was not complete. Often the lungs, which had an abnormal gray color, failed to collapse upon opening of the chest cavity. There was also evidence of consolidation of lung tissue and some residual alveolar damage.

The death patterns observed with HCl vapor and HCl aerosol were similar, with delayed death being observed in both cases. In general, more delayed deaths occurred in mice than in rats, but there was no significant shift between the vapor and aerosol exposures in the pattern of time to death.

The acute effects of exposure to HCl were similar to those observed with exposure to other pulmonary irritants such as OF_2 (reference 24), HF

(reference 25), ClF_5 (reference 25), and ClF_3 (reference 26). Deaths due to HCl were attributed primarily to its effect on the respiratory tract. There were few differences in observed symptomatology between exposure to HCl vapor and HCl aerosol. The results of this study seem to indicate little difference in the acute toxicity of HCl vapor and HCl aerosol in rats and mice, as the 95% confidence limits for each LC_{50} overlap in every case when comparing vapor to aerosol for the same length of exposure. The summary LC_{50} data are shown in table XX.

The effects of HCl vapor and HCl aerosol on rats and mice were similar to those reported by Machle et al. (reference 27) for exposure of rabbits and guinea pigs to HCl vapor. These workers reported that an exposure to 6.5 mg/liter (4416 ppm) for 30 minutes was 100% fatal to all rabbits and guinea pigs exposed. In the present study, exposure to HCl aerosol of 6.52 mg/liter (4432 ppm) for 30 minutes was fatal to 100% of the mice exposed.

An exposure of mice to 4076 ppm (6.0 mg/liter) HCl vapor for 30 minutes resulted in the death of 13 of 15 animals. This indicates that the toxicity of HCl as either a vapor or an aerosol is very similar in mice, rabbits, and guinea pigs; however, the actual exposures of rabbits and guinea pigs to HCl aerosol would have to be conducted to validate this premise. On the other hand, none of the rats exposed to 6.6 mg/liter (4481 ppm) HCl aerosol for 30 minutes died, and exposure to an HCl vapor concentration in this same range would be expected to kill only a small percentage of rats also. Therefore, rats are considerably more tolerant to HCl than any of the other three species mentioned here.

These experiments have shown that there is no significant difference between the acute toxic responses of rodents exposed to other gaseous or aerosol forms of HCl.

Table XX

Summary of Acute Toxicity Data for Exposure to HCl Gas and HCl Aerosol

	5-Minute LC_{50}		30-Minute LC_{50}	
	ppm	mg/liter	ppm	mg/liter
Aerosol				
Rat	31,008	45.6	5,666	8.3
Mouse	11,238	16.5	2,142	3.2
Gas				
Rat	40,898	--	4,701	-
Mouse	13,750	--	2,644	-

ACUTE INHALATION TOXICITY OF HYDROGEN BROMIDE

Hydrogen bromide (HBr) gas has not previously been subjected to toxicological examination. Because of its use in the chemical industry and consequent appearance in interstate transport the THRU was requested to determine its acute toxic effects. Acute toxicity experiments were conducted to define the 14-day LC_{50} resulting from a single one-hour exposure.

All exposures to HBr were made in a modified Rochester Chamber. The Rochester Chamber was equipped with sliding gasketed cage drawers which could be rapidly inserted and withdrawn from the chamber once the desired concentration of HBr had been reached within the chamber. The input air for these exposures was predried and metered at a constant rate of 11 cfm. The HBr was introduced into the input air immediately prior to its entry into the chamber. Contaminant concentrations within the chamber were monitored continuously during all exposures using a bromide ion specific electrode. This analysis was capable of measuring HBr concentrations of 50 to 5000 ppm, a range which bracketed all exposures used in the study. The hydrogen bromide concentrations to which the experimental animals were exposed were increased by a multiple of 1.26 to reach a level of 80-100% mortality. The LC_{50} values were based on the 14-day mortality data, and were calculated by the method of Weil (reference 22).

Male CFE (Sprague-Dawley derived) rats and male CF1 (ICR derived) mice weighing 200-300 grams and 20-30 grams, respectively, were used in these studies. The animals were held for 14 days in quarantine and subjected to quality control examinations to determine their suitability for toxicity experiments. The rats and mice were observed for visible signs of toxicity and mortality during exposure and for 14 days postexposure. The animals were weighed prior to exposure and at 3, 7, and 14 days postexposure. Gross pathology was performed on a representative group of animals following the 14-day observation period.

The one-hour inhalation LC_{50} for HBr vapor was 9.46 mg/m^3 (2858 ppm) for rats, and 2.69 mg/m^3 (814 ppm) for mice. The individual exposure data for this compound are shown in table XXI. Responses of the animals to HBr were consistently dose-related, and followed a general sequence of nose and eye irritation, labored breathing, gasping, and convulsions. This pattern of toxic signs is common to other severe pulmonary irritants such as HCl, HF, and ClF_5. The fur turned orange-brown in color during exposure, with the intensity of the color being directly related to the concentration in the chamber. There appeared to be a smoky haze around the animals during exposure, probably a result of the reaction of the HBr and the fur or the moisture on the fur.

Delayed deaths were observed with both species tested. During the 14-day postexposure period, the animals were prostrate and many suffered autolysis of exposed areas of skin, such as feet, tails, scrotum, and ears. Although the animals from the high mortality exposures were not weighed at three and seven days due to their moribund condition, dehydration and a resultant loss of weight was observed grossly during this period. Rats at the lowest exposure level (2205 ppm) demonstrated weight depression after three and seven days postexposure, but returned to a near-normal weight gain by 14 days. Mice at the lowest level (507 ppm) had normal weight gains during the entire 14-day period. Gross examination of rats at necropsy showed severe lung and liver congestion with pulmonary edema at the highest exposure level. Rats exposed to the lower HBr concentration tested had necrotic lesions on their feet and tails after 14 days.

Table XXI

Response of Rats and Mice to One-Hour Inhalation Exposures of Hydrogen Bromide

	Average Concentration (ppm)	Concentration Range (ppm)	Mortality Ratio
Rats	3822	3357-4155	10/10
	3711	3545-3855	7/10
	3253	2941-3454	6/10
	2759	2702-2871	4/10
	2328	2228-2451	4/10
	2205	2105-2304	1/10
Mice	1163	1108-1296	10/10
	1036	980-1130	9/10
	875	842- 920	7/10
	507	443- 554	0/10

Rats - LC_{50} = 2858 ppm (2581-3164)

Mice - LC_{50} = 814 ppm (707-947)

No gross pathology other than necrosis of tails was observed in mice surviving the 14-day postexposure period. Many rats and mice showed a milky opacity of the cornea at the end of exposure, which disappeared within 24 hours.

Table XXII compares the one-hour LC_{50} values for HBr, found in the present study, with those previously obtained for HCl and HF. Mice were the less tolerant species to all three of these halogen acid gases. Since the least resistant species must be considered when predicting man's response, these results indicate that the same precautions used when handling HF vapor should be exercised with HBr.

Table XXII

Comparative One-Hour LC_{50} Values (ppm) of Rats and Mice Exposed to HBr, HCl, or HF (ppm)

Species	HBr 60-Min. LC_{50}	HCl Estimated 60-Min. LC_{50}	HF 60-Min. LC_{50}
Rats	2858	2350	1278
Mice	814	1322	501

ACUTE TOXICITY OF HYDROGEN SULFIDE

Acute inhalation exposures to hydrogen sulfide (H_2S) were conducted in the THRU laboratories, again at the request of the Department of Transportation,

to clarify ambiguities in literature sources and to precisely define one-hour LC_{50} values for rats and mice. These values, compatible with current standards, would then be useful in toxicity classification of this compound.

The rodents used in this study were 200-300 gram male CFE (Sprague-Dawley derived) rats, and 20-30 gram male CF1 (ICR derived) mice, and were subjected to quality control examinations prior to use to insure that they were in good health.

The exposures were conducted in a 30-liter glass bell jar using an air flow rate of 30 liter/min. High purity H_2S gas was obtained from commercial sources and blended with the air supply stream to the bell jar exposure chambers.

The chamber exposure concentrations were monitored continuously using an ion specific sulfide electrode technique. The actual concentrations during exposure were unchanged from nominal, indicating little or no reactivity of H_2S with chamber materials.

As in previously described inhalation toxicity studies conducted for the Department of Transportation, the rodents were exposed in groups of 10 to H_2S concentrations increased by a factor of 1.26 until a concentration producing 80% mortality or greater was achieved using the method of Weil.

The toxic signs exhibited by rats and mice exposed to hydrogen sulfide vapors for one hour were gasping by both species, convulsions only in the mice, and death. Survivors of both species had normal weight gains during the 14-day

observation period. One mouse each from both the 800 ppm and 635 ppm exposed groups had a blocked urethral opening due to encrustation of the external orifice, and consequently their bladders were distended upon examination at 14 days. Rats surviving the acute exposure showed congestion and mottling of kidney and liver with moderate to severe fatty changes in the liver 14 days postexposure. The results from the one-hour exposures to hydrogen sulfide are shown in table XXIII. The one-hour LC_{50} values for rats and mice were 712 ppm (990 mg/m^3) and 634 ppm (881 mg/m^3), respectively, which places hydrogen sulfide in the "Highly Toxic" category.

ACUTE TOXICITY OF CHLORINE

The acute inhalation toxicity of chlorine (Cl_2) was reviewed by the THRU during the literature search phase of the reclassification of transportable chemicals. Although this chemical has found extensive commercial application in organic synthesis and water purification, the acute inhalation toxicity had not been defined in a manner suitable for comparison with other chemicals in a toxicity rating system. Consequently, one-hour LC_{50} studies for Cl_2 were conducted on rats and mice.

The rodents were exposed to Cl_2 in the 30-liter glass bell jar chambers to air concentrations of Cl_2 successively increased by a 1.26 factor as previously described for other inhalation studies (reference 22). Chlorine concentrations in the exposure chambers were continuously monitored using an ion specific electrode technique. There was a 20 to 30% loss of chlorine in the exposure system, probably due to wall effects.

Table XXIII

Acute Toxic Response of One-Hour Exposures of Rats and Mice to Hydrogen Sulfide

Measured Concentration (ppm)	Species	Mortality Response*
400	Rats	0/10
504	Rats	0/10
635	Rats	1/10
800	Rats	9/10
400	Mice	2/10
504	Mice	0/10
635	Mice	5/10
800	Mice	8/10

* Number dead/Number exposed.

LC_{50} values (95% confidence limits):

Rats - 712 (662-765) ppm
- 990 (920-1063) mg/m^3
Mice - 634 (576-698) ppm
- 881 (801-970) mg/m^3

Both rats and mice experienced immediate eye and nose irritation followed by lacrimation, rhinorrhea, and gasping after exposure to chlorine gas for one hour. Mice that survived at the lowest exposure level had subnormal weight gains during the postexposure observation period. Depressed growth rates were also seen in rats surviving the 268 ppm Cl_2 exposure. The most common finding at necropsy was mottling of liver tissue. The mortality resulting from these exposures is shown in table XXIV. The one-hour LC_{50} values for rats and mice were 293 ppm (850 mg/m^3) and 137 ppm (397 mg/m^3), respectively, which place chlorine in the "Extremely Toxic" category.

ACUTE INHALATION TOXICITY OF AMMONIA

The acute inhalation toxic response was reported by Boyd et al. (reference 28). They found an approximate LC_{50} in rabbits and cats to fall between 5000 and 10,000 ppm with death resulting from pulmonary edema and hemorrhage in the alveolar bed. They also found damage to the epithelial lining of bronchioles. The most frequent cause of death in man from exposure to ammonia is pulmonary edema. The concentrations of ammonia which are dangerous to man have been estimated by Henderson and Haggard (reference 29) to be in the range of 2500-6500 ppm.

The imprecision of the LC_{50} values described in previous studies made it difficult to reclassify this compound with respect to its toxicity potential. Consequently, the THRU was requested to determine the one-hour LC_{50} of ammonia for rats and mice.

Table XXIV

Effects of One-Hour Exposures to Chlorine on Rats and Mice

Measured Concentration (ppm)	Species	Mortality Ratio*	Mean Weight Gain of Survivors at 14-Days (grams)
213	Rats	0/10	13.2
268	Rats	3/10	- 6.2
338	Rats	8/10	**
427	Rats	10/10	-
Controls	Rats	-	21.4
122	Mice	3/10	- 7.7
159	Mice	8/10	**
193	Mice	10/10	-
Controls	Mice	-	1.6

* Number dead/Number exposed.
** Not enough survivors for comparison.

LC_{50} values (95% confidence limits):

Rats - 293 (260-329) ppm
- 850 (754-954) mg/m^3

Mice - 137 (119-159) ppm
- 397 (345-461) mg/m^3

The animals used were male CFE rats ranging from 200-300 grams and 20-30 gram male CF1 mice (ICR derived). The animals were exposed at each NH_3 concentration tested using the method of Weil previously discussed.

Chamber concentrations of ammonia were analyzed with an IR spectrophotometer. A calibration curve was prepared using bag samples and the actual concentrations were found to range from 25-40% lower than nominal concentrations.

The response of both rats and mice to ammonia vapors was immediate nasal and eye irritation followed by labored breathing and gasping. Convulsions were seen only in mice. Subnormal weight gains or actual weight losses were seen in both rats and mice during the 14-day observation period. At necropsy, the rats surviving the two highest exposure levels for 14 days had moderate mottling of the liver which the pathologist regarded as a probable fatty change. The mice which survived the two highest levels showed mild congestion of the liver at necropsy. No gross lesions were found in mice exposed to 3600 ppm, nor in rats exposed to 6210 ppm. The results of these exposures are shown in table XXV. One-hour LC_{50} values for rats and mice exposed to ammonia were 5100 mg/m^3 (7338 ppm) and 3360 mg/m^3 (4837 ppm) respectively. These values placed ammonia in the "Toxic" category.

TOXICITY SCREENING OF SPACE CABIN MATERIALS

The first set of Skylab materials was screened for 60-day chronic toxicity. Six Apollo materials which had not yet been tested were added to the 38 Skylab materials to form Skylab Group A. Ten gram portions of each

Table XXV

Effects of One-Hour Exposures of Ammonia on Rats and Mice

Measured Concentration (ppm)	Species	Mortality Ratio*	Mean Weight Gain of Survivors at 14-Days (grams)
6210	Rats	0/10	3.5
7820	Rats	8/10	**
9840	Rats	9/10	**
Controls	Rats	-	21.4
3600	Mice	0/10	- 0.2
4550	Mice	3/10	- 0.7
5720	Mice	9/10	**
Controls	Mice	-	1.6

* Number dead/Number exposed.
** Not enough survivors for comparison.

LC_{50} values (95% confidence limits):

Rats - 7338 (6822-7893) ppm
- 5100 (4840-5370) mg/m^3

Mice - 4837 (4409-5305) ppm
- 3360 (3064-3687) mg/m^3

material were freed of solvent if necessary by evaporating at room temperature. They were reweighed immediately before placing in the oven of the life-support loop. Numbers were assigned to the Skylab materials so that they could be more conveniently identified for toxicity testing. Some materials were deleted from the group to be tested for one of the following reasons:

1. the material had already been tested in the Apollo toxicity program;
2. after unsuccessful attempts on our part to obtain the material, NASA representatives indicated that it was no longer commercially available or was prohibitively expensive. At exposure termination, the materials in Group A were weighed again, and percent loss or gain calculated as shown in table XXVI.

Beginning on 16 December 1971 and ending on 14 February 1972, 20 male rats and 25 male mice were exposed to the combined gas-off products of all the materials in the oven which was held at 155 F. The system atmosphere was 68% O_2-32% N_2 at 5 psia pressure. The exposure chambers have been described in detail by Johnson (reference 30). Control animals were housed in a similar loop under the same experimental conditions except for contaminant. All animals were observed and weighed one week prior to exposure and at weekly intervals for four weeks postexposure. Necropsies were performed on half of the experimental animals two weeks postexposure and on the remainder four weeks postexposure. Individual rat body weight data are presented in tables XXVII and XXVIII while average weights of exposed and control mice are compared in table XXVIX. Figures 4 and 5 make graphic comparisons of growth rate between exposed and control rats and mice, respectively.

Table XXVI

Group A Apollo and Skylab Spacecraft Materials,
Weight Change during Experiment

	Initial Weight of Materials (grams)	Weight Change*	Percent Change*
		Apollo	
USAF Code No.			
108	10.0030	0.1454	1.46
111	45.0684	0.0188	0.04
208	10.0014	0.3010	3.01
437	10.0025	0.8736	8.73
465	2.3587	0.2640	11.1
938	10.0068	1.6802	1.68
		Skylab	
Toxicity Testing No.			
0001	10.0006	0.0220	0.22
0002	10.0066	0.5299	5.30
0003	10.0071	0.0017	0.02
0004	10.0071	0.1047	1.05
0005	10.0005	0.1863	1.86
0006	10.0095	0.1933	1.93
0008	9.9975	0.0027	0.27
0011	10.0027	0.0250	0.25
0013	10.0026	0.0135	0.13
0014	10.0096	1.6089	16.07
0016	10.0071	0.1073	1.07
0017	9.9590	0.6510	6.51
0018	2.1956	0.0630	2.87
0019	10.0008	1.8375	18.37
0020	2.0279	0.0805	3.97
0021	10.0000	0.1149	1.15
0022	4.7572	+0.0057	+0.12
0023	0.3200	+0.0010	+0.31
0024	10.0003	0.1522	1.52

Table XXVI (continued)

Skylab

Toxicity Testing No.	Initial Weight of Materials (grams)	Weight Change*	Percent Change*
0025	10.0012	0.1242	1.24
0026	10.0020	0.3730	3.73
0027	10.0008	0.2603	2.60
0028	10.0020	0.1576	1.58
0029	10.0022	0.0300	0.30
0031	10.0514	+0.0590	+0.59
0032	10.0029	0.2273	2.27
0034	10.0010	1.882	18.82
0035	5.4455	0.0802	0.15
0036	10.0035	0.0000	----
0037	10.0044	0.0719	0.72
0039	10.0000	0.0666	0.67
0040	10.0000	0.0466	0.47
0041	10.0011	0.3261	3.26
0042	10.0007	0.2238	2.24
0043	10.0130	0.4951	4.94
0044	5.1815	0.9842	18.99
0045	10.0039	+0.0045	+0.05
0047	10.0041	0.9165	9.16

*Weight changes are losses unless otherwise indicated.

Table XXVII

Growth of Control Rats Under Simulated Skylab Conditions

(grams)

Rat Number	1971		1972				
	12/10	12/16	2/14	2/21	2/28	3/6	3/13
55315	251	276	338	360	368	376	386
55321	253	277	355	359	371	375	389
55325	239	258	333	335	345	354	356
55326	232	255	328	337	342	350	354
55334	234	265	336	350	349	365	362
55341	249	274	338	355	358	371	377
55345	239	265	349	350	361	374	388
55350	269	294	382	393	400	400	419
55352	243	269	355	358	368	371	376
55353	260	291	363	370	379	381	396
55269	261	282	365	370	390		
55270	235	257	294	320	340		
55274	259	281	352	369	378		
55280	248	278	355	370	379		
55288	255	283	363	369	378		
55291	258	278	342	360	365		
55292	238	258	340	350	373		
55299	238	263	325	350	351		
55304	239	266	348	355	372		
55310	232	253	305	320	328		
Mean	247	271	343	355	365	372	380

Table XXVIII

Growth of Rats Exposed to Skylab Group A Materials

(grams)

Rat Number	1971		1972				
	12/10	12/16	2/14	2/21	2/28	3/6	3/13
55305	251	274	358	350	362	367	379
55306	253	286	419	410	427	432	448
55317	244	267	379	367	386	383	391
55322	243	270	355	360	375	380	384
55324	255	284	384	380	391	395	405
55335	252	281	391	382	392	394	404
55338	244	271	382	380	383	397	403
55339	249	278	369	355	369	370	383
55344	256	282	375	370	392	397	410
55351	254	279	373	382	396	402	408
55263	255	286					
55264	237	256	352	360	365		
55272	230	256	360	372	373		
55276	246	273	341	345	352		
55281	262	295	387	390	407		
55285	251	277	341	340	362		
55289	254	283	360	370	385		
55290	255	286	368	360	384		
55294	233	253	347	350	352		
55301	233	254	333	333	343		
Mean	248	275	367	366	379	392	396

Table XXVIX

Mean Weights of Exposed and Control Mice, Skylab Materials Study

(grams)

	1971		1972				
	12/10	12/16	2/14	2/21	2/28	3/6	3/13
			Control				
Mean Weights	28	30	32	33	35	36	37
Number of Mice	25	25	25	25	25	13	13
			Group A Exposed				
Mean Weights	29	31	37	38	38	37	39
Number of Mice	25	25	25	25	25	12	12

Table XXX

Mean Organ Weights of Rats Exposed to Skylab Materials

(grams)

	Heart	Lung	Liver	Spleen	Kidney
Controls	1.3	1.9	9.7	0.7	2.5
Exposed	1.3	1.8	9.8	0.7	2.6
			Necropsy Group B (4 Weeks Postexposure) N=10		
Controls	1.2	1.7	9.8	0.7	2.6
Exposed	1.2	1.8	9.9	0.7	2.7

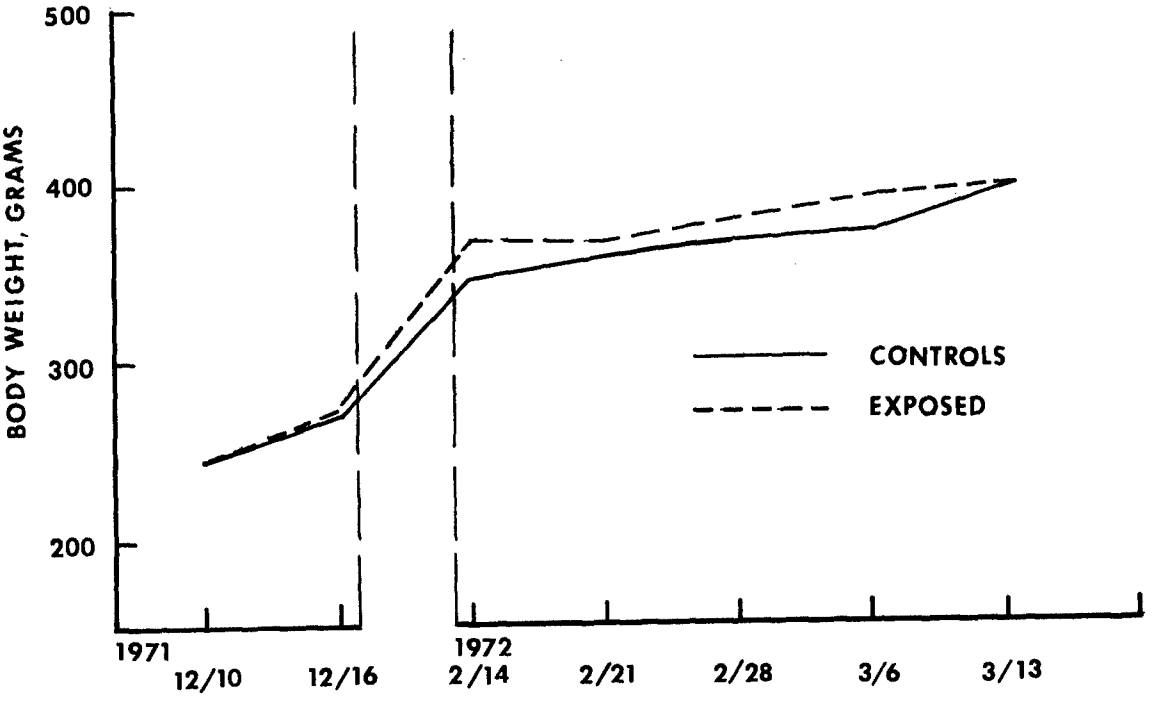


Figure 4

Growth Rate of Rats in Skylab Group A Study

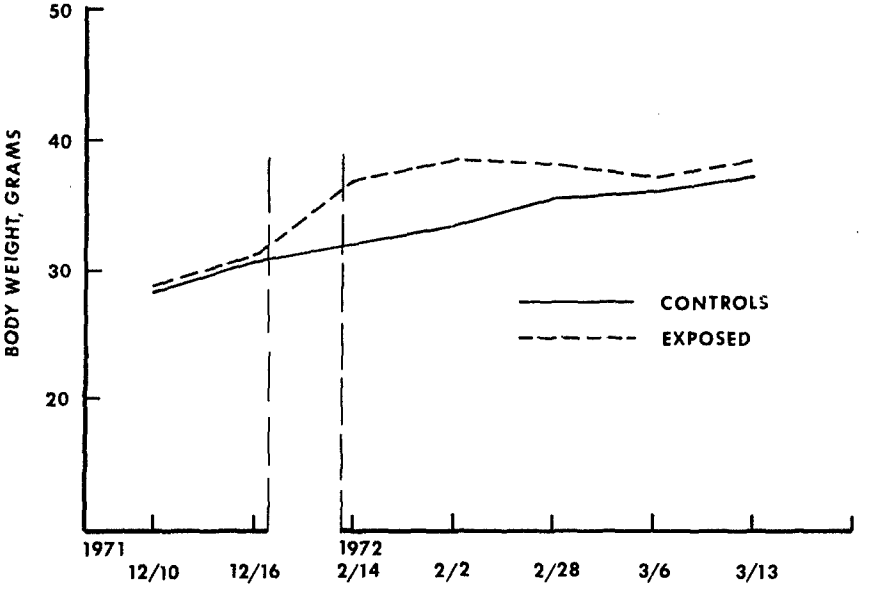


Figure 5

Growth Rate of Mice in Skylab Group A Study

Although there were significant differences between control and exposed rat and mouse mean body weights after the exposure, the weights of the exposed animals were always higher than those of the controls. Therefore, the weight difference at any period was not a deleterious effect of exposure to Skylab materials. There were no significant differences between control and exposed mean values of rat organ weights and organ to body weight ratios listed in tables XXX and XXXI.

Gas samples from the chamber environmental closed loop atmosphere and water samples from the condensation chillers were analyzed daily from beginning of exposure to the disappearance of contaminant peaks. The analyses were performed by gas chromatography using a flame ionization detector under the following conditions:

1. Column Packing - 5% Carbowax 20 M on Chromosorb W
2. Column Size - 11-Foot Length, 1/4" O. D., S. S.
3. Column Temperature - 105 C
4. Carrier Gas - N_2
5. Carrier Flow Rate - 36 cc/min.

Eight contaminant peaks were observed in the atmosphere samples. Although the contaminants were not identified unambiguously, compounds with retention times corresponding to those of the gas-off products were identified and used as standards.

Table XXXI

Mean Organ to Body Weight Ratios of Rats
Exposed to Skylab Materials

Necropsy Group A (2 Weeks Postexposure)
N=10

	Heart	Lung	Liver	Spleen	Kidney
Controls	0.374	0.521	2.724	0.185	0.709
Exposed	0.357	0.501	2.733	0.206	0.720

Necropsy Group B (4 Weeks Postexposure)
N=10

Controls	0.321	0.463	2.667	0.193	0.700
Exposed	0.321	0.453	2.563	0.173	0.686

Using the detector sensitivities of the compounds selected as standards, apparent concentrations of the gas-off products were calculated for each sampling period. These apparent concentrations are plotted against exposure times in days in figures 6 through 13. Concentrations of the compounds found ranged from 8 to 90 ppm with maxima occurring on the second day. The methane plots are difference curves; control minus exposed values. This procedure yielded negative values during the first seven days with a positive peak on the ninth day. This is not surprising since methane is primarily a metabolic excretion product and not a gas-off product from the materials.

Samples of water condensed from the life-support loop showed five peaks which appeared to be due to material gas-off products. These achieved maximum concentrations three to five days after start of the experiment and disappeared after seven days. Table XXXII lists compounds with retention times matching those of the peaks from the chiller water along with the maximum concentrations measured.

The chemical and toxicological evidence obtained in this exposure permits the conclusion that the gas-off products of the materials tested in Skylab Group A are nontoxic in the concentrations evolved under the conditions used.

Table XXXII

Maximum Concentrations of Substances Found in the Water Trap of the Skylab Exposure System

Retention Time (minutes)	Standard	Apparent Highest Concentration (ppm)
2.5	Acetone	12.5
2.8	Isopropyl alcohol	25.0
3.6	MIBK	1.0
4.4	Isobutyl alcohol	7.5
6.4	o-Xylene	4.1

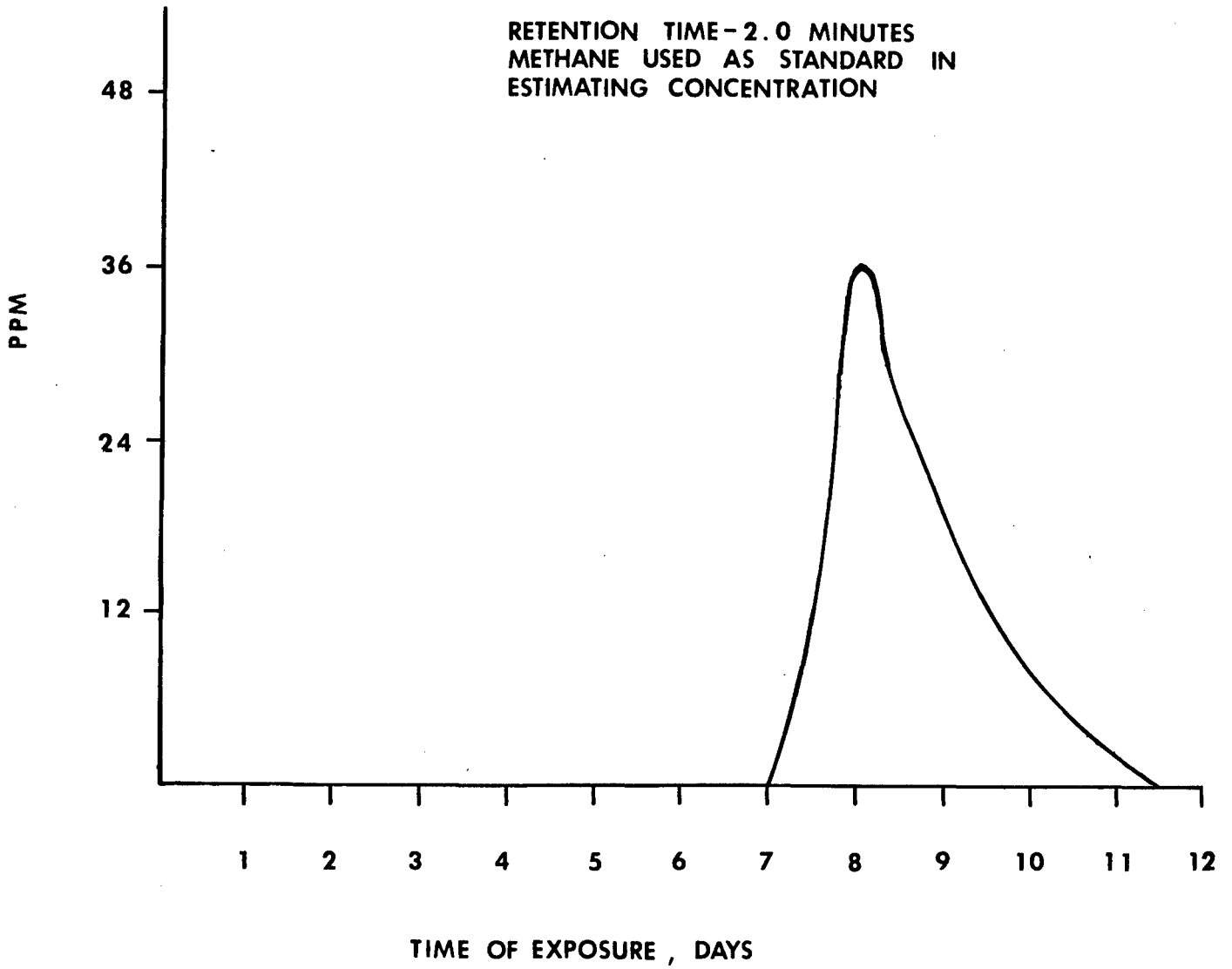


Figure 6

Skylab Materials Study; Relative Gas-Off Pattern of Contaminant No. 1, Group A

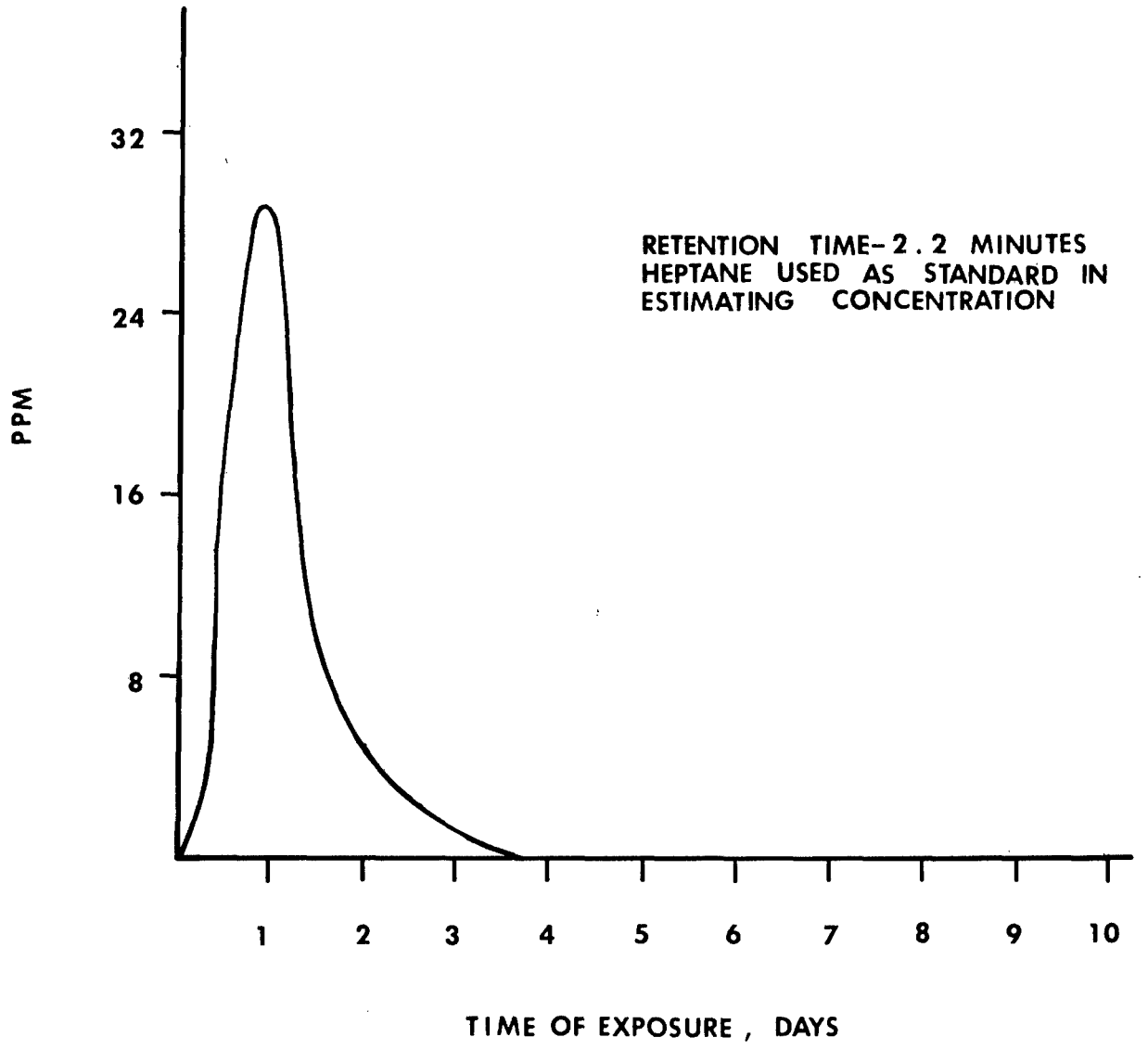


Figure 7

Skylab Materials Study; Relative Gas-Off Pattern of Contaminant No. 2, Group A

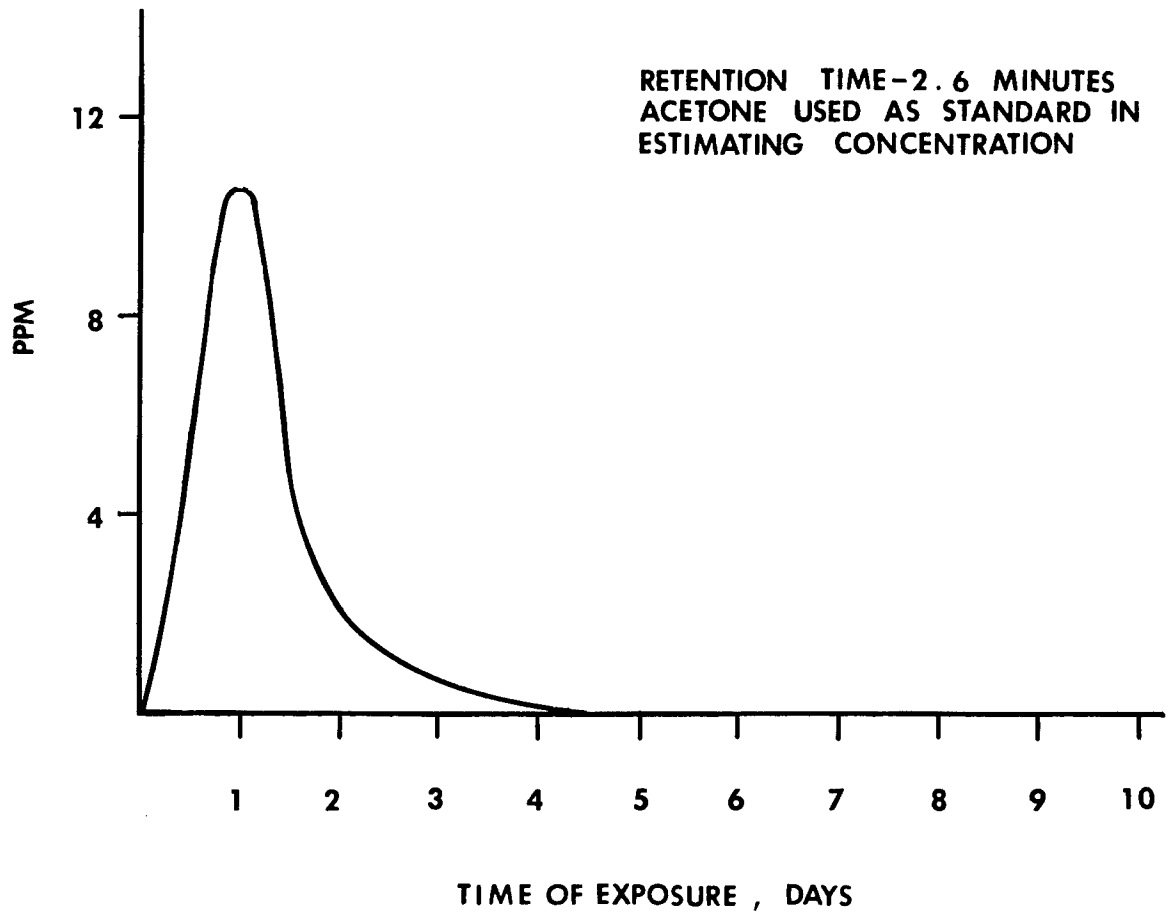


Figure 8

Skylab Materials Study; Relative Gas-Off Pattern of Contaminant No. 3, Group A

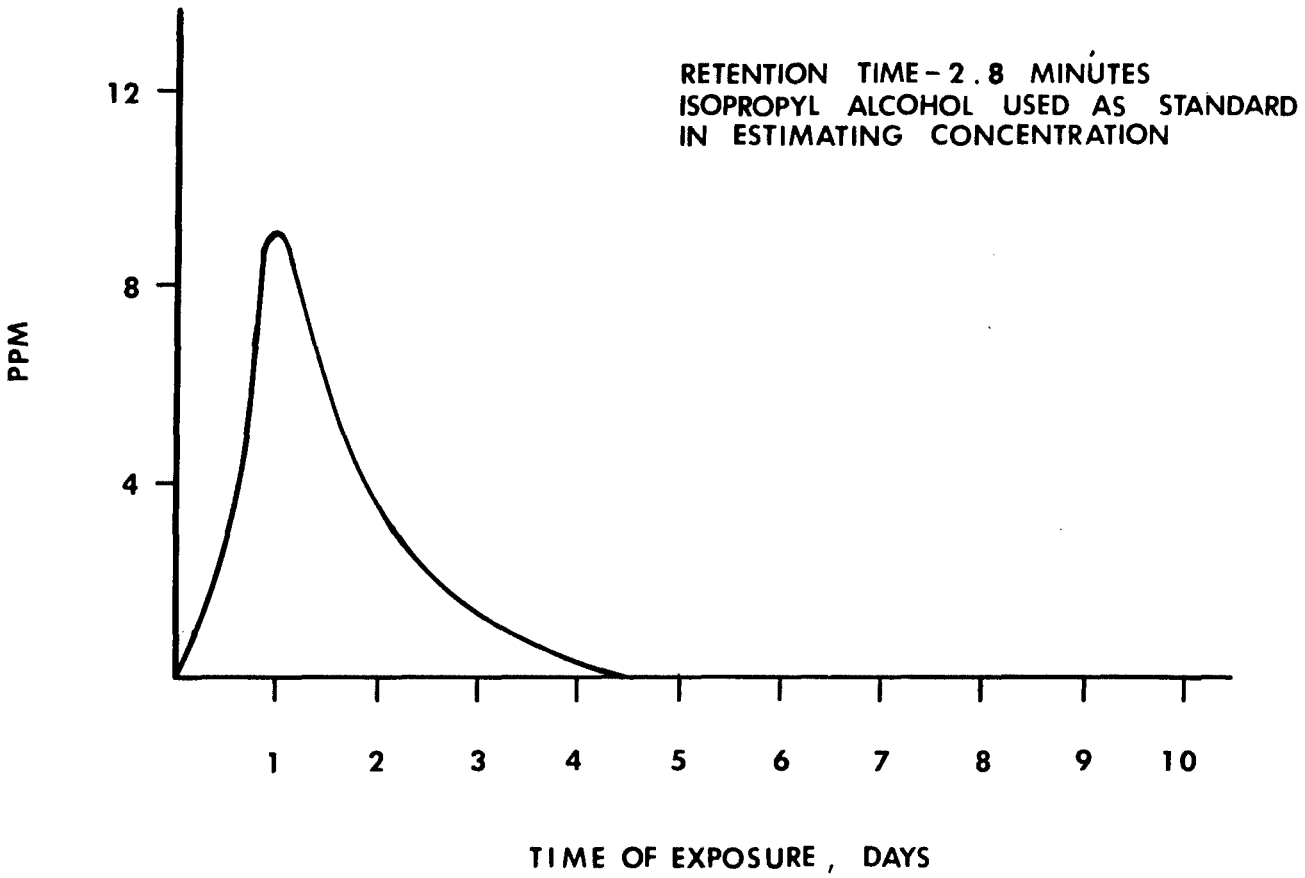


Figure 9

Skylab Materials Study; Relative Gas-Off Pattern of Contaminant No. 4, Group A

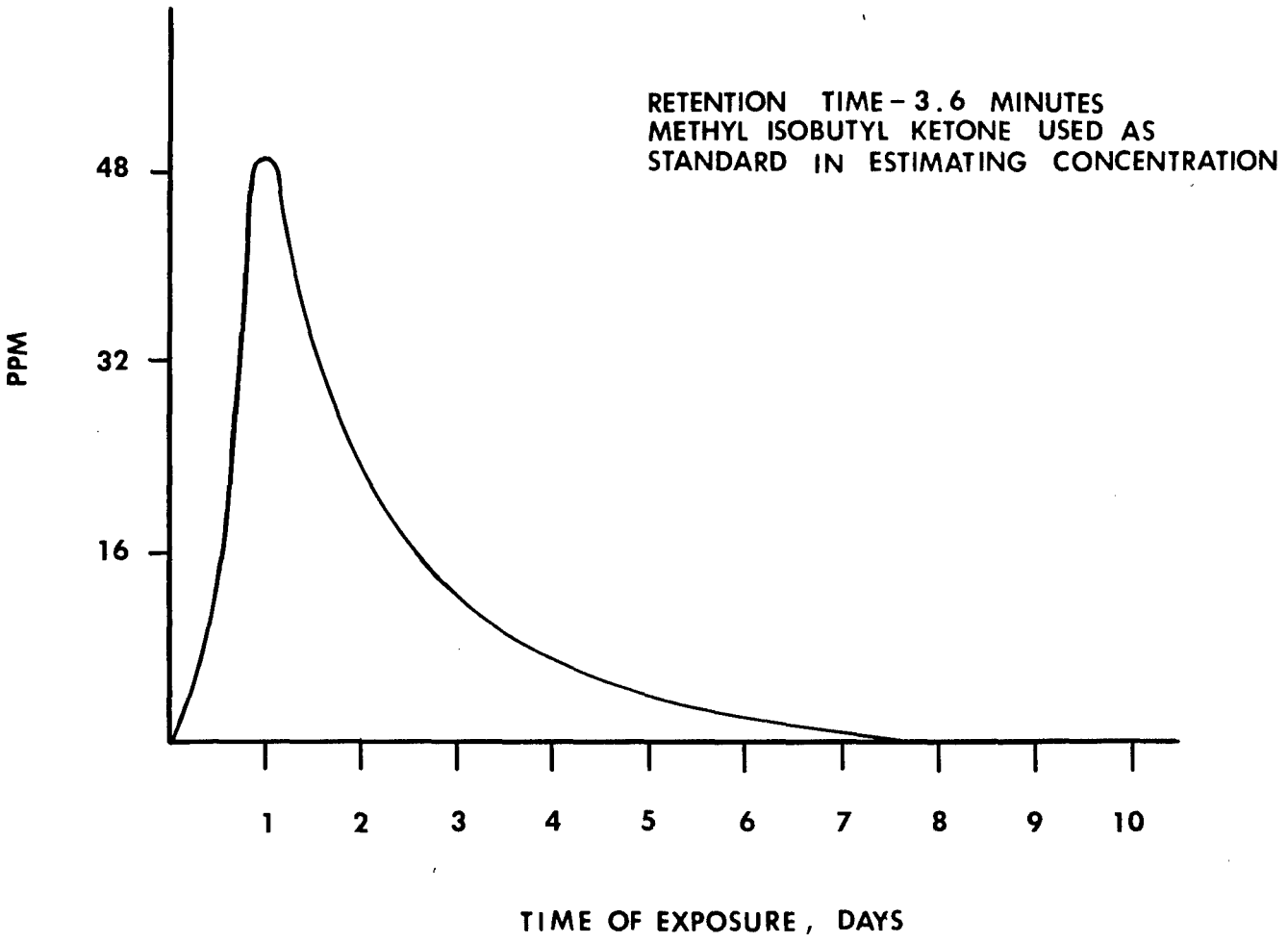


Figure 10

Skylab Materials Study; Relative Gas-Off Pattern of
Contaminant No. 5, Group A

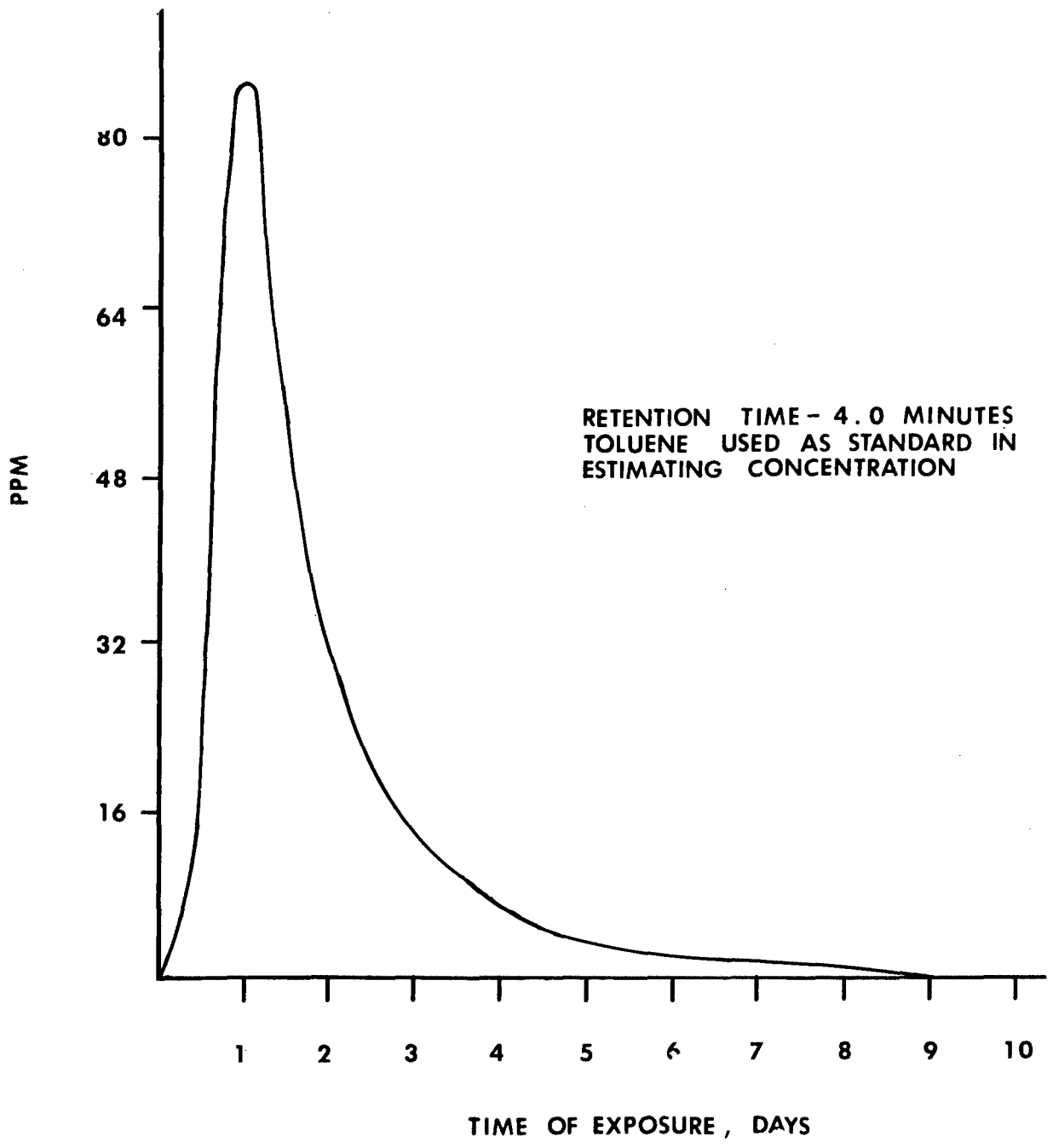


Figure 11

Skylab Materials Study; Relative Gas-Off Pattern of Contaminant No. 6, Group A

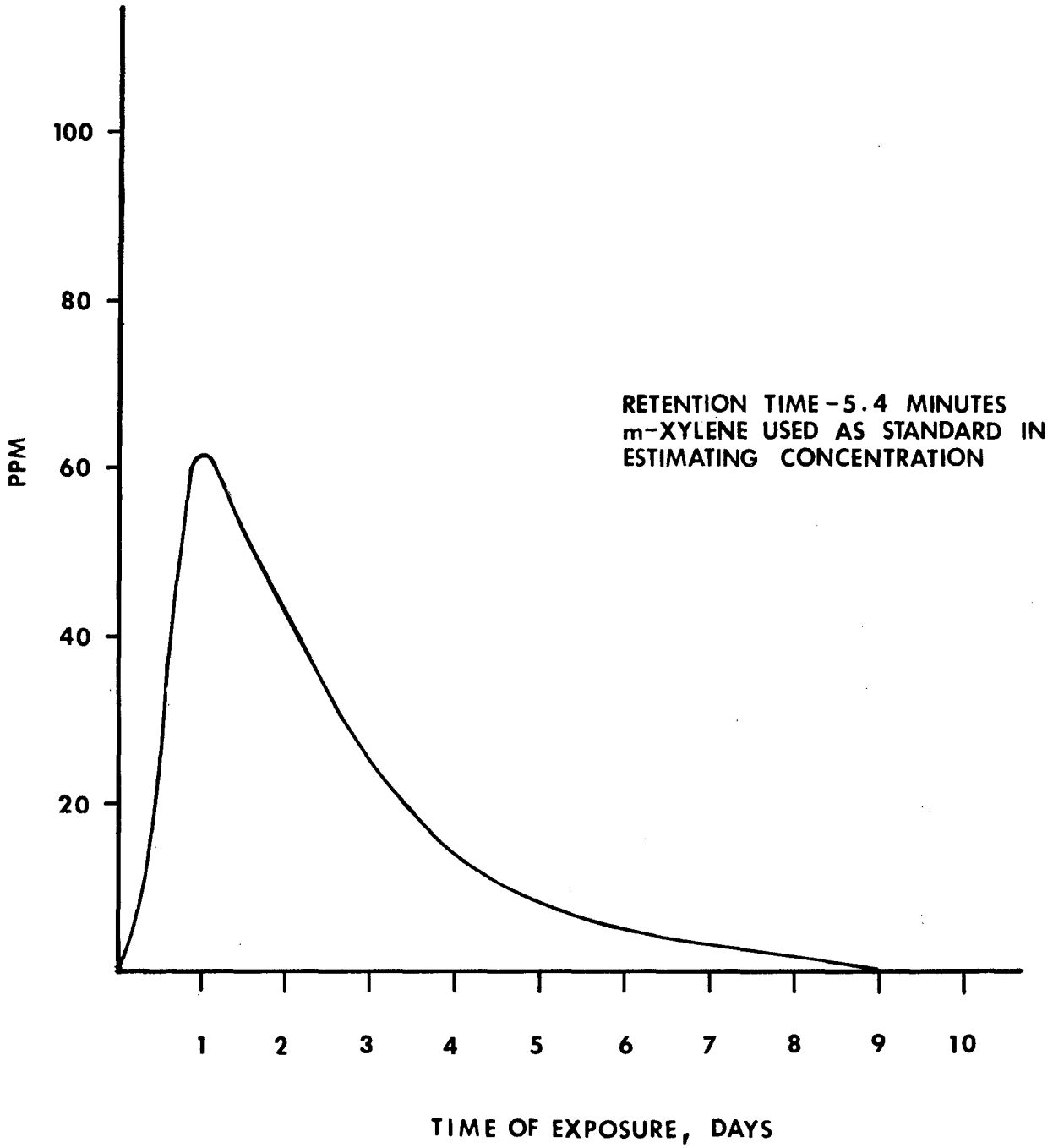


Figure 12

Skylab Materials Study; Relative Gas-Off Pattern of
Contaminant No. 7, Group A

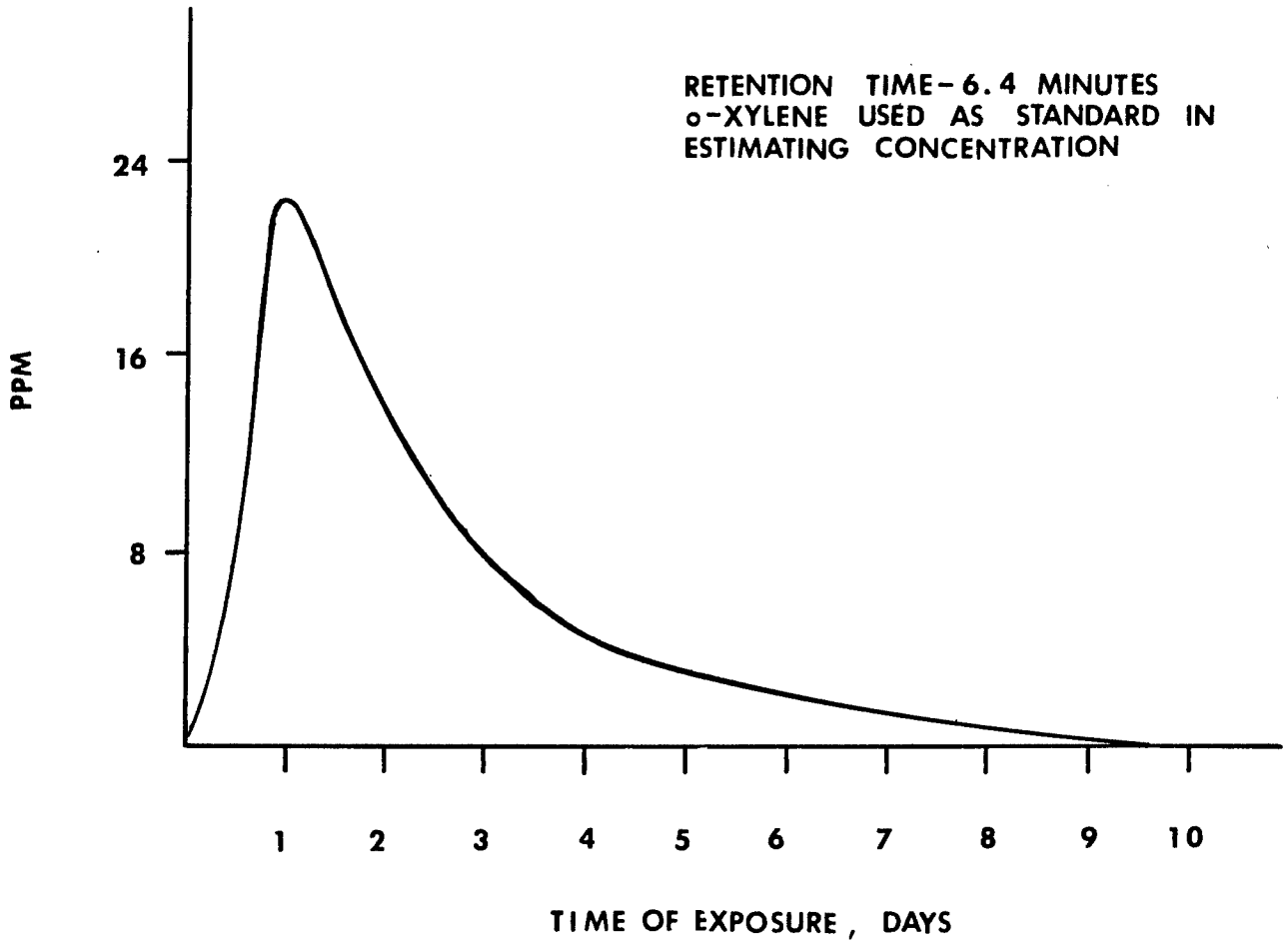


Figure 13

Skylab Materials Study; Relative Gas-Off Pattern of Contaminant No. 8, Group A

COAL TAR VOLATILES STUDY

During the current report period, a 90-day continuous aerosol study was initiated. The study was designed to investigate the potential carcinogenicity of volatile coal tar effluents of coke ovens. Careful examination of pulmonary tissues, liver, skin, and bladder will be made at termination of the experiment to determine any possible pathologic alterations resulting from the exposure challenge.

The animals are being exposed continuously for 90 days to aerosolized coal tar volatiles at concentrations of 0.2, 2.0, 10.0, and 20.0 mg/m^3. Control groups of each species are being maintained for comparison with test animals. The animals are observed daily for general appearance, behavior, signs of toxic stress, and lethality.

Experimental animals include female Sprague-Dawley yearling rats, male and female Sprague-Dawley weanling rats, male ICR mice, male CAF-1 mice, male Golden Syrian hamsters, and female New Zealand white rabbits. The hamsters and rabbits are being exposed only to the highest concentration (20 mg/m^3) while weanling and yearling rats and both groups of mice are being exposed to all contaminant levels.

The animal complement and chamber loads are as follows:

1. 4 Longley Chambers, one concentration level each.

   64 weanling rats (32 males, 32 females)

   64 yearling rats (all females)

   100 mice, male (50 CAF-1, 50 ICR)

2. Two Rochester Chambers (20 mg/m^3)

   24 rabbits (female)

   100 hamsters (male)

   18 yearling rats (female)

   9 weanling rats (male)

   9 weanling rats (female)

   25 CAF-1 mice (male)

   25 ICR mice (male)

3. Control Chambers

   82 weanling rats (41 female, 41 male)

   82 yearling rats (female)

   150 mice, male (75 CAF-1, 75 ICR)

   24 rabbits (female)

   100 hamsters (male)

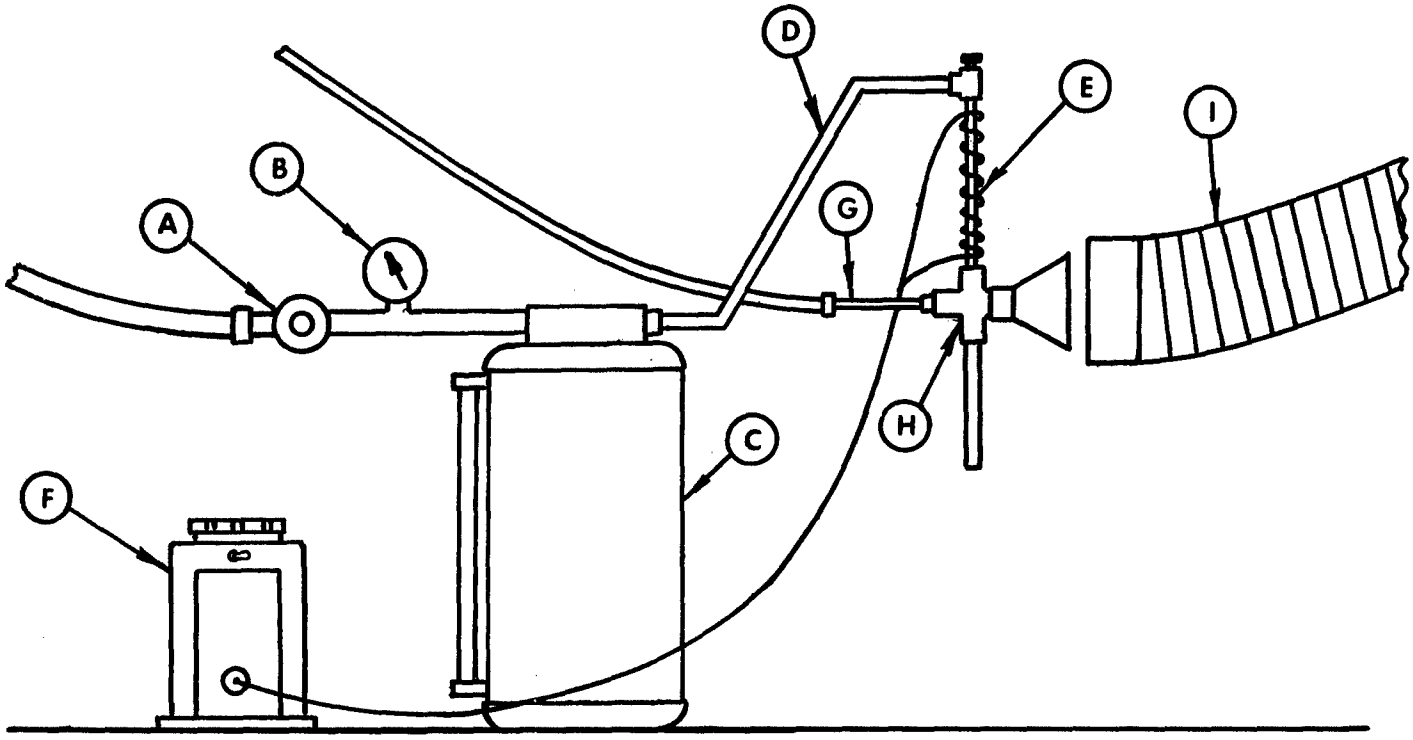
Ten percent of the hamsters, weanling rats, and yearling rats from the 20 mg/m^3 and control groups will be sacrificed at the termination of exposures. The remaining animals will be observed for a minimum of six months before termination of the study. All animals that die are subjected to gross pathological examination. In addition, any animal that dies from the 20 mg/m^3 exposure group will have sections of kidney, liver, and lung submitted to the chemistry section for extraction and analysis of fluorescent compounds. Histologic examination will include liver, lung, genito-urinary tract (including bladder), scrotum, skin, spleen, and bone marrow.

During the six-month postexposure observation period, differential blood cell counts will be performed on 10 rabbits and 10 rats each month (test and controls). Urine analysis for total phenols, creatinine, nitrogen, and solvent extractable fluorescent compounds will be performed on all species at sacrifice.

A fine aerosol of the coal tar is being generated by an apparatus pictured in figure 14. The generating device is made from a 1/8" stainless steel "cross" pipe fitting (H) with the exit opening enlarged to 3/4".

Stainless steel lines, 1/16" O. D., carry the coal tar and air to the generator where it is aerosolized and blown into the chamber air supply line (I). The generator air line is maintained at a constant pressure (35 psi). The quantity of coal tar entering the generator is controlled by the air pressure on the reservoir (C). The chamber concentration, therefore, can be regulated by either increasing or decreasing the pressure on the coal tar reservoir. Prior to entering the generator, the delivery line is subjected to mild heat to facilitate the aerosolization process.

Chamber concentrations are monitored using a combination of a hydrocarbon analyzer and a fluorometer. A standard curve is made using the results of the fluorometric sampling plotted against the readings of the hydrocarbon analyzer. Since the vapor-droplet ratio within the chamber changes occasionally, it is necessary to check the calibration of the analyzer periodically with fluorometric analysis. Fluorometric analysis only is used



(A) Coal Tar Pressure Regulator
(B) Coal Tar Pressure Gauge
(C) Coal Tar Reservoir
(D) Coal Tar Supply Line
(E) Coal Tar Delivery Line Heated with Nichrome Wire
(F) Variable Output Transformer (Variac)
(G) Generator Air Line
(H) Generator Nozzle
(I) Chamber Aerosol Delivery Line

Figure 14

Contaminant Generation System for Aerosolization of Coal Tar Volatiles

to monitor the 0.2 mg/m^3 exposure because the hydrocarbon analyzer is insensitive at this concentration. Chamber concentrations are recorded on an hourly schedule.

An aerosol particle size determination was performed on each chamber at the beginning of the study (6 determinations) following the procedure of Vooren and Meyer (reference 31). Particle sizing will be conducted hereafter on a monthly basis for each aerosol generator.

The exposures are interrupted approximately 15 minutes each day for routine animal maintenance. All leftover animal food is discarded and replaced with a fresh supply. The rodent cages in the Longley Chambers are rotated daily, the top cage going to the bottom and every other cage being moved up one level. The animal cages are changed on a weekly schedule.

This experiment is in progress and will be completed during the next report period.

SECTION III

FACILITIES

The support activities of the THRU essential to the operation of a research laboratory 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 mission of the laboratory. Included herein are special projects in analytical chemistry, training programs, computer program services and engineering modifications to the physical research facilities.

ANALYTICAL CHEMISTRY PROGRAMS

The chemistry department of the THRU performs the routine tasks of monitoring animal exposure chamber concentrations and special analyses on biological samples for non-routine clinical chemistry such as contaminant blood levels. Continuous monitoring methods of analysis are frequently not available for the Air Force materials subjected to toxicity investigations. Therefore, considerable effort is expended in the development and modification of analytical methods. These projects are the subject of this portion of the annual report.

BROMINE PENTAFLUORIDE (BrF_5)

The toxicity of BrF_5 is of interest to the Air Force because it is a member of the group of fluorinated oxidizer compounds which have potential use in rocket propulsion systems. Our previous experience with chlorine trifluoride indicated that the more reactive of these compounds are so unstable in moist

air that any accidental human exposure is to hydrofluoric acid (HF) rather than the oxidizer itself. It was, therefore, decided to investigate the stability of BrF_5 in air of 16-52% relative humidity (RH) and to determine its reaction products under these conditions.

A dilution system was designed and built to vaporize liquid BrF_5 under controlled temperature and pressure conditions and mix it with dry nitrogen or air of controlled RH to give desired concentrations. Using this system, concentrations of 250-1500 ppm BrF_5 were made up in dry nitrogen and infrared absorption curves run in Teflon® cells with silver chloride windows. The absorbances at 644 cm^{-1} were in close agreement with literature values demonstrating that no breakdown of BrF_5 had taken place under these conditions. Absorbances measured at 15 seconds to 15 minutes after dilution showed only small changes, indicating that BrF_5 is stable in dry nitrogen. This series of measurements is depicted in figure 15. It must be noted that the infrared measurement cell is not hermetically sealed and the small leakage of room air accounts for the slight decrease in the peak.

When dilutions were made up in air of 16% RH or greater, no infrared absorption band attributable to BrF_5 was detectable. Instead, a band appeared at 753 cm^{-1} and a brownish substance was deposited on the cell windows. A white aerosol appeared at the system exhaust under these conditions. Past experience indicates that this aerosol is HF mist which has no characteristic infrared absorption. The 753 cm^{-1} band remained after flushing the cell with air which demonstrated that it was associated with the solid on the windows.

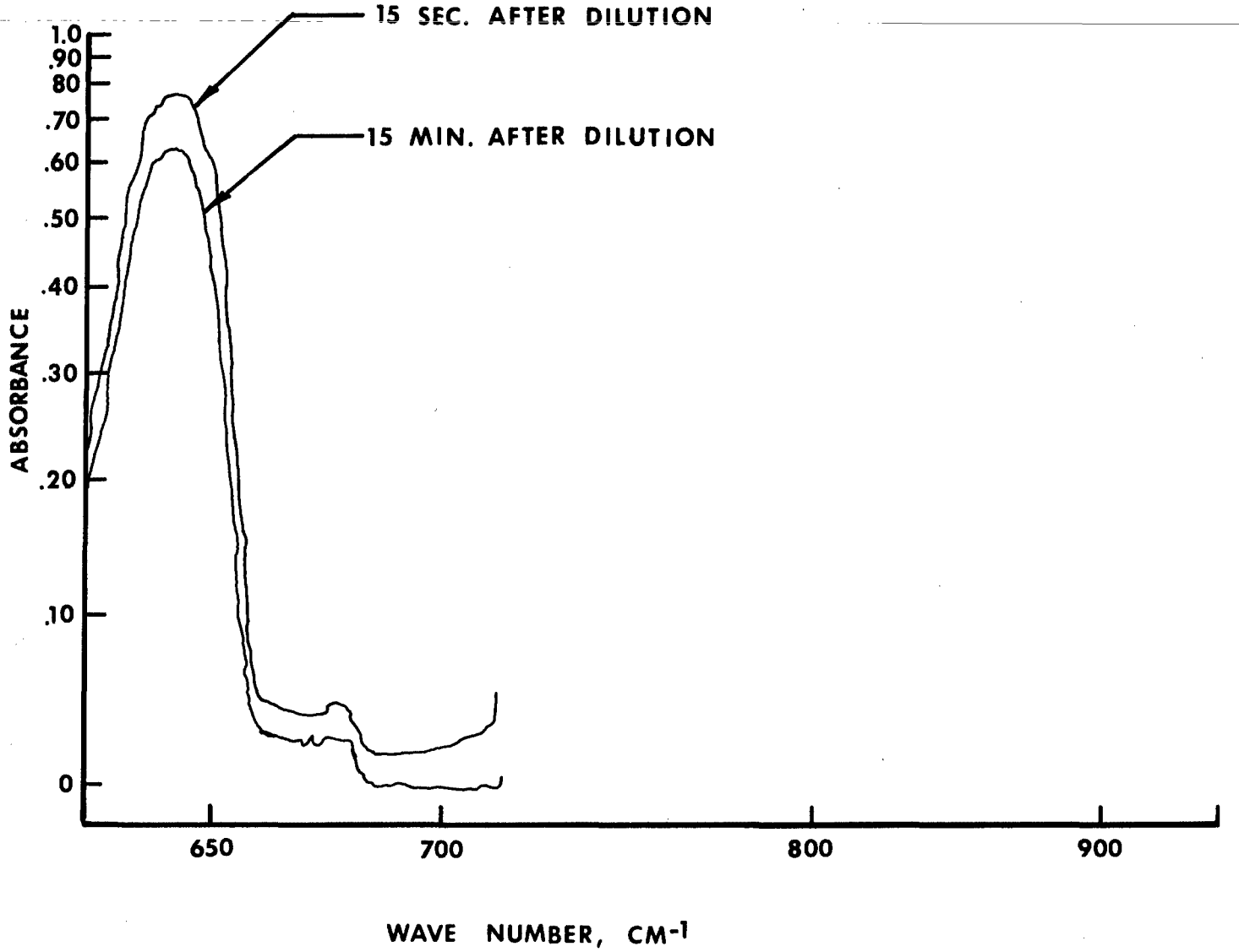


Figure 15

Infrared Time Scans of 2000 ppm BrF_5 in Dry Nitrogen

Figure 16 reproduces the infrared curves obtained with dilutions of 1000 ppm BrF_5 in dry nitrogen and 48% RH air. The typical peak at 644 cm^{-1} appears in dry nitrogen while it is completely lost in normal air to be replaced by the peak at 753 cm^{-1}. This latter peak remains even after flushing the cell, evidence that it is deposited on the cell windows.

A search of the infrared literature revealed that bromate (BrO_3^-) has a strong absorption band at 753 cm^{-1}. Examination of the stainless steel inlet tubes revealed a considerable amount of brownish material deposited as a fluffy powder. Chemical tests for iron proved positive, those for bromide and fluoride negative. A KBr disc of the powder showed strong hydroxyl bands in the infrared.

Inspection of all the evidence produced by this investigation leads to the following interpretation of the reaction of BrF_5 with air containing moisture: BrF_5 almost instantaneously reacts with moisture in air to form bromic acid ($HBrO_3$) and HF.

$$BrF_5 + 3H_2O \longrightarrow HBrO_3 + 5HF$$

The $HBrO_3$ forms bromate salts upon deposition on the infrared cell windows. The brownish powder appears to be hydrated ferric oxide resulting from the hydrolytic breakdown of the iron fluoride passivation film on the stainless steel surfaces.

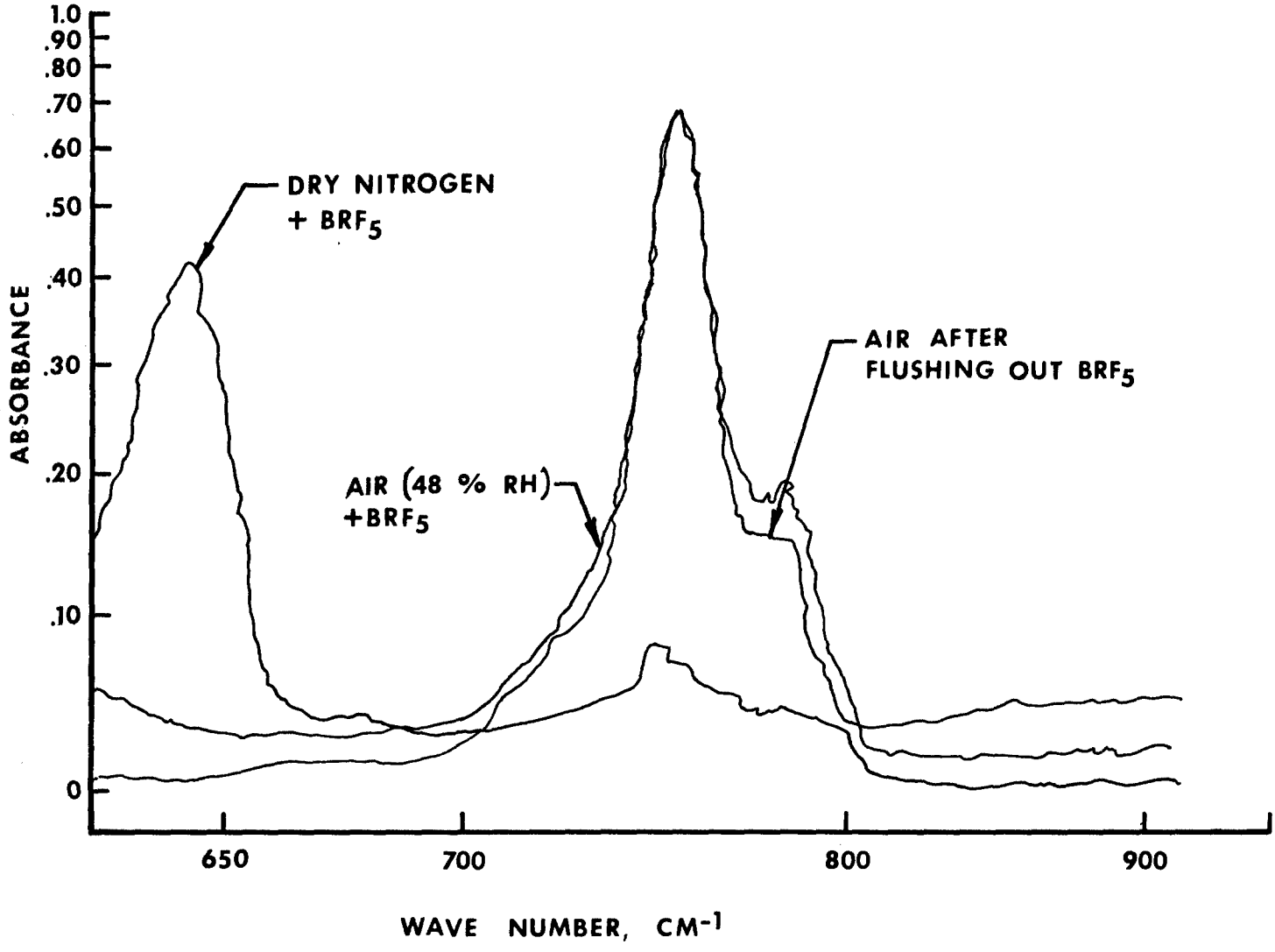


Figure 16

Infrared Scans of 1000 ppm BrF_5 in Dry Nitrogen and Air of 48% Relative Humidity

Since BrO_3^- ion is less toxic than F^- ion and HF is produced in 5 times the molar amount of $HBrO_3$, one can reasonably expect the toxicity of the mixture, and therefore of the BrF_5 in normal room air, to be due to HF. Because of the strong chemical evidence that exposures to BrF_5 would essentially be exposures to HF, it was decided that it would not be practical or productive to proceed with actual toxicological testing of this oxidizer.

MONOMETHYLHYDRAZINE (MMH), IN VITRO EFFECT ON BLOOD

Previous investigations (reference 32) of the in vitro production of methemoglobin by MMH on whole blood had not included mice. Therefore, experiments were performed on blood from this species and also on human, dog, monkey and rat blood to check results obtained in the earlier study.

All reactions were initiated in five milliliters of fresh whole blood (heparinized) with the addition of the monomethylhydrazine (MMH) solution. They were carried out at room temperature, aerobically, and constantly stirred. Aliquots were removed at convenient intervals and a modification of the method of Evelyn and Malloy (reference 33) for methemoglobin was performed immediately. The MMH solution was freshly prepared 0.1 M MMH in 0.2 N HCl. The approximate molar ratios of heme to MMH were 4/1, 3/1, 1/1, and 1/2. These are approximate since the hemoglobin content of the blood from different species is variable.

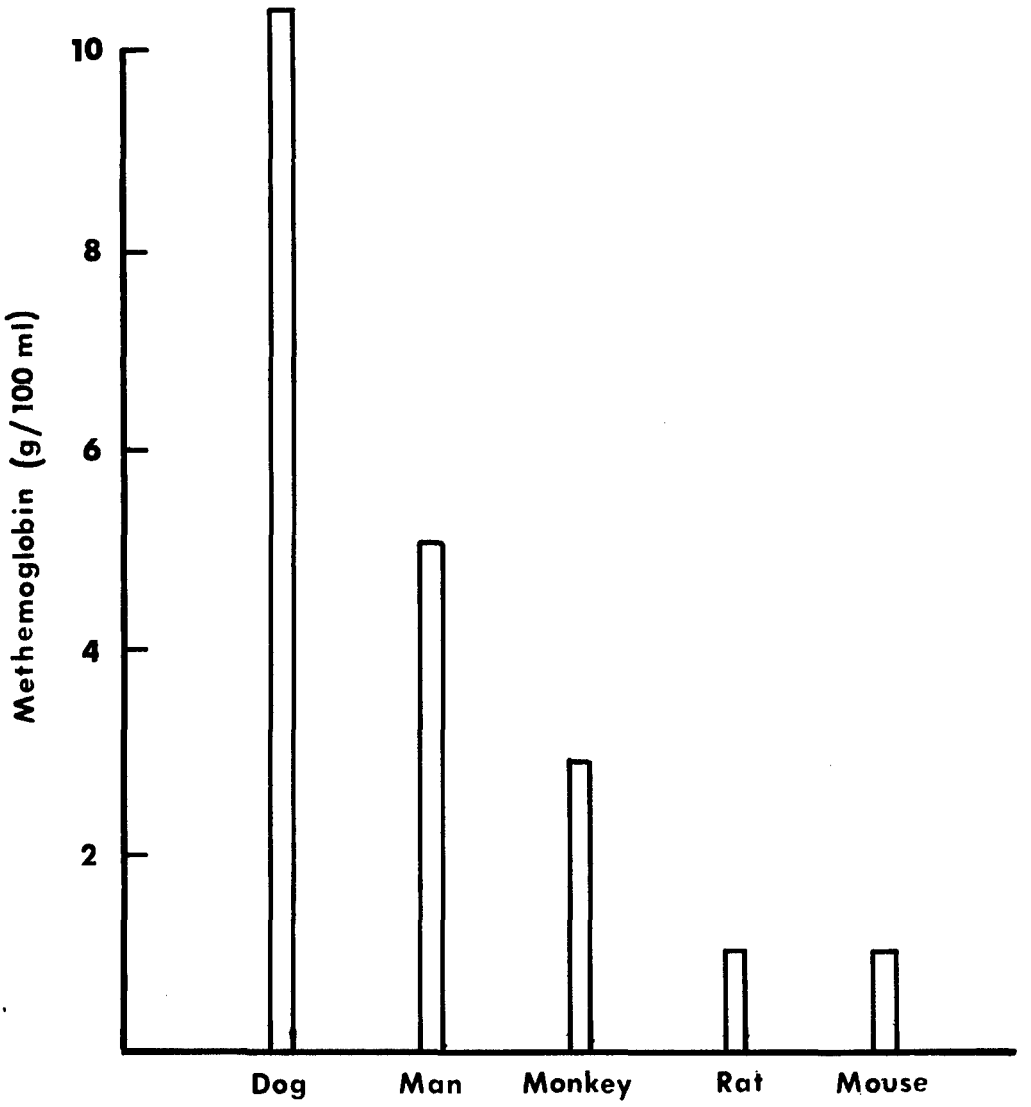
An initial study was performed of reaction conditions and concerned the use of HCl in the dilution of MMH. No significant differences with respect to methemoglobin production were noted whether 0.2 N HCl, 0.02 N HCl, or distilled water was used in freshly prepared MMH solutions. The 0.2 N HCl was maintained for the sake of continuity with previous work.

The results of this series of experiments are summarized in table XXXIII. Figure 17 compares the amount of methemoglobin produced in various species for a molar ratio of heme/MMH of 2/1. Overall, the results are similar to those obtained previously except that a large series of tests now show rat blood to be less sensitive to MMH than monkey blood. Mouse blood appears to be very similar to rat blood in its methemoglobinemic reaction to MMH.

Table XXXIII

Methemoglobin Produced in Blood after 1-Hour Aerobic Reaction with MMH at Room Temperature

Approximate Molar Ratio Heme/MMH	Methemoglobin (g/100 ml)				
	Dog	Man	Monkey	Rat	Mouse
4/1	7.4	3.4			0.7
2/1	10.4	5.1	2.9	1.0	1.0
1/1	14.5	6.7	4.5	2.1	1.5
1/2			5.0	1.8	



Reaction conditions: 2 μM Heme/1 μM MMH, in vitro, aerobic at room temperature for one hour.

Figure 17

Methemoglobin Formation in Blood of Various Species after In Vitro Reaction with MMH

DICHLOROMETHANE (CH_2Cl_2) IN URINE AND BLOOD

The method for analysis of CH_2Cl_2 in blood was detailed in the last annual report. It was adapted to urine to determine whether significant direct excretion of CH_2Cl_2 took place through the kidney during exposure to 5000 ppm CH_2Cl_2. The method is identical to that used for blood except that no anti-coagulant is required in this case. Since the urine samples were taken using catheters leading to polyethylene bags, diffusion of CH_2Cl_2 vapors from the chamber into the urine was a distinct possibility. Therefore, polyethylene bags containing water volumes equal to those expected for the urine samples were placed in the dome during the period of catheterization for use as blanks.

CH_2Cl_2 determinations were also carried out on the blood of dogs and monkeys continuously exposed to 25 and 100 ppm CH_2Cl_2. The absolute precision of this technique was required to be much higher than for 5000 and 1000 ppm. This was accomplished by using a standard addition of 1 ppm CH_2Cl_2 to the sample instead of 50 ppm as used previously. Under these conditions, 0.2 μg/ml CH_2Cl_2 was measurable in blood.

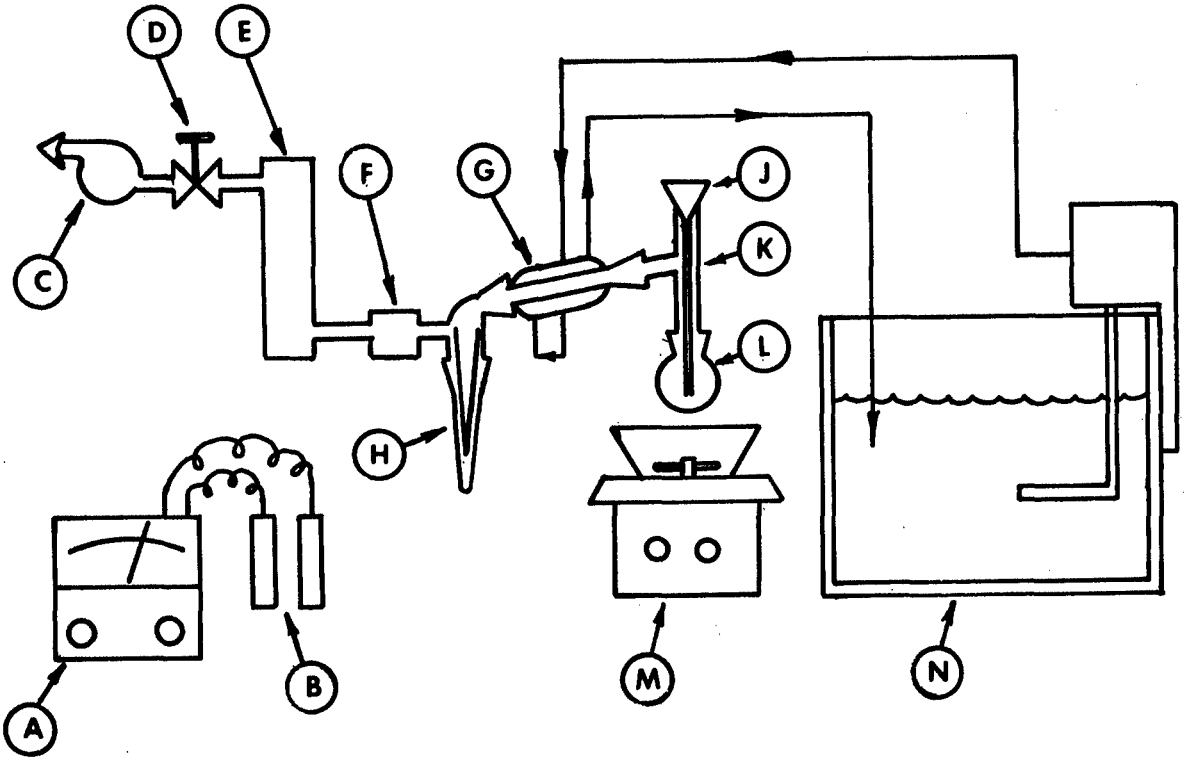
BONE FLUORIDE ANALYSIS AFTER EXPOSURE TO ClF_5

Bone represents a possible area for fluoride deposition after exposure to fluorinated oxidizers. Measurement of bone fluoride was, therefore, selected as one means of assessing the effect of single exposures to proposed emergency exposure limit concentrations of chlorine pentafluoride (ClF_5). The method selected was that of A. M. Pochomis and F. D. Griffith of Haskell Laboratory, E. I. duPont de Nemours and Company, Inc.

Femurs from control monkeys and those exposed to ClF_5 were treated with NaOH solution to remove all tissue and cartilage, rinsed in distilled water and air dried. The bones were then crushed, rinsed with distilled water to remove the marrow, and air dried. Further crushing was accomplished using two stainless steel plates and a 4500 pound press. All oil and grease were removed by soaking each sample in acetone several times and air drying.

Samples of bone weighing 50 mg were introduced into the distilling flask shown in figure 18. An oxidizing acid mixture of concentrated $HClO_4$ and concentrated H_2SO_4 in a 1:1 ratio was made up, and 5.0 ml of the mixture added to the distilling flask by means of the funnel shown in the figure. Distillation into the receiver was accomplished by placing the flask in an oil bath at 135 C for 20 minutes. A negative pressure was maintained on the system by the vacuum pump. The receiver was a microimpinger containing 6.0 ml of 0.2 M NaOH. After distillation, the condenser and microimpinger bubbler were rinsed into the impinger with a standard acetate buffer used in ion electrode measurements. The volume was brought to 10 ml with the acetate buffer, and fluoride ion concentration was measured using a specific ion electrode.

Calibration of the whole system was accomplished by weighing appropriate amounts of CaF_2 into the flask and going through the procedure. Figure 19 presents the curve obtained. It is obvious that most of the fluoride is not recovered. However, the response is linear with added fluoride and appears to be satisfactory for the analysis of bone.



(A) Ion Meter
(B) Electrodes
(C) Vacuum Pump
(D) Valve
(E) Rotometer
(F) Filter
(H) Impinger
(J) Funnel
(K) Distilling Head
(L) Sample Flask
(M) Stirrer and Oil Bath
(N) Water Bath and Pump

Figure 18

Analytical System for Analysis of Fluoride in Bone

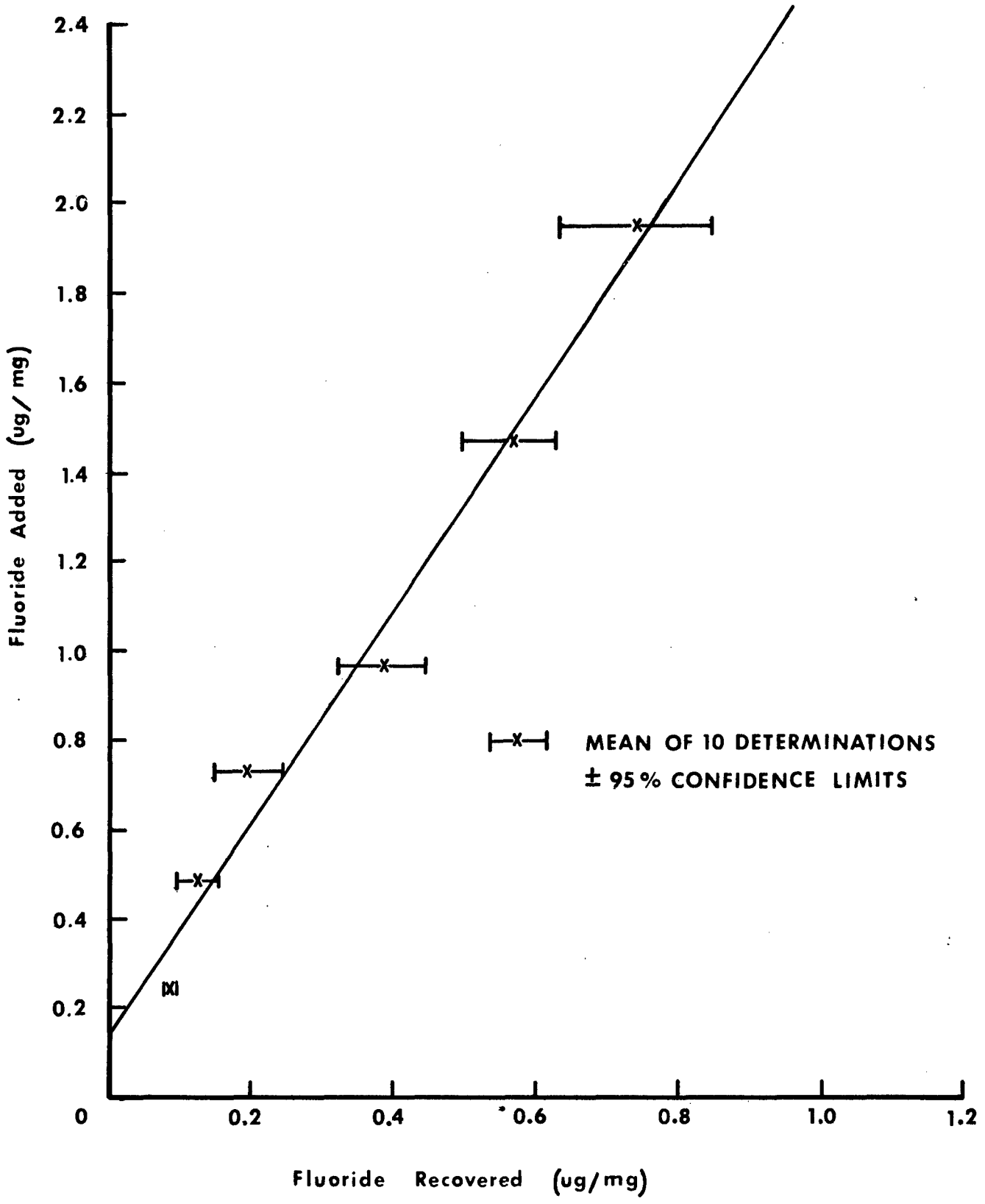


Figure 19

Calibration Curve - Recovery of CaF_2 Weighed into Distilling Flask

When monkey femurs were analyzed, the results shown in table XXXIV were obtained demonstrating that no buildup of fluoride in the bone had occurred.

Table XXXIV

Fluoride Concentrations of Monkey Femurs

ClF_5 Conc., ppm	Exposure Time minutes	Bone Fluoride Conc., mg/g
30	10	0.41 ± 0.08
23	30	0.37 ± 0.05
10.5	60	0.39 ± 0.08
Control		0.42 ± 0.11

FORMIC ACID IN URINE

Since Kuzelova and Vlasak (reference 14) had reported abnormally high concentrations of formic acid in the urine of workers exposed to CH_2Cl_2, it was decided to investigate the occurrence of urinary formic acid in dogs continuously exposed to 5000 ppm CH_2Cl_2. The method combined a modification of the standard AOAC (reference 34) steam distillation procedure with Grant's (reference 35) method for the microdetermination of formic acid.

Combined Method for Formic Acid in Urine

1. To a 60 ml urine sample, add 30 ml 20% phosphotungstic acid and 10 ml 1.0 N H_2SO_4.

2. Shake, allow time for complete precipitation and coagulation, and filter through a Buchner funnel.

3. Make acid to Congo Red paper, if necessary using conc. H_2SO_4 and add 75 ml to a steam distillation flask.

4. Steam distill for 30 minutes during which time 100 ml distillate should be collected. (If standard 0.1 N formic acid is distilled, acid recovery is 40.5%).

5. Add 80 mg magnesium ribbon to clean 10 ml test tube in ice bath and then 0.50 ml distillate from Step 4.

6. While keeping in ice bath add 0.50 ml conc. HCl in ten 50 μl aliquots, 1 to 3 minutes apart with stirring.

7. One minute after last HCl addition, add 1.50 ml chromotropic acid solution (0.6 g chromotropic acid + 20 ml H_2O + 180 ml conc. H_2SO_4).

8. Wrap in aluminum foil to protect from light, and heat for 30 minutes in a boiling water bath.

9. Centrifuge off white ppt. and read absorbance of supernatant at 570 mμ.

10. Multiply formic acid concentration determined in Step 9 by 5.0 to obtain concentration in urine.

A calibration curve was determined using a series of sodium formate dilutions and is shown in figure 20. When 15 μg/ml formic acid was added to urine and carried through the whole analytical procedure, a 97% recovery was obtained. Since the procedure depends upon reduction of formic acid to formaldehyde for color development, any formaldehyde originally present in the urine would interfere. Therefore, the procedure was carried out on urine from exposed dogs without the addition of magnesium ribbon to reduce formic acid. The absence of any measurable absorbance demonstrated that formaldehyde was not present.

CYANIDE (CN^-) ANALYSIS

Because of the increasing use of plastics such as polyurethane in aircraft interiors, interest is high in HCN as a possibly significant toxic product of aircraft fires. Pyrolysis of some nitrogen containing plastics does produce HCN, and this gas alone or in combination with CO may be a significant added danger in aircraft crashes with accompanying fire. Since the time available for evacuation during a fire is short, data are needed on the toxic effects of very short-term (2.5-7.5 minutes) exposure to HCN. In addition, it would be helpful to medical examiners and pathologists to know what concentration of blood CN^- is lethal in the presence of elevated carboxyhemoglobin levels. Therefore, short-term exposures of rodents to HCN are being planned, and a method for the analysis of blood CN^- is being developed.

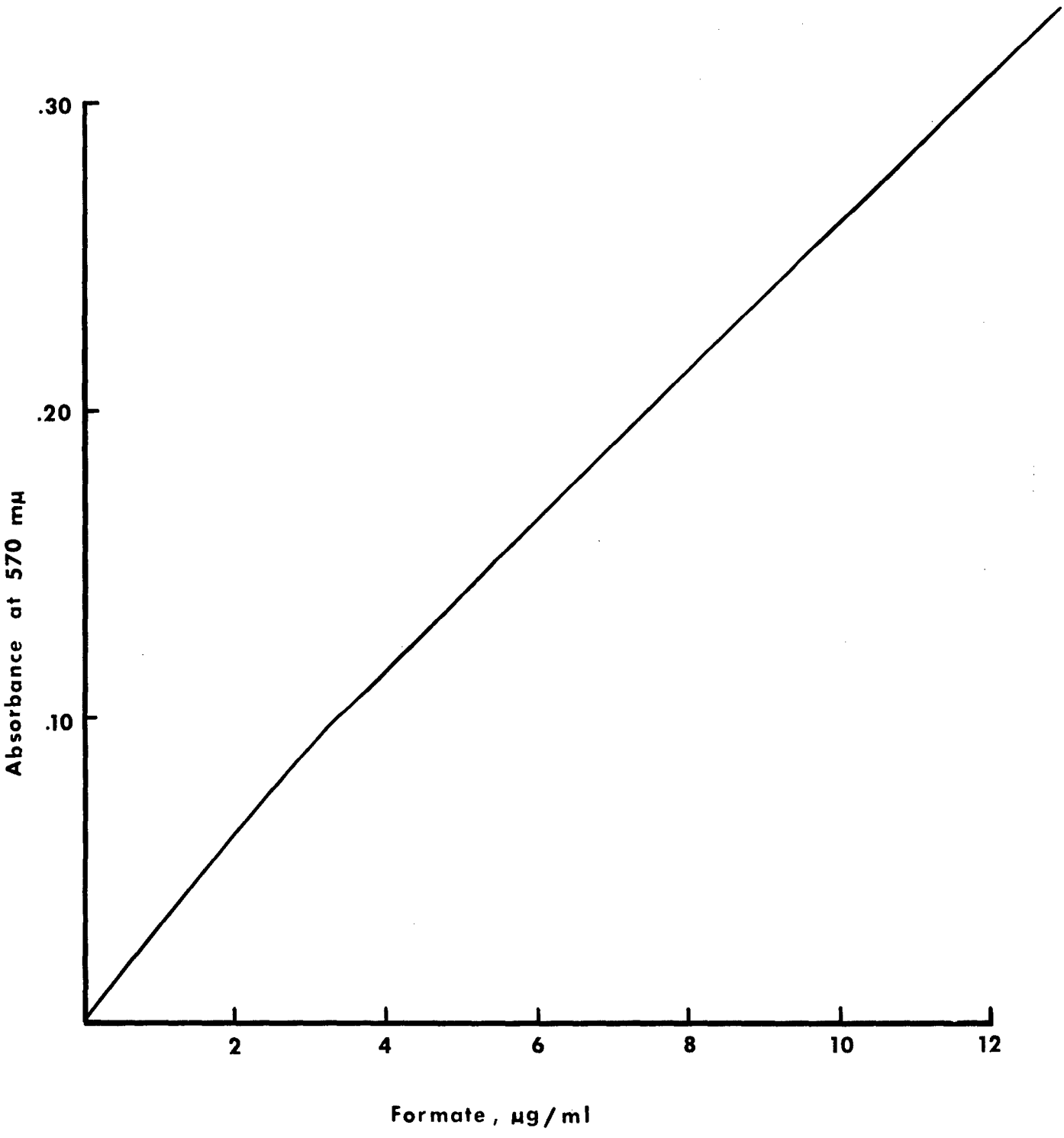


Figure 20

Absorbance at 570 mμ versus Formate Conc., μg/ml after Chromotropic Acid Color Development

A technique which appeared to hold promise is the use of the cyanide ion specific electrode for measurement after separation of HCN from the blood in a Conway diffusion dish. The method consists of pipetting 1.0 ml of rat whole blood into the outer ring of the diffusion dish. One ml of 0.10 N NaOH is pipetted into the center chamber of the Conway dish. The glass plate top is sealed on the dish using silicone grease. A small aperture is left to allow access to the outer ring. One-half ml of 10% H_2SO_4 is injected into the blood through this aperture, then the plate slid into place and the dish gently swirled to mix the blood and acid. The dishes are allowed to sit for 3.0 hours to permit the HCN liberated by the acid to diffuse and be absorbed by the base. After this time, the basic solution containing cyanide ion is pipetted to a micromeasurement dish. Cyanide ion concentrations are read using an electrode and a pH meter in the expanded millivolt position. The electrode pair is calibrated using standard cyanide solutions with total ionic concentrations similar to that expected in the samples.

Preliminary tests of the procedure have been made by adding sodium cyanide solutions to rat blood to give concentrations of 0.52, 1.04, 2.60 and 5.20 μg/ml CN^- in the blood. Results demonstrated that percent recovery decreased with increasing CN^- concentrations as shown in table XXXV. However, precision of the method appears to be quite good.

Table XXXV

Recovery of CN^- Added to Rat Blood

Known Conc. µg/ml	Found, µg/ml	Mean, µg/ml	% Recovery
0.52	0.46, 0.41, 0.46, 0.45	0.45	87
1.04	0.81, 0.75, 0.79, 0.81	0.79	76
2.60	1.49, 1.60, 1.78	1.62	62
5.20	2.64, 2.42, 2.60, 2.41	2.52	49

CARBOXYHEMOGLOBIN ANALYSIS

The discovery that carbon monoxide (CO) was produced in the blood of animals exposed to high concentrations of CH_2Cl_2 excited interest in the effects of continuous CH_2Cl_2 exposure at lower levels (25 and 100 ppm). In order to obtain dependable carboxyhemoglobin values for a number of species at these low concentrations, it was necessary to perform a large number of determinations which were not possible using the accurate but time-consuming gas chromatographic procedure. Therefore, a commercial instrument, the CO-Oximeter® was purchased to accomplish this task.

This instrument is a spectrophotometer which automatically dilutes whole blood, measures the absorbance at three very precisely determined wavelengths and calculates % oxygen saturation, % carbon monoxide saturation and gram % hemoglobin. However, the computer circuitry in the instrument is designed for human blood and gives erroneous results when used for other species. Calibration was therefore necessary for dog and monkey blood

using the previously developed gas chromatographic technique as standard. If reduced hemoglobin is present in animal blood, the CO-Oximeter® % CO reading is no longer a linear function of the carbon monoxide saturation, and calibration is a much more complex task. Since we were not interested in measuring % oxygen saturation, the simplest procedure was to saturate the blood with oxygen prior to reading in the CO-Oximeter®. This eliminates reduced hemoglobin and limits the number of species in blood to two; oxyhemoglobin and carboxyhemoglobin. Saturation of the blood with oxygen does not affect the carboxyhemoglobin content, since the blood has a much greater affinity for CO than for oxygen. Figure 21 details the calibration curves obtained for dog and monkey blood. It is seen that monkey blood gives values much closer to human than dog. This, of course, is not unexpected considering the phylogenetic relationships. It is also obvious that saturation of the blood with oxygen results in perfectly linear CO calibration curves. Routine examination of the instrument flow cell is necessary since blood clots may form and cause large errors in measurement.

Total hemoglobin, read directly on the CO-Oximeter®, appears to be quite accurate and precise. Ten replicate determinations were performed on dog and monkey blood by the CO-Oximeter® and the standard cyanmethemoglobin procedure. The means obtained using the CO-Oximeter® were 18.6 g% and 13.0 g% for dogs and monkeys respectively, compared to 18.5 g% and 12.6 g% obtained by the cyanmethemoglobin procedure.

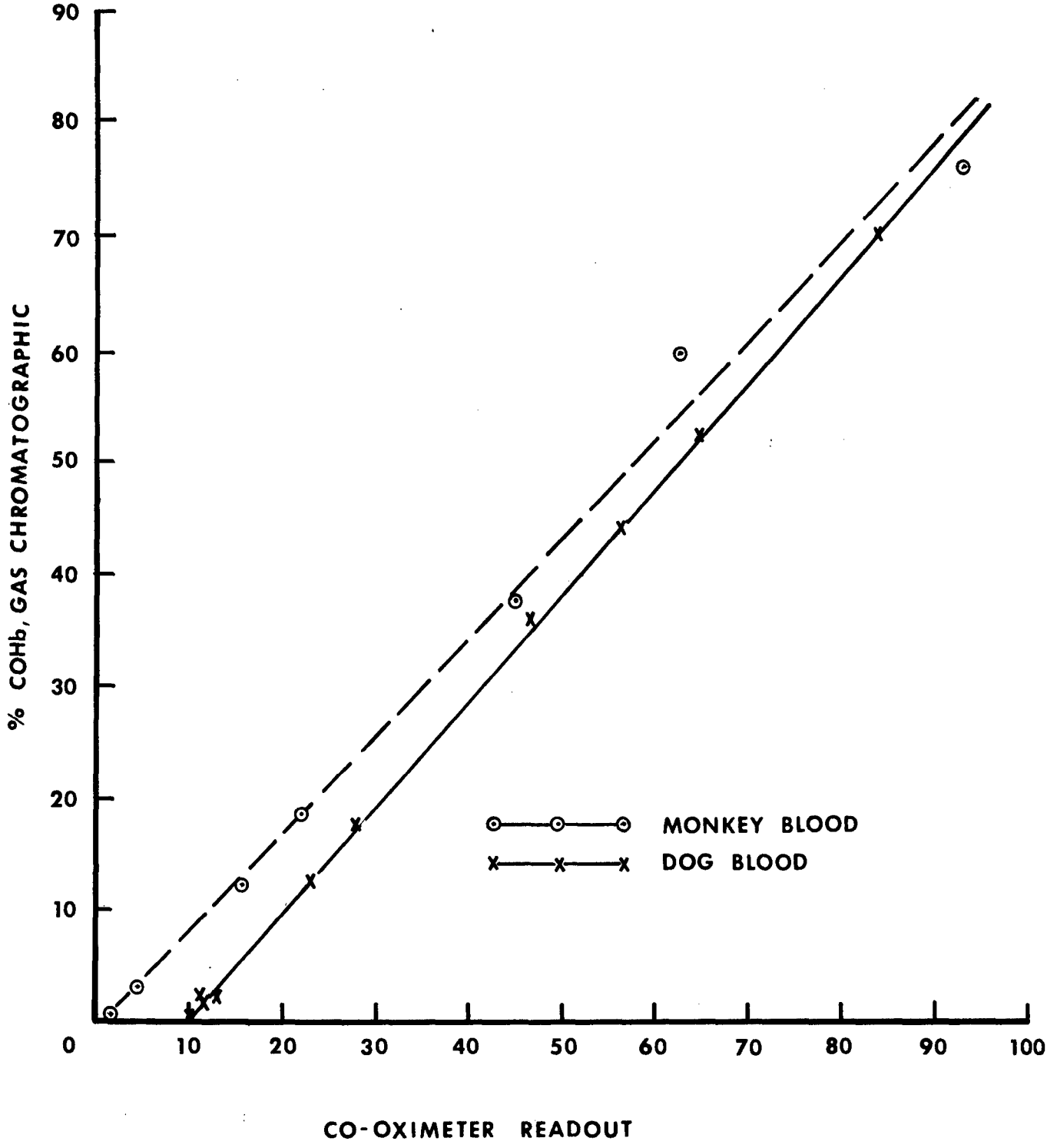


Figure 21

Calibration Curves, Gas Chromatographic % CO Saturation versus CO-Oximeter® Readout

COAL TAR VOLATILES (CTV)

The most difficult problem confronting the THRU in conducting exposures to a coal tar aerosol was the generation of the aerosol itself. Coal tar is a complex viscous mixture of coal pyrolysis products which contains a significant amount of dispersed solids. When commercial oil sprayers or foggers were used in attempts to produce satisfactory aerosols, frequent clogging of nozzles interrupted the generation. Commercial ultrasonic nebulizers worked satisfactorily for a short time, then failed. This probably occurred because the more volatile materials were being expelled from the nebulizer leaving a thick residue on the transducer which eventually caused it to stop.

Initial experimentation demonstrated that dilution of the coal tar with an equal volume of benzene decreased the viscosity sufficiently for the solids to be separated by centrifugation. The benzene added could then be removed from the tar by fractional distillation. When the product of these operations was aerosolized through a nozzle similar to the one described by Horton et al. (reference 36) no clogging took place, and a 7-day test at 20 mg/m^3 coal tar concentration was conducted without serious problems. It was, therefore, decided to separate the solids from the coal tar in this manner for conducting the 90-day exposure. Centrifugation took place in 100 ml bottles at 2500 rpm in an International Size 2 centrifuge. Distillation was carried out in one-liter batches using a three cap Snyder fractionationing column, removing everything coming off up to 85 C.

A sample of the solids removed from the coal tar was sent for characterization to an independent laboratory with long experience in the coke oven and coal tar field. Elemental analysis gave 92.33% carbon and 2.13% hydrogen by weight. This corresponds to a molar ratio of carbon to hydrogen of about 4/1. The separated solids therefore represent a material very close to pure carbon in chemical constitution. An ash determination on the solids showed 0.66% present after combustion.

A further responsibility of the THRU with regard to coal tar was the analytical separation of the tar into arbitrary groups such as polycyclic, aliphatic, phenols, quinones and neutrals. Initial experiments indicated that separation of the solids was a necessary prologue to further treatment if intractable emulsions were to be avoided. Therefore, 2 volumes of benzene were added to 1 volume of coal tar, mixed and centrifuged to remove the solids. The supernatant was extracted with 10% aqueous NaOH. Troublesome emulsions forming at this point, were broken up by immersing the separatory funnels in a 75 C water bath. After separation of the aqueous phase and washing, the organic phase was extracted with 20% aqueous H_2SO_4. Surprisingly, solids appeared to precipitate from the system and emulsions again formed. Heating to 75 C in the water bath broke the emulsion, but filtration was necessary to remove the solids. The solids were washed with benzene and dried. An infrared spectrum of the solids was recorded after making a KBr pellet. The infrared curve was analyzed by a computer with a large number of spectra in its memory bank and characterized as being most similar to 2, 4, 6 trimethyl quinoline sulfate.

Since extraction of coal tar solution with aqueous acid led to the formation of a precipitate, it was thought that a cleaner separation might be made if the basic materials in coal tar were precipitated using an acid solution in an organic solvent which was miscible with benzene. Therefore, ethyl ether was saturated with HCl gas to give approximately 20% w/v solution. About 1.5 ml of this solution was added to 20 ml of the 1 + 2 coal tar-benzene solution and a voluminous, easily filtrable brown precipitate was obtained. No acid reaction was produced on pH test paper after addition of the ether acid, but further addition of 1 ml more did produce a solution acid to the test paper and caused the precipitation of a black, slightly tarry precipitate. Infrared curves of the two precipitates were almost identical and similar to the quinoline-like curve which had been obtained previously.

Since precipitation of basic substances by HCl in ether appeared successful, an attempt was made to precipitate acidic substances in coal tar using alcoholic NaOH. A saturated solution of NaOH in absolute methanol was made up and added dropwise to a filtered benzene solution of coal tar. No precipitate formed and there appeared to be two liquid phases, i.e., the alcoholic NaOH was not soluble in benzene. This approach was therefore abandoned.

As a result of these experiments, a scheme for the separation of acidic and basic constituents of coal tar has been worked out:

1. Dissolve 1 volume coal tar in 2 volumes benzene, centrifuge, isolate solids.

2. Precipitate basic fraction from supernatant using ether HCl.

3. Extract acidic fraction from filtration using aqueous NaOH.

This work is still in progress and the results will be reported at a later date.

ALUMINUM PHOSPHIDE (AlP)

There are a number of metalic phosphides on the Department of Transportation list of transportable chemical agents. It is well known that these compounds are hazardous primarily because they generate phosphine (PH_3) on exposure to water or moist air. Aluminum phosphide was chosen as representative of these compounds, and experiments were run to determine the rate of generation of PH_3 from AlP in air of 50% RH.

No bubbles were observed when AlP was placed in water. It was necessary to make the solution acid (below pH 3.0) before noticeable generation of PH_3 took place. However, placing solid AlP in an air atmosphere of 55% RH resulted in a fairly linear rate of generation of PH_3 as measured by gas chromatography.

In order to follow the reaction of AlP with water vapor, 4 millimoles of AlP (232 mg) in a watch glass were placed in a 100-liter mylar bag containing air of 55% relative humidity. The relative humidity was determined with

a sling psycrometer before pumping into the bag. The reaction progress was followed by hot wire gas chromatographic analysis using a 4' x 1/4" SS Porapak Q column at 135 C and He flow rate of 40 cc/minute. The PH_3 peak from a 5 ml injection has a 1.3 minute retention time. The peak height was compared to standards prepared from pure PH_3 and air. The 100-liter bag containing AlP was continuously mixed to prevent local high concentration of PH_3 or low concentrations of water vapor.

Figure 22 plots the PH_3 generated with time under these conditions. In 55% RH air, PH_3 was formed at the rate of 2.5 mg/min/g AlP. If excess AlP were present in a confined space so that all the moisture in the 55% RH air were consumed, the final concentration of PH_3 could reach 4800 ppm.

ENGINEERING PROGRAMS

A comprehensive preventive maintenance program in action for the past two years has been very effective, resulting in no experimental time loss due to equipment malfunction. This has shifted expenditures from emergency repairs to preventive maintenance with an overall result of improved operation at no increase in cost.

Engineering modifications described in this section of the report have been made to upgrade experimental equipment, eliminate safety hazards, and simplify operational factors.

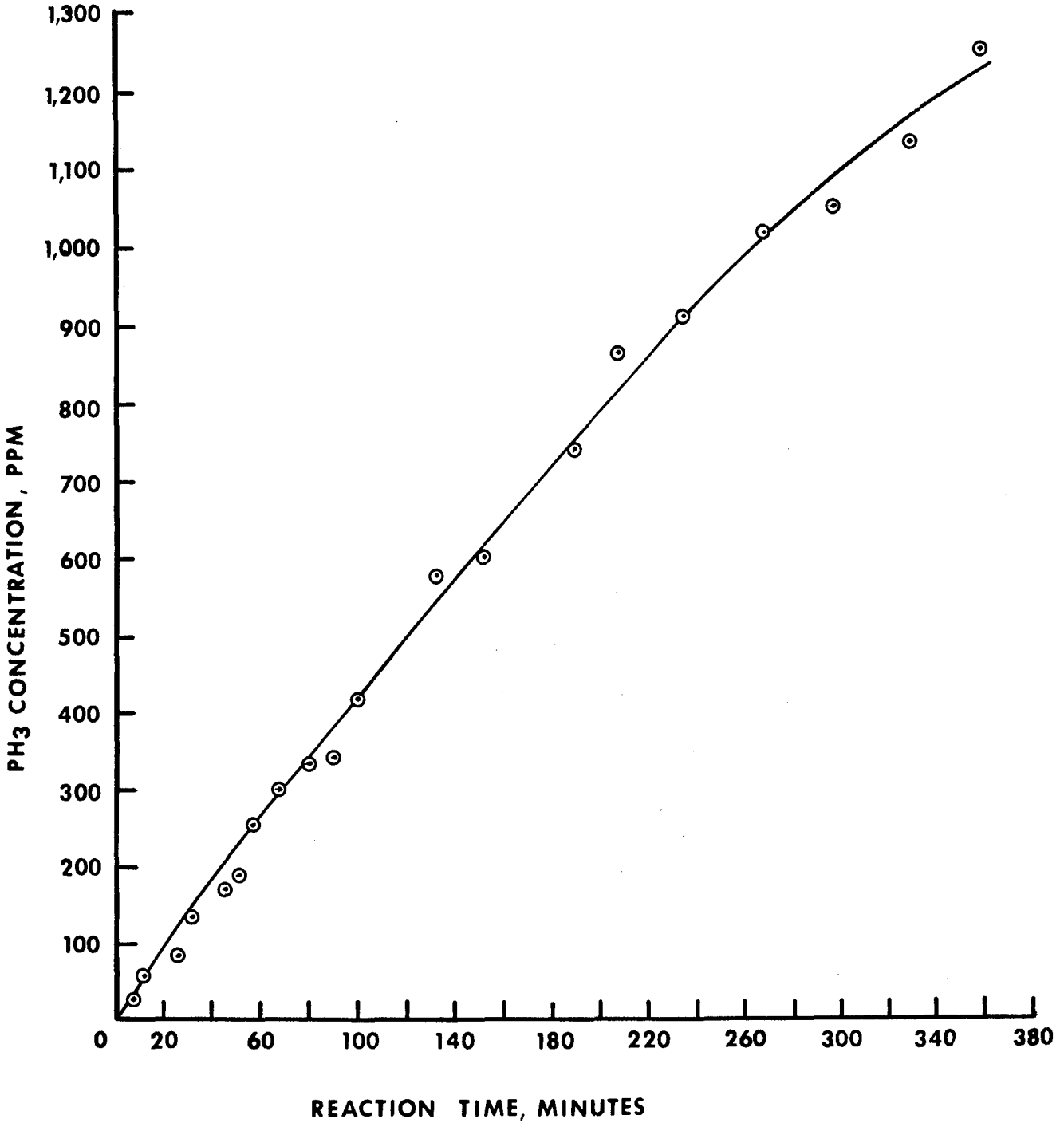


Figure 22

Reaction Rate of AlP with Water Vapor (55% RH Air)

EMERGENCY AIR AND BREATHING OXYGEN SUPPLY SYSTEM

The emergency air cylinders and emergency breathing oxygen cylinders were enclosed in a cabinet (figure 23) similar to the one used for instrument and span gas calibration (reference 37). The air cylinders supply the chamber pneumatic controls and dome O-rings and are automatically activated when a failure of the main air compressor occurs. The emergency air supply system is detailed in figure 24. The emergency breathing oxygen system, shown in figure 25, is automatically activated by a failure in the regular breathing oxygen supply from the LOX pad. With the completion of the emergency supply systems, a centralized location is provided for all compressed gas cylinders needed for dome operation during an emergency.

RELATIVE HUMIDITY BLOWER MOTORS

The RH monitoring and control system for the Thomas Domes includes a small DC electric fan for providing air flow across the wet-bulb thermometer to achieve accurate measurements. In the past this had proven to be a considerable maintenance problem due to frequent motor and bearing failure. After an exhaustive search a motor of equal size with oxygen compatible electrical specifications was selected as a replacement. A single motor was procured initially and installed for a test run. After trouble free operation for a period considerably longer than those experienced with the previous motors, they were deemed acceptable. The replacement motors were installed and have provided excellent service.

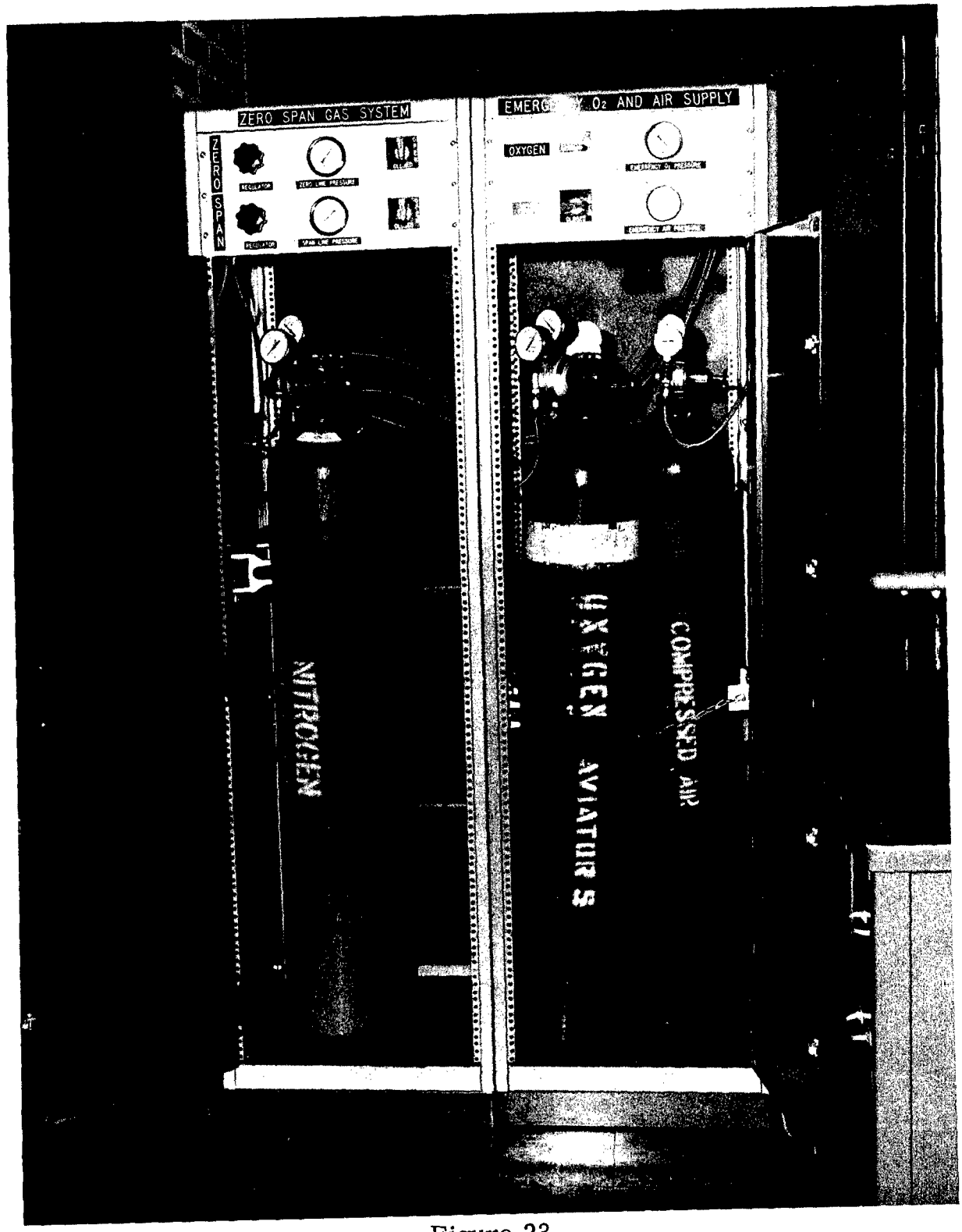


Figure 23

Emergency Air and Breathing Oxygen Supply System

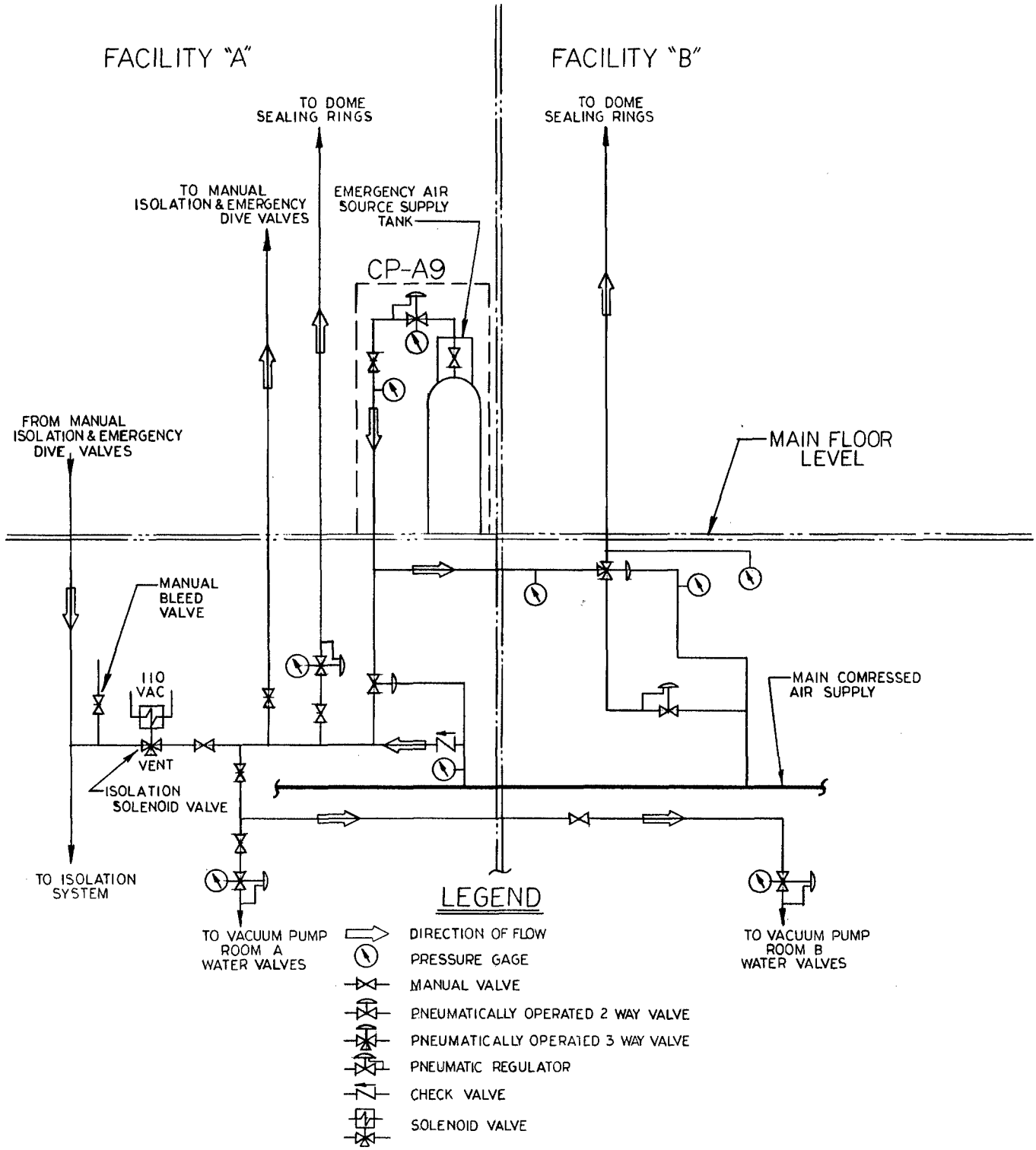


Figure 24

Auxiliary Air System Layout

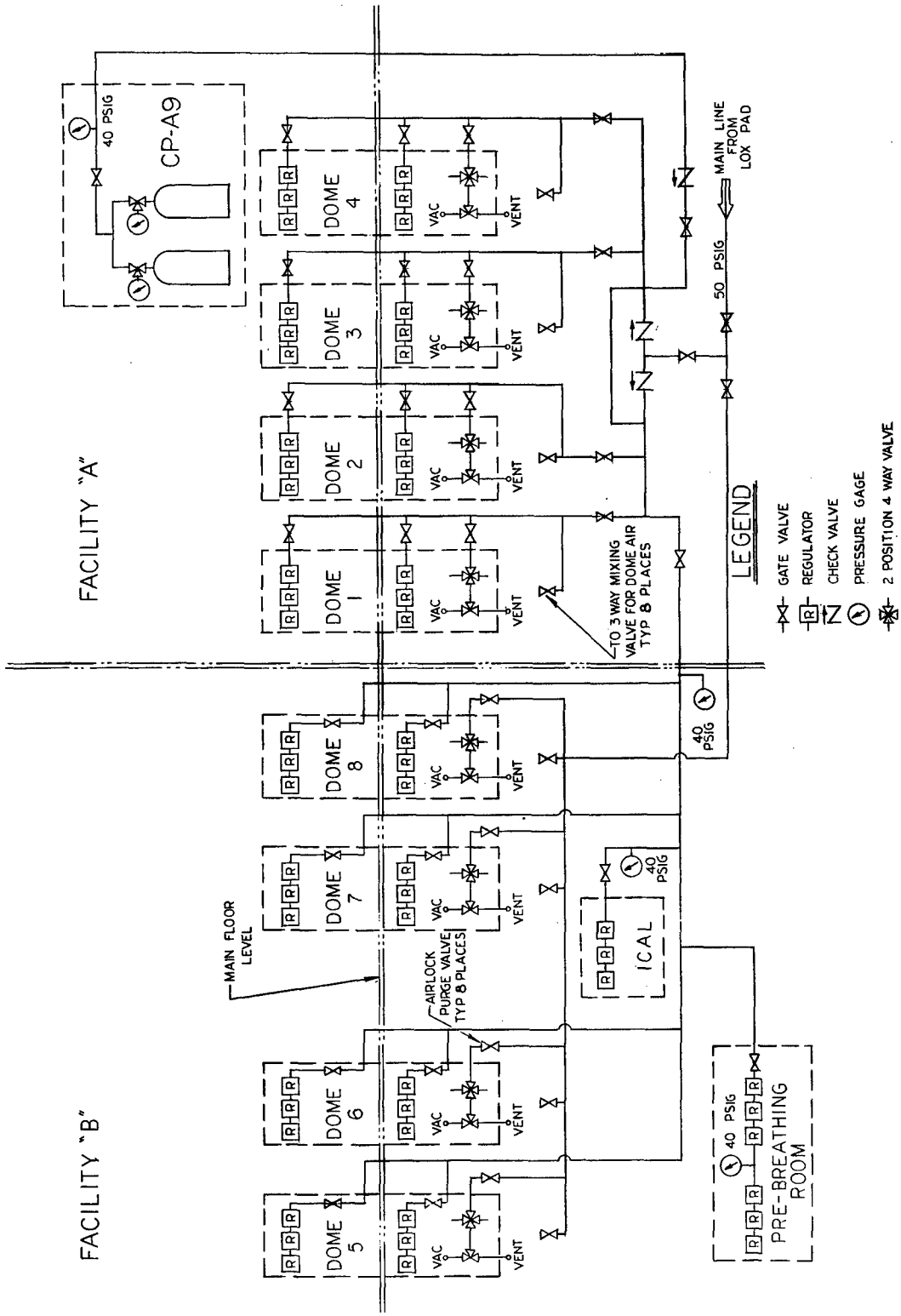


Figure 25

Emergency Breathing Oxygen System

LOAD CELL TEST STAND

The complete animal weighing system in the domes and animal holding rooms, described in last year's annual report, includes 22 load cells. Testing and calibrating these at their remote locations is difficult and time consuming. Therefore, a mounting support and rack for load cell testing was fabricated and installed adjacent to the main weighing console in Dome Room B. Load cells may now be brought in groups to this point for centralized calibration and testing. Details of the stand are shown in figure 26.

DIA-PUMP TEST STAND

A large number of atmospheric sampling pumps are used in the altitude exposure system for analysis of dome conditions. To facilitate ease of pump repair and maintenance and to reduce down-time, a stand was designed and fabricated to fit the special requirements of repairing these sample pumps. All required testing devices are included on the panel and sufficient room is provided to be used for spare parts storage in the bottom of the cabinet.

VACUUM PUMP MODIFICATIONS

Facility B vacuum pumps were initially installed with solenoid valves in their water supply lines to shut automatically when the pumps were stopped. Under this arrangement, continual problems were experienced with water leakage through the solenoids during times the pumps were off. Water flow through the cold pumps caused some corrosion which led to jamming of the

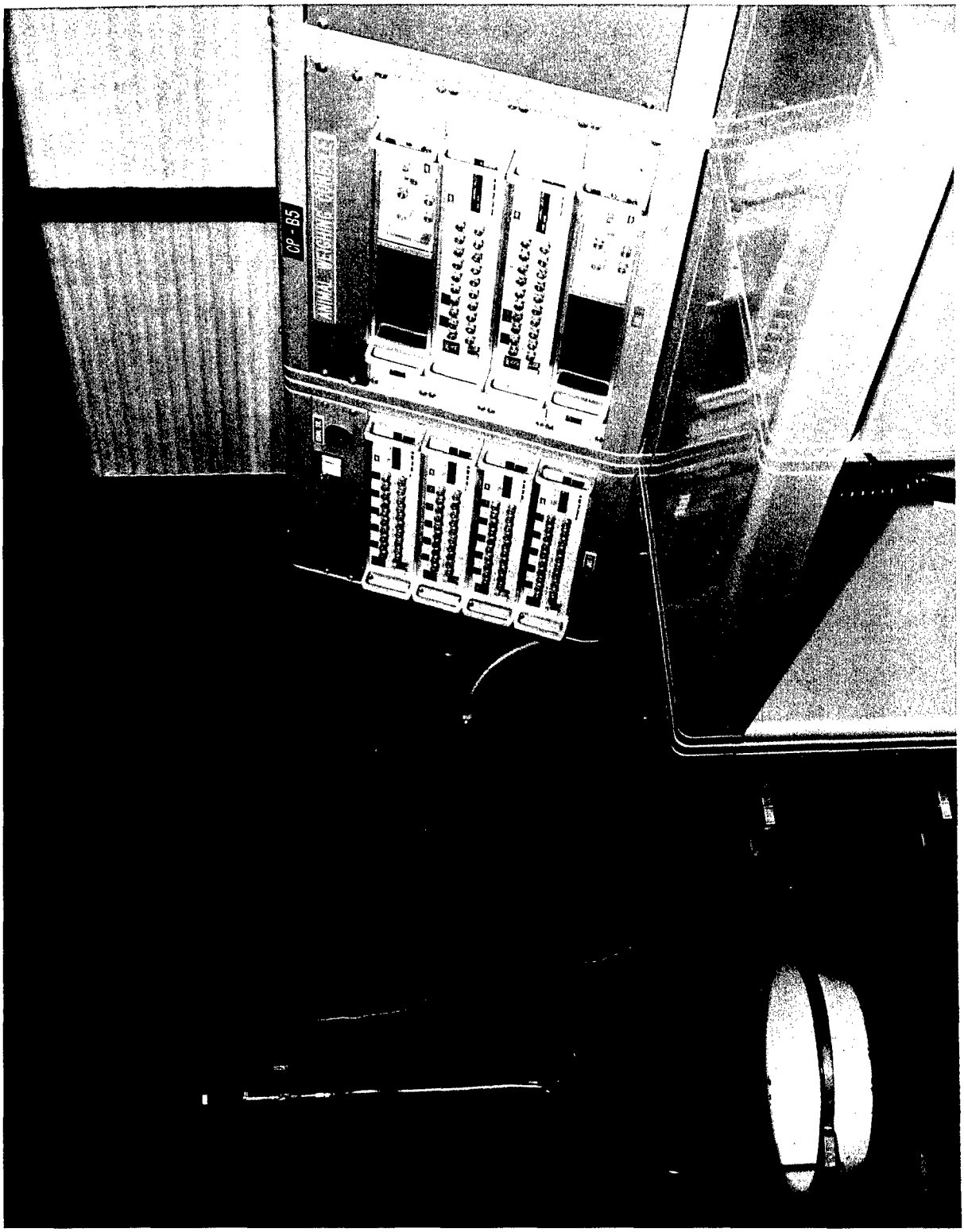


Figure 26

Load Cell Test Stand

pumps on restart. To achieve a more positive and reliable shut-off of water the solenoids were replaced by regular globe valves fitted with pneumatic operators. The pneumatic operators are air pressure activated by a solenoid valve wired to the vacuum pump starter switch. Each vacuum pump is supplied with a pneumatic-operated valve and solenoid arrangement. The emergency air supply system is automatically connected to the pneumatic operator if there is a loss of the primary compressor air supply. Details of the system are shown in figure 27.

Facility A vacuum pumps, as installed, had no check valves in the lines between the individual pumps and the domes being operated. Thus, the possibility existed that a non-running pump could provide leakage paths in the system or could run in reverse if the manual shut-off valve were opened. In order to prevent this, a swing check valve was installed in each inlet line. Because of space limitations vertical counter-balanced check valves were utilized. These were installed after a minor modification to the vacuum pump inlet plumbing.

Pneumatic operated globe valves were installed in the inlet water lines to each vacuum pump exactly as in Facility B to provide a positive shut-off in the water supply to each pump. These replaced manual valves originally installed.

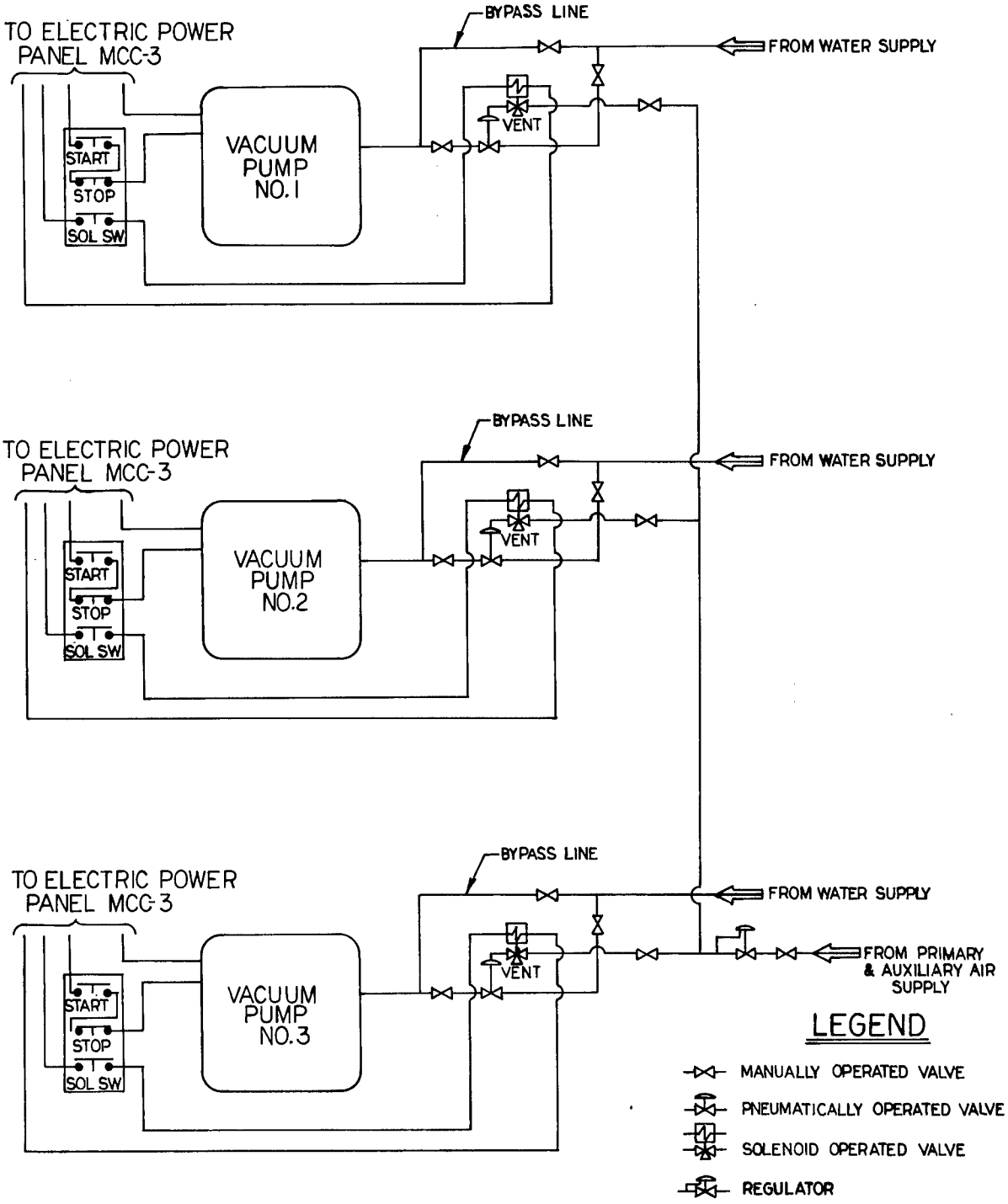


Figure 27

Vacuum Pump Solenoid Valve System - Facility B

MECHANICAL EQUIPMENT ROOM ALARM PANEL

Malfunctioning of building operating equipment located in the mechanical equipment room was annunciated on an alarm panel located only in that room. Both visual and sound alarms are activated; however, the panel is somewhat isolated from the more occupied areas of the building and subsequently the alarms could easily be unnoticed. The main alarm system associated with the domes is distributed throughout the building and it was therefore decided to interconnect the two alarm panels. A single point was selected on the main alarm panel and designated Mechanical Equipment Room. Equipment failures located in the mechanical equipment room are now annunciated throughout the building in the same manner as dome equipment failures.

AMBIENT WARNING LIGHT SYSTEM MODIFICATION

When experiments with hazardous materials are being conducted in the ambient laboratory, flashing red lights at all entrances illuminate signs reading "Hazard, Do Not Enter." The same controls activating the lights also turn on the air exhaust fan in the laboratory ceiling. Existing controls for this system were located outside the north and inside the south exits of the ambient laboratory. After experience in utilizing the system, it was felt that an additional control should be added convenient to the operating area. This control was therefore added adjacent to the Rochester Chambers on the west wall of the ambient laboratory.

THOMAS DOME HUMIDIFIER MODIFICATION

The steam supply system for control of dome relative humidity was modified by replacement of the control assemblies on each dome unit. The old assemblies were situated too close to the steam valves with the result that they were overheated by the steam passing through. This led to frequent diaphragm failure and steam leakage. The new control units were installed further away from the valves and provide positive steam shut-off using normal control signal pressure. The modified units have provided more reliable and accurate control of dome relative humidity with reduced maintenance requirements.

WELDER OUTLET DISTRIBUTION SYSTEM

In the past, needs frequently arose for use of the welder in areas of the facility remote from the single welder outlet in the machine shop. Many of these were difficult and some impossible to accommodate because of the extreme lengths of electrical cable necessary.

A distribution system was designed to extend the necessary power requirements to appropriate locations within the facility. Four 480 volt AC 50 amp outlets were installed in the following locations:

1. Motor Control Center 1 - Ambient Laboratory
2. Motor Control Center 2 - Basement, Facility A
3. Motor Control Center 4 - Basement, Facility B
4. North Wall of Dome Room B

Each outlet is supplied from an individual breaker and is labeled for welder use only. Locations of the outlets are shown in figure 28.

CONTAMINANT GENERATION HOOD - ROCHESTER CHAMBERS

A hood to contain contaminant generation apparatus was designed and fabricated for the Rochester Chambers. The hood was constructed to enclose the top structure of the contaminant bench that was fabricated previously for the ambient chambers. Electrical outlets are located in the bench superstructure which is constructed of Unistrut® support pieces enabling generation apparatus to be installed using standard fittings. The hood is shown in figure 29.

HOOD MODIFICATION - BUILDING 429 COAL TAR FRACTIONATION

A chemical hood in Building 429 was overhauled to provide the required ventilation and utilities required for coal tar fractionation. The hood as originally installed had insufficient air removal for the required face velocity. Face velocity was measured to be approximately 40 LFPM when the desired rate was between 100 and 150 LFPM. Evaluation of the existing blower and hood dimensions revealed that a replacement blower was needed. In addition, in the original installation the bottom exhaust opening in the hood had not been connected to the exhaust blower. The duct work connecting the hood to the blower was smaller in size than the inlet of the blower resulting in restriction of the inlet air flow. A new blower was procured of sufficient flow volume and suction pressure to achieve the theoretical face velocity desired. An exhaust plenum

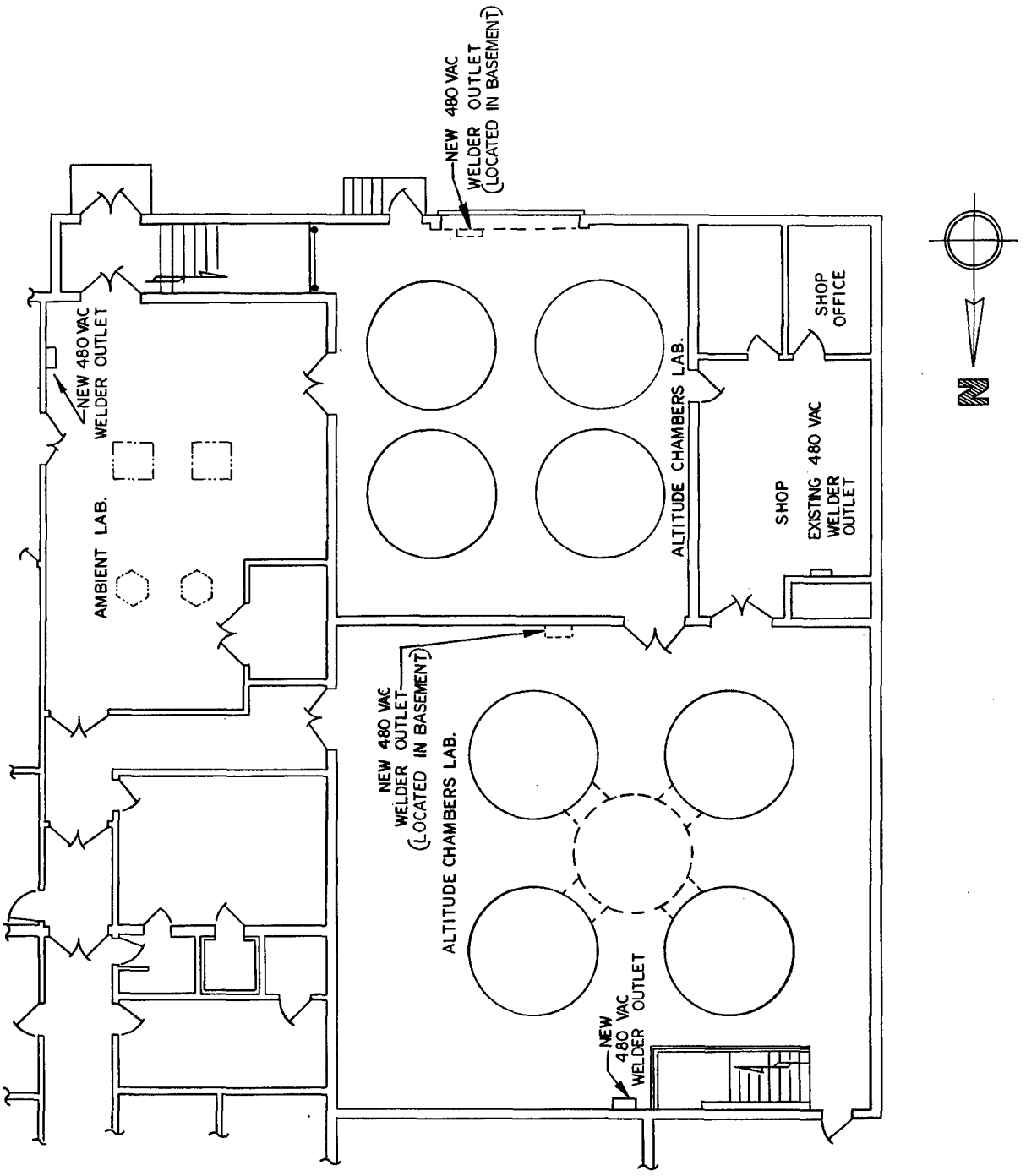


Figure 28

Welder Outlet Distribution System

Figure 29

Ventilation Hood - Rochester Chambers

was designed and fabricated to connect both bottom and top exhausts to the new blower. The interconnecting duct had the same diameter as the blower inlet. After installation of these items the hood had an operating face velocity of approximately 100-125 LFPM, which was satisfactory for the intended use. In addition, rods for support of distillation or extraction apparatus were designed and installed in the interior of the hood. Electrical lines, water shut-off valves, expansion joints and an emergency shower head were either renovated or replaced.

CYLINDER PAD EXTENSION AND RAMP

Several modifications were accomplished to the cylinder storage areas at the rear of the building. As reported in the last annual report two cylinder storage areas were constructed at the rear of the building. Moving and storage of cylinders was reported to be hazardous because of several different levels to enter the building. A concrete pad was constructed to extend both the length and width of the cylinder storage pad. Ramps are constructed to enable the moving of cylinders from ground level to the cylinder storage level and from this area to the first floor level of the building entrance.

AMBIENT CHAMBER MODIFICATIONS - CTV STUDIES

In preparation for the CTV studies, a number of projects were carried out to accommodate the high density of animal loading planned for the Longley and Rochester Chambers.

Fifty rodent and 12 rabbit cages were constructed. The cages are equipped with internal feeders and quick disconnect water assemblies so that the maximum amount of chamber space is utilized for animal storage. Support racks were also constructed in the Longley Chambers.

An independent animal watering system was installed in each of the ambient laboratory chambers. Each system consists of a filter, regulator, gage, valves and the required number of quick disconnect assemblies to fit all the cages. The new water lines were constructed using stainless steel tubing with welded connections where possible.

New rotating cage support bases were constructed for the Rochester Chambers using stainless steel mesh and angle. Each base consists of two seimicircular portions bolted at the diameter. Special ball bearings were installed on the radial arms of the base support to allow free rotation. Horizontally placed thrust springs on the radial arms adjust to slight irregularities in the circumference of the base.

To facilitate handling of the animals and cleaning of the chambers Rochester Chamber B was rotated to provide easy access to both doors. The original location of the door adjacent to the newly constructed contaminant generation hood had prevented its opening.

The existing ambient chamber filter boxes contained coarse filters for the removal of large particles and animal hair from the chamber exhaust air. The CTV aerosol particles are much too small to be captured by the coarse filter. Therefore, an absolute filter was installed downstream of the coarse filter to collect and remove coal tar from the exhaust air and protect the differential pressure flow transducer from clogging.

The six CTV aerosol exposure concentrations in the ambient chambers were to be analyzed using three hydrocarbon detectors, an arrangement which required the alternate delivery of samples from two experiments to each detector. To provide alternate sampling of 30-minute periods, three timer switching boxes were constructed and mounted between the chamber to be sampled. The boxes provide the capability of overriding the timer switch and sampling continuously from one chamber for calibration purposes. Indicator lights on the boxes show which of the two available points is being sampled. A typical unit is shown in figure 30.

CABIN MATERIALS SCREENING SYSTEM

Reexamination of the design of the cabin materials screening system from an engineering viewpoint revealed a number of areas where further optimization was possible. The chillers which condensed water vapor from the atmosphere in the closed loop for control of relative humidity were not operating at maximum efficiency for a number of reasons:

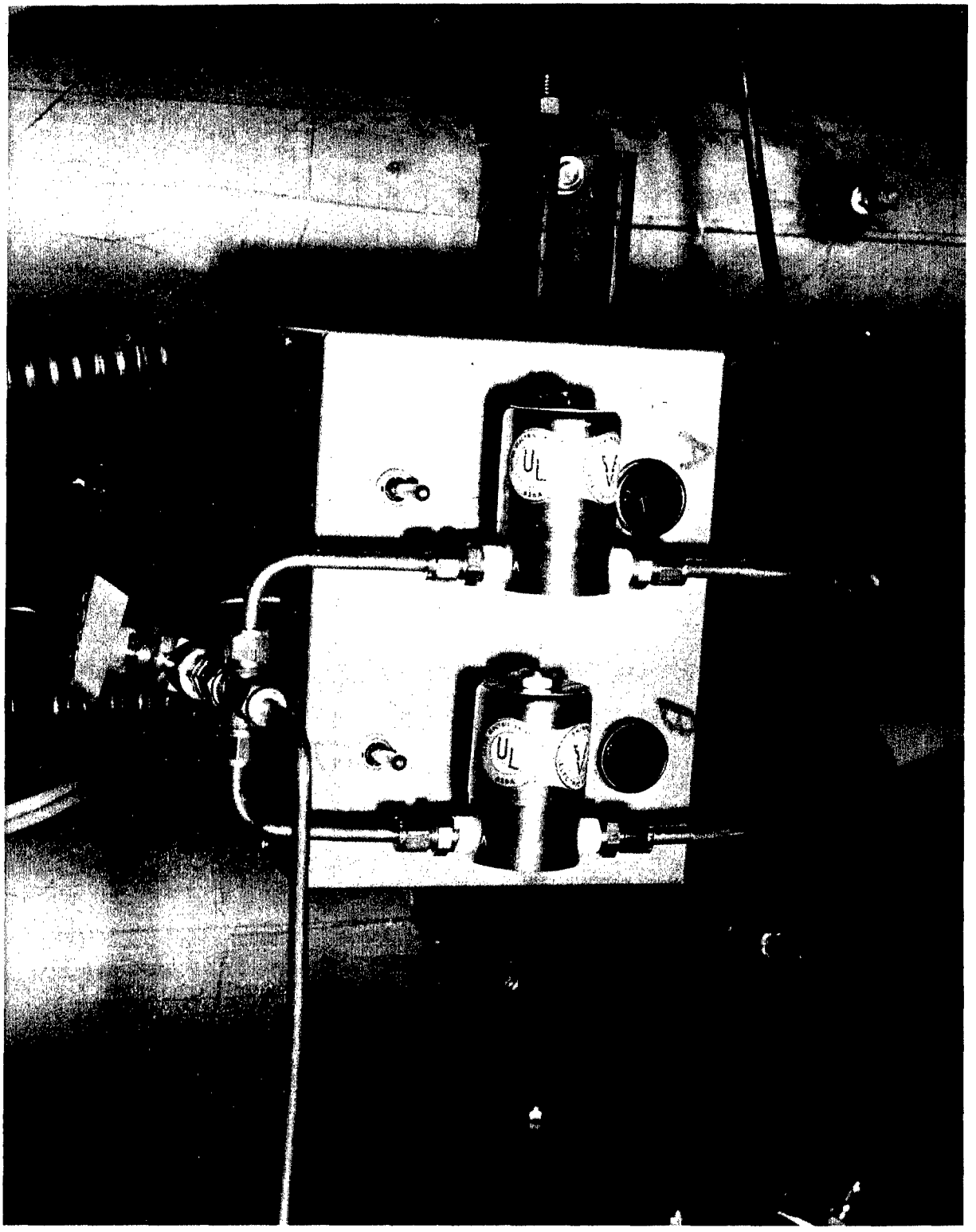


Figure 30

CTV Analyzer Sequential Switching Unit

1. They were located at a high point in the system leading to some carry-over of liquid condensate into low points instead of complete drainage.

2. The air was being directed through the chilled water channels and vice versa resulting in decreased efficiency.

3. Recalculation of the water vapor load in the loops revealed that one chiller did not have sufficient capacity to condense it satisfactorily.

Therefore, an additional chiller was added to each loop in parallel to the original; the pair of chillers was relocated to a low point in the system; and the water and air paths were reversed. Relocating the chillers to a low point in the system resulted in a higher static pressure on them. A design change in the chiller drip leg was necessary to achieve satisfactory drainage under the higher static pressure conditions.

The laminar flow element monitoring system atmosphere flow had been located at a low point, and condensate collection there resulted in erratic flow readings. The flow element was therefore relocated to a point higher than the chiller, placing it at a dry point in the system.

The ovens used to heat the samples of cabin materials were of the vacuum type which did not seal very well under the slight pressure difference between the Thomas Dome and the testing loop. To solve the leakage occurring around the oven doors, they were removed and replaced by an aluminum plate which was bolted to the oven after the materials were inserted.

In addition, the loops were examined carefully and all questionable components replaced. Testing of all three loops with full complements of rodents demonstrated that relative humidity was being controlled at satisfactory levels and that atmosphere leaks into or out of the loops met design criteria.

COMPUTER PROGRAM SERVICES

Problems with the new CDC 6600 Series computer continued into the current report year. Data reduction and analyses were delayed and often required program revision to achieve satisfactory results. These problems were finally resolved and data flow has improved substantially.

Major new additions to the biological data analysis systems during the past year included the programming and automated treatment of SMA-12® derived biochemical determinations. This program may, however, be abandoned due to conversion of analytical instrumentation to newer and more precise analyzers. Considerable effort was expended in the development of computer programs to fit several commercially available systems for treatment and

analysis of morphometric cytology data. These programs are useful in the quantitation of structural changes at both the cellular and organelle level where previously only qualitative changes could be described.

Property accountability data systems have been extensively revised during the past year in conjunction with the transfer of many items of installed equipment to Air Force accountability and actual relief from responsibility for outdated and defunct items of property.

TRAINING PROGRAMS

The Laboratory Operations and Animal Care Training Programs described in last year's annual report were continued this year as programmed. Phase I and Phase II training cycles were scheduled for newly hired chamber technicians and veteran technicians requiring refresher courses.

Monthly Emergency Training Procedures are unannounced deliberate equipment failures or simulated emergencies involving personnel in the Thomas Domes and exposure laboratory area. The following is a list of these emergency training procedures covered during this reporting period:

Date	Procedure	Personnel Participation*
July 1971	Fire in the Airlock with Entrants	A, B, C, E
August 1971	Vacuum Pump Failure	A
September 1971	Fire in the Dome with Entrant	A, B, C, E
October 1971	Liquid Oxygen Failure	A

Date	Procedure	Personnel Participation*
November 1971	Air Compressor Failure	A
December 1971	Rescue of an Incapacitated Dome Entrant in the Airlock	A, B, C, E
January 1972	Fire in the Exposure Area With Dome Entrant in the Airlock	A, B, C, E
February 1972	Vacuum Pump Failure	A
March 1972	Fire in Exposure Area During Entry	A,B, C, E
April 1972	Complete Power Failure	A
May 1972	Fire in Dome With Entrant	A, B, C, E

* A - Shift Operator

B - Safety Observer B

C - Safety Observer C

E - Dome Entrant

AMERICAN ASSOCIATION FOR LABORATORY ANIMAL SCIENCE (AALAS) CERTIFICATION PROGRAM

AALAS has instituted an educational and certification program to upgrade the quality of laboratory animal technicians. The levels of certification from lowest to highest are: Assistant Laboratory Animal Technican, Laboratory Animal Technician and Laboratory Animal Technologist. Requirements for certification at each level include experience in the field, study in a program designed by AALAS and examinations, both written and practical.

In order to provide chamber technicians with the opportunity for semi-professional status in the field and to equip the THRU with the most knowledgeable and efficient technicians possible, strong encouragement was given to chamber technicians to enroll in the program. The Veterinary Medical Division of AMRL had personnel qualified by AALAS to conduct the examinations, and they considerately agreed to provide that service. As result of these efforts, two technicians have been certified at the Laboratory Animal Technician level and five at the Assistant Laboratory Technician level.

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