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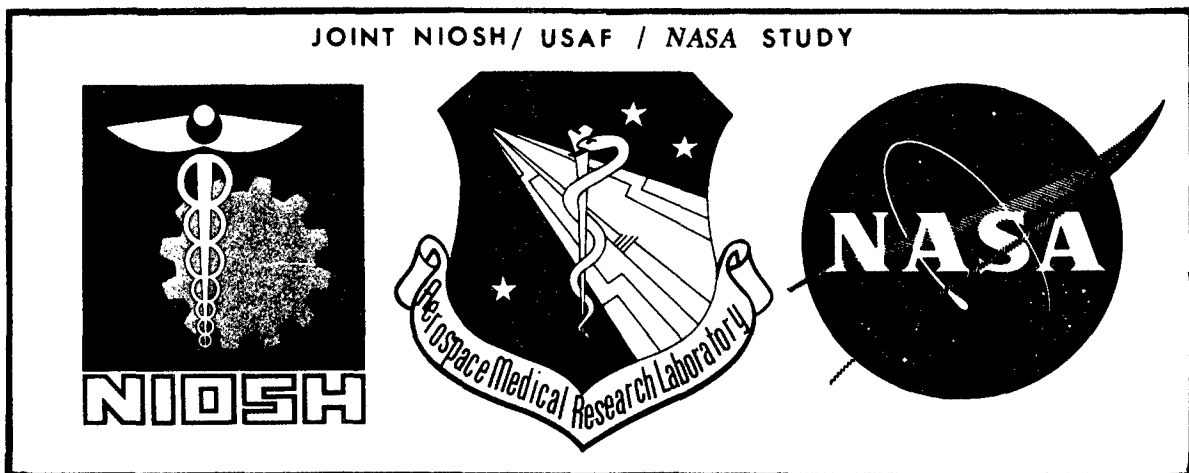
**TOXIC HAZARDS RESEARCH UNIT
ANNUAL TECHNICAL REPORT: 1974**

J. D. MacEWEN

E. H. VERNOT

UNIVERSITY OF CALIFORNIA

JULY 1974



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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The activities of the Toxic Hazards Research Unit (THRU) for the period of June 1973 through May 1974 are reviewed in this report. Acute inhalation toxicity experiments were conducted on benzonitrile, deuterium fluoride and fluomine. Chronic toxicity studies were conducted with an aircraft fuel JP-4 and constituents of JP-9. Chronic studies of coal tar aerosols and hydrazine were also continued. Oral and percutaneous toxicity determination and skin irritation and		

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sensitization studies were conducted on a number of fuel additives and photographic chemicals.

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PREFACE

This is the eleventh annual report of the Toxic Hazards Research Unit (THRU) and concerns work performed by the Department of Community and Environmental Medicine of the University of California, Irvine on behalf of the Air Force under Contract No. F33615-73-C-4059. This document constitutes the second report under the current contract and describes the accomplishments of the THRU from June 1973 through May 1974.

The current contract for operation of the Laboratory was initiated in 1972 under Project 6302 "Toxic Hazards of Propellants and Materials," Task 01 "Toxicology" Work Unit No. 63020113. K. C. Back, PhD, Chief of the Toxicology Branch was the technical contract monitor for the Aerospace Medical Research Laboratory.

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

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SECTION I

INTRODUCTION

The Toxic Hazards Research Unit (THRU) is a research team composed of an interdisciplinary group of University of California, Irvine toxicologists, chemists, statisticians and engineers supported by Air Force pathologists, veterinarians and medical technologists. This document constitutes the eleventh annual report of the THRU which began in June 1963 to operate an inhalation toxicology laboratory for the investigation of potentially hazardous chemicals and materials of interest to the Air Force and other governmental agencies.

During the first six years of operation the primary research efforts of the THRU were concentrated on obtaining information about health hazards of spacecraft flight, and the biological data obtained have been used as criteria for establishing emergency and continuous exposure limits as well as engineering design safety factors. As the space program has decreased the THRU has placed more emphasis on biological data useful for solution of inhalation toxicity problems encountered in aircraft operations and 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.

The research facilities used by the THRU consist of animal exposure chambers and supporting laboratories. The chamber facilities consist of two types, each performing a separate function. 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 (Fairchild, 1967; MacEwen, 1965; MacEwen and Geckler, 1966; MacEwen and Vernot, 1968, 1969, 1970; Thomas, 1968).

With the comprehensive scientific team and exposure resources described above the THRU can and does conduct realistic simulation of human exposures to contaminants causing adverse health effects. These exposures, carefully monitored and controlled using continuous analytical techniques, are provided to multiple animal species for assurance of safe exposure limits for humans. The animals used in these experiments are continuously observed and monitored visually and with regularly scheduled biochemical and physiological measurements.

As part of its contract responsibilities, THRU presents an annual technical conference to disseminate new toxicological information to Air Force, other governmental and industrial scientists. This year's conference presented 30 technical papers and had as a keynote theme "The Toxic Substances Control Act" with emphasis on its potential impact upon environmental health and safety. Other sessions were concerned with chemical carcinogenesis and the toxicity of halogenated hydrocarbons and missile fuels and oxidizers. Seven papers were presented by University of California Faculty and staff members. The open forum discussion following each session resulted in significant contributions of additional technical information and scientific exchange. The conference, held 16 October through 18 October 1973, drew 149 participants including speakers. Next years conference planning is well underway and will be held in Spetember 1974.

A University wide Scientific Management Team (SMT) was formed during the first year of the current contract. This team represents the professional fields of occupational medicine, pharmacology, pathology, industrial hygiene, neuropharmacology, physiology, biochemistry, management sciences, and biostatistics thus providing greater scientific depth of expertise and consultative advice to the THRU operations than it has had in past years. Three formal meetings of the entire Scientific Management Team were held during the year at the Irvine, Davis and Berkley campuses to review scientific protocols and experimental results and in this manner participate directly in the research programs of the THRU. Individual faculty research programs to be conducted at THRU were also designed and reviewed at these meetings and will soon be initiated as part of the University's privilege of partial use of THRU facilities. Additional meetings of smaller groups of the SMT were held periodically to implement special activities such as an in depth study of the management and operations of the THRU with a view toward improved operational efficiency, utilization of resources and effective personnel practices.

Other meetings were held to implement new research programs on environmental effects of pollutants on air, soil and water quality. These research programs, being conducted at various University of California campuses will be described in a separate report.

SECTION II

RESEARCH PROGRAM

The research function of the THRU is a continuing activity which has been conducted independent of contract years or contractors. Experiments are usually in progress at the beginning and end of each report period. Experiments that were initiated and completed during the past year and were of sufficient magnitude to merit separate technical reports are only summarized in this document. This year's research program was conducted on a broad range of chemicals and includes inhalation studies on aircraft fuels, coke oven effluents, deuterium fluoride and a variety of fuel and lubricant additives. Range-finding studies and acute oral toxicity studies were conducted on several compounds. Skin irritation and sensitization studies were also performed on new Air Force materials.

Chronic Inhalation Toxicity of JP-4 Jet Fuel

Accidental overexposure of workers to JP-4 vapors indicated the need for a Threshold Limit Value (TLV) for this jet fuel based on experimental data. A lack of existing animal toxicity information necessitated the design of a test to define the chronic toxic effects of low levels of JP-4 vapors on several species of laboratory animals. Toxicity data from this study could then be used to either predict safe exposure levels or provide input for the design of a subsequent test to determine an industrial TLV for JP-4. Application of this TLV would serve to prevent health hazards to those individuals charged with handling JP-4 jet fuel either in storage or in the field.

JP-4 is a complex mixture of aliphatic and aromatic hydrocarbon compounds defined in terms of physical and chemical characteristics, and including various additives, all of which meet the requirements of Military Specification MIL-J-5624E. Upper limits for some of the constituents are detailed in the military specification as shown in table 1.

TABLE 1. JP-4 CONSTITUENTS LISTED IN
MILITARY SPECIFICATION MIL-J-5624E

<u>Constituent</u>	<u>Maximum Concentration</u>
Sulfur	0.4% (by wt.)
Mercaptan Sulfur	0.001% (by wt.)
Aromatics	25.0% (by vol.)
Olefins	5.0% (by vol.)
Various Butyl Phenol Antioxidants	24% mg/liter
Aliphatic Diamine Metal Deactivators	5.8% mg/liter

Obviously, these constituents represent only a fraction of the total content of JP-4 jet fuel, the remainder consists of unspecified hydrocarbon compounds in the kerosene boiling range.

The American Conference of Government Industrial Hygienist's (ACGIH) guidelines indicate that a single TLV for gasoline and/or petroleum distillates is not applicable but that the total aromatic hydrocarbon content should determine the suitable TLV (Elkins et al., 1963). In essence, the TLV of hydrocarbon-type fuels should be calculated as those vapor concentrations of the fuel yielding no more than 25 ppm benzene (current OSHA industrial TLV for benzene is 25 ppm).

The toxic effects of chronic benzene exposure to laboratory animals and humans are too numerous to list, but have been well documented and reported by Browning (1965). A summary of the salient hematological and clinical chemical manifestations of chronic benzene poisoning in animals is shown in table 2.

TABLE 2. SOME HEMATOLOGY AND CLINICAL CHEMISTRY CHANGES ASSOCIATED WITH CHRONIC BENZENE INTOXICATION IN LABORATORY ANIMALS

<u>Increased</u>	<u>Decreased</u>	<u>Other</u>
Lymphocytes	Hemoglobin	Bone Marrow:
Eosinophiles	Red Blood Cells,	Erythroid hyper-
Monocytes	White Blood Cells	plasia and matura-
Nucleated Red Blood Cells	Platelets	tion arrest in
Reticulocytes	RBC Life Span	erythrocytic and
Serum Bilirubin	Serum Alkaline Phos-	granulocytic series.
Serum Lactic Dehydro-	phatase	
genase		

Aksoy et al. (1972) also report an increase in the red blood cell osmotic fragility in humans subjected to long-term benzene exposure.

Several acute toxicity tests, with JP-4 jet fuel, were performed in this laboratory as antecedents to a chronic toxicity investigation. Single oral doses of JP-4 diluted in corn oil were administered to rats and mice with the resultant mortality ratios shown in table 3.

TABLE 3. SINGLE DOSE ORAL TOXICITY OF JP-4 JET FUEL
IN RATS AND MICE

<u>Dose (mg/kg)</u>	<u>Mortality Ratio</u>	
	<u>Rats</u>	<u>Mice</u>
125		0/3
250		0/3
500	0/3	2/3
1000		1/3
2000	0/3	
4000	0/3	
8000	0/3	

No rat deaths were produced in JP-4 oral doses up to 8000 mg/kg. A subsequent saturated vapor inhalation test of 6 hours duration, to an estimated JP-4 concentration of 38 mg/l, resulted in poor coordination and convulsions in several of the rats tested but no mortalities.

Gas chromatographic analysis of liquid JP-4 by THRU chemists indicated the measured concentration of benzene to be 0.8% by weight. Because of its variable composition, the average molecular weight of JP-4 is unknown. JP-4 concentrations, therefore, are expressed in terms of their total hydrocarbon content and measured as mg/liter. Utilizing a hydrocarbon analyzer, we determined that the JP-4 vapor concentration of 5.0 mg/l total hydrocarbon contained 25 ppm benzene.

The present 6-month study, designed to properly assess the inhalation hazard associated with chronic exposure to JP-4 vapors, includes four species

of laboratory animals which are being exposed to two total hydrocarbon concentrations of the fuel, 5.0 mg/l (25 ppm benzene), and 2.5 mg/l (12.5 ppm benzene). Additionally, a positive control group, exposed to 25 ppm benzene, and an air exposed control group are also being maintained. All exposures are intermittent industrial-type exposures of 6 hours per day duration repeated for 5 consecutive days per week.

Test and control groups consist of 6 beagle dogs (2 female, 4 male), 4 rhesus monkeys (1 male, 3 female), 50 male Sprague-Dawley rats and 40 female CF-1 mice. Each group of animals is housed in a separate Thomas Dome operated at 40 CFM airflow and 710 mm Hg pressure to avoid leakage of the fuel vapors.

JP-4 for this study is supplied by the Air Force in 55 gallon steel barrels. All fuel received by our laboratory is representative of that found in actual use situations and conforms to the previously listed military specifications.

The system used to introduce JP-4 vapors into the Thomas Domes is shown in figure 1. Liquid JP-4 is pumped under pressure from a 55 gallon supply drum (not illustrated). It passes through a glass flowmeter to a heated glass evaporator from which an air stream carries JP-4 vapors into the main air supply for the exposure dome. Excess JP-4 not vaporized in the evaporator is drained into a receiving tank where it is collected. Thermocouples are placed at the top and bottom of the glass evaporator to sense any hazardous increase in temperature and to activate both an alarm and a solenoid valve system which cuts off the fuel supply.

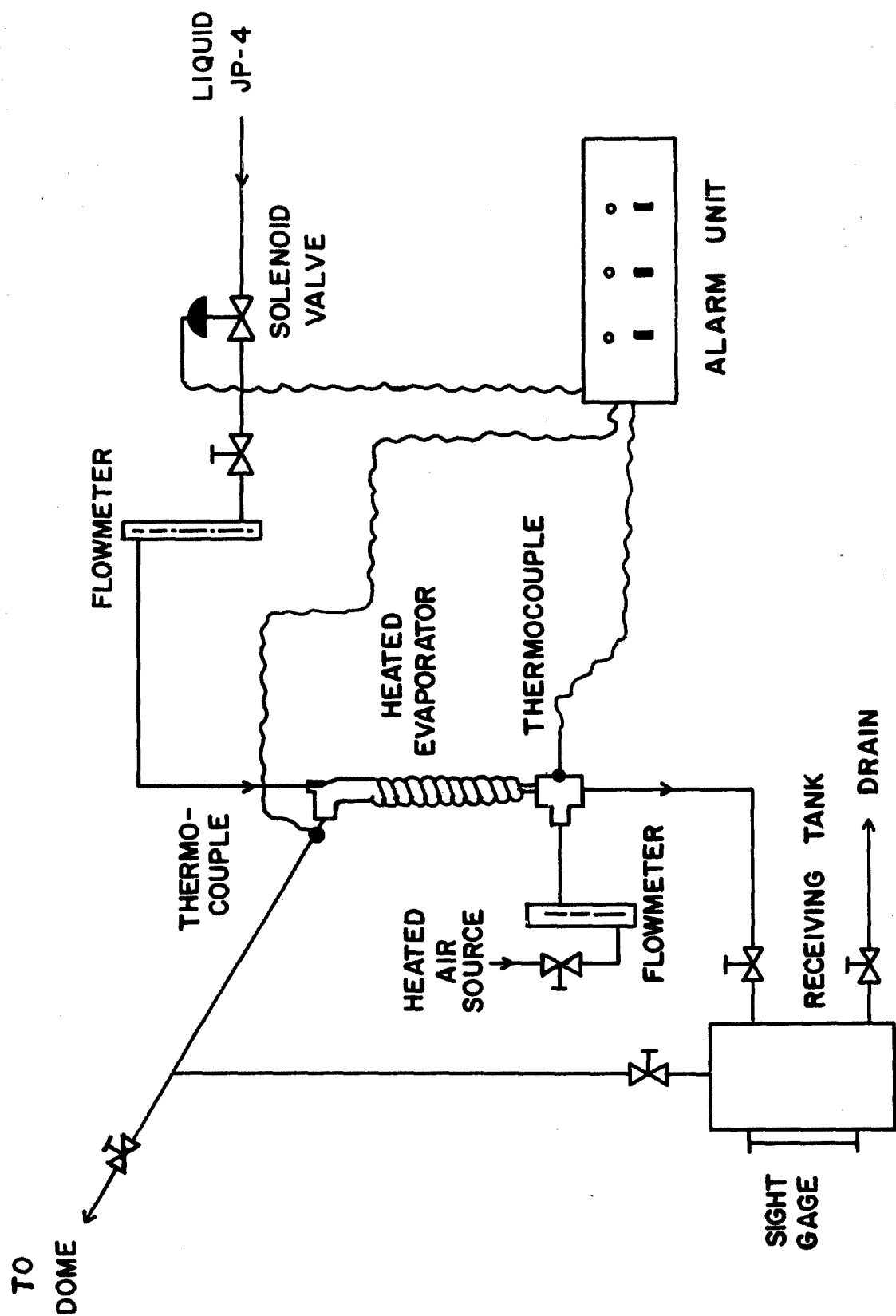


Figure 1. JP-4 vapor introduction system.

Benzene vapor generation for the positive control exposure is achieved simply by use of an infusion pump and glass syringe. Liquid benzene is metered through a "T" fitting to a copper line where vaporization takes place. This air stream is then injected into the dome air supply system for mixing and introduction into the breathing zone of the experimental animals.

Continuous analysis of JP-4 chamber concentrations is achieved by pumping air samples from each exposure dome into total hydrocarbon analyzers calibrated with known concentrations of propane. Propane has the same detection sensitivity as JP-4 vapor. This sample system is shown in figure 2. Quantitation of benzene content of the JP-4 vapors in the domes is made by gas chromatographic analysis. Benzene vapors within the positive control dome are continuously monitored using a total hydrocarbon analyzer calibrated with known benzene standards.

The series of parameters selected to measure the chronic toxicity of JP-4 vapors and effects of benzene at the industrial TLV include biweekly hematology and clinical chemistry tests on dogs and monkeys and biweekly body weight measurements on dogs, monkeys, and rats. The dog and monkey clinical chemistry regimen being utilized is shown in table 4.

Bone marrow from 20 rats serially sacrificed (5 per experimental group) at 8 and 16 weeks was examined for myeloid/erythroid (M/E) ratios. Blood samples, taken by cardiac puncture, were collected from these rats for hematocrit, hemoglobin and RBC determinations before sacrifice and subsequent gross and histopathological examination.

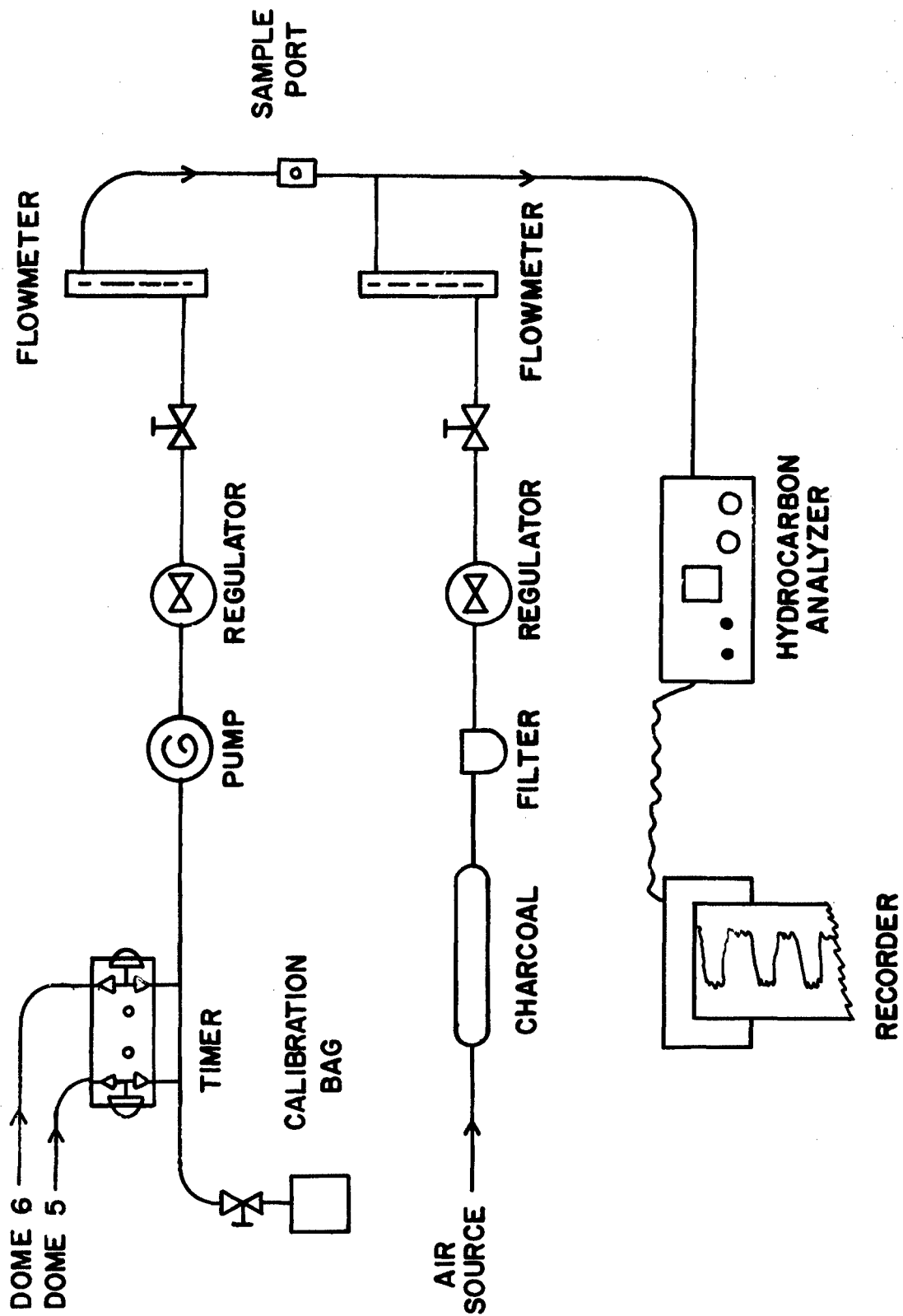


Figure 2. JP-4 atmospheric concentration sampling system.

TABLE 4. CLINICAL HEMATOLOGY AND CHEMISTRY TESTS
PERFORMED ON DOGS AND MONKEYS EXPOSED TO JP-4
AND BENZENE VAPORS

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

Signs of toxic stress are recorded as well as numbers of animals that die and times to death. Organ weights will be obtained on rats to be sacrificed at the conclusion of the study. Gross and histopathological examinations were made on all animals that died or were sacrificed during the study, and will be performed on animals that will be killed at termination of the study.

Activity depression in dogs during the initial three weeks of the exposure was one of the two toxic signs noted thus far in the study. The activity of dogs being exposed to either benzene or JP-4 vapors was depressed when compared to their controls. JP-4 and benzene exposed dogs, if not sleeping, were quiescent and prostrate during periods of exposure. The controls, however, remained highly active during a comparable time period. Activity depression

in exposed monkeys was evident although not as pronounced as in the dogs. No similar effects were seen in either rats or mice. By the end of the first month of exposure, all dogs and monkeys were exhibiting normal activity patterns. After two weeks of exposure, emesis was noted from one male and one female dog in the 5.0 mg/l JP-4 exposed group. The fluid expelled by the male animal contained large quantities of bile while that of the female contained some traces of blood. Emetic activity has not been noted to occur again since that initial episode.

There have been no dog or monkey deaths to date in either the exposed or the control groups. There have been rodent deaths in both the 25 ppm benzene and the 5.0 mg/l JP-4 exposed groups. A JP-4 exposed rat and one JP-4 exposed mouse died after 4 months of exposure. A benzene exposed positive control rat was sacrificed during the sixth month of exposure after a mammary tumor ruptured. Two benzene exposed mice and one JP-4 exposed mouse died after six months of exposure. Gross pathological examination of the 5.0 mg/l JP-4 exposed mouse that died after 6 months of exposure indicated a left lung abscess, pale and blotchy liver, and blood in the abdominal cavity and uterus. With the exception of the benzene exposed rat with the mammary tumor, no gross lesions were noted in any of the other rodents dying from exposure to either 25 ppm benzene or 5.0 mg/l JP-4 vapors.

The mean body weights of all groups of exposed dogs did not differ significantly from controls at any time during the study. After 12 weeks of exposure, however, a notable but not statistically significant rise in the mean

group body weight of the 5.0 mg/l exposed dogs was observed and continued until the eighteenth week of exposure (figure 3). There were no significant differences between the growth rates of any of the exposed monkeys and the control group (figure 4). Growth curves for exposed rats, shown in figure 5, illustrate completely normal growth rates for all groups in the study.

Variable, sex dependent hematologic effects, from chronic benzene exposure, are discussed by Browning (1965). There is some supportive evidence to indicate that women and at least two species of female laboratory animals are more susceptible to the effects of benzene than are males.

Biweekly routine hematological measurements, in all exposed dogs and monkeys, both male and female, showed no significant differences from their respective control values. Only scattered values were found to be statistically different from controls and these differences could not be attributed to either benzene or JP-4 exposure. Blood indices for all dogs and monkeys, calculated from their biweekly hematocrit, hemoglobin, and RBC values, did not indicate any difference in test animals when compared to corresponding controls.

Biweekly RBC osmotic fragility testing was performed using a modification of the method described by Davidsohn et al. (1969). Initially, only two female dogs and two female monkeys from each dome were sampled. An increase in the RBC osmotic fragility in female dogs exposed to 5.0 mg/l JP-4 was noted between the twelfth and twenty-second weeks of exposure (table 5).

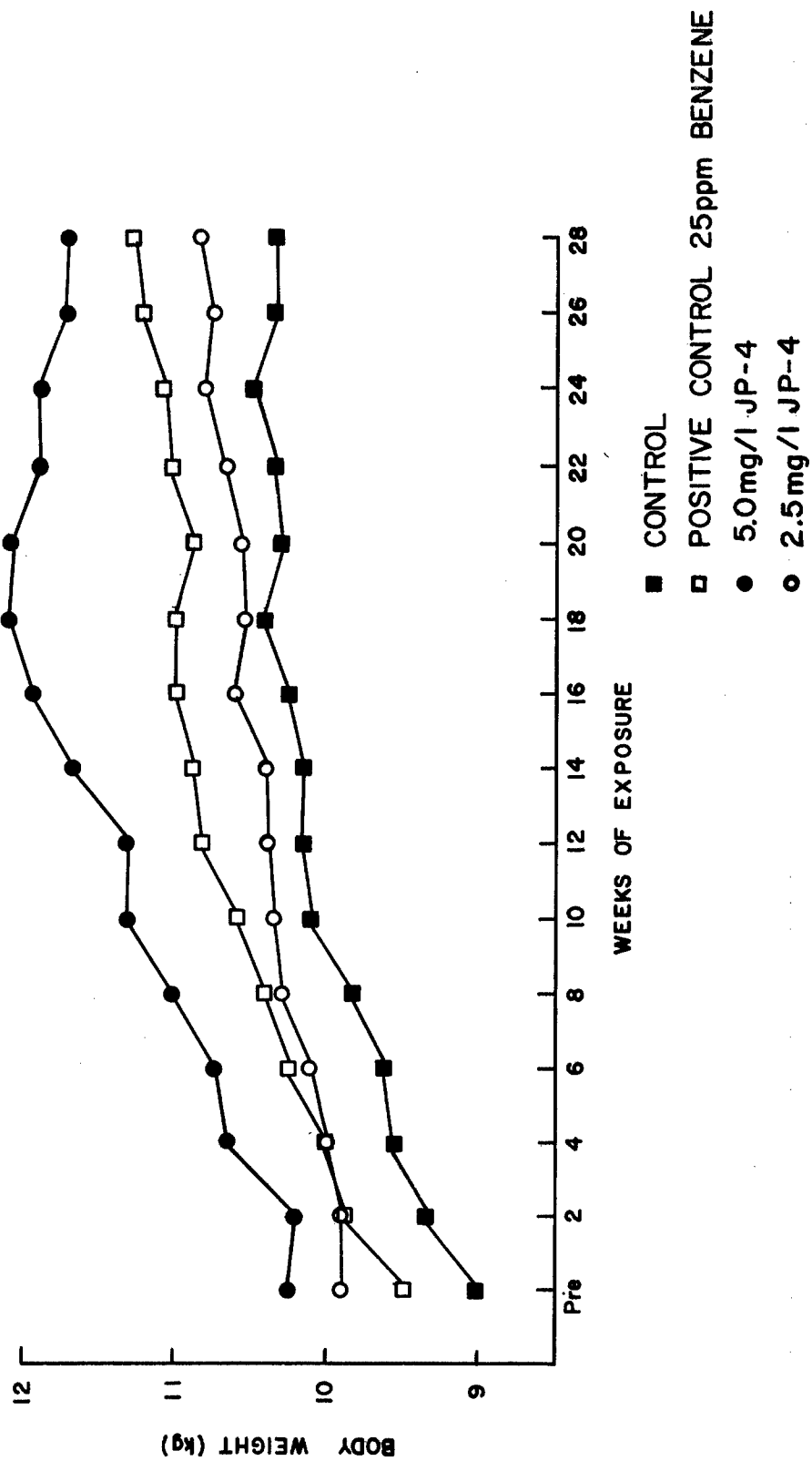


Figure 3. Effect of exposure to JP-4 or benzene on dog growth.

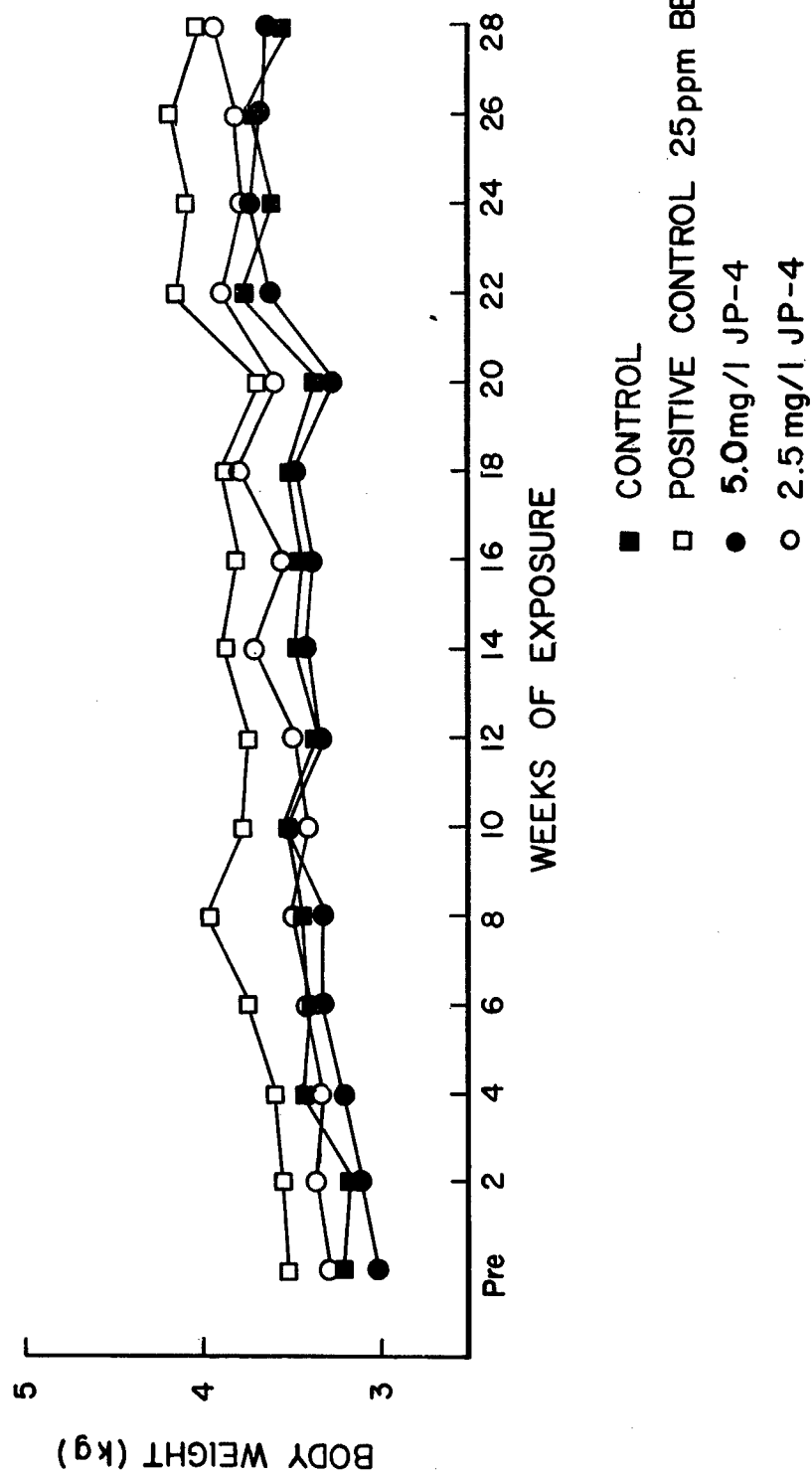


Figure 4. Effect of exposure to JP-4 or benzene on monkey growth.

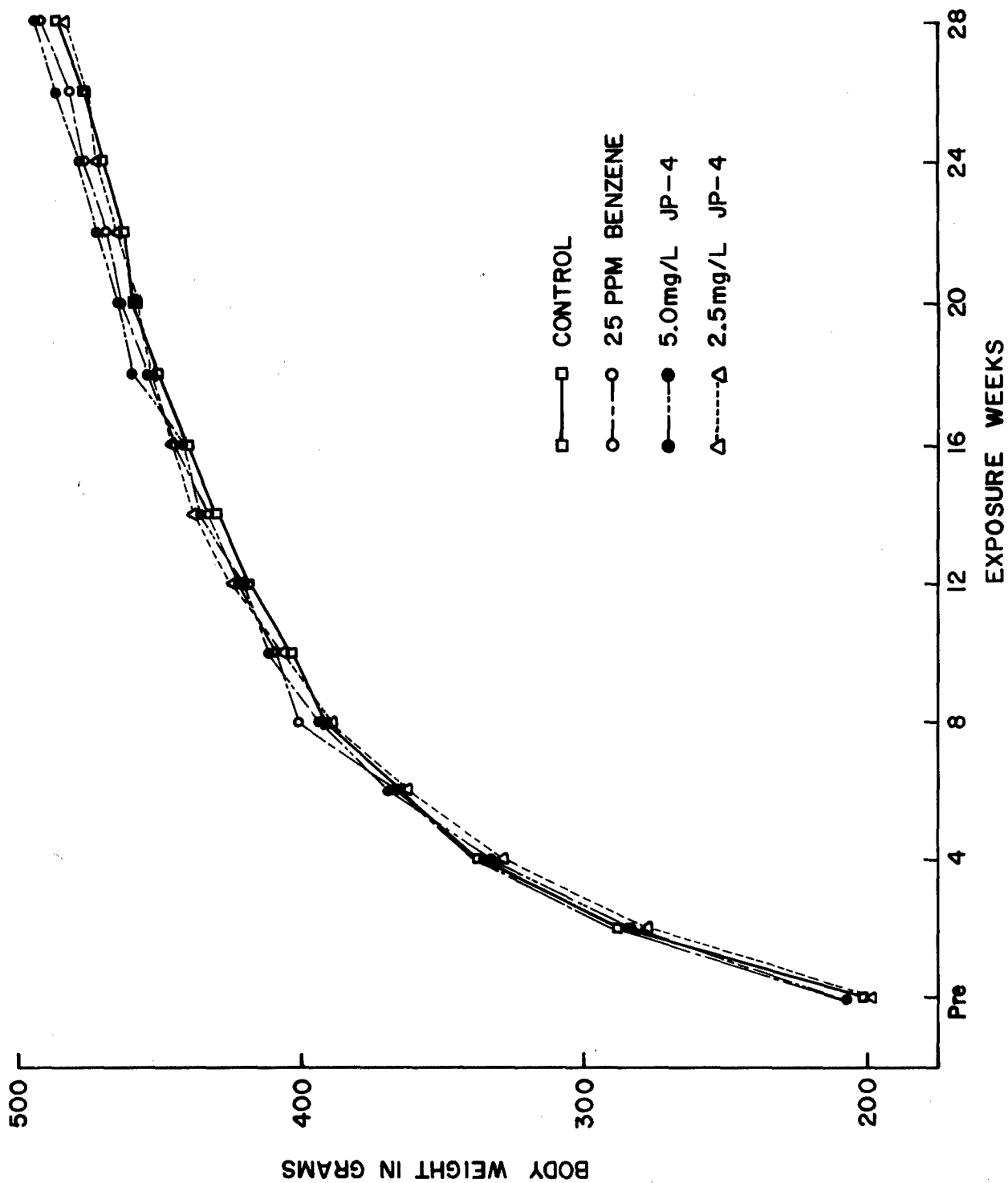


Figure 5. Effect of exposure to JP-4 or benzene on rat growth.

TABLE 5. EFFECT OF EXPOSURE TO JP-4 OR BENZENE ON
RBC FRAGILITY IN FEMALE DOGS

% Salt Solution:	% Hemolysis							<u>Exposure</u>
	<u>0.55</u>	<u>0.50</u>	<u>0.45</u>	<u>0.40</u>	<u>0.35</u>	<u>0.30</u>	<u>0.20</u>	
<u>Exposure Week</u>								
0	0.0	0.0	2.0	46	83	95	97	Control Group
2	0.2	0.6	4.9	42	87	95	97	
4	0.2	0.7	7.4	56	93	97	99	
8	0.0	2.3	19.8	61	93	96	98	
12	0.0	1.9	17.0	58	93	97	98	
16	0.0	2.5	15.6	64	94	96	96	
20	0.0	0.2	6.1	37	90	98	99	
22	0.0	0.4	6.1	43	93	96	100	
24	0.0	0.9	7.5	48	94	97	-	
26	0.0	0.0	3.7	31	89	98	99	
28	0.0	0.7	4.3	36	86	97	99	
0	0.0	1.2	9.9	55	89	95	97	Positive Control 25 ppm Benzene
2	0.0	0.7	5.0	42	83	96	98	
4	0.0	1.1	5.8	36	87	97	99	
8	0.3	1.7	15.5	40	89	97	98	
12	0.0	1.4	9.9	40	85	97	97	
16	0.2	1.5	10.4	40	86	97	98	
20	0.0	0.8	8.3	37	83	97	98	
22	0.0	1.2	7.1	27	78	95	99	
24	0.0	0.9	7.5	38	90	97	-	
26	0.0	1.9	6.6	41	90	93	98	
28	0.3	1.4	11.1	49	89	98	100	

TABLE 5. (Continued)

% Salt Solution:	% Hemolysis							<u>Exposure</u>
	<u>0.55</u>	<u>0.50</u>	<u>0.45</u>	<u>0.40</u>	<u>0.35</u>	<u>0.30</u>	<u>0.20</u>	
<u>Exposure Week</u>								
0	0.0	2.3	14.5	72	97	98	100	5.0 mg/liter Total Hydrocarbons
2	0.0	0.5	11.3	58	93	98	99	
4	0.0	2.6	18.8	66	94	97	98	
8	0.6	1.9	16.0	63	95	98	97	
12	0.2	1.5	20.3	76	96	98	98	
16	0.2	7.5	41.2	87	96	97	98	
20	1.5	13.1	46.9	86	96	98	99	
22	0.2	12.6	46.3	86	97	98	99	
24	0.1	4.9	34.6	83	95	99	-	
26	0.0	3.7	31.8	72	96	96	98	
28	0.2	1.8	14.0	58	93	96	100	
0	0.0	0.3	2.2	23	72	95	99	2.5 mg/liter Total Hydrocarbons
2	0.3	0.6	4.5	30	84	96	98	
4	0.0	1.3	9.0	46	89	97	97	
8	0.0	0.9	7.4	40	90	98	98	
12	0.2	0.7	13.6	58	93	97	98	
16	0.0	1.8	17.7	62	72	98	99	
20	0.0	2.4	11.7	50	89	96	98	
22	0.0	1.6	10.4	49	90	97	98	
24	0.0	1.4	9.2	49	90	95	-	
26	0.0	0.4	9.2	46	86	96	98	
28	0.0	2.1	12.3	51	82	96	99	

The fragility increase occurred between the 0.50 and 0.40% saline solutions used in the fragility assay. There was no evidence of hemolytic effects noted in any of the hematological parameters tested at the same time the RBC fragility measurements were made. Figure 6 shows the female dog RBC fragility data for all test and control groups at 0.45% saline solution for all sampling periods up to the writing of this report. Increased fragility in the 5.0 mg/l JP-4 exposed female dogs diminished after 22 weeks of exposure and returned to normal by 28 weeks of exposure.

Because of the absence of any evidence of a concurrent anemia accompanying the increased RBC fragility, the study was extended for an additional 60 days in an effort to determine the etiology of the increased fragility.

Beginning at 22 weeks of exposure all dogs in the experiment, male and female, were sampled for RBC fragility measurements on a biweekly basis. Table 6 lists the average RBC fragilities for males and females in each of the three test and the control groups, as measured at 0.45% saline concentration, from 22 weeks to the time of this reporting. The increase in fragility in the 5.0 mg/l JP-4 exposed female dogs reflects the greatest change from control, but there may be an effect on the male beagles being exposed to both concentrations of JP-4 vapor. Although male dogs generally exhibit an increase in fragility over control values, there does not appear to be a dose response relationship, i.e., increased fragility in 5.0 mg/l JP-4 exposed male dogs is not greater than that measured in the 2.5 mg/l JP-4 exposed male animals. Because data on RBC fragility values does not exist before 22 weeks of

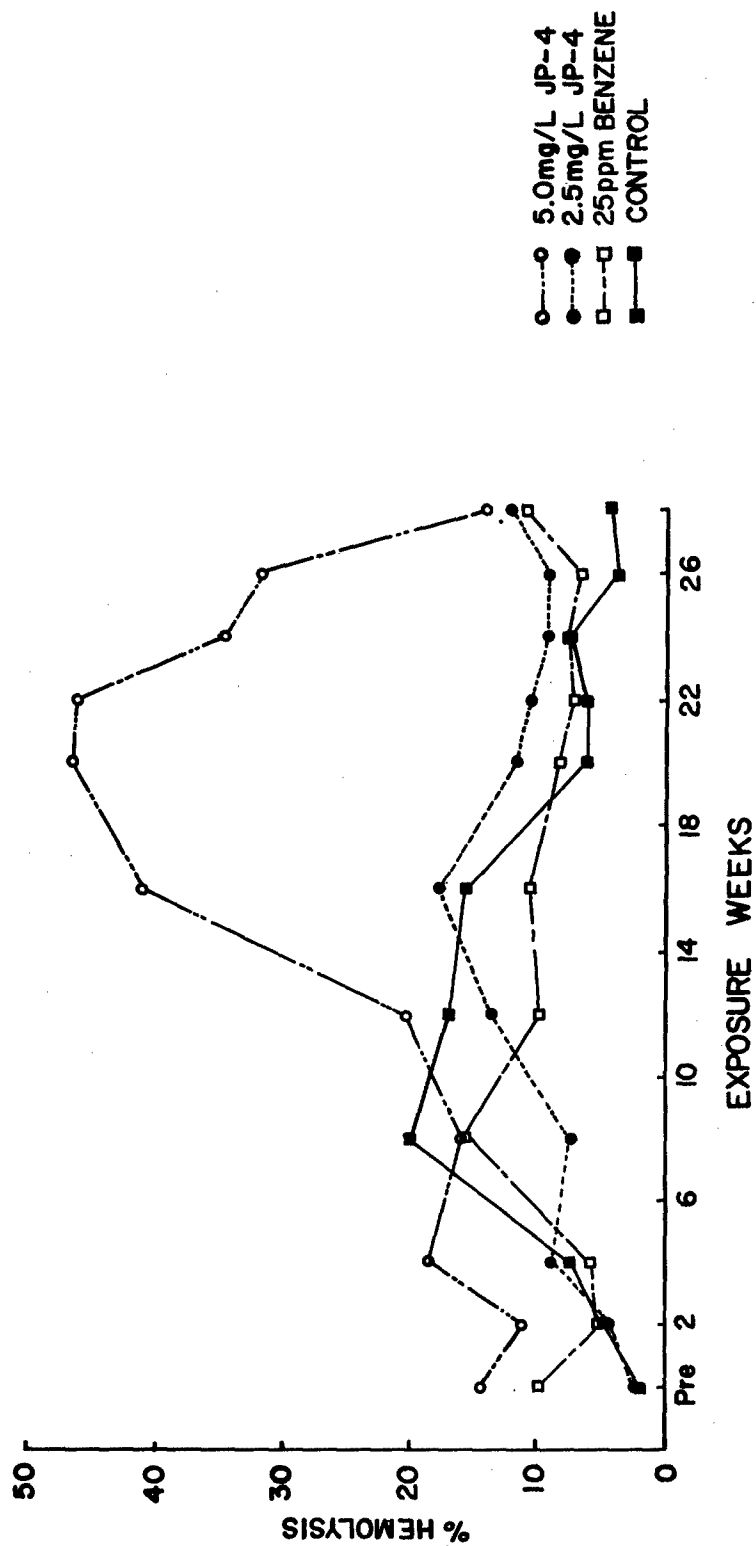


Figure 6. Effect of exposure to JP-4 or benzene on RBC fragility in female dogs at a 0.45% saline concentration.

exposure for the male dogs, it is impossible to assess the fragility effects before this point in time for them.

TABLE 6. AVERAGE RBC FRAGILITY VALUES FOR MALE AND FEMALE BEAGLE DOGS AT AN 0.45% SALINE CONCENTRATION

		<u>% Hemolysis</u>			
		<u>Control</u>	<u>25 ppm Benzene</u>	<u>5.0 mg/l JP-4</u>	<u>2.5 mg/l JP-4</u>
22 weeks	♂	4.4	8.8	16.9	18.4
	♀	6.1	7.1	46.3	10.4
24 weeks	♂	6.3	12.6	13.7	19.8
	♀	7.5	7.5	34.6	9.2
26 weeks	♂	5.6	8.6	6.2	14.7
	♀	3.7	6.6	31.8	19.2
28 weeks	♂	4.5	6.9	3.7	9.6
	♀	4.3	11.1	14.0	12.3

Hematology measurements including hematocrit, hemoglobin and RBC determinations, made on the 5 rats sacrificed from each group at 8 and 16 weeks, failed to show any statistically significant difference from controls. Bone marrow samples from these same animals revealed M/E ratios which were comparable to control values.

Examination of clinical chemistry data, which consisted of 8 separate determinations collected on a regular biweekly schedule for dogs and monkeys, revealed no significant changes in monkeys which could be attributed to exposure conditions. Dog serum glucose values, however, shown in figure 7,

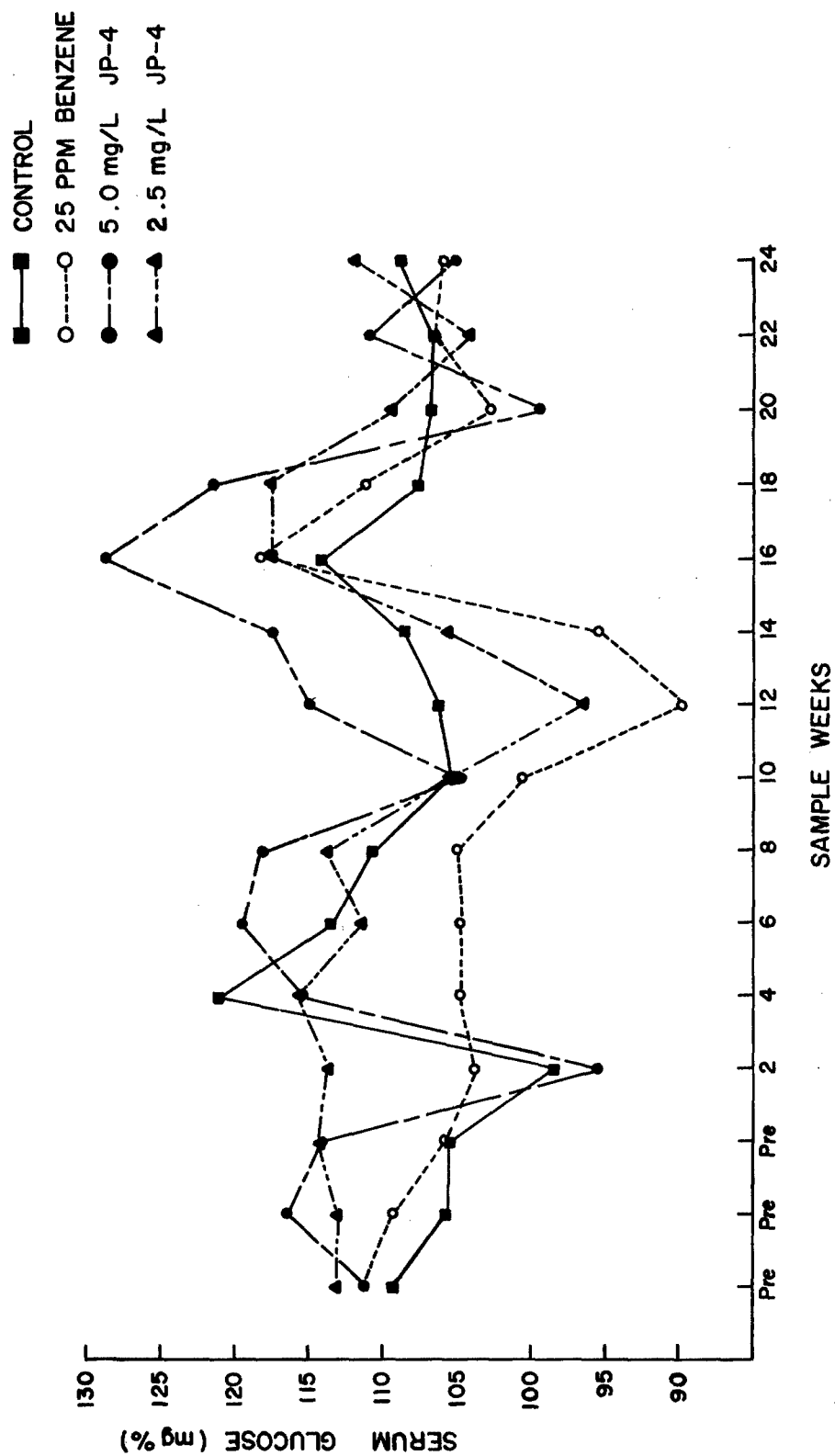


Figure 7. Effect of exposure to JP-4 or benzene on dog serum glucose levels.

are statistically significantly higher than control values at 12, 16, and 18 weeks of exposure for the animals exposed to 5.0 mg/l JP-4 vapors. This rise in serum glucose levels occurred concurrently with the apparent increase in growth rate manifested by the 5.0 mg/L JP-4 dogs detailed previously and shown in figure 3. No other dog clinical chemistry parameters resulted in significant changes which could be related to exposure of either JP-4 or benzene vapors.

This study was extended for sixty days to include additional blood studies which would aid in the elucidation of mechanisms accountable for the limited hematological and clinical chemical changes observed. Three additional assays were included to supplement the routine battery of tests already being performed. These additional tests included methemoglobin determinations on control and 5.0 mg/l JP-4 exposed dogs, bone marrow studies on iliac crest samples of control and 5.0 mg/l JP-4 exposed dogs and red blood cell density distribution determinations on all exposed and control dogs.

Bone marrow and red blood cell density distribution studies have not yet been completed and cannot be reported at this time. Methemoglobin measurements, however, taken on all control and 5.0 mg/l JP-4 exposed dogs at 26 weeks of exposure failed to demonstrate any difference in test values versus those of the controls.

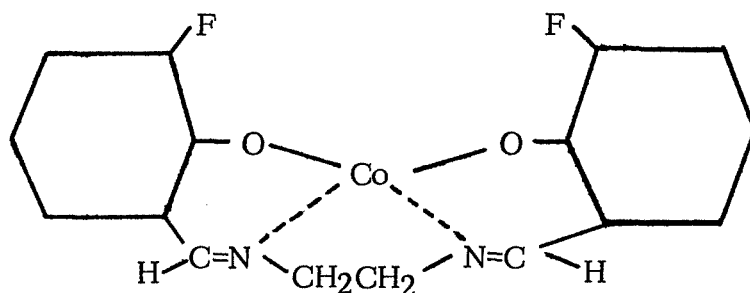
Exposure to JP-4 jet fuel vapors has produced increased RBC fragility in female dogs exposed to 5.0 mg/l concentrations for 6 hours per day, 5 days per week, for up to six months. This same effect was not seen in female

beagles exposed to 2.5 mg/l JP-4 vapors or 25 ppm benzene at the same time periods (figure 6).

Mechanisms responsible for the effects reported are as yet unidentified and must await completion of the bone marrow and red blood cell density distribution studies as well as gross and histopathological examination of all test animals at the conclusion of the study.

Mammalian Toxicity of Fluomine Dust

Fluomine [cobalt-bis(3-fluorosalicylaldehyde)-ethylenediimine], when activated, is capable of selectively absorbing oxygen from air and, upon heating, will release pure molecular oxygen. There are several chelates which are capable of absorbing molecular oxygen including salcomine [bis(salicylaldehyde) ethylenediimine cobalt (II)], the parent compound of fluomine. However, fluomine far exceeds the capabilities of the others to absorb oxygen while in a solid form. This property renders it useful as a possible component in the life support systems of high altitude aircraft or spacecraft. The structure of fluomine is sketched below:



A search of the current literature failed to reveal any information concerning the mammalian toxicity of this compound. However, Coon et al. (1942) reported on the toxicity of salcomine powder inhalation on mice. They report 1 of 6 mice dying following inhalation of 390 mg/m³ dust for 5.5 hours and 4 of 6 mice dying after 6 hours at 1000 mg/m³. Exposure to salcomine dust resulted in severe irritation to the tracheo-bronchial system and the lungs. The lungs were hyperemic and contained edema fluid in the peripheral portions of the lobules.

The compound was tested for eye irritation using New Zealand Albino rabbits and varying doses of fluomine suspended in normal physiological saline. Twenty-four hours prior to use, the rabbits eyes were examined for corneal lesions using a 1% solution of fluorescein dye.

Equal numbers of left and right eyes were tested using the following method. The lower lid was pulled away from the eye and 0.1 ml of the fluomine suspension was instilled into the lower conjunctival sac. The lid was held open for a few seconds then raised to close with the upper lid. The eyes were not washed following dosing.

The eyes were examined at 1, 24 and 72 hours and again at 7 days after application of the fluomine. Staining with fluorescein was done on any of the eyes which showed irritation after 24 and 72 hours and 7 days. Grading of eye irritation was done following the standard method of Draize (1944) which provides a maximum total numerical score derived from the sum of corneal, conjunctival and iris irritation.

A patch-test method was done to measure the degree of primary skin irritation of fluomine on the intact and abraded skin of New Zealand albino rabbits. All rabbits were clipped of hair on the backs and flanks 24 hours prior to exposure. The abrasions were minor incisions through the stratum corneum, but not sufficiently deep to disturb the derma or to produce bleeding. These were made in a "tic, tac, toe" pattern with a syringe needle used to make the incisions.

One-half gram of fluomine was applied to each area, the intact skin and the abraded skin, and covered by a one-inch square of surgical gauze, two layers thick. The gauze patches were held in place with strips of non-irritating elastic tape. The entire area was then covered with a rubber dental dam strip, secured with more elastic tape. The patches remained in place on the rabbits for 24 hours. During that time, the rabbits wore leather restraining collars to prevent disturbance of the patch area, while allowing the rabbits freedom of movement and access to food and water during the test period.

After 24 hours, the wrap and patches were removed and the test areas evaluated for irritation using the Draize table (table 7) as a reference standard. Readings were also made at 72 hours (48 hours after the first reading). The values for erythema and eschar formation were added to the combined values for edema formation. The mean of these values is the primary irritation score.

TABLE 7. EVALUATION OF SKIN REACTIONS*

	<u>Value^(a)</u>
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Edema formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extended beyond exposed area)	4

(a) The "value" recorded for each reading is the average value of the six animals subject to the test.

*Draize et al. (1944).

Fluomine was administered orally to rodents as a suspension in corn oil. The suspensions were kept in a turbulent state prior to dosing by stirring them on a magnetic stirring platform. Glass syringes with 18 gauge intubation needles were used to administer the suspensions to the rodents. The animals were fasted for at least 16 hours prior to dosing to allow for more uniform absorption since the amount of food in the stomach varies greatly from animal to animal in the unfasted condition.

Five rats and 5 mice were dosed at each level and the LD₅₀ with its 95% confidence limits was calculated using the moving average interpolation method of Weil (1952) and/or the probit analysis method of Finney (1952).

Deaths which occurred during the 14 days immediately following the administration of the single dose were included in the final mortality tally.

Groups of 10 rats and 10 mice were exposed to varying concentrations of airborne fluomine dust particles for 1-hour and 6-hour periods and observed for 14 days postexposure to determine mortality and/or signs of toxic stress. The concentrations were established using a Wright Dust Feeder® and adjusting gear ratios to vary the amount of suspended dust entering the 120 liter chamber. A constant flow of 20 liters of air per minute was maintained through the chamber. Concentrations were measured by gravimetric sampling using a 47 mm membrane filter with a mean pore size of 0.45μ .

A particle size analysis was performed on the dust sampled from the 120 liter inhalation chamber using a plastic overlay on a microphotograph similar to the procedure of Vooren and Meyer (1971). The chamber air sample was drawn through a filter at 5 liters/minute (370 cm/minute), a rate calculated to be capable of collecting particles up to 35 microns in diameter.

After sampling, the filter was covered with immersion oil and the slide placed under the microscope. Photographs taken of the filter and of a stage micrometer were enlarged to the same degree. The particles were then measured using a plastic overlay on which concentric circles had been drawn corresponding to particle diameters of 1, 2, 5, 10 and 20 microns. These measurements were calculated directly from the microphotograph of the stage micrometer. A pinhole was made in the center of the circles of the template, enabling the technician to pierce each particle image as counted.

A total of 455 particles were measured by the above method (table 8). No attempt was made to classify the particles below one micron in diameter and they were included in the one micron group. Seventy-one percent of all particles were five microns or less in diameter and, therefore, of respirable size.

TABLE 8. PARTICLE SIZE DISTRIBUTION OF FLUOMINE DUST

<u>Particle Size Range (Microns)</u>	<u>Number of Particles</u>	<u>Percentage of Particles</u>	<u>Cumulative Percentage of Particles</u>
<1	88	19.3	19.3
1-2	94	20.7	40.0
3-5	144	31.7	71.7
10	72	15.8	87.5
11-20	37	8.1	95.6
>20	20	4.4	100.0

A similar examination of particle size was done on fluomine which had been ground in a mortar and used for a high concentration level dust exposure and for the guinea pig inhalation-sensitization study. The results of this particle sizing are shown in table 9. A total of 381 particles were measured with approximately 82% of these within the respirable range.

TABLE 9. PARTICLE SIZE DISTRIBUTION
OF FLUOMINE DUST AFTER GRINDING

<u>Particle Size Range (Microns)</u>	<u>Number of Particles</u>	<u>Percentage of Particles</u>	<u>Cumulative Percentage of Particles</u>
<1	132	34.6	34.6
1-2	93	24.4	59.0
2-5	87	22.8	81.8
5-10	50	13.1	94.9
10-20	15	3.9	98.8
>20	4	1.0	99.8

A modified Landsteiner (1937) technique was used to determine whether fluomine would cause an antigen-antibody reaction in male albino guinea pigs. The experimental group consisted of 18 guinea pigs. Another group of three guinea pigs was used to determine the primary irritation properties of the compound.

The primary irritation test consisted of injecting 0.05 and 0.10 ml quantities of an 0.1% suspension of fluomine in peanut oil into the closely clipped scapular and sacral areas of the three guinea pigs. Similar injections of peanut oil alone were also made. The injection sites were examined at 24 and 48 hours. The intradermal injections of the 0.1% suspension caused inflammatory reactions resulting in this concentration being considered a primary irritant. A repeat of the process using an 0.01% suspension did not

cause a response that could be considered as irritating and, thus, this concentration was used for the intradermal sensitization regimen.

The sensitization test was started on a Monday with the guinea pigs being weighed and clipped on the scapular areas. An injection of 0.05 ml of the 0.01% suspension of fluominewas made intradermally into the upper right scapular area of each animal. A similar injection of peanut oil was made concurrently into the upper left scapular area. The injections were examined at 24 and 48 hours and the numerical scores (determined as shown in table 10) recorded.

TABLE 10. GRADING OF SKIN REACTIONS IN THE GUINEA PIG SENSITIZATION TEST

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

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

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

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

The guinea pigs were rested for three weeks (incubation period), weighed, and given a challenge dose of 0.05 ml of the 0.01% suspension into the lower right scapular area. A control injection of peanut oil was made into the lower left scapular area. The reactions were again read after 24 and 48 hours.

The grading system is designed so that the intensity of the skin reaction is represented by a proportionate numerical value and also that any reaction elicited by the vehicle (peanut oil) is subtracted from the reaction elicited by the test substance and vehicle combined.

The rodents used in these experiments were 25 to 30 gram CF-1 mice, 250 to 400 gram CFE (Sprague-Dawley derived) rats, and 450-600 gram albino short-hair (Hartley strain) guinea pigs. All of the rodents were obtained from Carworth Farms, Incorporated. New Zealand albino female rabbits purchased from Pel Freeze, Inc., weighing between four and five pounds, were used for the eye and skin irritation studies. Quality control studies on all species during the quarantine period showed the animals to be in good health.

To define the effects of fluomine dust, a range-finding toxicity study was performed on rodents. With the exception of one inhalation exposure and the inhalation sensitization study, the fluomine was used in the same form as received. The LD₅₀% and LC₅₀% values were based on 14-day observation periods.

To determine the oral LD₅₀ in rats and mice, fluomine was administered as a suspension in corn oil. The oral LD₅₀ of fluomine in the male mouse is 123 mg/kg while in the male rat it is 187 mg/kg as shown in Tables 11 and 12. Most deaths occurred during the first 24 hours postexposure with the latest deaths occurring at 7 days postexposure.

TABLE 11. ACUTE SINGLE DOSE ORAL TOXICITY OF
FLUOMINE FOR MALE MICE

<u>Dose, mg/kg^(a)</u>	<u>Mortality Ratio^(b)</u>	<u>Days to Death^(c)</u>
400	5/5	1, 1, 1, 1, 4
200	5/5	1, 1, 1, 4, 4
100	1/5	6

LD₅₀ and Confidence Limits = 123(93-167) mg/kg

(a) Administered as a suspension in corn oil.

(b) Number died over number dosed.

(c) "1" indicates any death within 24 hours after dosing.

TABLE 12. ACUTE SINGLE DOSE ORAL TOXICITY OF
FLUOMINE FOR MALE RATS

<u>Dose, mg/kg^(a)</u>	<u>Mortality Ratio^(b)</u>	<u>Days to Death^(c)</u>
400	5/5	1, 1, 3, 5, 7
200	3/5	1, 5, 7
100	0/5	-
50	0/5	-

LD₅₀ and Confidence Limits = 187(129-270) mg/kg

(a) Administered as a suspension in corn oil.

(b) Number died over number dosed.

(c) "1" indicates any death within 24 hours after dosing.

Gross examination of animals that died within 24 hours of dosing revealed distended blood-filled stomachs in mice but not in rats. Microscopic examinations showed areas of necrosis of the lymphocytes within the germinal centers of the spleen. This was seen in both rats and mice examined. This lesion is not normally found in our rodents and is considered to be a result of the chemical insult.

Into one eye of each of six rabbits 0.1 ml of a 33% (w/w) suspension of ground fluomine in saline was instilled with the other eye serving as a control. Under these conditions, fluomine proved to be extremely irritating to the conjunctivae, causing marked chemosis and considerable discharge. Due to severe swelling of the conjunctivae and nictitating membranes, examination of corneal and iris tissue was impossible except in one case where definite corneal opacity was noted at 72 hours.

This was followed by the installation of a 3% (w/w) suspension into the eyes of six rabbits. A comparison of the results of the two tests is shown in table 13. As can be seen in the table, the 1-hour and 24-hour responses are comparable for the two mixtures tested. The values after 24 hours, however, indicate the higher concentration resulted in a degree of irritation of increased duration and severity. No residual fluomine particles were noted after 24 hours in those eyes treated with the 3% suspension. Undissolved fluomine was observable in eyes receiving the higher dose up to 7 days after treatment. The difference noted is probably due to the amount of retained particles capable of solubilizing in the ocular fluid and serving to provide a constant source of chemical irritation.

TABLE 13. IRRITATION RESPONSE FROM INSTILLATION OF
FLUOMINE IN RABBIT EYES

<u>Concentration of Suspension</u>	<u>Draize^(a) Scores</u>			
	<u>1 hour</u>	<u>24 hours</u>	<u>72 hours</u>	<u>7 days</u>
33%	4.3	12.7	23.3	52.5
3%	1.3	8.7	1.0	0

(a) Draize et al., 1944.

The characteristics of the irritation indicated that it was chemical in nature rather than mechanical abrasion, which implied that some solubilization of the fluomine was occurring to provide the irritant. In order to test this hypothesis, fluomine was milled with physiological saline for 1.5 hours and filtered to yield a 0.71 g/100 ml solution. This solution was tested for eye irritation potential by installation of 0.1 ml into one eye of each of six

rabbits. Examinations at 1 hour, 72 hours, and 7 days were negative, indicating that the solution, as tested, was nonirritating to the eyes.

The result cast some uncertainty on the original interpretation of fluomine eye irritancy residing in the soluble portion, but it is possible that the fluomine solution was quickly washed out of the eye while the solids were retained on the conjunctiva providing a continuous source of soluble material. However, mechanical abrasion cannot be ruled out as a contributing factor. Further testing would be necessary to define the relative contributions of both forms to eye irritancy.

Rabbit skin irritation tests show fluomine to be a mild to moderate irritant, more potent in areas of abrasion. This would be consistent with the findings of extreme irritancy to the eye.

Six-hour and one-hour acute inhalation exposures were given to groups of 10 rats and 10 mice in a 120 liter plexiglas chamber. The measured exposure concentrations and the resulting mortality are shown in table 14. The six-hour LC_{50} is 112 mg/m^3 for rats and 473 mg/m^3 for mice. The one-hour LC_{50} for rats was 712 mg/m^3 while it was not possible to generate high enough concentrations of fluomine to produce deaths in the mice at this time period.

Gross examination of the rats that died following exposure revealed fluomine particles in the trachea and in the lungs. Microscopic examination of the animals revealed the following pathology: The entire mucosa of the

TABLE 14. MORTALITY RESPONSE OF RATS AND MICE TO 1-HOUR
AND 6-HOUR INHALATION EXPOSURES TO FLUOMINE DUST

Rats		Mice	
<u>Conc., mg/m³(a)</u>	<u>Mortality Ratio(b)</u>	<u>Conc., mg/m³(a)</u>	<u>Mortality Ratio(b)</u>
<u>Six Hours</u>			
407	10/10	464	6/10
185	10/10	195	1/10
104	3/10	94	1/10
49	0/10		
LC ₅₀ and 95% C.L. = 112 (81-163) mg/m ³		LC ₅₀ and 95% C.L. = 416 (222-780) mg/m ³	
<u>One Hour</u>			
890(c)	9/10	890(c)	0/10
750	7/10	750	0/10
507	0/10	507	0/10
LC ₅₀ and 95% C.l. = 712 (639-792) mg/m ³			

(a) Concentration measured gravimetrically.

(b) Number died over number dosed.

(c) This exposure was done with milled fluomine dust.

nasal cavity showed erosion and sloughing of the epithelium with a severe, purulent inflammatory response. The trachea also showed an intense inflammation with necrosis of the epithelium which extended downward into the glandular tissue.

The lungs showed signs of perivascular edema, intraalveolar edema, and congestion with diffuse focal areas of lymphoid hyperplasia. Larger air spaces were often filled with a proteinaceous material. The liver showed periacinal congestion with dilation of the sinusoids.

The guinea pig intradermal sensitization study was done using an 0.01% suspension, one-tenth the standard concentration, of fluomine in peanut oil. Sensitization responses were obtained in 16 of 18 animals with a high mean reaction score indicating that fluomine is a potent sensitizer for guinea pigs by the intradermal route.

Since the normal route of exposure to this material will be by inhalation, guinea pigs were exposed to a series of inhalation exposures to see if a sensitization response would be elicited. This was performed following the same regimen as the previous sensitization study, with inhalation exposures substituted for the intradermal injections. Milled fluomine powder was used in this series of exposures.

Table 15 lists the number of animals exposed and the concentrations received during this study. The first 2-hour exposure of guinea pigs to 30 mg/m³ fluomine dust resulted in the deaths of six animals. New animals replaced these prior to the next exposure of 12 mg/m³ for two hours.

Following this, eight more of the original group died. The exposure time was then reduced to one hour for the remainder of the exposures without further loss of animals. To determine whether sensitization response could be elicited after only a few exposures, an additional group of three guinea pigs was added for the last three inhalation exposures.

TABLE 15. INHALATION EXPOSURE REGIMEN FOR GUINEA PIG SENSITIZATION STUDY

<u>Date</u>	<u>Exposure Time, Hrs.</u>	<u>Fluomine Conc., mg/m³</u>	<u>No. of Guinea Pigs in Group</u>		
			<u>1</u>	<u>2</u>	<u>3</u>
11/7	2	30	20		
11/9	2	12	14	6	
11/12	1	12	6	6	
11/14	1	12	6	6	
11/16	1	12	6	6	3
11/19	1	12	6	6	3
11/21	1	12	6	6	3
12/12	1	15	6	6	3

To the three groups listed in table 15, were added groups 4 and 5, consisting of six guinea pigs each, remaining from the original intradermal sensitization study. These animals did not receive any of the dust inhalation exposures. Three weeks after the last inhalation sensitization exposure and 2 months after intradermal challenge for groups 4 and 5, the following treatments were given:

Groups 1, 4 and controls: a 1-hour inhalation exposure to 15 mg/m³ fluomine dust.

Groups 2, 3, 5 and controls: An intradermal injection of 0.05 ml of an 0.01% suspension of fluomine in peanut oil.

All animals in the inhalation challenge group exhibited eye and nose irritation. The severity of symptoms was equal in all groups, and none of the guinea pigs showed any signs of anaphylactic response after 3-4 hours. Three of the six guinea pigs in group 2 showed a sensitization response to intradermal injections of fluomine. These three exhibited a mean reaction score of 72. At 48 hours, the response had subsided, giving a mean reaction score of 41. No response to intradermal challenge was seen on the part of group 3 or controls. Contrastingly, in group 5, sensitization responses were found in four of the six guinea pigs at 24 hours and five of the six at 48 hours. The mean reaction scores at these time periods were 600 and 405 respectively. The conclusions of the study may be summarized in the following way:

- I. Guinea pigs exposed 6 or 7 times to fluomine dust demonstrate a mild to moderate sensitization response to an intradermal challenge. However, 3 inhalation exposures do not elicit this response.
- II. Guinea pigs do not show a sensitization or anaphylactic type response when challenged by an inhalation exposure of fluomine dust.
- III. Intradermal challenge presented two months after termination of a fluomine intradermal sensitization study elicits a strong reaction.

Single peroral doses of fluomine are toxic to both rats and mice at very low levels. The mouse appears to be more susceptible to the irritating properties of the compound, evidenced by the lower LD₅₀ and the incidence of bloated, blood filled stomachs found in the mice but not seen in any of the rats.

Fluomine particles are highly irritating to the eyes of rabbits. The compound causes severe irritation to the conjunctivae resulting in chemosis and marked swelling. If the particles are not removed from the eye, they provide a source of constant irritation to the eye and surrounding membranes. A similar irritation is produced when the compound is in contact with broken or abraded skin. Little or no effect was noted on unbroken skin.

Single inhalation exposure LC₅₀ results indicate that fluomine is highly toxic by this route. Rats are more sensitive than mice but not nearly as sensitive as guinea pigs which died after a single two-hour inhalation exposure to 30 mg/m³. The toxicity appears to be directly proportionate to the increase in animal size tested. This may be directly related to the different respiration rates, volumes, and size of the pulmonary passageways of the three species.

Fluomine dust has a highly irritating effect on the entire respiratory systems of all species tested. Also, a systemic action is evidenced by the effect on livers and the necrosis of the lymphoid elements within the germinal centers of the spleen.

The dust of this compound is a potent sensitizer by the intradermal route to guinea pigs. Although an anaphylactic-type response was not elicited

by the guinea pigs given an inhalation challenge, an intradermal injection showed that it is possible for the animals to produce antibodies if exposed to repeated inhalation of low concentrations of fluomine.

If the assumption that the larger nares and passageways allow for more and larger particles to reach the lower respiratory tract, then man could be more severely affected than any of the species tested. Presumptively, until metabolism is studied, man must be considered to be as vulnerable as the most sensitive species, the guinea pig. Respiratory, oral, ocular and dermal contact of this compound should be avoided until hygienic guidelines are established. Consideration should be given to the overall production and use hazard of this compound. Chronic inhalation studies will be initiated in the near future to define a safe exposure level for workmen installing, regenerating and repairing the fluomine systems for oxygen supply.

Acute Toxicity Studies on Cluster Marker Residue (Burned Misch Metal)

These studies were conducted to determine the acute toxicity of burned misch metal, the major component found in the residue of a cluster marker of interest to the U. S. Air Force. Toxicology data from this study is to be used in an environmental impact assessment of the material in addition to providing occupational guidelines for human exposure.

A testing laboratory report on the quantitative and qualitative analysis of cluster marker residue indicates the major components to be oxides of

lanthanum (61.6%) and zinc (27.0%). Cochran et al. (1950) reported an oral LD₅₀ for lanthanum oxide in rats of > 10,000 mg/kg. Although an intraperitoneal (ip) LD₅₀ for lanthanum oxide was not determined, five other lanthanum salts demonstrated ip LD₅₀ values one order of magnitude lower than their respective oral LD₅₀'s.

The results reported herein evaluate the acute toxicity of burned misch metal by use of the following tests:

1. Single Dose Oral LD₅₀ in Rats and Mice
2. Primary Skin Irritation in Rabbits.

Male CFE (Sprague-Dawley derived) rats, 5 per group, ranging in weight from 200-300 grams and male CF-1 mice, 5 per group, ranging in weight from 20-30 grams were orally dosed with tap water suspensions of burned misch metal, for LD₅₀ determinations. The misch metal was pulverized with a mortar and pestle and used to prepare suspensions such that pre-calculated doses could be given with dose volumes of 0.01 ml per gram of body weight. Homogeneity of the suspension was insured by use of a magnetic stirrer using teflon-coated stirring bars. Experimental animals were fasted for at least 16 hours prior to administration of the test material with a glass syringe and special oral dosing needle. Rats and mice were weighed individually at the time of dosing to determine the proper dose volume. Test animals were observed for 14 days immediately following the administration of the single oral dose. Any deaths occurring during this observation period were included in the final mortality figures.

A single dose oral LD₅₀ for burned misch metal could not be determined in either rats or mice using the standard oral dosing techniques. Doses administered at or below 2000 mg/kg failed to produce deaths in rats. Quantities of suspended misch metal needed for doses greater than 2000 mg/kg eventually plugged the syringe and needle preventing testing at those levels. Doses at or below 4000 mg/kg resulted in only one mouse death. Administration of doses greater than 4000 mg/kg to the mice was also prevented by clogging of the needle and syringe. Because of the reduced quantity of suspended material needed for a given mouse dose versus that used to treat a rat at the same dose level, higher dose levels could be attained in the mouse series before clogging of the syringe and needle occurred.

Oral toxicity data for rats and mice and the toxicity classification category for mice are shown in table 16. The toxicity classification system used is detailed in Back et al. (1972), page 2.

TABLE 16. MORTALITY RESPONSE OF RATS AND MICE TO SINGLE ORAL DOSES OF BURNED MISCH METAL

	<u>Dose (mg/kg)</u>	<u>Mortality Response (No. Dead/No. Dosed)</u>	<u>Toxicity Classification</u>
Rats	4000	Could not administer	
	2000	0/5	
	1000	0/5	
Mice	8000	Could not administer	Nontoxic*
	4000	1/5	
	2000	0/5	

Mice - LD₅₀ > 5000 mg/kg (estimated)

*14-Day Single Dose Oral LD₅₀ > 5000 mg/kg.

Six female New Zealand white rabbits, 4-5 lbs., were tested according to the methods described by Draize et al. (1944) to determine the primary skin irritation potential of burned misch metal. This patch test method is utilized to evaluate the degree of primary irritation produced on intact and abraded skin.

Using 0.5 gram quantities, the cluster marker residue was applied to the designated test areas and covered with a 2-inch square of surgical gauze several layers thick. The patches were taped in place for 24 hours after which time test areas were examined for irritation using the Draize et al. (1944) table as a reference standard.

Twenty-four and 72-hour examinations of areas tested with the burned misch metal failed to show any skin irritation effects in either the intact or abraded skin. There was no evidence of erythema or eschar formation which would be indicative of primary irritation.

Burned misch metal has been shown by analysis to contain large amounts of lanthanum oxide (61.6%). Although oral LD₅₀'s could not be determined for burned misch metal, the lack of mortality in rats, as well as the limited mouse mortality, indicate that this material is relatively nontoxic by ingestion. Other investigators have shown, however, that some soluble salts of lanthanum exhibit biologically significant, increased toxicities when administered by either intraperitoneal (10 times more toxic than oral) or intravenous (100 times more toxic than oral) modes.

When tested according to standard evaluation methods, burned misch metal did not demonstrate any potential as a corrosive material capable of producing primary skin irritation in rabbits.

Coal Tar Aerosol Studies

The inhalation exposure portions of three 90-day coal tar volatile aerosol studies were completed during the past year. The animals were exposed continuously for 90 days to the aerosolized coal tar at concentrations of 10, 2, and 0.2 mg/m³. The pilot coal tar aerosol study, detailed in a previous annual report (MacEwen and Vernot, 1972) is shown in figure 8. Control groups of each species were maintained for each study and used for comparison with the test animals. The animals were observed daily for general appearance, behavior, signs of toxic stress and lethality. With the exception of 10% of the rats and hamsters and the groups of mice scheduled for serial sacrifice, all animals are being maintained for lifetime observation in the postexposure animal facilities.

The experimental animals, chamber loading and aerosol generation method detailed in the last annual report (MacEwen and Vernot, 1973) remain the same. Postexposure animal sacrifices and hematology evaluations were also unchanged throughout these three studies.

Due to aerosol generation difficulties in the pilot study, the solids were removed from the coal tar to reduce the viscosity. The pilot study coal tar aerosol also did not contain a light oil fraction of the coke oven distillate which

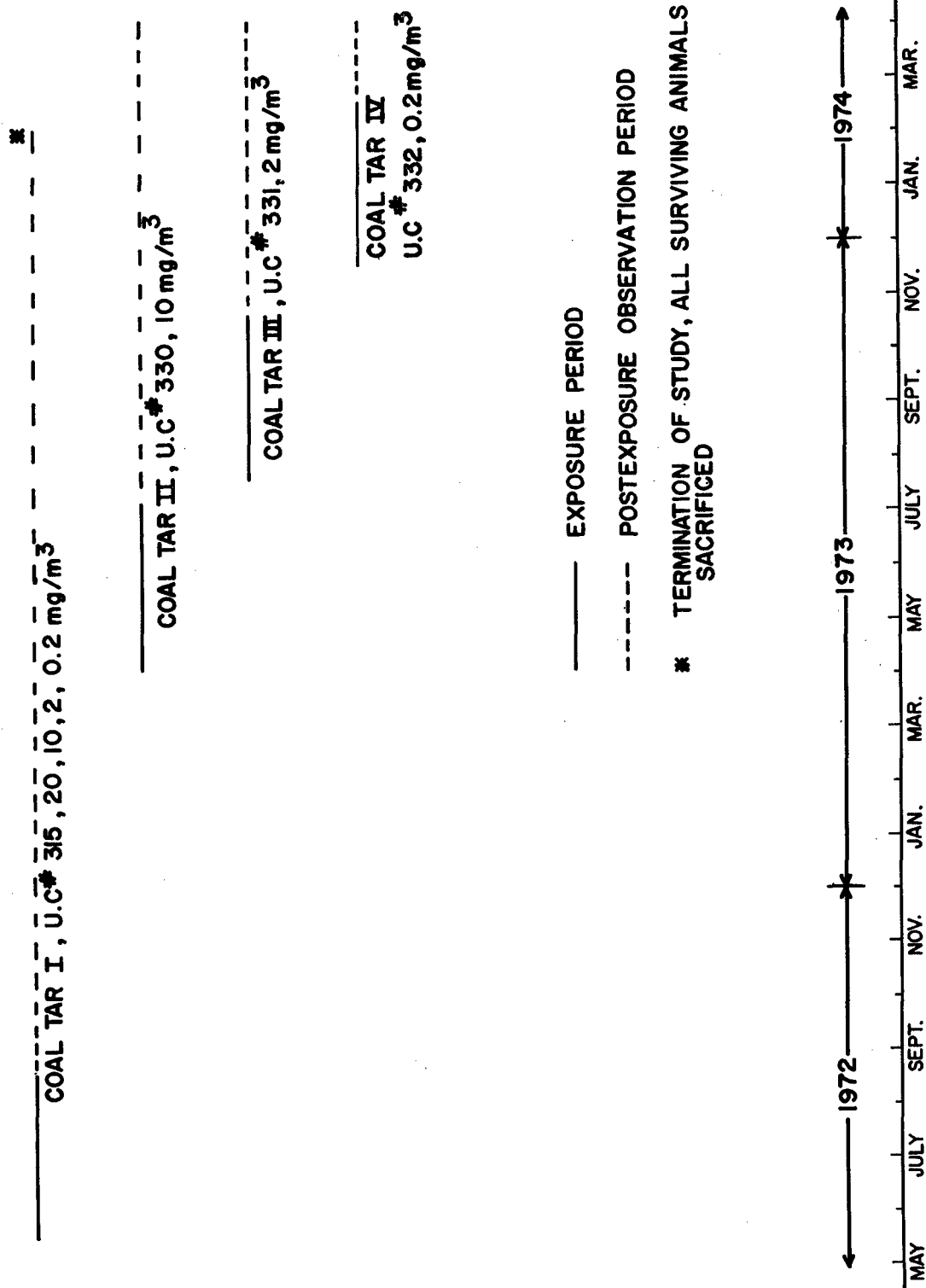


Figure 8. Schedule of coal tar aerosol studies.

is normally removed late in the separation process. This light oil fraction is also known as the BTX fraction and consists primarily of benzene, toluene and xylene.

The three subsequent exposures to 10, 2 and 0.2 mg/m³ contained the entire complex mixture of coke oven effluent (solids and BTX fraction) to avoid the possibility of eliminating the active agent or agents responsible for cancer induction. Gaseous coke oven effluents such as carbon monoxide, sulfur dioxide, and hydrogen sulfide were not included in the exposure mixture.

An aerosol particle size determination was performed on each chamber monthly during the study following the procedure of Vooren and Meyer (1971). Table 17 is a summary of the particle size analysis for all three studies. A minimum of 97% of all droplets were five microns or less in diameter and in most cases, 99% of the droplets were within this respirable range.

TABLE 17. SUMMARY OF PARTICLE SIZE ANALYSES
DURING COAL TAR AEROSOL STUDIES

Exposure Concentration	Month of Exposure	Percentage of Droplets 5 Microns or Less in Diameter			
		Chamber A	Chamber B	Chamber C	Chamber D
10 mg/m ³	1	98.8	100.0	98.4	98.5
	2	99.7	99.8	99.7	99.7
	3	98.9	99.8	99.1	99.5
2 mg/m ³	1	100.0	99.5	99.0	99.7
	2	100.0	98.6	99.7	99.1
	3	99.9	98.3	99.0	98.7
0.2 mg/m ³	1	100.0	99.6	99.1	97.3
	2	99.4	99.7	99.7	99.8
	3	99.0	99.6	99.5	99.4

The chamber concentrations were analyzed using gravimetric sampling to trap the aerosol droplets on a membrane filter. The fluorescent materials were dissolved from the filter with toluene and the fluorescence measured with a Turner fluorometer. Sampling was done on an hourly basis for the first two weeks of each study, then every two hours when it was determined that the concentrations were stable. The final mean chamber concentrations for each study are shown in table 18. The consistency of these concentrations demonstrates the excellent control that was possible with this aerosol generation system.

TABLE 18. MEAN AEROSOL CONCENTRATIONS ACHIEVED
DURING 90-DAY EXPOSURES TO COAL TAR VOLATILES

Nominal Conc. (mg/m ³)	Measured Concentration, mg/m ³			
	<u>Rochester A</u>	<u>Rochester B</u>	<u>Longley C</u>	<u>Longley D</u>
10	10.0	9.8	10.0	9.9
2	2.0	2.1	2.1	2.0
0.2	0.20	0.20	0.21	0.20

The benzene concentration in the exposure chambers was carefully monitored throughout the 10 mg/m³ study and at the start of the 2 mg/m³ study to ensure that the Threshold Limit Value (TLV) for this solvent was not exceeded. The present TLV published by the American Conference of Governmental Industrial Hygienists (1972) is 80 mg/m³ based on a 40-hour work week. Considering the animals in these studies are exposed continuously (168 hours per week or four times the TLV time period), the limit was considered to be one-fourth of 80 or 20 mg/m³ on a time weighted basis.

The benzene concentrations of the 10 mg/m³ coal tar exposure ranged between 5.8 and 19.0 mg/m³ with an overall mean concentration of 10.6 mg/m³. The benzene concentration in the 2.0 mg/m³ coal tar exposure ranged from 2.7 to 4.7 mg/m³ with an overall mean of 3.8 mg/m³. When it was determined that the benzene concentration in the chambers was well below the established limits, monitoring was terminated.

Body weights were measured on the weanling rats and the rabbits during the exposure portion of each study and on all animal groups during the post-exposure period on a monthly schedule. The weanling rats were in the rapid growth phase during exposure and, therefore, were the most useful group for demonstrating any adverse effects on the normal growth rate. The rat growth rates plotted in figures 9, 10, and 11 are illustrative of the coal tar induced effects found in all of the other species. Most species showed a growth pattern similar to that demonstrated by the female weanling rats in that they continued to show a significant difference in mean weight for six or more months postexposure. A dose response relationship is best seen in comparing the mean weight gains of the male weanling rats. The statistical difference in body weight disappeared sooner, in the postexposure period, with successively lower exposure concentrations. The one species that did not follow the pattern of the other animals was the hamster. Figures 12, 13, and 14 show that the hamster growth was adversely affected during exposure to 10 mg/m³ aerosol but not affected at 2 or 0.2 mg/m³ coal tar aerosol. In fact, the 0.2 mg/m³ exposed hamsters outgained the control hamsters throughout the study.

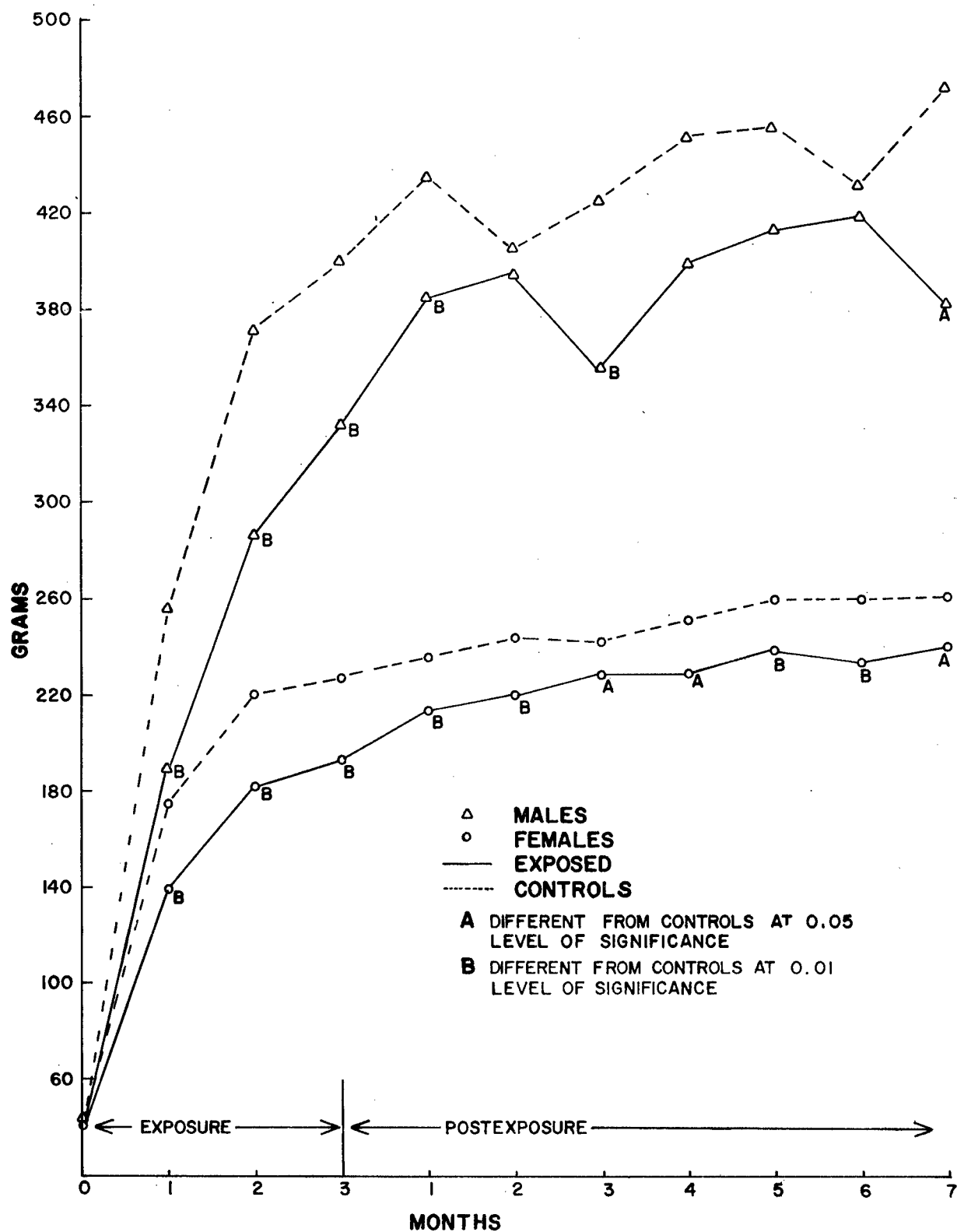


Figure 9. Effect of 90-day continuous exposure to 10 mg/m³ coal tar aerosol on growth of weanling rats.

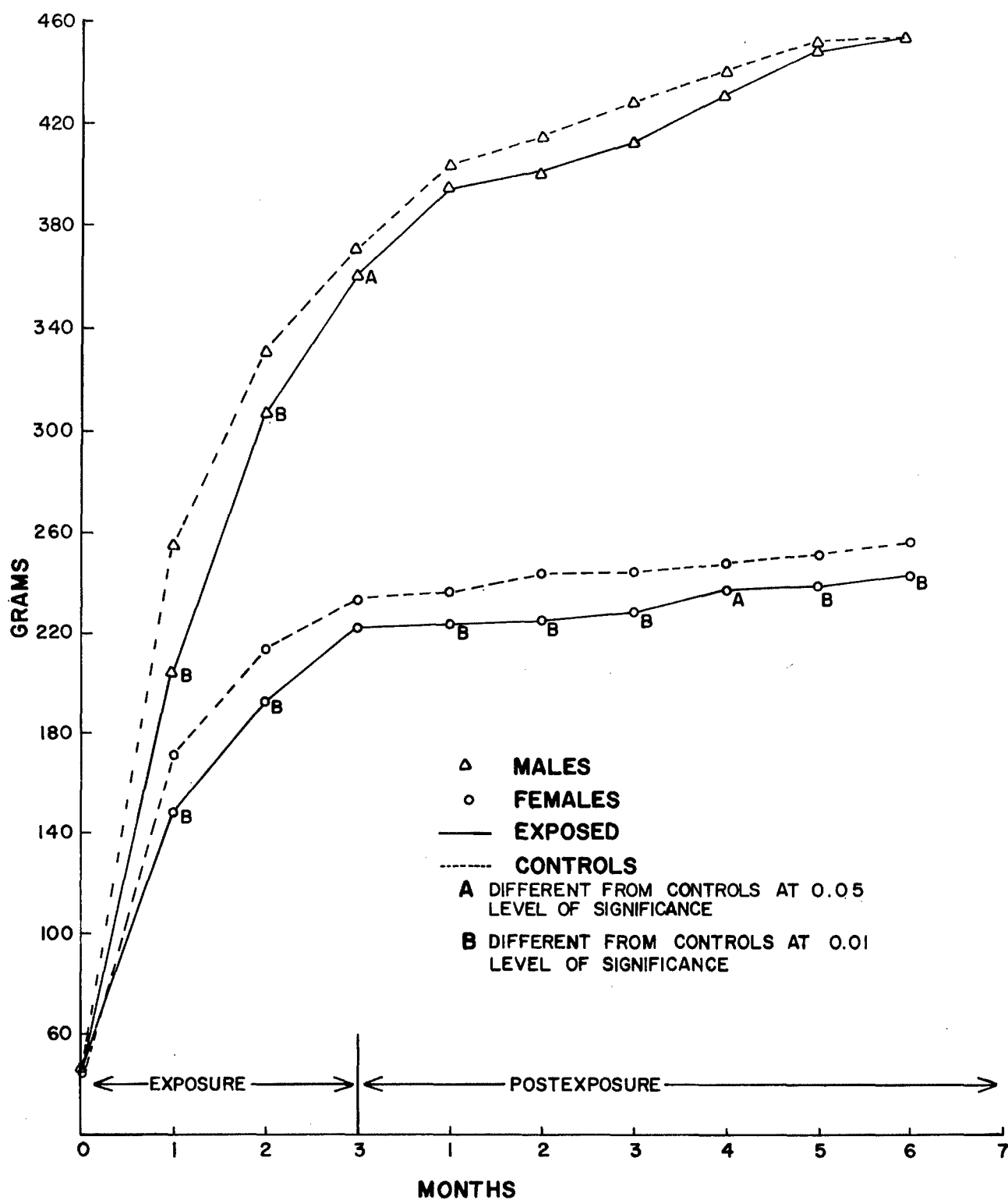


Figure 10. Effect of 90-day continuous exposure to 2 mg/m³ coal tar aerosol on growth of weanling rats.

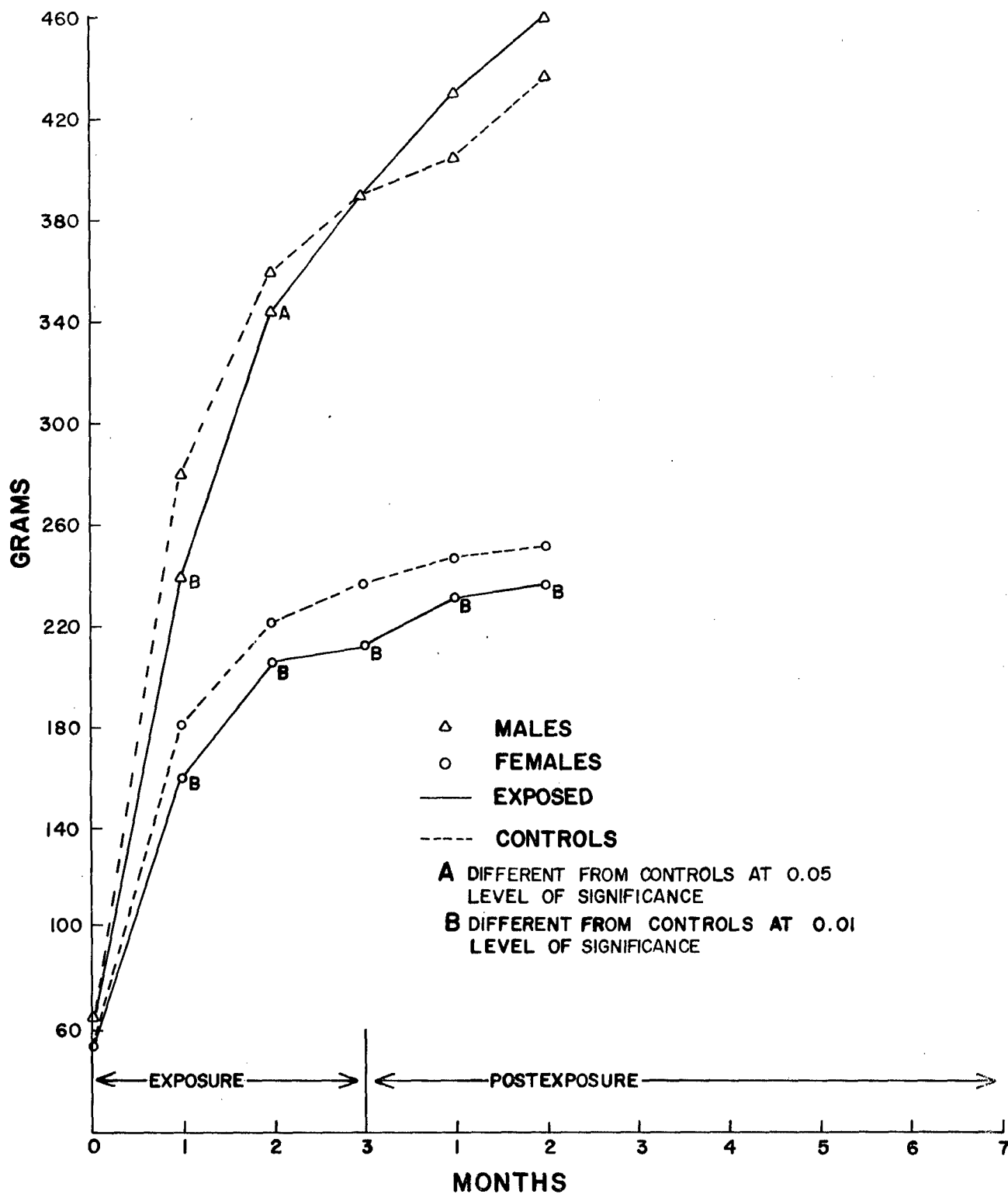


Figure 11. Effect of 90-day continuous exposure to 0.2 mg/m^3 coal tar aerosol on growth of weanling rats.

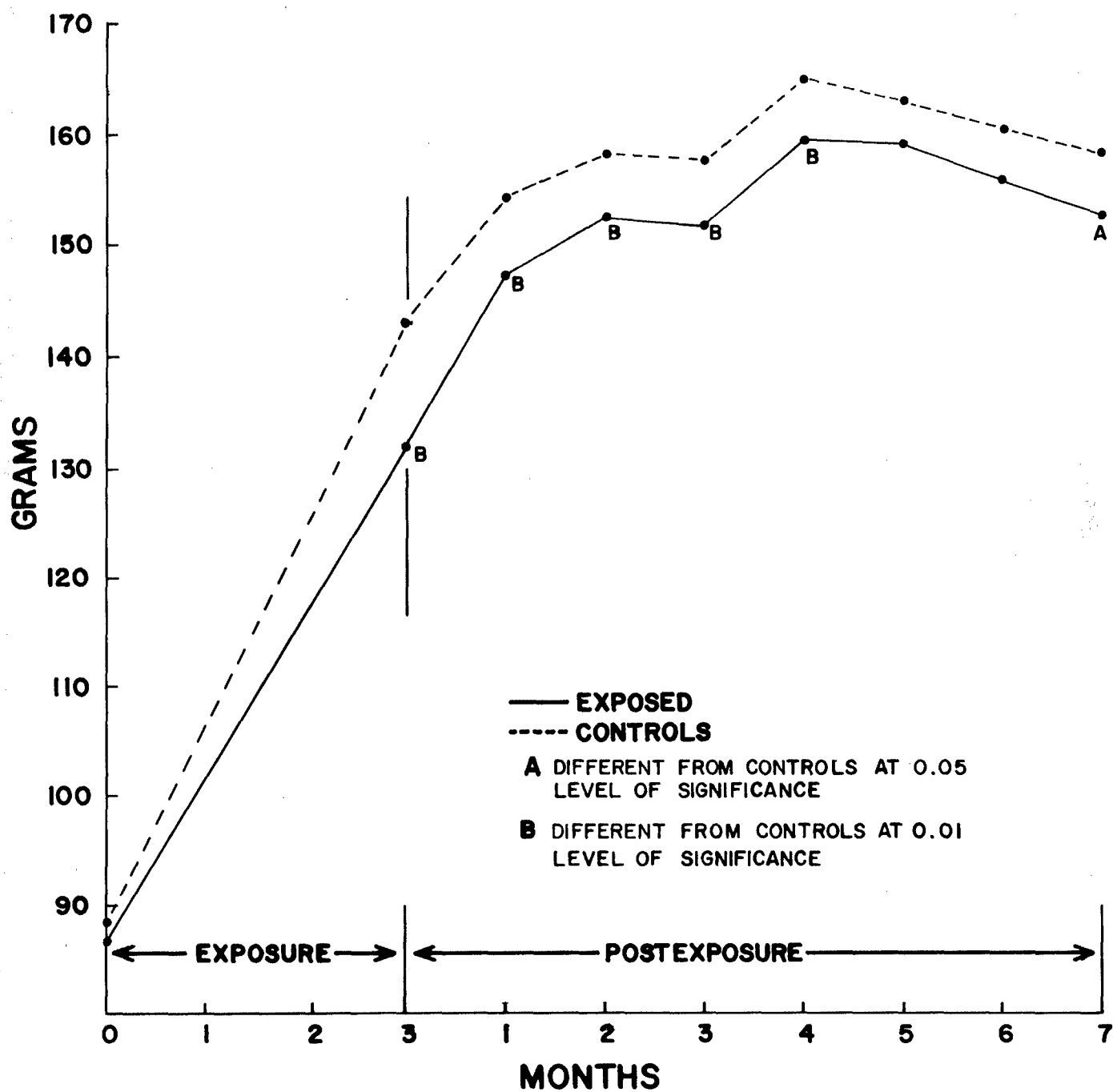


Figure 12. Effect of 90-day continuous exposure to 10 mg/m³ coal tar aerosol on growth of male hamsters.

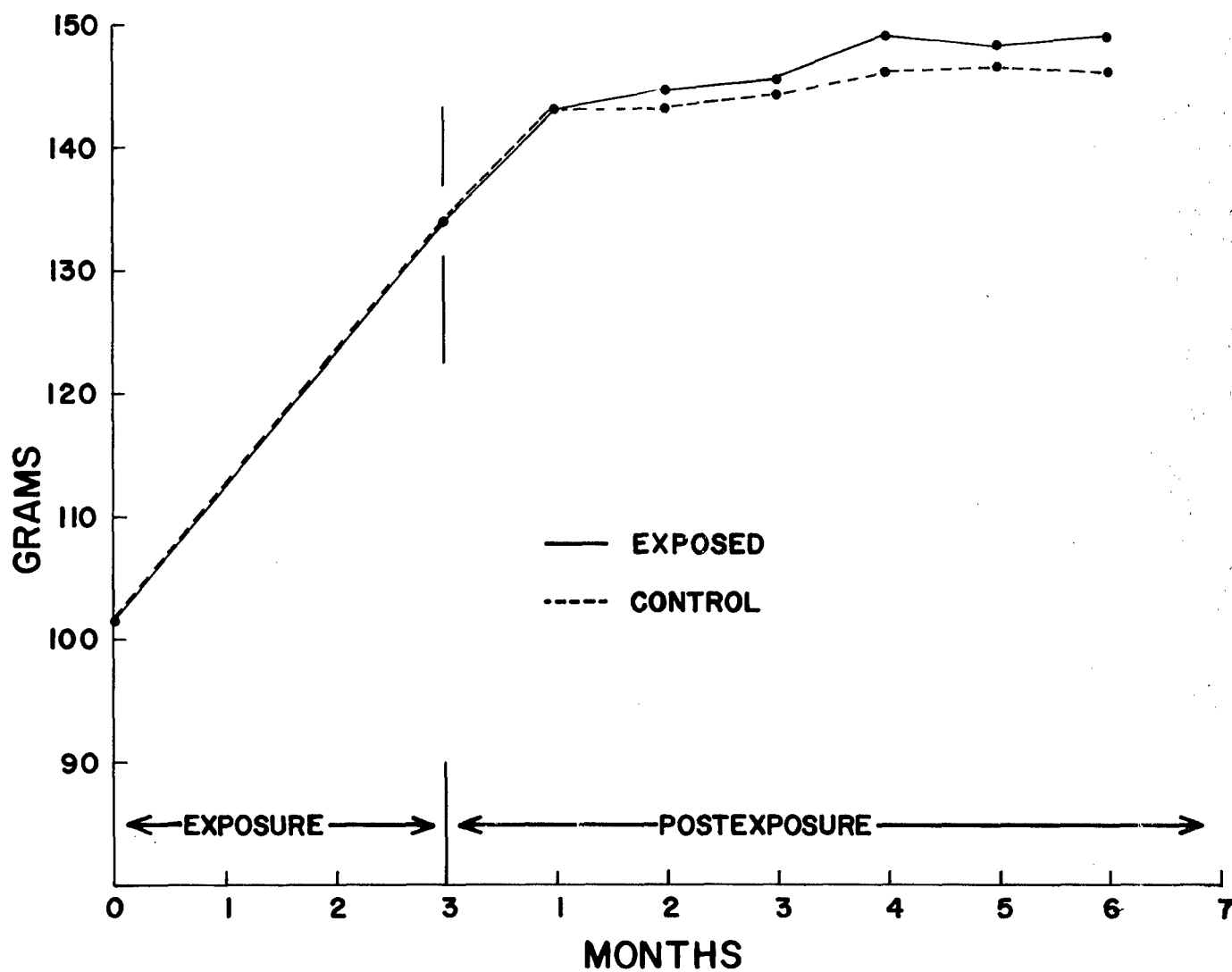


Figure 13. Effect of 90-day continuous exposure to 2 mg/m³ coal tar aerosol on growth of male hamsters.

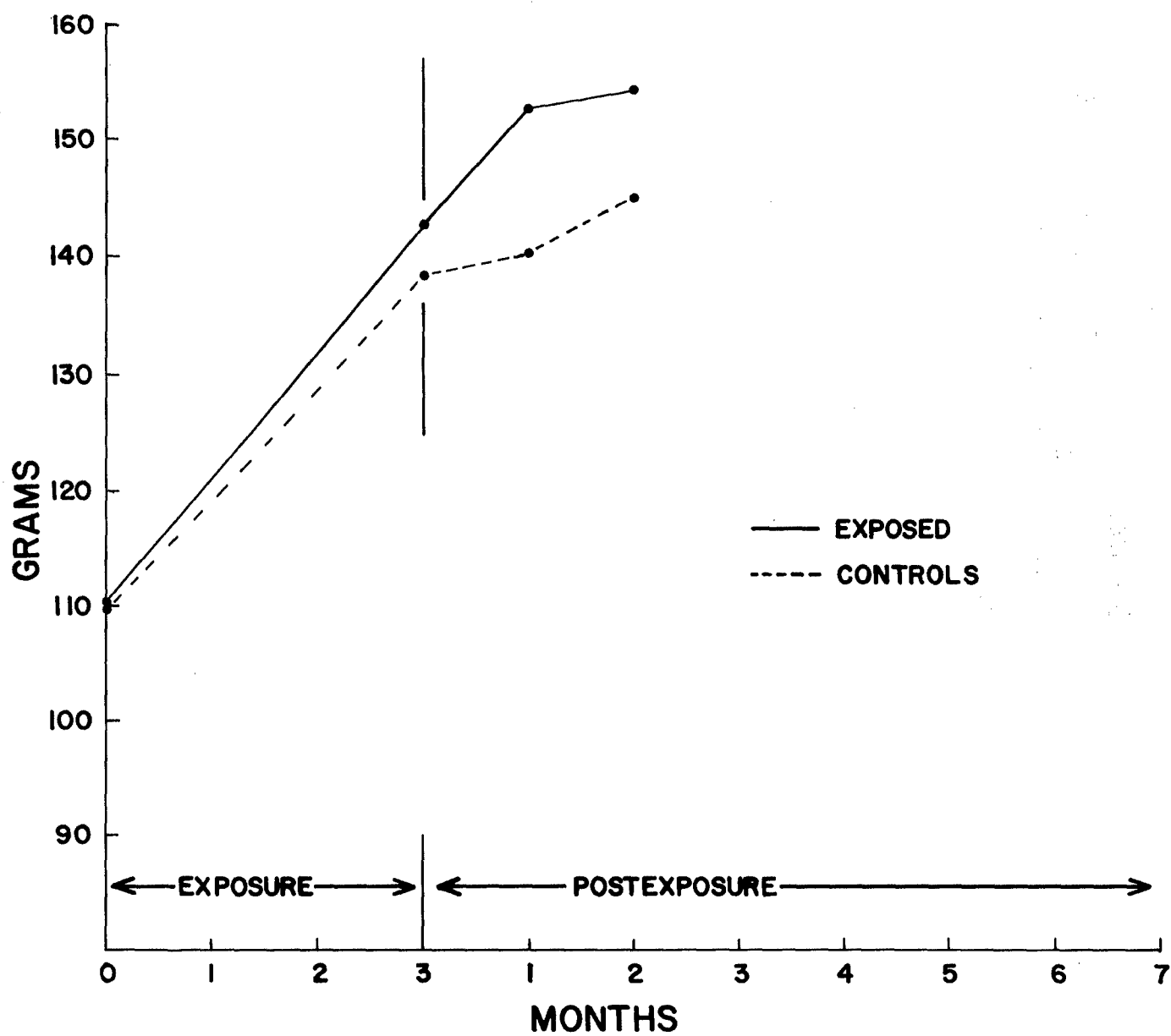


Figure 14. Effect of 90-day continuous exposure to 0.2 mg/m³ coal tar aerosol on growth of male hamsters.

After one and seven days and monthly thereafter, five exposed CF-1 mice and five controls, selected randomly by a computer program prior to start of the study, were sacrificed and submitted to pathology for gross and histological examination of tissues. These mice had a 1" x 1" patch of skin (fur intact) removed from the backs and submitted along with a section of lung for analysis of fluorescent compounds. Fluorescence was also measured on sections of lung removed from the rats and hamsters sacrificed at the conclusion of the exposure portion of each study.

Figure 15 shows the mean fluorescence values for each of the studies. A definite dose response relationship is demonstrated in this graph. A rapid drop in fluorescence can be seen after one month postexposure when better than 50% of the amount of fluorescent compounds has been cleared from the lungs. Clearance continues at a lesser rate during the subsequent monthly examinations, but fluorescence is still detectable 8 months postexposure in the 10 mg/m³ mice.

A summary of lung fluorescence values obtained at termination of the exposures to 10, 2 and 0.2 mg/m³ coal tar aerosol is presented in table 19. If coal tar lung clearance rates were constant, the fluorescence values for each animal strain would reflect the 10/2/0.2 ratio of the aerosol concentration. The male weanling rats and CF-1 mice show this pattern while the others do not. However, the departures from the ratios for the other strains are not so great as to lead to rejection of the hypothesis of constant clearance rates for all the concentrations studied. One thing that is consistent in all three studies is the high fluorescence values of the yearling rats. This may be

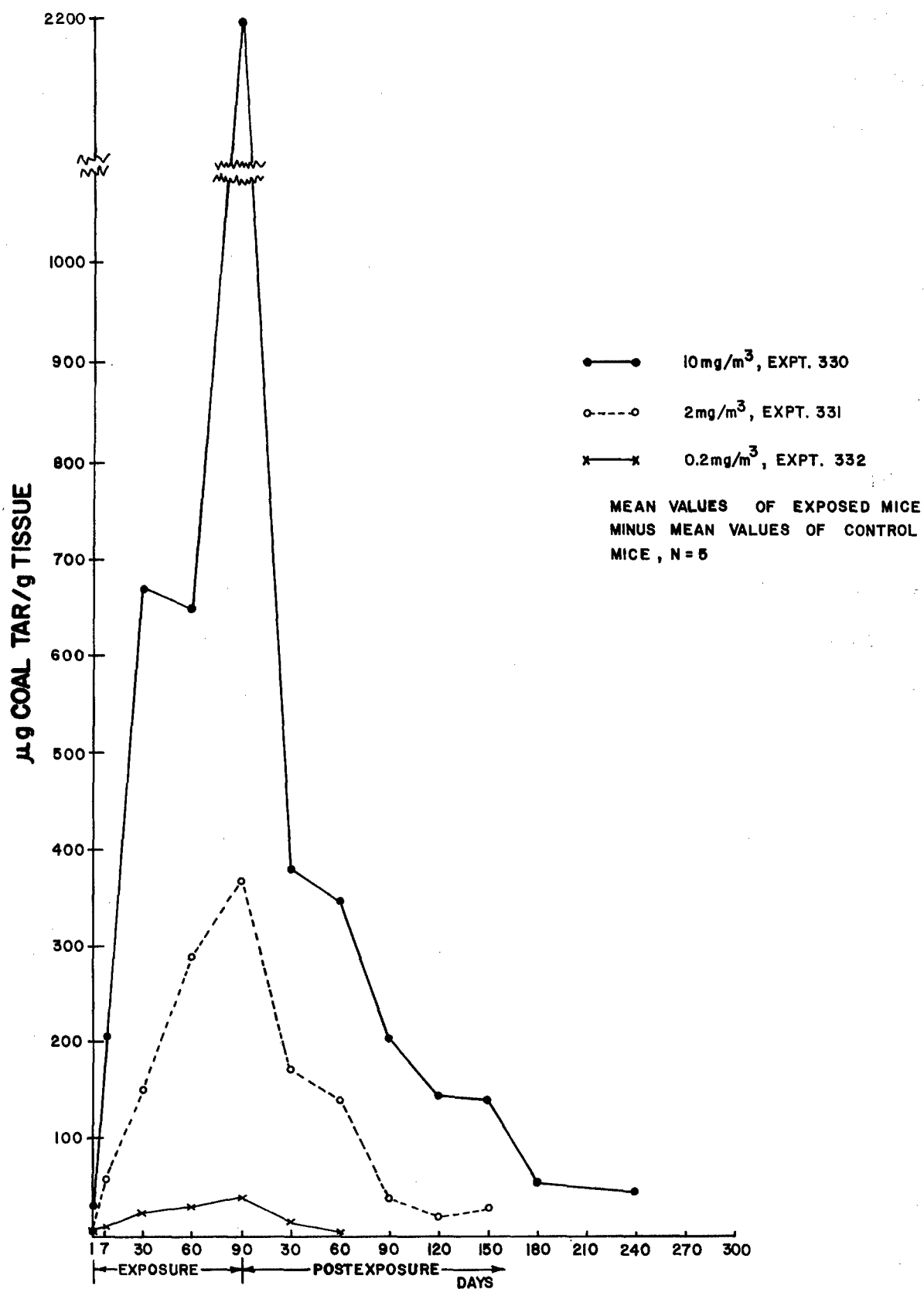


Figure 15. Lung tissue fluorescence in mice.

explained by the fact that they are the only species which entered the studies as full grown adults. It is possible that the lung clearance mechanisms are not as efficient in older animals as in younger animals.

TABLE 19. SUMMARY OF LUNG FLUORESCENCE VALUES
FOUND AT THE CONCLUSION OF 90-DAY
COAL TAR AEROSOL EXPOSURES

<u>Species</u>	Number per Group	Lung Fluorescence*			Ratio† 10/2/0.2
		10 mg/m ³	2 mg/m ³	0.2 mg/m ³	
Hamsters, ♂	10	1759	531	38	6.5/2/0.14
Weanling Rats, ♂	4	2142	457	54	9.2/2/0.23
Weanling Rats, ♀	4	958	439	68	4.3/2/0.31
Yearling Rats, ♀	8	2685	1078	132	4.8/2/0.24
CF-1 Mice, ♂	5	2182	366	39	11.8/2/0.21

*Mean values of exposed animals less the mean values of their respective controls, expressed as μg of coal tar/g of tissue.

†Calculated after normalization of 2 mg/m³ fluorescence values to 2.

Hide fluorescence peaked early during the exposure portions of each study (table 20). The mice do not preen effectively during the early portion of the study when being stressed by the effects of the contaminant and the change in environment. After a period of adjustment, the amount of coal tar removed is approximately equivalent to the amount being deposited. Within one month postexposure, no fluorescence can be detected on the fur or skin of the mice.

TABLE 20. SUMMARY OF HIDE FLUORESCENCE OF CF-1 MICE
DURING AND AFTER EXPOSURE TO COAL TAR AEROSOLS

<u>Aerosol Conc.</u>	<u>($\mu\text{g}/\text{cm}^2$)</u>					
	<u>1 Day</u>	<u>7 Days</u>	<u>30 Days</u>	<u>60 Days</u>	<u>90 Days</u>	<u>30 Days Postexposure</u>
10 mg/m ³	26.9	34.9	19.2	23.8	21.6	0
2 mg/m ³	2.4	9.3	7.6	4.4	9.9	0
0.2 mg/m ³	0.4	1.6	4.9	1.0	3.8	0

Monthly differential blood cell counts were done, starting one month postexposure, on 10 rats and 10 rabbits in the pilot coal tar study. Complete hematology with blood indices was done on 10 rats and 10 rabbits monthly during the postexposure period in the 10, 2 and 0.2 mg/m³ studies. Also, complete hematology was done on the CF-1 mice serially sacrificed during and after exposure in the latter studies.

There were no statistical differences in blood parameters measured in any of the species tested when compared to their respective controls. Post-exposure examination of the 0.2 mg/m³ animals has just begun and results are not yet available.

The inhalation of coal tar aerosol appears to stimulate the proliferation of alveolar macrophages in the lung which contain the deposited material in the form of microglobules, brownish-black in color. Most of these macrophages are found within the lumen of the alveoli or attached to the alveolar wall. A lesser number were found in the interstitium and in peribronchial lymphoid areas. Their numbers were proportional to the concentration level to which they had been exposed. Hamsters tended to show less pigmentation than any other species. The pigment decreased in amount rapidly after exposure although some did remain until termination of the pilot study.

Descriptions of the skin tumors found in the pilot study were detailed in the last annual report. Those skin tumors found in the subsequent coal tar aerosol studies were of the very same nature. Table 21 shows the cumulative numbers of tumors for each concentration and mouse strain from the coal tar pilot study. There appears to be a predisposition for the CF-1 strain of mouse to develop skin tumors, at least at the 10 mg/m³ and lower concentrations. The only JAX mice that showed skin tumor development were those exposed to 20 mg/m³. A definite dose response relationship can be seen in the incidence of CF-1 mouse skin tumors. No skin tumors were found in the following groups: JAX mice, 10, 2, 0.2 mg/m³ and control groups; CF-1 mice, 0.2 mg/m³ and control groups.

TABLE 21. SUMMARY OF MOUSE SKIN TUMORS FOUND POSTEXPOSURE TO THE COAL TAR AEROSOL INHALATION PILOT STUDY

Days Postexposure	Cumulative Number of Skin Tumors*			
	CF-1 20 mg/m ³	JAX 20 mg/m ³	CF-1 10 mg/m ³	CF-1 2 mg/m ³
93	2 (36) [†]	6 (27)	0 (8)	0 (25)
128	6 (18)	7 (11)	1 (7)	0 (20)
148	6 (18)	7 (10)	1 (4)	1 (19)
170	7 (15)	7 (8)	2 (4)	1 (18)
186	7 (14)	8 (8)	2 (4)	1 (15)
199	9 (14)	8 (8)	3 (4)	1 (15)
213	9 (14)	8 (7)	3 (3)	1 (15)
227	9 (13)	8 (7)	3 (3)	1 (13)
242	9 (12)	8 (6)	3 (3)	1 (10)
256	9 (10)	8 (6)	3 (3)	1 (10)
279	9 (10)	8 (6)	3 (3)	1 (8)
291	9 (9)	8 (6)	3 (3)	1 (8)
310	10 (6)	8 (4)	3 (3)	1 (6)
338	10 (4)	8 (4)	3 (3)	1 (6)
371	10 (2)	8 (3)	3 (1)	1 (2)
387	10 (2)	8 (3)	3 (1)	2 (2)
400	10 (2)	9 (3)	3 (1)	2 (2)
415	10 (2)	10 (3)	3 (1)	2 (2)

* No skin tumors were found on the following groups: JAX, 10 mg/m³; JAX, 2 mg/m³; CF-1, 0.2 mg/m³; JAX, 0.2 mg/m³; CF-1, controls; JAX, controls.

[†]Number in () is the total number alive and examined at this time period.

Summaries of the mouse skin tumors found after 90-day exposure to 10 and 2 mg/m³ coal tar aerosol are shown in tables 22 through 25. Beginning at six weeks postexposure, the animals were examined biweekly. Mouse skin tumors were found during the first inspection of both the 10 and 2 mg/m³ groups. No skin tumors have been found in the 0.2 mg/m³ group after 11 weeks postexposure in any species. After approximately 20 weeks postexposure, a definite dose-response relationship can be seen in the number of skin tumors on CF-1 mice in the 10 mg/m³ versus the 2 mg/m³ groups. A hint of the continued dose-response relationship is the fact that no tumors have yet been found in the 0.2 mg/m³ concentration level. However, it is still too early in the postexposure period to draw any conclusions on the latter exposure group.

Skin tumors were found in the JAX mice at both the 10 and 2 mg/m³ levels. The numbers of mice have been small and there does not appear to be any kind of a dose-response effect. It does appear that JAX mice take a longer time than the CF-1 strain to develop skin tumors.

A skin tumor was found on a 10 mg/m³ exposed hamster 41 weeks postexposure. This is the first of this species to develop a skin tumor. Although this tumor has not been examined histopathologically, grossly it resembles the skin tumors described for the mice.

Alveolargenic carcinomas were found in 9 of 50 controls and 17 of 47 high level mice in a biased sample of animals sacrificed early in the pilot study program. Histopathologic examination of all tissue from the pilot study

TABLE 22. SUMMARY OF SKIN TUMORS FOUND IN CF-1 MICE
EXPOSED TO 10 mg/m³ COAL TAR AEROSOL

No. of Weeks Post- exposure	New Tumors		Cumulative Number of Tumors		Total Number Examined	
	Group A	Group B	Group A	Group B	Group A	Group B
6	3	0	3	0	55	109
8	0	3	3	3	53	109
10	0	0	3	3	52	98
12	5	0	8	3	52	94
14	0	0	8	3	52	88
17	0	15	8	18	52	87
21	12	9	20	27	49	67
23	7	8	27	35	47	63
25	2	2	29	37	47	63
27	4*	3	33(25)	40	47	63
29	2	4	35(26)	44	45	58
31	3	1	38(28)	45	41	55
33	0	4	38(25)	49(35)	39	53
35	0	3	38(24)	52(29)	39	43
37	1	1	39(23)	53(29)	37	39
39	0	1	39(22)	54(26)	33	35
41	0	2	39(20)	56(22)	28	28
43	2*	1*	41(19)	57(15)	23	24

Group A = Unclipped mice (original N = 75).

Group B = Clipped mice for pulmonary pathology (original N = 150).

*A control CF-1 mouse was found with a tumor on this date.

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

TABLE 23. SUMMARY OF SKIN TUMORS FOUND IN JAX MICE
EXPOSED TO 10 mg/m³ COAL TAR AEROSOL

<u>No. of Weeks Postexposure</u>	<u>New Tumors</u>	<u>Cumulative Number of Tumors</u>	<u>Total Examined</u>
6	0	0	43
8	0	0	43
10	0	0	43
12	0	0	43
14	0	0	43
17	0	0	42
21	0	0	42
23	1	1 (1)	42
25	0	1 (1)	42
27	1	2 (2)	41
29	1	3 (3)	41
31	0	3 (3)	41
33	0	3 (3)	41
35	0	3 (3)	41
37	0	3 (3)	41
39	0	3 (3)	41
41	1	4 (2)	38
43	1	5 (3)	38

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

TABLE 24. SUMMARY OF SKIN TUMORS FOUND IN CF-1 MICE
EXPOSED TO 2 mg/m³ COAL TAR AEROSOL

No. of Weeks Post- exposure	New Tumors		Cumulative Number of Tumors		Total Number Examined	
	Group A	Group B	Group A	Group B	Group A	Group B
6	3	2	3	2	75	124
8	1	0	4	2	75	123
10	0	3	4	5	75	120
12	0	1	4 (4)	6	75	94
14	0	0	4 (4)	6	74	82
16	0	0	4 (4)	6	69	73
18	1	0	5 (5)	6 (4)	69	54
20	0	3	5 (4)	9 (6)	66	46
22	4	0	9 (8)	9 (4)	66	37
24	0	0	9 (7)	9 (4)	61	31
26	0	0	9 (7)	9 (2)	60	25
28	0	1	9 (7)	10 (2)	52	24

Group A = Unclipped mice (original N = 75).

Group B = Clipped mice for pulmonary pathology (original N = 150).

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

TABLE 25. SUMMARY OF SKIN TUMORS FOUND IN JAX MICE
EXPOSED TO 2 mg/m³ COAL TAR AEROSOL

<u>No. of Weeks Postexposure</u>	<u>New Tumors</u>	<u>Cumulative Number of Tumors</u>	<u>Total Examined</u>
6	0	0	65
8	0	0	65
10	0	0	65
12	0	0	64
14	0	0	62
16	1	1 (1)	59
18	0	1 (1)	59
20	0	1 (1)	54
22	2	3 (3)	54
24	0	3 (3)	51
26	0	3 (2)	50
28	0	3 (2)	47

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

has not yet been completed. Incidence of lung tumors was scattered throughout the intermediate exposure levels. All tumors were identical in their microscopic morphology. They were well circumscribed but nonencapsulated. Metastases were uncommon and mitotic figures were rare. The essential cell is cuboidal to low columnar and usually uniform in shape. Ultrastructurally, the tumor cells contain numerous Type C virus particles.

Histopathology on the serially sacrificed mice from the 10, 2 and 0.2 mg/m³ studies has been contracted to another source and results are not yet available. Therefore, it is not known what incidence of lung carcinomas may exist in these groups. No lung tumors have been found on any of the animals during gross examination.

Acute Toxicity Studies on Four Amine Compounds

These studies were conducted to determine the acute toxicity of four amine compounds in use, or proposed candidates, as oil and lubricant additives. These compounds are:

Primene JM-T Amine Salt

N-phenyl- α -naphthylamine

p, p'-Diocetyldiphenylamine

Octylphenyl- α -naphthylamine.

Primene JM-T Amine Salt is a long chain aliphatic ammonium salt of chloromethylphosphorous acid with a molecular weight range of 286 to 342. The lack of adequate toxicity information on these compounds necessitated their testing

in this laboratory for determination of potential toxicity hazards to Air Force personnel responsible for their handling and use.

The results reported herein represent a toxicity screening designed to evaluate the acute toxicities of these four chemicals by use of the following tests:

1. Single Dose Oral LD₅₀ in Rats and Mice
2. Primary Skin Irritation in Rabbits
3. Skin Sensitization in Guinea Pigs.

Male CFE (Sprague-Dawley derived) rats, 5 per group, ranging in weight from 200-300 grams and male CF-1 mice, five per group, ranging in weight from 20-30 grams were orally dosed with corn oil solutions of the four test chemicals for LD₅₀ determinations. Experimental animals were fasted for at least 16 hours prior to administration of a chemical with a glass syringe and special oral dosing needle. Solutions of the materials were prepared such that precalculated doses could be given using dose volumes of 0.01 ml per gram of body weight. Rats and mice were weighed individually at the time of testing to determine the proper injection volume.

Test animals were observed for 14 days immediately following the administration of the test agent. Any deaths occurring during this observation period were included in the final mortality figures. Mortality data was treated statistically using the moving average interpolation method of Weil (1952) for LD₅₀ and 95% confidence limits determinations.

The single dose oral LD₅₀'s for the Primene salt were 1866 mg/kg for rats and 536 mg/kg for mice. N-phenyl- α -naphthylamine produced single dose oral LD₅₀'s of 1625 mg/kg in the rat and 1231 mg/kg in the mouse. P,p'-dioctyldiphenylamine and octylphenyl- α -naphthylamine could not be given in sufficient quantities to cause death in either rats or mice. Doses greater than 8000 mg/kg could not be administered at reasonable dose volumes because the resultant mixture was a thick paste.

Oral toxicity data and toxicity classification categories for rats treated with the four amines are shown in table 26. Corresponding information for mice is listed in table 27. The toxicity classification system used is detailed in Back et al. (1972), page 2.

Six female New Zealand white rabbits, 4-5 lbs., were tested according to the methods described by Draize et al. (1944) to determine primary skin irritation potential for each of the four test materials. This patch test method is utilized to evaluate the degree of primary irritation produced on intact and abraded skin by assigning numerical scores to skin reactions based on the amount of erythema and edema found at 24 and 72 hours after application of a test substance. Averages of these scores are used to derive a primary irritation index. Compounds producing an index of 2 or less are only mildly irritating whereas those with indices of 2-5 are moderate irritants and those above 6 are to be considered severe irritants.

Twenty-four and 72-hour postexposure examinations of animals tested with N-phenyl- α -naphthylamine and p,p'-dioctyldiphenylamine failed to

TABLE 26. MORTALITY RESPONSE OF RATS TO SINGLE ORAL DOSES OF FOUR AMINE COMPOUNDS

<u>Compound</u>	<u>Dose (mg/kg)</u>	<u>Mortality Response (No. Dead/No. Dosed)</u>	<u>Toxicity Classification*</u>
Primene JM-T Amine Salt	4000	5/5	Toxic
	2000	3/5	
	1000	0/5	
	500	0/5	

LD₅₀ and 95% CL = 1866 mg/kg (1289-2702)

N-phenyl- α - phenylamine	4000	5/5	Toxic
	2000	4/5	
	1000	0/5	
	500	0/5	

LD₅₀ and 95% CL = 1625 mg/kg (1201-2197)

p, p'-Dioctyldi- phenylamine	8000	0/5	Nontoxic
	4000	0/5	

Octylphenyl- α - naphthylamine	8000	0/5	Nontoxic
	4000	0/5	

*Toxic - 14-Day Single Dose Oral LD₅₀ 50-5000 mg/kg
Nontoxic - 14-Day Single Dose Oral LD₅₀ >5000 mg/kg.

TABLE 27. MORTALITY RESPONSE OF MICE TO SINGLE ORAL DOSES OF FOUR AMINE COMPOUNDS

<u>Compound</u>	<u>Dose (mg/kg)</u>	<u>Mortality Response (No. Dead/No. Dosed)</u>	<u>Toxicity Classification*</u>
Primene JM-T Amine Salt	2000	5/5	Toxic
	1000	4/5	
	500	3/5	
	250	0/5	

LD₅₀ and 95% CL = 536 mg/kg (332-864)

N-phenyl- α - naphthylamine	4000	5/5	Toxic
	2000	5/5	
	1000	1/5	
	500	0/5	

LD₅₀ and 95% CL = 1231 mg/kg (910-1665)

p, p'-Dioctyldi- phenylamine	8000	0/5	Nontoxic
	4000	0/5	
Octylphenyl- α - naphthylamine	8000	0/5	Nontoxic
	4000	0/5	

*Toxic - 14-Day Single Dose Oral LD₅₀ 50-5000 mg/kg
Nontoxic - 14-Day Single Dose Oral LD₅₀ >5000 mg/kg.

demonstrate any irritation effects in either the intact or abraded skin. There was no evidence of erythema or eschar formation which would be indicative of primary irritation.

Octylphenyl- α -naphthylamine treatment resulted in very slight erythema and edema, at 24 hours, on one of six test animals and only very slight edema on another rabbit at that same time interval. No signs of irritation were noted at the 72-hour examination. The responses were only noted on the abraded skin. The primary irritation score produced from this material was 0.33 indicating that octylphenyl- α -naphthylamine is only very mildly irritating.

All six test animals treated with Primene JM-T Amine Salt demonstrated signs of erythema, at 24 hours, that ranged from very slight to severe. Five of the six rabbits showed edema in the test patch areas. Examination at 72 hours showed that the erythema and edema was slightly reduced, but severe necrosis was present in the entire area treated with the chemical. Necrotic changes led to the subsequent formation of a thick, "leathery" eschar which persisted for several weeks after the test. Tissue damage was sufficient to cause formation of scar tissue in both intact and abraded test areas. The primary irritation score produced by this chemical was 4.5, indicative of moderate irritation. This score, however, is based entirely on erythema and edema formation, as the Draize method only applies to these two parameters. In consideration of the necrotic changes resultant from treatment with this chemical, the Primene salt should be designated a severe irritant.

The degree of primary skin irritation, in rabbits, produced by each of the four amines tested is summarized in table 28 below:

TABLE 28. PRIMARY SKIN IRRITATION EFFECTS PRODUCED IN RABBITS BY FOUR AMINE COMPOUNDS

<u>Compound</u>	<u>Degree of Irritation</u>
Primene Salt	Severe
N-phenyl- α -naphthylamine	None
p,p'-Dioctyldiphenylamine	None
Octylphenyl- α -naphthylamine	Very Mild

Eighteen male, albino guinea pigs, 450-600 grams, were tested to determine the sensitization potential of the four amine compounds. The method employed for use was a modification of the Landsteiner Guinea Pig Sensitization Test (1967). This method tests whether repeated intradermal injections of small amounts of a test material, followed by an appropriate incubation period have the potential for producing an antigen-antibody response. Numerical scores are derived from measurements of the reaction at injection sites (wheal) 24 and 48 hours after a challenge injection of the test material is administered. Reaction scores are based on the intensity of the skin reaction and are represented by a proportionate numerical value indicative of the sensitization response in the test animal.

None of the four amine compounds tested showed potential for sensitization.

A summary of the test results from toxicity screening of the four materials is shown in table 29.

TABLE 29. SUMMARY OF TEST RESULTS FROM TOXICITY SCREENING OF FOUR AMINE COMPOUNDS

<u>Compound</u>	<u>Oral Toxicity Classification</u>	<u>Skin Irritation Produced</u>	<u>Sensitization Potential</u>
Primene JM-T Amine Salt	Toxic	Severe	None
N-phenyl- α -naphthylamine	Toxic	None	None
p, p' -Diocetyldiphenylamine	Nontoxic	None	None
Octylphenyl- α -naphthylamine	Nontoxic	Very Mild	None

The acute toxicity screening tests applied to the four amine compounds have shown that 2, N-phenyl- α -naphthylamine and the Primene salt, have a low order of oral toxicity while the others are relatively nontoxic by this route.

Primene JM-T Amine Salt is a severe skin irritant and should be handled with special care to prevent skin contact.

While these studies have evaluated the acute toxic effects of the test agents, nothing is known about their potential for producing chronic pathologic changes or carcinogenesis.

Acute Inhalation Toxicity of Benzonitrile

Male CFE rats and CF-1 mice were exposed to air substantially saturated with vapors of benzonitrile in a 30 liter glass chamber. The animals were exposed for either two or four hours and the survivors observed for 14 days

postexposure. Gross and micropathology was performed on representative animals following death or upon sacrifice after a 14-day observation period.

The benzonitrile vapor saturated air was produced by passing air through a fritted disc bubbler immersed in 200 ml of the test material following the method of Smyth and Carpenter (1944). The air flow through the bubbler was 10 liters per minute with the resultant saturated vapor then passing into the 30 liter glass exposure chamber. The amount of time necessary to achieve 95% saturation in the exposure chamber was nine minutes.

There is little information on the toxicity of benzonitrile in the literature. The lethal dose for mice of 180 mg/kg by the subcutaneous route was reported in the Archives of International Pharmacodynamie (1904). The Chemical Hygiene Fellowship of Carnegie-Mellon University (Carpenter, C.P.) report an oral LD₅₀ of 0.71 ml/kg (720 mg/kg) when administered undiluted to male rats. Death resulted in rats when inhaling substantially saturated vapor of benzonitrile (estimated at 950 ppm) for eight hours. A 4-hour inhalation period did not result in any rat deaths.

The reagent grade benzonitrile used in this study is a colorless liquid whose pertinent physical properties are as follows: molecular weight, 103; boiling point at 1 atmosphere, 190.7 C; boiling point at 100 mm, 123.7 C; density 15/15, 1.010; vapor pressure at 25 C, 0.7 mm. Air saturated with benzonitrile at 20 C and 760 mm contains 920 ppm vapor. At these same conditions, 1 ppm = 0.00421 mg/liter and 1 mg/liter = 237.4 ppm. The sample used in this study was obtained from Coulter CMS, Inc., Cincinnati, Ohio.

The benzonitrile chamber concentration was continuously analyzed using a total hydrocarbon analyzer. The instrument was initially calibrated with several standard gas bags containing 450 and 900 ppm benzonitrile in air.

A group of six male rats were exposed to the saturated vapor for four hours. Groups of mice were exposed for four and two hours. The rat exposure resulted in no deaths at 700 ppm for four hours, and exposure of the same duration resulted in 100% mouse mortality. One mouse of a group of seven died following a two-hour exposure (table 30).

Both species showed irritation of the extremities during the first hour of exposure. This was followed by labored breathing and poor coordination which occurred after 60 and 90 minutes in mice and after three hours in rats. Prostration occurred in both species, the mice after 2-1/2 hours and the rats after 3-1/2 hours of exposure. Following the 14-day observation period, five of the six rats showed a subnormal weight gain. Mouse weights were not recorded during the observation period.

TABLE 30.
SUMMARY OF BENZONITRILE VAPOR INHALATION
EXPOSURES TO MALE RATS AND MICE

<u>Species</u>	<u>Nominal Conc. (ppm)</u>	<u>Measured Conc. (ppm)</u>	<u>Measured Conc. (mg/l)</u>	<u>Time (hrs.)</u>	<u>Mortality Ratio</u>	<u>Time to Death</u>
Rats	900	-	-	4	0/6	-
Mice	900	700	2.95	4	10/10	3@0 days*; 3 @ 1 day, 4@ 2 days
Mice	900	890	3.75	2	1/7	1@ 2 days

*One mouse was dead after 3-1/2 hours of exposure, the other two were dead at the conclusion of exposure.

Gross pathology on the mice that died during exposure and on both species when sacrificed following the 14-day observation period failed to show any abnormal lesions which could be attributed to benzonitrile exposure. However, when examined microscopically, the rat lungs contained multifocal areas of lymphoid hyperplasia with foamy macrophage accumulations. The lungs of all mice examined were congested with accompanying edema. The mice exposed for four hours also showed hepatic congestion and sinusoidal dilation.

Upon completion of our tests, additional toxicity data was received in a personal communication (Colo, 1974) which is presented below:

<u>Test</u>	<u>Results</u>
Acute oral toxicity study - albino rats	LD ₅₀ = 1.0 g/kg
Acute dermal toxicity study - albino rats	LD ₅₀ = 1.2 g/kg
Acute aerosol inhalation study - albino rats (4-hour exposure)	8.0 mg/l air: 3/10 dead 0.8 mg/l air: no effect
Eye irritation study - albino rabbits	mildly irritating
Primary skin irritation study - albino rabbits	nonirritating

When trying to predict man's response, we must consider the least resistant species which is the mouse. These tests indicate that saturated vapors of benzonitrile would not constitute a hazard to man provided the insult did not continue for a prolonged period of time, i. e., more than thirty minutes.

The Biological Effect of Continuous Inhalation Exposure of 1, 1, 1-Trichloroethane (Methyl Chloroform) on Animals

Studies were conducted to evaluate the effects of continuous exposure to 1, 1, 1-trichloroethane on hepatic morphology and function and to compare these effects with those produced by methylene chloride (dichloromethane) reported in a previous study (MacEwen et al., 1972; Haun et al., 1972; and Weinstein et al., 1972). The primary aspect of comparison was to determine environmental concentrations of each compound that would produce a similar biological response, i. e., a comparable increase in liver triglycerides over control levels.

A preliminary 14-day test was conducted using mice only, to establish exposure concentrations for the 100-day continuous exposures. In the preliminary test, mice were exposed to 100 and 250 ppm and serially sacrificed at 3, 7, 10 and 14 days for liver triglyceride determinations and tissue stains for fat deposition. The mice exposed to the highest concentration of 1, 1, 1-trichloroethane (TCE) showed no significant liver triglyceride changes while an occasional animal showed increased hepatic fat accumulation after 14 days exposure. We, therefore, selected 250 and 1000 ppm TCE as our exposure concentrations for the 100-day continuous exposure study.

Groups of 4 monkeys, 180 mice, 8 dogs and 40 rats each were housed in Thomas Domes. The three groups of animals were exposed to 250 ppm TCE, 1000 ppm TCE and clean air respectively. The clean air exposed animals served as controls for the test animals.

Ten mice were removed from each group on a weekly basis for gross examination, liver fat stains and liver triglyceride determinations. Rats, dogs, and monkeys were weighed on a biweekly schedule for evaluation of growth patterns. The rats were sacrificed at the end of the exposure period for organ weight measurement, liver fat stains and complete histopathologic evaluation.

Blood samples were collected from dogs and monkeys at the time of weighing and the following biweekly determinations made:

TABLE 31. CLINICAL TEST SCHEDULE FOR LARGE ANIMALS
EXPOSED TO 1, 1, 1-TRICHLOROETHANE

HCT	Albumin/Globulin
HGB	Total Protein
RBC	BUN
WBC	Glucose
Reticulocyte Count	Alkaline Phosphatase
Sodium	SGOT
Potassium	Creatinine
Cholesterol	Chloride
Calcium	SGPT
Inorganic Phosphorus	Serum Triglycerides
Total Bilirubin	

Since dichloromethane had been shown to be partially metabolized to carbon monoxide, carboxyhemoglobin measurements were made on samples taken after 2 weeks exposure. Blood levels of TCE were determined during the third, fifth, ninth and thirteenth weeks of exposure.

The exposures were completed on 12 November 1973 and final animal weights and blood samples were measured at that time except for 2 sets of mice from each experimental group that were examined at 2 and 4 weeks

postexposure to determine reversibility of any measured effects. Tissue samples were harvested for histopathologic examination and fat stains of frozen liver sections taken at weekly intervals.

Continuous exposure of mice to 1000 ppm TCE produced a significant increase in fat droplets which were evident in centrilobular hepatocytes. A slight increase was also seen at the 250 ppm TCE concentration while control mice showed very little fat accumulation. A semiquantitative measurement of fat accumulation was made on individual mouse liver sections stained with oil red "O" and the mean values are presented in table 32. There was no evidence of hepatocyte necrosis, inflammation or fibrosis in any of the mouse livers examined. In mice, almost all fatty change was microglobular and was in a centrilobular distribution while in monkeys, the majority of the fat was in Kupffer cells and only a trace of microglobular fat was present in hepatocytes of all groups, control and exposed. Minimal microglobular fat was present in rather diffuse distribution in both the rats and dogs and was similar in both exposed and control groups.

Mouse liver weights and liver to body weight ratios are shown in table 33. There was little, if any, real effect at the 250 ppm exposure level but a consistent and statistically significant increase in liver to body weight ratios was seen in the 1000 ppm TCE exposure group of mice. Variation of liver triglyceride values from mouse control values in the 250 ppm TCE exposure group was spotty but was highly significant at 1000 ppm. The increase in liver triglycerides at 1000 ppm TCE was relatively constant throughout the exposure period.

TABLE 32. A SEMIQUANTITATIVE MEASURE OF FAT ACCUMULATION
IN MOUSE LIVER AFTER 100 DAYS CONTINUOUS INHALATION
EXPOSURE TO 1,1,1-TRICHLOROETHANE

(Mean Values, 3 Mice Per Group)

<u>Week of Exposure</u>	<u>Scale Value 1 to 5+</u>		
	<u>Control</u>	<u>250 ppm</u>	<u>1000 ppm</u>
1	0.7	1.7	2.0
2	0.5	0.8	2.3
3	0.3	0.5	1.7
4	0.0	1.0	2.3
5	0.0	0.2	2.0
6	0.2	0.8	1.7
7	0.3	0.3	2.7
8	0.3	0.5	1.3
9	0.3	0.8	1.7
10	0.2	0.5	1.7
11	0.2	0.3	1.0
12	0.2	0.0	1.0
13	0.0	0.5	0.8
14	0.5	0.5	1.0
<u>Post- exposure</u>			
2	0.2	0.2	0.3
4	0.0	0.2	0.0

<u>Scale Criteria</u>	<u>Description</u>
1+	Fatty change, marked, up to 5 cell diameters from border of central vein.
2+	Fatty change, marked, up to 10 cell diameters from border of central vein.
3+	Fatty change, marked, greater than 10 cell diameters from central vein with many confluent areas and some tendency for macroglobular fat.

TABLE 33. THE EFFECT OF 100-DAY CONTINUOUS INHALATION EXPOSURE TO 1,1,1-TRICHLOROETHANE ON MOUSE LIVER

Week of Exposure	Liver Weights Grams			Ratio of Liver/Body Weight			Liver Triglyceride mg/g		
	Con- trol	250 ppm	1000 ppm	Con- trol	250 ppm	1000 ppm	Con- trol	250 ppm	1000 ppm
1	1.7	1.8	2.0	5.8	6.1	7.3**	4.0	6.0*	-
2	1.7	1.9*	2.4*	5.6	6.3*	8.1*	5.6	5.9	22.5**
3	1.9	2.0	2.2	5.7	5.8	7.0**	6.2	3.8*	29.7**
4	2.0	1.9	2.3**	5.9	5.8	7.5**	4.9	7.5**	29.5**
5	1.9	1.9	2.1	5.6	5.8	6.5*	7.5	7.3	38.8**
6	1.9	2.0	2.1	5.5	6.0	6.5**	6.9	9.5	20.8**
7	2.0	2.1	2.6**	5.6	5.9	7.4**	7.5	6.3	40.9**
8	1.8	2.2**	2.3	5.3	6.6**	7.1**	6.9	10.4	21.4*
9	2.0	2.3	2.6**	5.5	6.2*	7.7**	4.4	6.2	28.7*
10	2.1	2.2	2.3	5.7	6.2	7.1**	5.4	6.2	24.7**
11	2.4	2.3	2.5	6.1	6.2	7.3**	6.4	4.5	19.7*
12	2.1	2.2	2.8**	6.0	6.0	7.7**	3.9	3.9	18.3**
13	2.4	2.5	2.5	6.0	6.6	7.3**	3.9	6.3	12.5
14	2.3	2.3	2.5	5.9	6.2	7.2**	4.8	6.3	16.8**
Post-exposure									
2	2.4	2.6	2.2	6.0	6.4	5.9	3.8	4.1	5.0
4	2.3	2.4	2.5	6.2	6.3	7.1	3.4	3.5	2.6

* Significant at the 0.05 level.

** Significant at the 0.01 level.

After 2 weeks postexposure the liver to body weight ratios were no longer different from control values indicating the observed fatty liver change was a reversible effect as was also observed in fat stains of hepatic tissue.

Rat growth rates were identical in the control and 250 ppm TCE exposed group, and the growth of the 1000 ppm TCE exposed rats was not significantly different, although the group mean was approximately 10 grams lower from the sixth week on. Growth rates for three rat groups are depicted in figure 16. Rat liver to body weight ratios were also significantly increased at the 1000 ppm exposure level but the 250 ppm exposure group was similar to control animals.

Carboxyhemoglobin levels were measured in dogs and monkeys after 2 weeks exposure. The measured values were identical in control and test animals as shown in table 34.

TABLE 34. MEAN CARBOXYHEMOGLOBIN CONCENTRATIONS IN DOGS AND MONKEYS EXPOSED TO 1, 1, 1-TRICHLOROETHANE (% COHb)

<u>Exposure Level</u>	<u>Dogs</u>	<u>Monkeys</u>
Control	0.6%	0.9%
250 ppm	0.6%	0.8%
1000 ppm	0.5%	*

*Sample lost

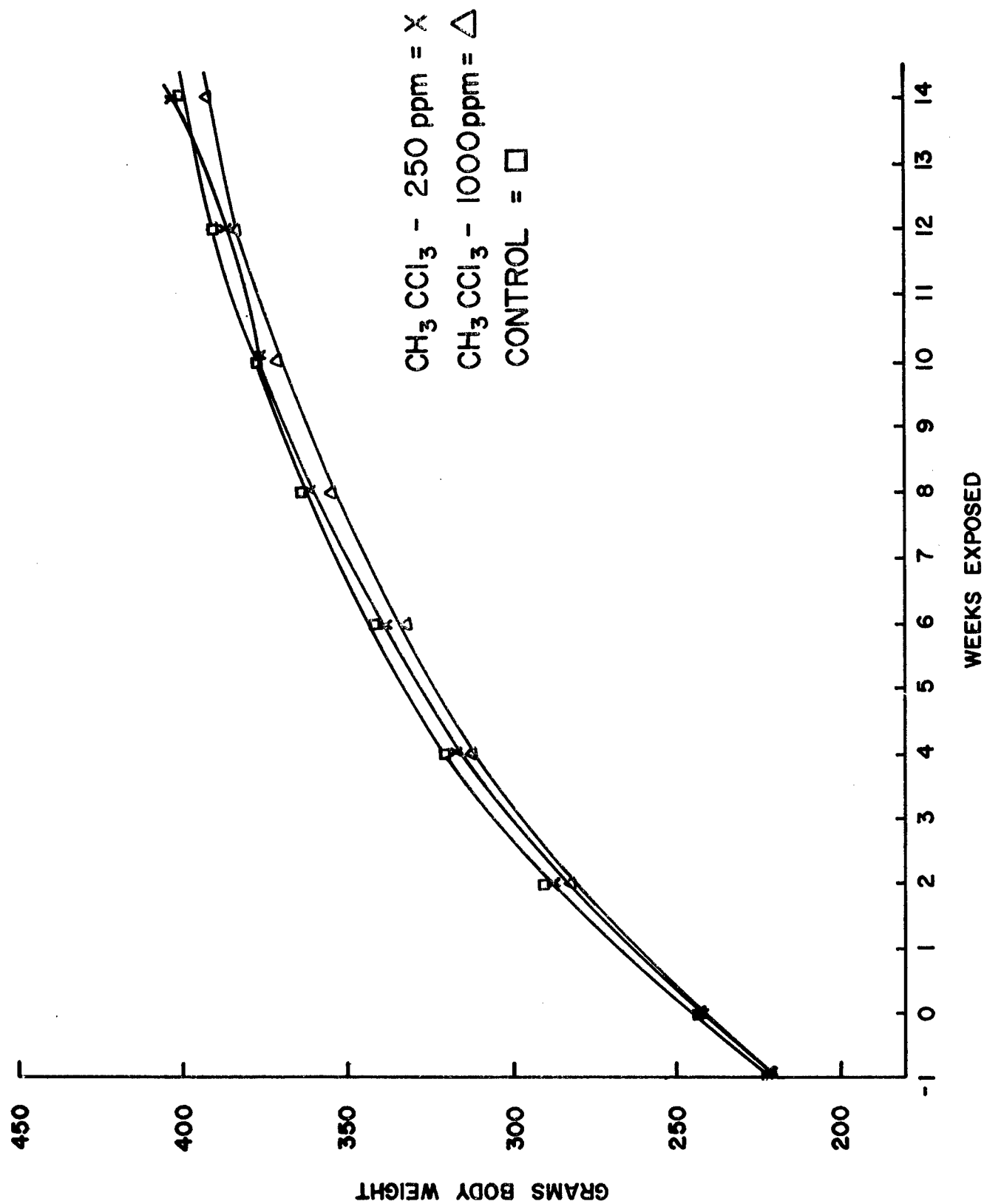


Figure 16. Effect of continuous 1,1,1-trichloroethane exposure on rat growth.

The 1,1,1-trichloroethane levels in dog and monkey blood were variable from one sampling period to another but ranged from 4 to 6 times higher in the 1000 ppm TCE exposure group when compared with the 250 ppm group. The mean blood concentrations of unchanged 1,1,1-trichloroethane are shown in table 35.

TABLE 35. 1,1,1-TRICHLOROETHANE CONCENTRATIONS IN BLOOD
($\mu\text{g}/\text{gram}$)

Exposure Level	<u>Dogs</u>				<u>Monkeys</u>			
	<u>Weeks</u>				<u>Weeks</u>			
	<u>3</u>	<u>5</u>	<u>9</u>	<u>13</u>	<u>3</u>	<u>5</u>	<u>9</u>	<u>13</u>
Control	0	0	0	0	0	0	0	0
250 ppm	11.3	16	9.2	17	4.0	14	3.2	4.4
1000 ppm	75	46	38	75	33	48	17	30

Although various clinical chemistry and hematology parameters fluctuated occasionally, there were no clinically significant differences between the control dogs and monkeys and their TCE exposed counterparts.

Electron microscopy (McNutt et al., 1974) revealed that cytoplasmic alterations were most severe in centrilobular hepatocytes in the 1000 ppm group, and were mild to minimal in the 250 ppm group. These alterations consisted of vesiculation of the rough endoplasmic reticulum with loss of attached polyribosomes, increased smooth endoplasmic reticulum, microbodies and triglyceride droplets. Some cells had ballooned cisternae of the rough endoplasmic reticulum.

Focal hepatocyte necrosis peaked at the 12th week of exposure where it was present in 40% of the mice exposed to 1000 ppm TCE. This necrosis was associated with an acute inflammatory infiltrate and hypertrophy of Kupffer cells.

The most significant lesion in the rats was the presence of chronic respiratory disease (CRD) which was found in 12 of 40 controls, 28 of 40 rats in the 250 ppm group, and in 17 of 40 rats exposed to 1000 ppm TCE.

The only other lesion of consequence was the presence of focal areas of tubular dilatation in the kidney. Since this was found at approximately the same incidence in the controls and experimentals (19 of 40 controls, 22 of 40 rats exposed to 250 ppm, and 21 of 40 exposed to 1000 ppm), it was interpreted as being unrelated to the exposure.

No evidence of fatty infiltration of the liver was observed in the experimental rats, although 2 of 40 controls showed this change.

No lesions were observed in dogs and monkeys which could possibly be related to the exposure. In fact, very few lesions, other than an occasional intestinal nematode, were observed in any of the dogs. The only significant lesion observed in monkeys was the presence of pulmonary acariasis, which was noted in both the controls and experimentals.

The experimental findings indicate that the pathological alterations observed with 1, 1, 1-trichloroethane are similar to those observed with dichloromethane except for different time courses of the effects and different

degrees of recovery. A ten-fold greater atmospheric concentration of 1, 1, 1-trichloroethane is required to produce the minimal liver changes found at 100 ppm of dichloromethane. Thus, we have achieved an exposure level of TCE producing a similar effect to dichloromethane which in further experiments should allow direct comparisons between these two solvents.

Monomethylhydrazine in Drinking Water Studies With Golden Syrian Hamsters

The chronic effects of exposure to very low vapor concentrations of monomethylhydrazine (MMH) have been described by MacEwen and Haun (1971) and shown to be manifested as a dose related hemolytic anemia in dogs and monkeys exposed to the compound either continuously or on an intermittent basis. Exposure to higher vapor concentrations of MMH produces severe renal damage in dogs (Sopher, 1967; Van Stee, 1965) but only subcellular morphogenic kidney changes in monkeys (George et al., 1968). Kroe (1971) described hepatic changes in mice and dogs which included hemosiderosis, periportal cholestasis and bile duct proliferation.

Recent studies by Toth (1972, 1973) ascribe carcinogenic activity to MMH when administered continuously in the drinking water of Swiss mice and Golden Syrian hamsters as a .01% solution. Preliminary studies to an experiment designed to test the reproducibility of these carcinogenic effects in hamsters were reported in last year's annual report (MacEwen and Vernot, 1973).

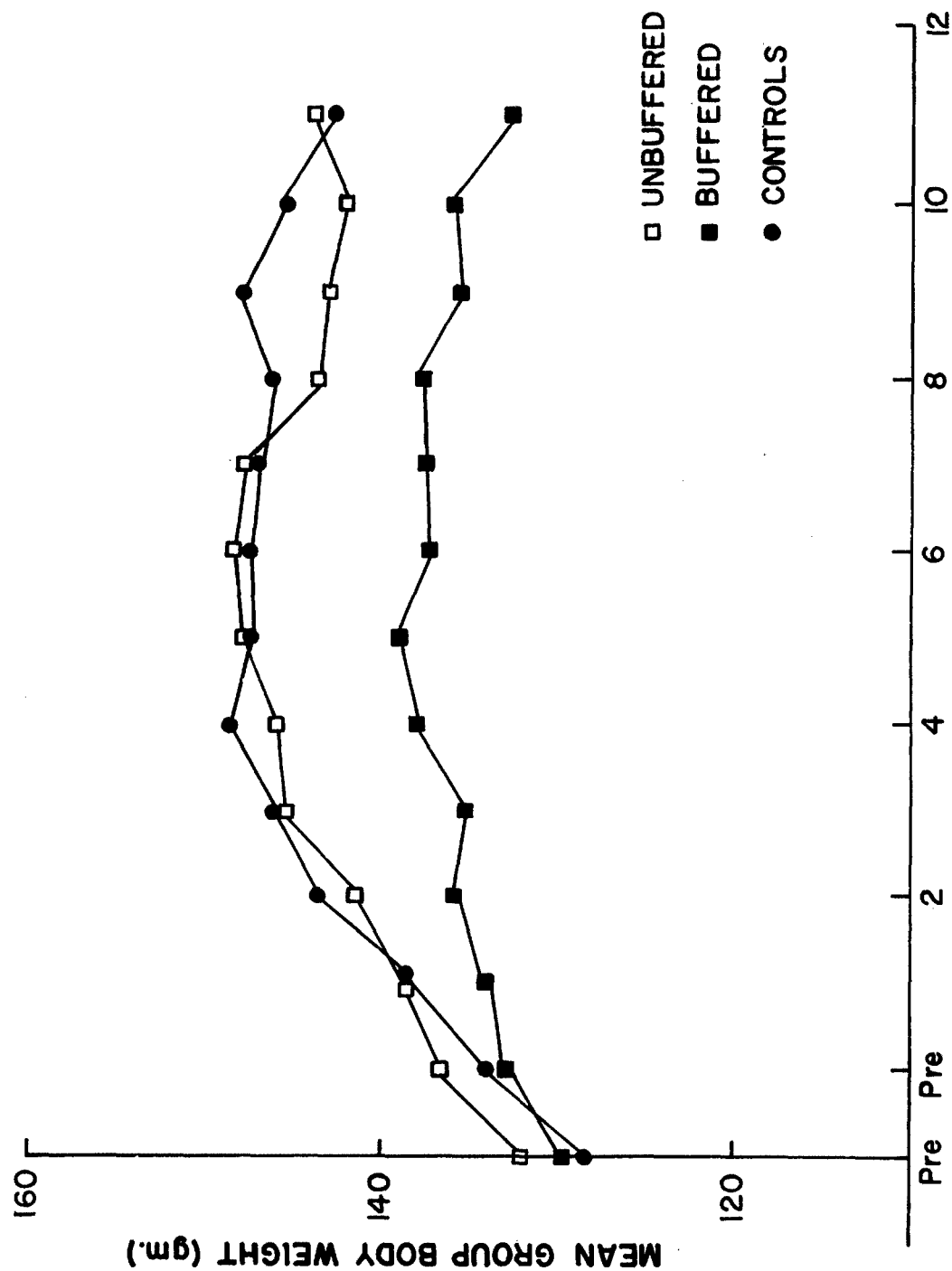
The present investigation involves two groups of male Golden Syrian hamsters which are being maintained, for life, on an MMH-drinking water solution. The various groups in this study and their respective drinking solutions are as follows:

- | | |
|-----------------------|---|
| <u>Control</u> | - drinking water adjusted to pH 3.5 with HCl |
| <u>Buffered MMH</u> | - 0.01% MMH in drinking water adjusted to pH 3.5 with HCl |
| <u>Unbuffered MMH</u> | - 0.01% MMH in drinking water not pH adjusted. |

Each of the test groups consists of 30 hamsters, 10 of which are to be used for serial sacrifices scheduled to occur at 15, 18 and 21 months of the study. The control animals number 20, as serial sacrifices are not scheduled for this group.

The pH adjustment of the drinking water is necessary to insure stability of the MMH in water for at least 24 hours. Without stabilization, oxidation of MMH begins almost immediately. Drinking solutions are prepared daily utilizing a 5 liter glass volumetric flask and individual premeasured aliquots of MMH and HCl contained in glass vials. Water bottles are filled on a daily basis. Complete filling of the bottles is attempted to minimize air content which could hasten decomposition of MMH in solution.

All animals are gang caged, with 5 hamsters per cage, and supplied laboratory chow ad libitum. Test and control hamsters are weighed on a monthly schedule. Water consumption measurements are determined for the 24-hour period preceding a scheduled weighing and allow for calculation of



MONTHS OF TREATMENT

Figure 17. Effect of MMH in drinking water on hamster weights.

the nominal MMH dose ingested on a daily basis. Figure 17 shows the body weight profile for each of the groups up to 11 months of the test. The hamsters drinking the buffered MMH solution exhibited a depressed growth pattern, in comparison to controls, very early in the study. This difference is statistically significant from the third month through the eleventh month. The unbuffered and control groups maintained comparable growth patterns up to the seventh month of treatment. The body weight depression seen in the unbuffered group between 8 and 10 months is notable but not statistically significant. The downward trend in body weight, for all groups, at about 8 months of treatment is a function of the age of the hamsters. These animals were 4 months old at the start of the study and by 8 months of treatment had maximized body weights and reached approximately one-half their life expectancy. Control hamsters, of the same age, from the coal tar study (Coal Tar II) are exhibiting this same pattern.

For the initial 4 months of treatment, the group administered the unbuffered solution consumed slightly less water than either the control group or the group drinking the buffered solution. The alkalinity of the unbuffered solution may have been unpalatable, therefore requiring a short period of acclimatization. Monthly, 24-hour water consumption measurements corrected to ml/g of body weight are shown in table 36.

TABLE 36. TWENTY-FOUR HOUR AVERAGE CONSUMPTION
OF WATER AND WATER CONTAINING MMH BY HAMSTERS

<u>Treatment Month</u>	<u>ml H₂O/g Body Weight</u>		
	<u>Unbuffered MMH</u>	<u>Buffered MMH</u>	<u>Control</u>
1	.074	.079	.084
2	.063	.074	.075
3	.083	.098	.095
4	.068	.084	.094
5	.087	.087	.092
6	.074	.080	.079
7	.094	.101	.102
8	.091	.083	.083
9	.085	.074	.122
10	.090	.078	.080
11	.107	.099	.112

This data could not be treated statistically as water consumption values are group averages based on the gang caging arrangement and mean group body weight and do not represent individual animal determinations. The overall differences in water consumption, however, do not appear to be significantly different. Calculated daily doses of MMH based on the above water consumption data are shown in table 37.

TABLE 37. CALCULATED 24-HOUR MMH DOSE TO HAMSTERS
BASED ON WATER CONSUMPTION

<u>Treatment Month</u>	<u>mg MMH/kg/day</u>	
	<u>Unbuffered MMH</u>	<u>Buffered MMH</u>
1	6.5	6.9
2	5.6	6.5
3	7.3	8.6
4	6.0	7.4
5	7.7	7.7
6	6.5	7.1
7	8.3	8.9
8	7.9	7.3
9	7.4	6.4
10	7.9	6.9
11	9.4	8.7
	$\bar{x} = 7.3$	$\bar{x} = 7.5$

The average daily dose for the two groups, as calculated from the monthly 24-hour water consumption determinations, is almost identical. These data indicate that the nominal MMH dose being ingested by both the unbuffered and buffered groups is approximately one-third the single oral dose LD₅₀ for MMH in hamsters (25.8 mg/kg) as determined in preliminaries to this study and described in last year's annual report (MacEwen and Vernot, 1973).

Hematology determinations on a representative number of hamsters from each of the three groups were done at 8 months of treatment. A non-destructive bleeding method, utilizing ocular orbital puncture, was used to provide blood samples for measurement of hematocrit and RBC values. The average hematology data from five hamsters sampled in each group are shown in table 38.

TABLE 38. EIGHT MONTH AVERAGE HEMATOLOGY VALUES
FOR MMH EXPOSED HAMSTERS

	<u>Control</u>	<u>Unbuffered MMH</u>	<u>Buffered MMH</u>
RBC (millions)	8.74 (7.90-9.29)	6.65 (5.66-7.08)	6.77 (6.48-6.99)
HCT (Vol %)	52 (47-57)	44 (38-49)	45 (43-48)

The two groups ingesting MMH in their drinking water exhibit abnormally lower RBC and HCT values when compared to controls. The proximity of the values for both test groups indicates that the erythropoetic effects are comparable for ingestion of either the unbuffered or the buffered MMH drinking solution. Similar effects are described by Haun (1970) from a hemolytic anemia seen in dogs and monkeys exposed to 5 ppm MMH vapors daily for six months.

Hamster deaths for the initial 11 months of this study have been few. Mortality information is presented in table 39.

TABLE 39. CUMULATIVE MORTALITY OF HAMSTERS
DRINKING MMH SOLUTION

<u>Group</u>	<u>Mortality Ratio</u>	<u>Time to Death</u>
Control	1/17*	45 weeks
Unbuffered MMH	2/20	35 weeks, 45 weeks
Buffered MMH	1/20	45 weeks

*Three hamsters escaped after clean cage change.

Gross pathological examination of dead animals yielded unspectacular findings. The hamster from the buffered group exhibited pulmonary congestion with pneumonia suspected as the causative factor. All other animals examined were free of gross pathological lesions. Histopathological examination for microscopic lesions has not yet been completed; therefore, results cannot be reported at this time.

With the exception of the depressed growth pattern exhibited by the hamsters drinking the buffered MMH solution and anemia in both test groups, there have been no outward manifestations of MMH toxicity. These experiments are continuing.

A Six-Month Chronic Inhalation Toxicity Study of the Biological Effects of JP-9 Constituents

A new aircraft fuel has been developed for extending flight ranges before refueling. The fuel, designated JP-9, is a mixture of three primary ingredients, namely, RJ-4, RJ-5 and methylcyclohexane. RJ-4 and RJ-5 are

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

The acute and chronic toxicity studies on methylcyclohexane have been reported by Treon et al. (1943). Acute exposures of rabbits to inhaled concentrations of methylcyclohexane above 10,000 ppm (≈ 40 mg/liter) caused significant weight loss, narcosis and convulsions while a concentration of 15,227 ppm was fatal in slightly over one hour. Repeated daily, 5-hour exposures of rabbits to concentrations of 1162 ppm or lower for periods up to 10 weeks produced no measurable or observable signs of toxicity.

Some of the physical chemical properties of RJ-4 and RJ-5 are shown in table 40. RJ-4 is a mixture of isomeric dimers of perhydromethylcyclopentadiene. RJ-5, also known as "Shelldyne H", is a mixture of reduced dimers of bicycloheptadiene. To examine the acute inhalation hazard and to obtain experimentally determined saturation concentrations as an aid in the selection of vapor levels for the chronic study, groups of six rats each were

exposed for 6 hours to essentially saturated vapors of each compound. No adverse effects were seen during exposure. Pathologic examination after 14-day postexposure observation showed no abnormalities. Peroral doses of 4 g/kg RJ-5 in corn oil were not lethal to a group of 3 rats; however, 2 of 3 mice succumbed to a 250 mg/kg dose.

TABLE 40. PHYSICAL CHEMICAL PROPERTIES OF RJ-4 AND RJ-5

	<u>RJ-4</u>	<u>RJ-5</u>
Empirical Formula	C ₁₂ H ₂₀	C ₁₄ H ₂₀
Molecular Weight	164	188
Boiling Point (°F)	431	522
Vapor Pressure (70 F)	0.354 mm Hg	0.025 mm Hg
Density (70 F)	0.925 g/ml	1.0813 g/ml

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

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

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

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

Although expected to be low, the toxicity of the two chemicals under study is unknown except for the minimal information mentioned earlier. Personnel working with these materials are avoiding skin contact and inhalation. The vapor generation apparatus and chemical supplies are in ventilated hoods and the areas around the chambers have been designated as "no smoking" zones.

The chamber concentrations of RJ-4 and RJ-5 are continuously monitored using hydrocarbon analyzers. The generation and monitoring techniques, including safety precautions, were identical to those described for JP-4.

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

All exposed animals are being observed for signs of toxic stress as well as mortality. Gross and histopathologic examinations are made on all dead animals. Body weights of dogs, monkeys and rats are being measured on a biweekly schedule. Table 41 shows the reduced battery of clinical hematology and chemistry tests performed on blood samples taken from dogs and monkeys on a biweekly basis. A complete battery of clinical laboratory tests was made at the start and will also be made at the completion of the exposures. These tests include, in addition to those shown in table 41, creatinine, chlorides, cholesterol, BUN, total inorganic phosphorus and bilirubin. At final blood sampling or sacrifice of the large animals, additional blood samples will be drawn for identification and refrigerated storage of serum. These "banked" serum samples will be stored until histopathology reports have been received and reviewed.

TABLE 41. CLINICAL BLOOD TESTS PERFORMED ON RJ-4, RJ-5 EXPOSED AND CONTROL DOGS AND MONKEYS

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

Ten weeks of the 6-month exposure have been completed. An examination of the clinical blood test results collected over this time period for dogs and monkeys show no abnormalities or trends to adverse hematological effect. There have been four animal deaths in the study. One male monkey

died during the seventh week of exposure to RJ-5. Pathology revealed death was due to gastric dilatation of unknown etiology, but believed to be unrelated to exposure. One mouse in the RJ-4 exposure was sacrificed due to an injury during the fourth week of the study. One mouse and one rat, controls, died during the 10 week period.

Mean body weights of exposed monkeys were normal when compared with controls. However, growth depression was noted in rats and dogs exposed to RJ-4 and RJ-5. The growth rate of the three rat groups is shown in Figure 18. Noticeable is the apparently subnormal gain from 2 weeks forward for both exposed groups. The mean weights of the RJ-4 exposed animals are statistically different from control values at all time periods. The weights of the RJ-5 exposed rats, however, are not. At 10 weeks, the mean weight of the RJ-4 rat group is 22 grams less than control and the RJ-5 rat group is 11 grams less than control. It is interesting to note that the weight depression effect is broadening as exposure time increases. The odors of RJ-4 and RJ-5 are very noticeable even after purging of the chambers with air after each exposure period. Odor threshold for both materials have been estimated to be in the parts per billion range. Analytical measurement will be made to determine laboratory atmospheric levels of both chemicals. It was theorized that the chemical odors were causing appetite suppression in rats resulting in growth suppression. Food consumption measurements over a 3-day period during the 10th week of exposure failed to confirm this theory.

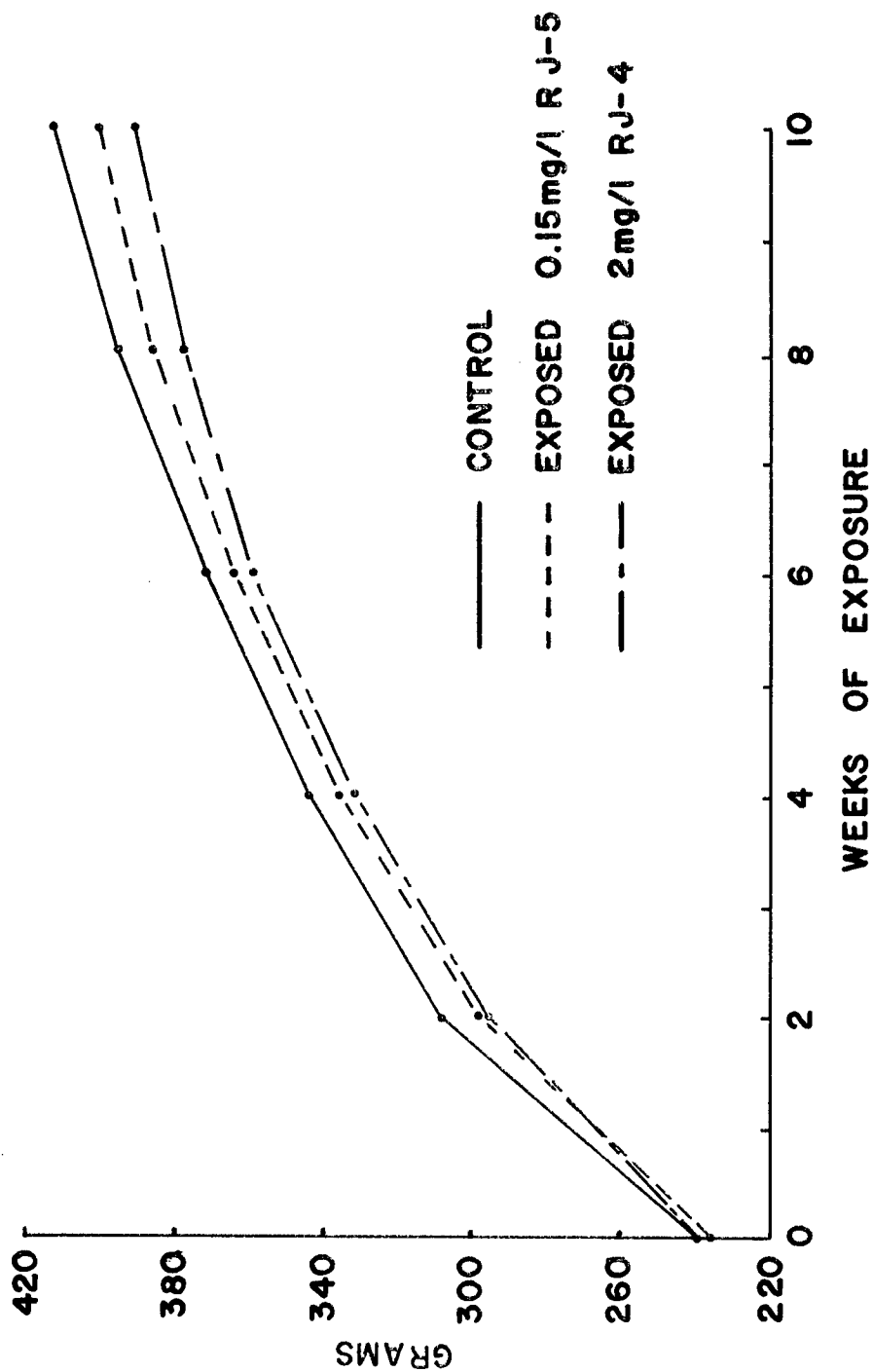


Figure 18. Effect of chronic inhalation exposure to RJ-4 and RJ-5 vapors on rat growth rate.

Comparable subnormal weight gains occurred in the dogs exposed to RJ-4 and RJ-5. Examination of the biweekly mean body weight data revealed that both exposed groups gained less weight than the controls at all sampling periods through 10 weeks. The control mean weight gain of dogs used as controls was 1.37 kg compared to 0.45 kg for both exposed groups of dogs after 10 weeks of exposure.

Overall assessment of current experimental results from the JP-9 Constituents Study suggests no need for additional clinical tests at this time. The experiments are being continued and the final results will be reported at a later date.

Acute Toxicity Studies on Triphenyl Stibine and 1,1-bis(p-dimethylaminophenyl)-ethylene (D-290)

Studies were conducted to determine the acute toxicity of the photographic compounds triphenyl stibine and 1,1-bis(p-dimethylaminophenyl)-ethylene in laboratory animals.

Occupational exposure guidelines for antimony and antimony compounds, including stibine (SbH_3), are known. The industrial threshold limit value (TLVs[®] Threshold Limit Values for Chemical Substances in Workroom Air, 1973) for this element and its compounds via airborne exposure is set at 0.5 mg/m^3 . An oral LD_{50} of 100 mg/kg for antimony and an intraperitoneal LD_{50} of 250 mg/kg for triphenyl stibine, both in the rat, have been reported

(Toxic Substances List, 1973). No toxicological information, however, was available for 1,1-bis(p-dimethylaminophenyl)-ethylene.

The results reported herein evaluate the acute toxicities of these two chemicals by use of the following tests:

1. Single Dose Oral LD₅₀ in Rats and Mice - triphenyl stibine and 1,1-bis(p-dimethylaminophenyl)-ethylene
2. Single Intraperitoneal (ip) Injection LD₅₀ in Rats - triphenyl stibine and 1,1-bis(p-dimethylaminophenyl)-ethylene
3. Primary Skin Irritation in Rabbits - 1,1-bis(p-dimethylamino-phenyl)-ethylene
4. Skin Sensitization in Guinea Pigs - 1,1-bis(p-dimethylamino-phenyl)-ethylene.

Male CFE (Sprague-Dawley derived) rats, 5 per group, ranging in weight from 200-300 grams and male CF-1 mice, 5 per group, ranging in weight from 20-30 grams were orally dosed with corn oil solutions of the two test chemicals, for LD₅₀ determinations. Experimental animals were fasted for at least 16 hours prior to administration of the chemical with a glass syringe and special oral dosing needle. Solutions of the materials were prepared such that precalculated doses could be given using dose volumes of 0.01 ml per gram of body weight. Rats and mice were weighed individually at the time of dosing to determine the proper injection volume.

Test animals were observed for 14 days immediately following the administration of the single oral dose. Any deaths occurring during this

observation period were included in the final mortality figures. Mortality data were treated statistically using the moving average interpolation method of Weil (1952) for LD₅₀ and 95% confidence limit determinations.

The single dose oral LD₅₀ for triphenyl stibine was 187 mg/kg for rats and 650 mg/kg for mice. Oral toxicity data and toxicity classification categories, for rats and mice, are shown in table 42. The toxicity classification system used is detailed on page 2 of Back et al. (1972).

TABLE 42. MORTALITY RESPONSE OF RATS AND MICE
TO SINGLE ORAL DOSES OF TRIPHENYL STIBINE

	<u>Dose (mg/kg)</u>	<u>Mortality Response (No. Dead/No. Dosed)</u>	<u>Toxicity Classification</u>
Rats	400	5/5	Toxic*
	200	3/5	
	100	0/5	
	50	0/5	
Mice	1600	5/5	Toxic*
	800	4/5	
	400	0/5	

Rats - LD₅₀ and 95% CL = 187 mg/kg (129-270)

Mice - LD₅₀ and 95% CL = 650 mg/kg (472-895)

*Oral, 14-Day Single Dose LD₅₀ 50-5000 mg/kg

A single dose oral LD₅₀ for 1, 1-bis(p-dimethylaminophenyl)-ethylene could not be determined using the standard oral dosing techniques. Doses

administered at or below 8000 mg/kg failed to produce deaths in rats and resulted in only one mouse mortality. Doses greater than 8000 mg/kg could not be administered at reasonable dose volumes due to the thickness of the test preparation. Oral toxicity data and toxicity classification categories for rats and mice treated with this material are shown in table 43.

TABLE 43. MORTALITY RESPONSE OF RATS AND MICE
TO SINGLE ORAL DOSES OF
1, 1-BIS(P-DIMETHYLAMINOPHENYL)-ETHYLENE

	<u>Dose (mg/kg)</u>	<u>Mortality Response (No. Dead/No. Dosed)</u>	<u>Toxicity Classification</u>
Rats	8000	0/5	Nontoxic*
	4000	0/5	
Mice	8000	1/5	Nontoxic*
	4000	0/5	

Rats - LD₅₀ >8000 mg/kg (estimated)

Mice - LD₅₀ >8000 mg/kg (estimated)

*Oral, 14-Day Single Dose LD₅₀ >5000 mg/kg

Unfasted, male CFE (Sprague-Dawley derived) rats, 5 per group, ranging in weight from 200-300 grams were used for ip LD₅₀ determination. Solutions of the test material were prepared in corn oil such that precalculated doses could be administered using injection volumes of 0.01 ml per gram of body weight. Single injections were made into the abdominal cavity using a glass syringe and 20 gauge needle. Rats were weighed individually at the time of dosing to determine the proper injection volume.

Test animals were observed for 14 days immediately following the single ip injection. Any deaths occurring during this observation period were included in the final mortality figures. Mortality data was treated as in the oral LD₅₀ determination using the moving average interpolation method of Weil (1952).

The difficulties associated with the oral administration of 1, 1-bis(p-dimethylaminophenyl)-ethylene, in doses sufficient to produce rat mortalities, were again demonstrated in the ip series. The diameter of the needle used for ip injections was much smaller than that of the oral dosing needle, preventing the injection of a dose greater than 4000 mg/kg. This inability to inject reasonable dose volumes of the compound sufficient to produce mortalities, precluded the determination of an ip LD₅₀ for 1, 1-bis(p-dimethylaminophenyl)-ethylene.

The single-injection ip LD₅₀, in rats, for triphenyl stibine was 168 mg/kg. Table 44 lists the mortality results with ip challenges of various doses.

TABLE 44. MORTALITY RESPONSE OF RATS TO SINGLE IP INJECTIONS OF TRIPHENYL STIBINE

<u>Dose (mg/kg)</u>	<u>Mortality Response (No. Dead/No. Dosed)</u>
400	5/5
200	3/5
100	1/5

LD₅₀ (95% Confidence Limits) = 168 mg/kg (95-299)

The LD₅₀ for a single ip injection of this compound (168 mg/kg) is essentially the same as that determined from a single oral dose (187 mg/kg), indicating that the stomach pH does little to retard the rate of absorption in the animal and that no diminution in toxic response should be expected from the ingestion of triphenyl stibine as compared to other modes of entry.

Six female New Zealand white rabbits, 4-5 lbs., were tested according to the methods described by Draize et al. (1944), to determine the primary skin irritation potential of 1,1-bis(p-dimethylaminophenyl)-ethylene. This patch test method is utilized to evaluate the degree of primary irritation produced on intact and abraded skin.

Using 0.5 gram quantities, 1,1-bis(p-dimethylaminophenyl)-ethylene was applied to the designated test areas and covered with a 2-inch square of surgical gauze several layers thick. The patches were taped in place for 24 hours after which time test areas were examined for irritation using the Draize et al. (1944) table as a reference standard.

Twenty-four and 72-hour examinations of areas tested with 1,1-bis(p-dimethylaminophenyl)-ethylene resulted in negative skin irritation effects on both intact and abraded skin. There was no evidence of erythema or eschar formation which would be indicative of primary irritation.

When tested according to standard evaluation methods, 1,1-bis(p-dimethylaminophenyl)-ethylene did not demonstrate any potential as a corrosive chemical capable of producing primary skin irritation.

Eighteen male, albino guinea pigs, 450-600 grams, were tested to determine the sensitization potential of 1,1-bis(p-dimethylaminophenyl)-ethylene. The method employed for use was a modification of the Landsteiner Guinea Pig Sensitization Test (1967). This method provides a test for determining if properly spaced, repeated, minute intradermal injections of a test material, followed by an appropriate incubation period lead to an antigen-antibody response upon challenge. Numerical scores are derived from measurements of the reaction at injection sites (wheal) 24 and 48 hours after a challenge injection of the test material is administered. Reaction scores are based on the intensity of the skin reaction and are represented by a proportionate numerical value indicative of the sensitization response in the test animal. Ranges of scores used in this test and their significance are shown below:

MEAN REACTION SCORES AND THEIR SIGNIFICANCE

<u>Score</u>	<u>Sensitizing Potential of Chemical</u>
0-25	None
26-99	Slight
100-200	Moderate
> 200	Severe

When tested in accordance with the methods described above, 3 of 18 guinea pigs produced reaction scores which would be indicative of a sensitization of 65 indicating that 1,1-bis(p-dimethylaminophenyl)-ethylene has, at most, a slight potential for sensitization.

Evaluation of the acute toxicity of triphenyl stibine, by single oral dose LD₅₀ and single intraperitoneal LD₅₀ determinations in rats and mice, indicates that this compound is toxic. A summary of resultant LD₅₀ information is shown below in table 45.

TABLE 45. LD₅₀ VALUES FOR RATS AND MICE
CHALLENGED WITH TRIPHENYL STIBINE

<u>Administration Mode</u>	<u>Species Tested</u>	<u>LD₅₀ with 95% C.L. (mg/kg)</u>
Oral	Rat	187 (129-270)
Intraperitoneal	Rat	168 (95-299)
Oral	Mouse	650 (472-895)

The rat oral and ip LD₅₀ values for triphenyl stibine, as determined in this test, are similar to that reported for antimony via oral administration (Toxic Substances List, 1973). Based on their comparable toxicities the existing TLV for airborne exposure to antimony 0.5 mg/m³ should also be applied for use with triphenyl stibine until inhalation data becomes available.

The inability to produce rat and mouse deaths with 1,1-bis(p-dimethylaminophenyl)-ethylene indicates that this compound is nontoxic as tested. Additionally, this material is not a primary skin irritant and demonstrates only very slight potential as a sensitizing agent.

The Effects of 6-Month Chronic Low Level Inhalation Exposures to Hydrazine on Animals

Hydrazine (N_2H_4) is a highly reactive reducing agent which is widely used as an intermediate in organic synthesis and either singly or in combination with other hydrazines such as 1,1 dimethylhydrazine or methylhydrazine as a missile propellant. It is also used extensively as a corrosion inhibitor in boiler feed water. Hydrazine is a colorless liquid with a molecular weight of 32.05, density of 1.008 g/ml and a vapor pressure of 14.4 mm Hg at 25 C.

Hydrazine is a strong convulsant at high doses but may cause central nervous system depression at lower doses. Its toxicity and pharmacologic effects are detailed in a comprehensive review by Clark et al. (1968). Animals may die acutely of convulsions, respiratory arrest, or cardiovascular collapse within a few hours of an acute exposure by any route of administration, or may die 2 to 4 days later of liver and kidney toxicity (Weir et al., 1964; Witkin, 1956). Jacobson et al. (1955) reported the 4-hour inhalation LC_{50} value as 252 ppm (330 mg/m³) for the mouse and 570 ppm (750 mg/m³) for the rat.

House (1964) exposed monkeys, rats and mice to a hydrazine concentration of 1.0 ppm continuously for 90 days. Though mortality was very high, some animals survived the experiment. Ninety-six percent of the rats and 98% of the mice died during the exposure while monkeys proved to be the most resistant species with only a 20% mortality.

Comstock et al. (1954) exposed dogs, in separate experiments, to 5 and 14 ppm. Both dogs survived the repeated 6-hour exposures to 5 ppm hydrazine for 6 months and 2 of 4 dogs lived after 194 six-hour exposures to 14 ppm. Two of four dogs died during the third and fifteenth weeks in a debilitated condition. The dog that died during the fifteenth week had a severe convulsive seizure prior to death. Prior to death, both dogs showed signs of anorexia and general fatigue. Changing diets and forced feedings resulted in the survival of the remaining two dogs.

The present Threshold Limit Value (TLV) published by the American Conference of Governmental Industrial Hygienists (1973) for N_2H_4 is 1 ppm or 1.3 mg/m³.

To compare the effects of repeated 6 hour per day, 5 days per week (industrial type) exposures with continuous exposures of equivalent concentrations and to evaluate the safety factor of the current TLV, four concentration levels were selected for the 26-week exposure of four animal species. The concentrations selected were: 1.0 ppm and 0.2 ppm for continuous exposures and 5.0 ppm and 1.0 ppm for intermittent daily exposures. These concentrations would result in the following CT (concentration x time) values:

1.0 ppm continuous	=	168 ppm-hours per week
5.0 ppm intermittent	=	150 ppm-hours per week
1.0 ppm intermittent	=	30 ppm-hours per week
0.2 ppm continuous	=	33.6 ppm-hours per week.

Thus, the 1.0 ppm continuous and the 5.0 ppm intermittent studies would be relatively equivalent doses and the 1.0 ppm intermittent and 0.2 ppm continuous would also be comparable.

Four exposed groups and a control group were used in these experiments. Each consisted initially of 8 male beagle dogs, 4 female rhesus monkeys, 50 male Sprague-Dawley rats and 40 female CF-1 mice. The animals were monitored throughout the 6 months of exposure with biological measurements made at biweekly intervals. These measurements consisted of hematology and clinical chemistry values, body weights, physical examinations, and on selected animals bone marrow examinations were conducted. The details of the experimental methodology and findings were presented by Haun et al. (1973) with the exception of the results in groups of rats and mice held for long-term postexposure observation. Ten rats and 10 mice from each experimental and control group were set aside at the end of the 6-month exposure period and maintained in an animal holding room.

The effects of chronic inhalation of hydrazine are dose related regardless of the nature of exposure, i. e., intermittent or continuous. The highest hydrazine dose caused approximately 40% deaths in mice within the first two-month exposure while the TLV dose equivalents caused approximately 5% mortality.

Although mice were not weighed, rats exhibited a dose related growth rate depression and dogs exposed to hydrazine showed weight loss at the highest dose levels.

The most significant or noticeable signs of stress occurred in the case of the dogs exposed to 1 ppm continuously. Weight loss was very noticeable in these dogs, and although we did not measure food consumption, it was obviously reduced. Anorexia continued with progressive emaciation until about 16 weeks when some recovery occurred in the surviving dogs. One dog in this group experienced tonic convulsions on 3 separate occasions, once after 3 months of exposure, then once in the morning and once in the afternoon of the same day after 5 months of exposure. These findings were consistent with those reported by Comstock et al. (1954).

In animals held postexposure, weight differences between control and exposure groups became insignificant by four weeks.

There were no abnormal findings in clinical chemistry and hematology measurements made on monkeys and rats. Dogs, however, had a hydrazine dose related depression of red blood cell counts, hemoglobin values and hematocrits, red blood cell fragility was decreased and there was little or no reticulocytosis before the fifth month of exposure at which time the dogs continuously exposed to 1 ppm N_2H_4 had a sharp depression of RBC count accompanied by reticulocytosis. At autopsy, this group of dogs were the only group of any species to demonstrate erythropoietic activity as measured by a decreased myeloid/erythroid ratio in bone marrow.

The results of gross and histopathologic examination of mice that died during exposure showed that death was probably due to hydrazine hepatotoxicity. At sacrifice, moderate to severe fatty liver change was a consistent finding in mice from all exposure levels. Monkey livers showed slight to moderate fat accumulation. Perhaps compromising part of this information is the fact that control monkeys also showed some degree of fatty liver change. Malnutrition, the result of nonspecific hydrazine toxicity caused the death of 2 dogs in the 1 ppm continuous exposure. At sacrifice, dogs exposed to the TLV concentration showed no abnormalities but dogs from the high doses had fatty livers. Since one dog in the 1 ppm continuous exposure group convulsed during exposure, the brains of this dog and 3 others in the same group were perfused at sacrifice. Histology revealed no CNS lesions. Two dogs each from the high concentration experiments were sacrificed at 6 weeks postexposure. All were described as being essentially normal animals.

Organ weights of exposed rats, monkeys and dogs were not statistically different from control values. In the case of the rats, the depressed growth rates resulted in increased organ to body weight ratios to which no biological significance can be attributed.

There were no significant pathologic changes in rats except in the case of the 5 ppm intermittent exposure group. Of the 30 rats, 19 had chronic bronchopneumonia. Whether this condition was due to a hydrazine pulmonary irritation or pathogens present, or the former predisposing the

rats to the latter, is difficult to say. The net effect, however, was that 10 rats from this group retained postexposure, showed no weight recovery as demonstrated by the other exposed groups. Consequently, the infection, we reasoned, spread to other rat groups during storage in the same room and within 6-8 weeks following exposure termination, 50% of the rats were dead. The number of deaths were distributed rather evenly in the exposed groups and in the controls as well. Consequently, none of the rats survived long enough for conclusions to be drawn about the carcinogenic potential of hydrazine on this species.

Approximately half the mice in each group were alive 1 year post-exposure. At necropsy, non-neoplastic lesions were found in the CF-1 mice with approximately equal frequency in both experimental and control groups. An occasional mouse had a mammary gland adenoma, but since these are normally found with an incidence of 5-10% in mice, they were considered to be unrelated to the hydrazine exposure. For similar reasons, a single small squamous papilloma found in one exposed mouse was not considered significant.

The tumor incidences shown in table 46 are believed significant for two reasons. First, alveolargenic carcinomas are found in higher, dose related, frequencies among exposed mice than in controls. Second, lymphosarcoma and the uncommon malignant hepatoma are absent from controls but occur in mice exposed to the higher dose.

TABLE 46. TUMOR INCIDENCE IN MICE ONE YEAR AFTER
CHRONIC INHALATION EXPOSURE TO HYDRAZINE
(6-MONTH EXPOSURE PERIOD)

<u>Exposure</u>	<u>Alveolargenic Carcinoma</u>	<u>Lympho- sarcoma</u>	<u>Hepatoma</u>	<u>Number of Mice With Tumors</u>
<u>High Dose</u>				
1.0 ppm Continuous	5/9	2/9	1/9	6/9
5.0 ppm Intermittent	5/6	0/6	0/6	5/6
<u>Low Dose</u>				
0.2 ppm Continuous	3/8	0/8	0/8	3/8
1.0 ppm Intermittent*	2/6	0/6	0/6	2/6
Control Group	1/8	0/8	0/8	1/8

*Current Threshold Limit Value (TLV)

Alveolargenic carcinomas are "normally" found in older mice with a frequency of about 10% and we have found this rate of tumors in this experiment as well as in other concurrent experiments in this laboratory using the CF-1 strain mice. In previous studies, using electron microscopy, these tumors have been shown to contain Type C virus particles. The virus particle is thought to be the probable etiologic agent for these spontaneous alveolar carcinomas. Another significant finding is the metastatic

extension of the hepatoma to the spleen and metastasis of alveolargenic carcinomas to heart and rib cage respectively in two other hydrazine exposed mice. These findings should be confirmed in additional experiments exposing large numbers of animals of several species to hydrazine.

An Acute Inhalation Toxicity Study on Deuterium Fluoride

This study was conducted to determine the acute inhalation toxicity of deuterium fluoride in rats and mice. Deuterium fluoride (DF), the deuterated analogue of hydrogen fluoride (HF), is currently being used in gas laser systems. The lack of available toxicity information on DF necessitated its testing for determination of potential toxicity hazards to personnel responsible for the operation of systems using this material.

Deuterium fluoride is a colorless liquid which boils at 18.6 C and has a vapor pressure of 0.93 psi at 17 C. The corresponding values for HF are 19.4 C and 0.89 psi for boiling point and vapor pressure respectively.

The results reported herein measured the acute toxicity of DF by determination of 60-minute inhalation LC₅₀ values for rats and mice. A similar series of tests using HF is also detailed to facilitate a comparison of the toxicities of these two compounds and to determine whether occupational exposure guidelines for HF are applicable.

Male CFE (Sprague-Dawley derived) rats, 5 per group, ranging in weight from 250-300 grams and female CF-1 mice, 10 per group, ranging

from 30-35 grams were exposed to various vapor concentrations of DF and HF. The experimental tests, designed to facilitate LC₅₀ determinations, consisted of 60-minute dynamic flow exposures using a specially constructed 120 liter rectangular Plexiglas® chamber. This chamber was fitted with a sliding cage drawer which allowed for the rapid insertion and withdrawal of the test animals. Test animals were observed for toxic signs and deaths for 14 days after exposure to either of the test compounds.

Generation of DF and HF was achieved by use of a method similar to the commercial preparation method for HF:



D₂SO₄ or H₂SO₄ was mixed with equimolar amounts of CaF₂ and heated in a Monel reaction flask. Control of the generation rate was achieved by heating the mixture to higher or lower temperatures. A 500 cc/min carrier flow of dry nitrogen was passed through the Monel vessel and carried the reaction product (DF or HF) to the exposure chamber.

Continuous analysis of chamber concentration was provided by passing a sample of the test atmosphere through an aqueous reagent absorber, with subsequent measurement of F[⊖] in the absorber using an ion specific electrode mounted in a flow cell.

Statistical analysis of mortality data for LC₅₀ calculation was done using the BMD035 Biomedical Computer Program, Biological Assay - Probit Analysis Methods.

The 60-minute LC₅₀ values for mice exposed to HF and DF were 324 ppm and 456 ppm respectively. Mortality response data and LC₅₀ information for mice are shown in table 47.

TABLE 47. MORTALITY RESPONSE OF MICE EXPOSED TO INHALED DF OR HF FOR 60 MINUTES

<u>Deuterium Fluoride (DF)</u>		<u>Hydrogen Fluoride (HF)</u>	
<u>Conc. (ppm)</u>	<u>Mortality Response (No. Dead/No. Exposed)</u>	<u>Conc. (ppm)</u>	<u>Mortality Response (No. Dead/No. Exposed)</u>
229	0/10	351	0/10
311	3/10	438	5/10
352	9/10	505	9/10
409	9/10	518	6/10
478	10/10	633	10/10
LC ₅₀ and 95% CL = 324 ppm (301-349)		LC ₅₀ and 95% CL = 456 ppm (426-489)	

The 60-minute LC₅₀ values for rats exposed to various concentrations of DF and HF were 1095 ppm and 966 ppm respectively. Mortality response data and LC₅₀ information for rats are listed in table 48.

TABLE 48. MORTALITY RESPONSE OF RATS EXPOSED
TO INHALED DF OR HF FOR 60 MINUTES

<u>Deuterium Fluoride (DF)</u>		<u>Hydrogen Fluoride (HF)</u>	
<u>Conc.</u> <u>(ppm)</u>	<u>Mortality Response</u> <u>(No. Dead/No. Exposed)</u>	<u>Conc.</u> <u>(ppm)</u>	<u>Mortality Response</u> <u>(No. Dead/No. Exposed)</u>
965	1/5	848	0/5
1031	2/5	1097	3/5
1158	4/5	1576	5/5
1439	5/5		

LC₅₀ and 95% CL = 1095 ppm (1008-1189) LC₅₀ and 95% CL = 966 ppm (785-1188)

There were a number of toxic signs noted both during and after exposure to vapors of DF and HF. Mice, exposed to both compounds, exhibited eye and nose irritation, respiratory distress and erythema of exposed areas of skin. Signs of toxicity noted in rats exposed to both DF and HF were eye and nose irritation, lacrimation, rhinorrhea, salivation, respiratory distress and erythema. Corneal opacity often resulted from exposure to both chemicals. Postexposure observations indicated similar signs of toxicity in rats and mice surviving exposure to both DF and HF, which included dyspnea and severe eye and nose irritation with crusting of the eyes and nares.

The majority of mouse deaths from exposure to either of the test compounds occurred within 24 hours postexposure. Several delayed deaths, up to 6 days postexposure, were noted in mice exposed to HF vapors. Rat deaths peaked at 2 days postexposure with several delayed deaths occurring up to 6 days postexposure from both DF and HF exposed animals.

Histopathological examination of mice sacrificed after the 14-day observation period indicated scattered areas of intraalveolar microhemorrhage in animals exposed to both DF and HF. Additionally, the DF exposed mice exhibited diffuse pulmonary congestion at all concentrations tested. The only residual congestion noted in 14-day surviving mice exposed to HF was found in the animals receiving the two highest concentrations of the vapor.

Fourteen day rat survivors exhibited a similar pattern of histopathological lesions in both DF and HF exposed animals. Peribronchiolar lymphoid hyperplasia, vascular congestion and multiple areas of intraalveolar microhemorrhage were common findings in rats exposed to both compounds. No lesions were noted in any of the other major organs examined.

This series of 60-minute inhalation exposures of rats and mice to various atmospheric concentrations of DF and HF produced results indicating that the acute toxicities of these two materials are comparable.

A summary of LC₅₀ values for both species challenged with each of the test materials is shown in table 49.

TABLE 49. COMPARISON OF RAT AND MOUSE LC₅₀ VALUES
DETERMINED FROM 60-MINUTE INHALATION
EXPOSURES TO DF OR HF

<u>Species Tested</u>	<u>Compound</u>	<u>LC₅₀ and 95% CL</u>
Mouse	DF	324 ppm (301-349)*
Mouse	HF	456 ppm (426-489)*
Rat	DF	1095 ppm (1008-1189)
Rat	HF	966 ppm (785-1188)

*Indicates statistically significant difference.

Although there is a statistically significant difference between the two mouse LC₅₀ values, this difference is not biologically significant in the sense that it might imply a greater acute hazard for DF.

Histopathological examination indicates that the pathological changes resultant from exposure to DF or HF are similar in both rats and mice. It appears that the mechanism for cellular destruction is comparable for both compounds and that pulmonary damage is the major toxic manifestation of DF intoxication.

Based on the data obtained in these experiments, the following conclusions may be drawn:

1. The current EEL values for HF are equally applicable for DF.
2. Current environmental health practices used for control of HF exposures are suitable for DF exposures.
3. Since normal intake of deuterium ion in drinking water and food exceeds the amount of deuterium that could be taken into the body by inhalation of DF at the TLV for HF (3 ppm) by a factor greater than 30 fold, the industrial TLV for HF is also applicable for this compound.

The Determination of a 60-Minute LC₅₀ for Hydrogen Chloride on Rodents

Sixty-minute exposures of rats and mice to hydrogen chloride vapors were conducted as part of preliminary tests for a planned study to determine Emergency Exposure Limits (EEL) concentrations for a combination of

hydrogen chloride (HCl), hydrogen fluoride (HF), and alumina (Al₂O₃). This combination is found in combustion products formed during the firings of certain rocket and missile engines.

A series of 60-minute rodent lethality tests is to be conducted to examine the effects of combined exposures to HCl, HF and Al₂O₃ before EEL exposures begin. The lack of existing 60-minute HCl toxicity data necessitated the conduct of a series of rat and mouse exposures to determine an LC₅₀ at this time limit.

All exposures to HCl vapor were conducted in one quadrant of a Longley chamber equipped with a sliding gasketed cage drawer which permitted easy insertion and withdrawal of the test animals. Hydrogen chloride vapors were metered from a steel cylinder to the exposure chamber via a glass rotometer. Input air to the chamber was predried and maintained at a constant rate of 10 CFM for all exposures. Continuous analysis of chamber concentration was provided by passing a sample of the test atmosphere through an aqueous reagent absorber, with subsequent measurement of Cl⁻ in the absorber using an ion specific electrode mounted in a flow cell.

Male CFE (Sprague-Dawley derived) rats, 10 per group ranging in weight from 200-300 grams and female CF-1 mice, 10 per group ranging from 30-35 grams were exposed to selected vapor concentrations of HCl. Test animals were observed for toxic signs and mortalities, during and for 14 days after exposure to the test compound. Animals dying from exposure were submitted for gross and histopathological examination. Representative

numbers of survivors were sacrificed after the 14-day observation period for gross and histopathological examinations.

The 60-minute LC₅₀ values for rats and mice exposed to HCl vapors were 3124 ppm and 1108 ppm respectively. Statistical analysis for LC₅₀ calculation was done using the BMD035 Biomedical Computer Program, Biological Assay - Probit Analysis Methods. Mortality response data and LC₅₀ information for rats and mice are shown in table 50.

TABLE 50. MORTALITY RESPONSE OF RATS AND MICE
EXPOSED TO VAPORS OF HCl FOR 60 MINUTES

	<u>Conc. (ppm)</u>	<u>Mortality</u>	<u>Time to Death</u>
Rats	1813	0/10	-
	2585	2/10	5 days, 8 days
	3274	6/10	1 during, 4 days, 2 @ 5 days, 8 days, 13 days
	3941	8/10	1 during, 1.5 hrs., 6 days, 3 @ 7 days, 8 days, 10 days
	4455	10/10	7 during, 3 days, 4 days, 7 days
LC ₅₀ and 95% CL = 3124 ppm (2829-3450)			
Mice	557	2/10	2 days, 8 days
	985	3/10	2 @ 4 days, 13 days
	1387	6/10	3 days, 5 @ 4 days
	1902	8/10	1 during, 2 days, 2 @ 3 days, 4 days, 2 @ 5 days, 6 days
	2476	10/10	1 during, 2 @ 1 hr., 3 days, 2 @ 4 days, 2 @ 5 days, 6 days, 8 days

LC₅₀ and 95% CL = 1108 ppm (874-1404)

Toxic signs noted during exposure were comparable to those observed in the 5-minute and 30-minute HCl vapor LC₅₀ tests, as previously determined by Darmer et al. (1972). Toxic signs included eye and nose irritation and

labored breathing in rats and mice with salivation, lacrimation and rhinorrhea also manifest in the rats.

The time to death pattern seen in these 60-minute exposures, when compared to the previous work at lower time limits, indicates that the number of delayed deaths is proportional to the length of exposure, i. e., 60 min. > 30 min. > 5 min.

Gross pathological examination of animals dying from exposure showed pulmonary congestion and intestinal hemorrhages in both rats and mice, with rats also exhibiting thymic hemorrhages. Surviving animals sacrificed after the 14-day observation period demonstrated no gross lesions in mice but multifocal areas of red hepatization in lungs of rats and occasional engorged or pale livers.

A summary of the now existing HCl vapor toxicity information as determined in this laboratory is shown in table 51.

TABLE 51. SUMMARY OF ACUTE TOXICITY DATA
FOR EXPOSURE TO HCl VAPOR

	<u>5-Minute LC50 (ppm)*</u>	<u>30-Minute LC50 (ppm)*</u>	<u>60-Minute LC50 (ppm)</u>
Rat	40,898 (34,803-48,272)	4701 (4129-5352)	3124 (2829-3450)
Mouse	13,750 (10,333-18,283)	2644 (2264-3086)	1108 (874-1404)

*Work of Darmer et al. (1972).

() = 95% CL.

In comparing the 5-minute and 30-minute LC₅₀'s for HCl determined previously to the 60-minute value, it is apparent that Haber's Law (Concentration X Time = K) is applicable to HCl in both species of rodents tested.

	<u>5 min. CT</u>	<u>30 min. CT</u>	<u>60 min. CT</u>
Rats	204,490 ppm min.	141,030 ppm min.	187,440 ppm min.
Mice	68,750 ppm min.	79,320 ppm min.	66,480 ppm min.

SECTION III

FACILITIES

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

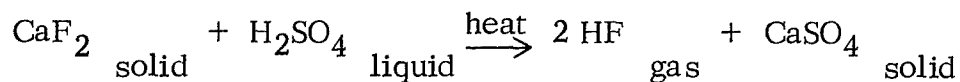
Analytical Chemistry Programs

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

Deuterium Fluoride

The THRU was requested to conduct comparative studies of the acute toxicity of deuterium fluoride (DF) and hydrogen fluoride (HF). The cost of pure DF was so high that the option of purchasing the pure material for introduction into inhalation chambers was rejected and a decision made to synthesize DF for immediate introduction.

There were several possible processes available that would produce high percentage yields of anhydrous DF. The direct combination of hydrogen or deuterium atoms with fluorine atoms thermally was discussed by Benton (1964). Olah and Kuhn (1959) showed that the deuterolysis of fluorosulfonic acid would give high yields of anhydrous gas. Earlier work by these same authors (1956) discussed the deuterolysis of SOF_3 , COF_2 , HCOF , CH_3COF to yield deuterium fluoride. All these processes suffered from the drawback that the reactants were appreciably volatile and potentially toxic. Therefore, the commercial process for the production of HF was investigated as a means of supplying DF for our experiments. This process involves the heterogeneous reaction of solid calcium fluoride (CaF_2) with concentrated sulfuric acid (H_2SO_4) at 250 C (Simons, 1950).



The analogous reaction for the formation of deuterium fluoride gas is:

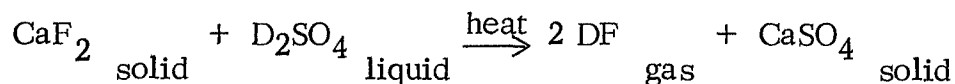


Figure 19 is a schematic of the apparatus used in this study. The Monel[®] flask contained a bed of CaF_2 which was constantly stirred. Heat was applied to the flask and D_2SO_4 was delivered from a glass syringe by a monodrum feeder. The gas generated was purged from the reaction flask by dry nitrogen to a chamber where it was diluted with a stream of dry air. Samples were taken from this chamber for analysis of fluoride ion concentration by ion selective electrode. The electrode analysis was accomplished by scrubbing 500 ml/min of chamber sample free of fluoride ion with 1.7 ml/min buffered acetate absorber solution. All chemicals used for generation and analysis were of reagent grade quality. The D_2SO_4 used was 96-98% pure by weight and contained 99 atom % deuterium.

An initial experiment was set up to test the laboratory generation of HF using H_2SO_4 . The reaction flask, containing 100 g CaF_2 , was heated to 175 C with a N_2 purge of 500 ml/min. The monodrum feeder, with a 20 ml glass syringe containing 15 ml of 96% H_2SO_4 , delivered 0.9 ml/min, which was sufficient to produce a chamber concentration of 2800 ppm HF. Results showed that the original intention of controlling generation rates by regulation of acid flow to the reaction flask was not feasible. Examination of the flask contents demonstrated that a crusty layer had formed covering the CaF_2 , indicating that the poor generation rates observed were the result of the inability of the acid to reach a fresh surface of CaF_2 .

A new approach was investigated. It was observed that as the reaction flask was cooled to room temperature, there was an apparently corresponding

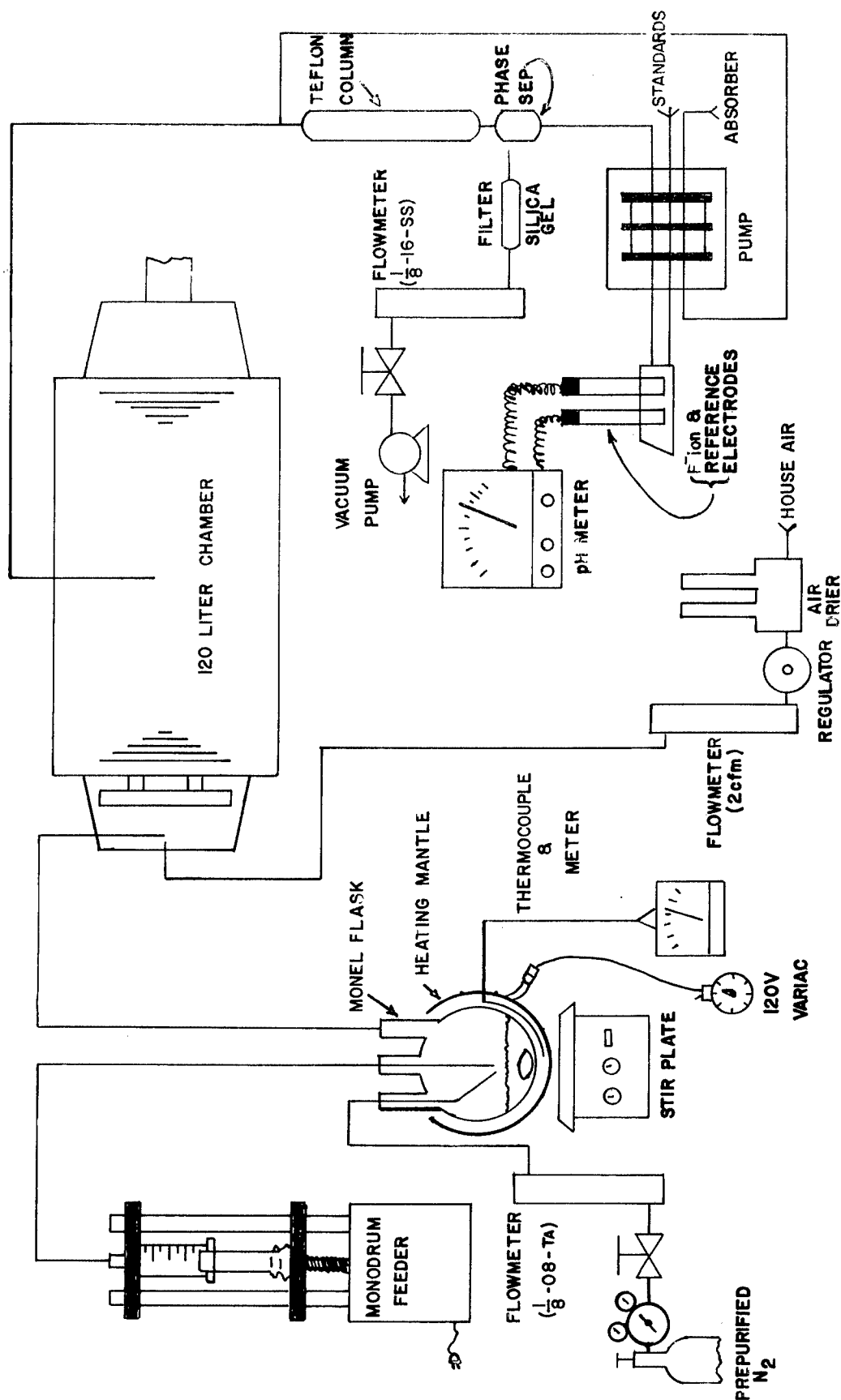


Figure 19. Schematic diagram of the deuterium fluoride generation, monitoring and animal exposure system.

decrease in chamber concentration. The monodrum feeder was eliminated from the system in Figure 19. A pool of the H_2SO_4 was placed in the reaction flask and gently stirred under a 500 ml/min flow of dry nitrogen. The CaF_2 was added to the flask which was then sealed. It was felt that by varying the reaction temperature, the rate of generation of HF gas could be maintained at the desired levels.

The first experiment used 50 g of CaF_2 added to 53 g of the acid, equimolar quantities. The temperature of the flask was maintained at 24 C. The chamber maintained a concentration of 400 ppm for four hours. Further experiments were performed where the flask temperature was increased in steps and the chamber concentration monitored. These data are shown below:

TABLE 52. DEPENDENCE OF CHAMBER HF CONCENTRATION ON REACTION FLASK TEMPERATURE

Temperature °C	Chamber Concentration, ppm			
	Expt. #1	Expt. #2	Expt. #3	Expt. #4
24	400	400		400
26			500	
55			950	925
100				1625
155		2500	2500	2500

Each concentration level was maintained for a period of at least one hour. All of these experiments were run with equimolar amounts of materials. In order to test the effect of varying the relative quantities of reactants, an experiment was run with twice the molar equivalent of acid, and one with nine times the molar equivalent of the acid. These data along with that from the table, are shown in Figure 20.

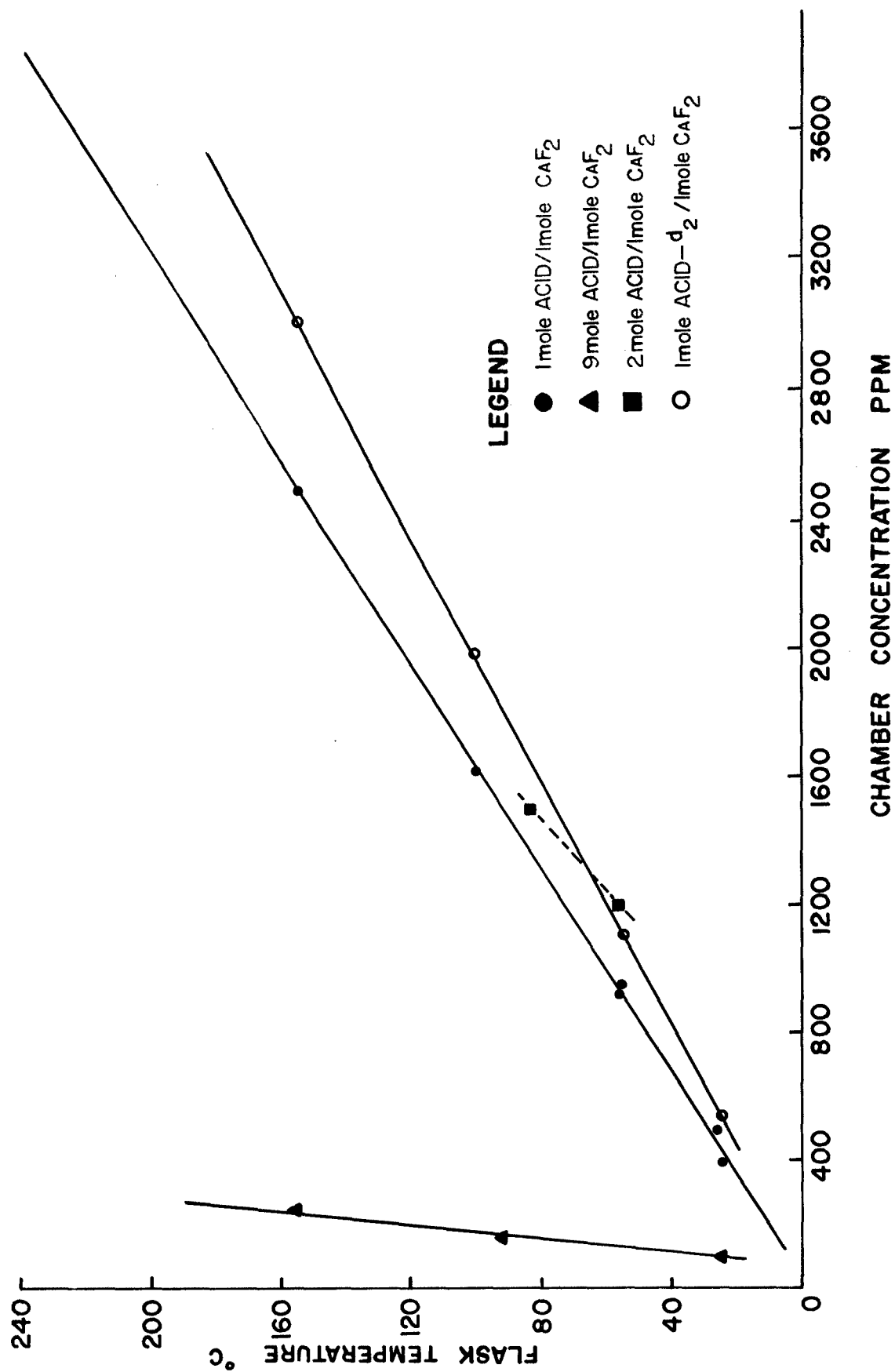


Figure 20. Variation in chamber HF concentration with flask temperature.

Equimolar amounts of CaF_2 and H_2SO_4 gave the widest range of concentration over a specific temperature range, and were felt to be the concentration of choice. It was found that levels of DF generated from equimolar amounts of the D_2SO_4 and CaF_2 were higher at any given temperature than for the non-deuterated material. This is also shown in Figure 20, with these data being taken during animal exposures to deuterium fluoride.

Samples were also taken from the reaction flask to determine the actual presence of DF by mass spectral analysis. Deuterium fluoride spectra compared to HF spectra indicated that the reaction in the flask between D_2SO_4 and CaF_2 was indeed producing the deuterated fluoride.

Use of Ion Selective Electrodes in Inhalation Toxicology

Mr. M. G. Schneider of the chemistry department presented a paper on this subject at the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy in Cleveland, 5 March 1974. Since it integrates all the applications of ion selective electrodes in the THRU, it is included in this report.

The THRU has performed a number of investigations into the toxicity of materials which are analyzable by ion selective electrodes. Many of these experiments were done for various Federal agencies which entered into inter-agency agreements with the Air Force for carrying out the work at the THRU facilities.

The Department of Transportation desired to set criteria for the handling and shipping of hydrogen sulfide (H_2S) and hydrogen bromide (HBr), while the Air Force was concerned with the handling and storage of oxidizers such as chlorine pentafluoride (ClF_5). The Federal Aviation Agency was concerned with the possible effects from the production of hydrogen chloride (HCl), hydrogen fluoride (HF) and hydrogen cyanide (HCN) during aircraft cabin fires. The Air Force was further concerned with the effects of formation of HCl aerosol during solid rocket fuel burning.

Exposure periods of five to sixty minutes were necessary to determine the acute effects of these materials when inhaled, and the toxicologist required analytical methods for exposure chamber monitoring that were continuous and rapid. Of the analytical tools available for use in our laboratory, the ion-selective electrodes provided a means of measuring the above mentioned chemical species when in solution. These electrodes met our requirements since they responded rapidly to changes in solution concentration, were reproducible, were easily calibrated and were stable with respect to calibration.

The method used to measure a compound in air involved the continuous absorption of that species to form an ion in solution. Figure 21 is a schematic of the system used. A known volume of chamber air was mixed with a known volume of the absorber solution in a glass or Teflon[®] scrubber column. The chamber sample was measured by flowmeter and the absorber solution volume was controlled by a peristaltic pump using different internal diameter pump tubing. Solution from

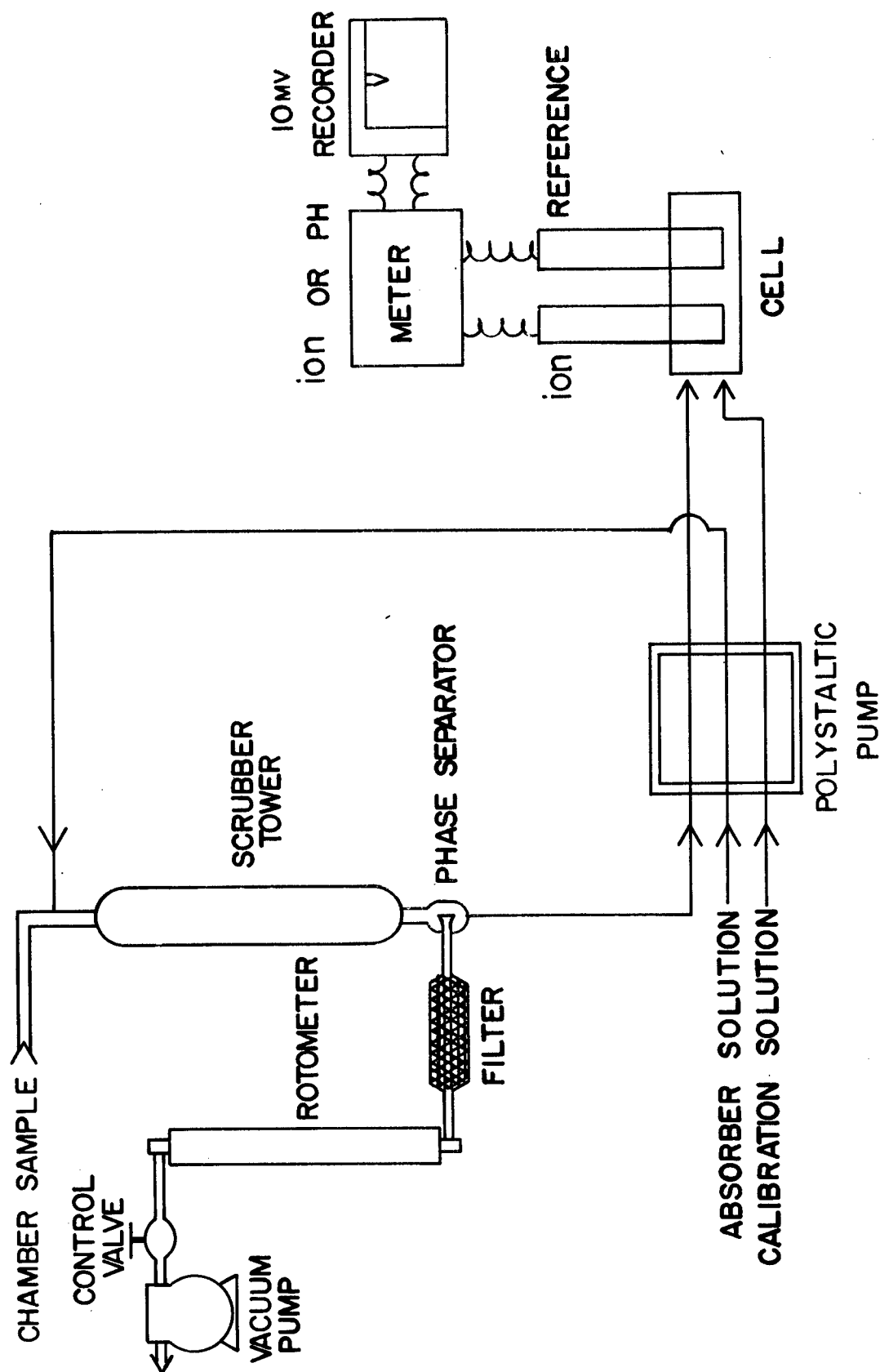


Figure 21. System for analysis of chamber contaminant concentration by specific ion electrode.

the phase separator was pumped to a cell containing the ion and reference electrodes. The most effective flow cell, in our experience, has been a 3-inch length of 1/2-inch bore PVC tubing. Electrode response was read from an ion meter or an expanded millivolt pH meter. Calibration standards were pumped to the electrodes, at the same rate as the unknown solution, prior to each series of measurements. The rate of flow through the peristaltic pump tubing was observed to decrease, by as much as three percent, over a period of several weeks. Thus, it was necessary to determine the exact rate prior to each measurement series. The equation at the bottom of Figure 21 was used to convert the ion solution concentration to species air concentration as parts per million. This relationship was also useful in determining operational parameters such as flow rates and pump tubing sizes since, for the required range in air concentration, it was desirable to have the ion concentration of the solution being analyzed fall in the linear range of electrode response.

Table 53 lists the parameters selected for measurement of HF or HBr. The absorber solution was buffered at pH 6.8 and pumped at 1.6 and 1.8 ml/min respectively. The exposure chamber was sampled at 20 and 400 ml/min respectively. The HF exposure levels ranged from 0.1 to 10 percent HF in air, which corresponded to an ion concentration range of from 10^{-3} to 10^{-1} molar fluoride ion. The HBr chamber concentration ranged from 0.01 to 1 percent, corresponding to 10^{-3} to 10^{-1} molar bromide ion measured. The electrode calibration standards covered the range of ion concentrations analyzed and were prepared using the salts of the ion of interest in the absorber solution.

TABLE 53. EXPERIMENTAL CONDITIONS FOR
MEASUREMENT OF HYDROGEN FLUORIDE AND HYDROGEN BROMIDE

<u>Species</u>	<u>HF</u>	<u>HBr</u>
Absorber Solution	0.07 M NaOAc-3H ₂ O 0.17 M NaCl 0.0004 M Na ₃ Citrate	0.07 M NaOAc-3H ₂ O 0.17 M NaCl 0.0004 M Na ₃ Citrate
Absorber Rate	1.6 ml/min.	1.8 ml/min.
Chamber Sample Rate	20 ml/min.	400 ml/min.
Chamber Concentration	0.1% to 10%	0.01% to 1.0%
Ion Concentration Analyzed	10 ⁻³ M F [⊖] to 10 ⁻¹ M F [⊖]	10 ⁻³ M Br [⊖] to 10 ⁻¹ M Br [⊖]
Calibration Standards	NaF in Absorber	KBr in Absorber
Recovery ± Relative Standard Deviation	99 ± 2%	100 ± 6%

Percent recovery was determined on the basis of measurement of known concentrations of HF or HBr in mylar bags of 50-liter capacity. Ten bag standards at each of several points in the chamber concentration range were measured.

Table 54 lists the parameters used for analysis of ClF_5 . ClF_5 is an interhalogen which is known to hydrolyze to fluoride ion in solution in the presence of bicarbonate. The level of bicarbonate in the absorber was calculated to be in excess of the highest ClF_5 level. These data are from separate studies and are presented as such here. Recoveries were based on standards prepared in polyethylene bags of 10-liter capacity. While the recovery rates varied with air concentration, they proved to be very reproducible.

The parameters for HCl analysis are listed in Table 55 as a gas and as a gas/aerosol mix. The scrubber tower was placed inside the exposure chamber for the aerosol measurement. This helped eliminate loss of HCl adsorbed on sample line walls.

Table 56 lists the experimental conditions for measurement of HCN and H_2S . Because of some uncertainty concerning the sodium sulfide water of hydration, the calibration standards were titrated with standard silver nitrate with the endpoint read using the sulfide ion electrode.

The method described here has a response lag of three minutes and the time from first response to maximum is one minute. This leads to a small amount of concentration averaging. However, changes of one percent were easily discerned.

TABLE 54. EXPERIMENTAL CONDITIONS FOR
MEASUREMENT FOR CHLORINE PENTAFLUORIDE

<u>Species</u>	<u>ClF₅</u>	<u>ClF₅</u>
Absorber Solution	0.07 M NaOAc-3H ₂ O 0.17 M NaCl 0.0004 M Na ₃ Citrate 0.006 M NaHCO ₃	0.07 M NaOAc-3H ₂ O 0.17 M NaCl 0.0004 M Na ₃ Citrate 0.018 M NaHCO ₃
Absorber Rate	2.1 ml/min.	1.7 ml/min.
Chamber Sample Rate	1000 ml/min.	1000 ml/min.
Chamber Concentration	0.0001% to 0.01%	0.01% to 0.1%
Ion Concentration Analyzed	10 ⁻⁴ M F [⊖] to 10 ⁻² M F [⊖]	10 ⁻³ M F [⊖] to 10 ⁻¹ M F [⊖]
Calibration Standards	NaF in Absorber	NaF in Absorber
Recovery ± Relative Standard Deviation	47 ± 7% to 64 ± 2%	64 ± 2% to 86 ± 2%

TABLE 55. EXPERIMENTAL CONDITIONS FOR
MEASUREMENT OF HYDROGEN CHLORIDE GAS AND AEROSOL

<u>Species</u>	<u>HCl</u>
Absorber Solution	Double Distilled Water
Absorber Rate	1.6 ml/min.
Chamber Sample Rate	20 ml/min.
Chamber Concentration	0.10% to 10.0%
Ion Concentration Analyzed	10^{-3} M $\text{Cl}^{\ominus}$ to 10^{-1} M $\text{Cl}^{\ominus}$
Calibration Standards	NaCl in Absorber
Recovery $\pm$ Relative Standard Deviation	$98 \pm 3\%$

TABLE 56. EXPERIMENTAL CONDITIONS FOR
MEASUREMENT OF HYDROGEN CYANIDE AND HYDROGEN SULFIDE

<u>Species</u>	<u>HCN</u>	<u>H₂S</u>
Absorber Solution	0.10 M NaOH	1.0 M NaOH
Absorber Rate	1.6 ml/min.	1.7 ml/min.
Chamber Sample Rate	20 ml/min.	500 ml/min.
Chamber Concentration	0.001% to 0.10%	0.01% to 1.0%
Ion Concentration Analyzed	10^{-5} M $\text{CN}^{\ominus}$ to 10^{-3} M $\text{CN}^{\ominus}$	10^{-3} M $\text{S}^{(2)\ominus}$ to 10^{-1} M $\text{S}^{(2)\ominus}$
Calibration Standards	NaCN in Absorber	Na ₂ S in Absorber
Recovery $\pm$ Relative Standard Deviation	$100 \pm 2\%$	$90 \pm 8\%$

In summary, this method allows rapid, reproducible and precise analysis of exposure chamber environments during inhalation exposures, and at a reasonable cost in terms of utilization of space, equipment and analyst time. The method described here should be easily applicable to the measurement of many chemical species in an atmospheric environment by selection of suitable ion-specific electrodes.

Analysis of Coal Tar Chamber Atmospheres

The coal tar used for the second series of animal exposures contained 16% by volume of light oil, a benzene, toluene, xylene mixture. Because of this, it was necessary to determine that the TLV of benzene was not being exceeded during the exposure. Since animals were being continuously exposed, a chamber concentration of 20 mg/m³ of benzene was considered to be equivalent to the TLV of 80 mg/m³ during a 6-hour day. Gas samples taken from each chamber were analyzed once a day for the first two weeks and thereafter biweekly for the 10 mg/m³ exposure. The results are indicated in Table 57. The chambers containing 2 mg/m³ coal tar were analyzed once each for the first two days of the study then sampling was discontinued inasmuch as the benzene level never exceeded 4.7 mg/m³.

TABLE 57. BENZENE CONCENTRATIONS IN CHAMBER ATMOSPHERES CONTAINING 10 mg/m³ COAL TAR AEROSOL

Chamber	Benzene Concentration (mg/m ³)			
	A	B	C	D
Mean	10.4	13.2	9.5	9.4
Range	5.8-17.2	7.5-19.0	6.0-17.4	6.0-16.1
N	32	32	32	32

Mean for all chambers: 10.6 mg/m³.

The method of analysis for benzene utilized a dual flame ionization detector gas chromatograph. Columns, 1/4" O.D. and 3' in length, contained 60/80 mesh Chromosorb 103 operated at a temperature of 165 C. Nitrogen at a sufficient flow rate to complete the analysis within 5 minutes was the carrier gas of choice. The chromatograph was periodically calibrated using standard vapor samples of benzene made in 320 cc glass sample bulbs.

The extensive use of toluene for the fluorometric analysis of chamber coal tar aerosol concentration resulted in a disposal problem which was solved by cleaning the toluene for reuse. The toluene was passed through an activated charcoal column, 4' in length and 1" in diameter. The cleaned material was checked for fluorescence using "Spectro-Quality" toluene as a standard.

Tissue Coal Tar Analysis

The method of tissue coal tar analysis detailed in last year's annual report (MacEwen and Vernot, 1973) was time consuming, largely due to the insolubility of water in the extracting solvent, toluene. Extraction in a separatory funnel was required after homogenization, and troublesome emulsions often formed at this point. It was found that acetone extracted coal tar from tissues as efficiently as toluene, dehydrating the tissue in the process so that simple filtration yielded a clean acetone extract. With this change, and the substitution of an electrically operated homogenizer for the manual technique previously used, analysis of coal tar in tissues became much more precise and rapid at low coal tar levels.

Fractionation of Crude Coal Tar

During the year, sufficient quantities of all coal tar fractions were isolated for delivery to the National Institute for Occupational Safety and Health (NIOSH) for carcinogenesis testing. A preliminary separation scheme was presented in the last annual report (MacEwen and Vernot, 1973). The final separatory technique used in obtaining the fractions delivered to NIOSH is as follows:

A. Step 1

Three volumes of benzene were added to one volume of coal tar and the mixture centrifuged. About 15% by weight of solids was removed by this procedure. The precipitate was washed in benzene and the benzene then used for dilution of more crude tar. The precipitate is designated as Fraction A in the general scheme of separation (Figure 22) while the benzene solubles are Raffinate I. The various separated fractions shown in Figure 22 are defined in Table 58. The benzene insoluble portion was not extensively studied, but when ashed in air, yielded a residue of 0.4% by weight of the sample. No distinguishing IR spectra were obtained from the powder. Some fluorescent material could still be dissolved out readily after four washes in benzene. The dark brown material produced after Soxhlet extraction of the insoluble portion could be pulverized into a dry powder.

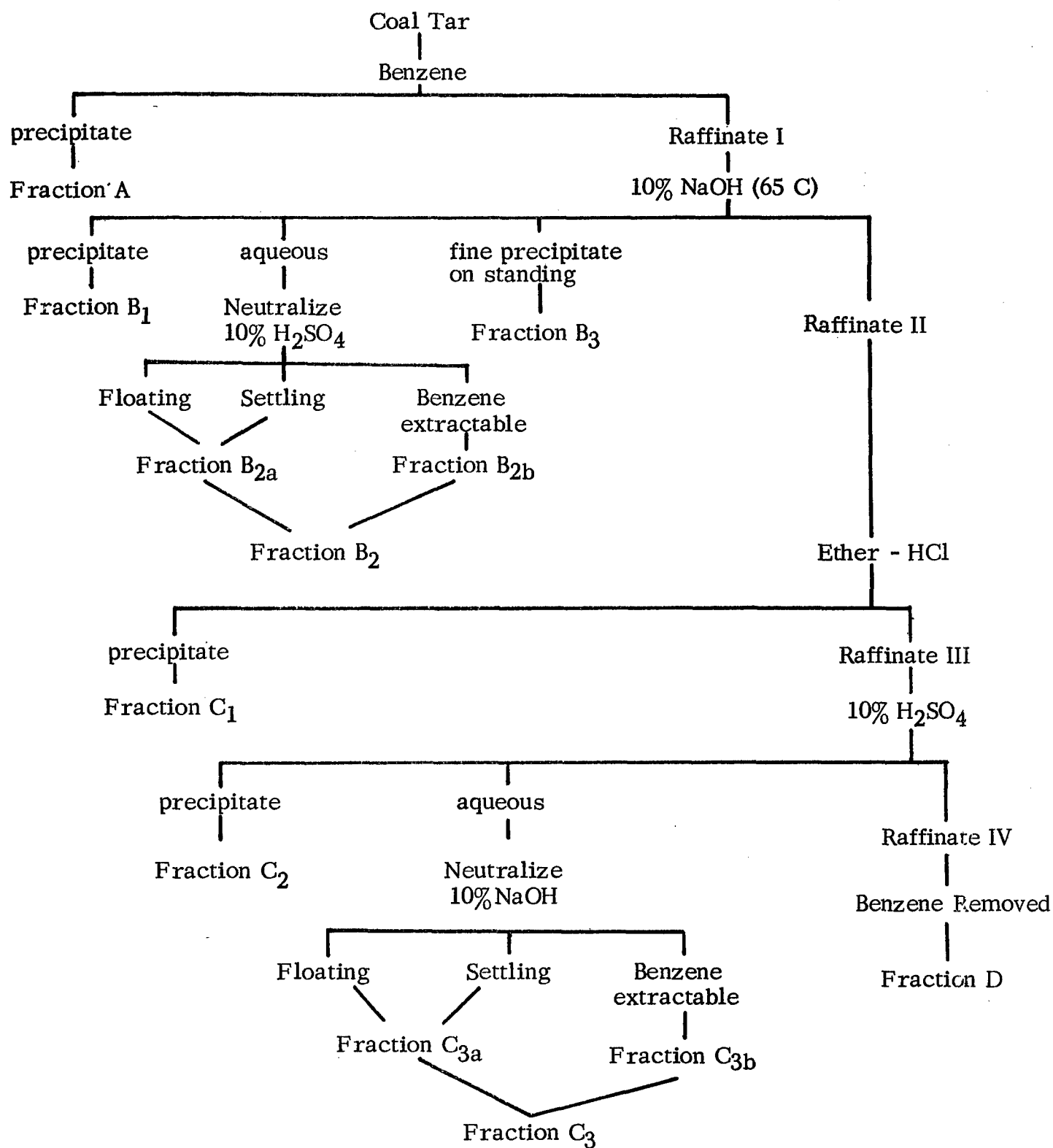


Figure 22. Simplified scheme of coal tar fraction separation.

TABLE 58. IDENTIFICATION OF COAL TAR
FRACTIONS SEPARATED

Fraction A	-	Benzene Insolubles
Fraction B	-	Tar Acids
B ₁	-	Precipitate on Addition of 10% NaOH Solution
B ₂	-	Recover from alkaline extract on neutralization
		B _{2a} - recovered directly
		B _{2b} - recovered by benzene extraction
B ₃	-	Further Precipitation in Raffinate
Fraction C	-	Tar Bases
C ₁	-	Precipitate on Addition of Ether-HCl
C ₂	-	Precipitate on Addition of 10% H ₂ SO ₄
C ₃	-	Recovered from acid extract on neutralization
		C _{3a} - recovered directly
		C _{3b} - recovered by benzene extraction
Fraction D	-	Tar Oils (benzene removed)

B. Step 2, Separation of Tar Acids - B Fractions

A counter current double extraction was performed using a 2:1 ratio of clarified coal tar-benzene (Raffinate I) to 10% NaOH solution. To prevent emulsification, both reactants were preheated to 65-70 C. This operation resulted in a 3-phase system, an aqueous solution, a benzene solution and a tarry precipitate. The latter appeared mainly at the solution interface but also readily adhered to glassware. The precipitate following recovery was both water and benzene washed then stored. It is designated as B₁. These washings, as all washings in this separation, are returned to a previous step where materials removed will not be lost.

The benzene and aqueous phases were then separated, filtered, and washed. The benzene phase was now held for further separation and designated as Raffinate II. Some precipitation occurred during storage and this precipitate was designated B₃.

The aqueous phase was then titrated to a final pH below 6 using 10% sulfuric acid. Heavy and light tar oil-like materials separated from the water phase and were recovered by centrifugation. These were recombined to form Fraction B_{2a}. The remaining water phase was then extracted with benzene (10:1 - water:benzene, again using a counter current double extraction). On concentration, the benzene extract was designated B_{2b}.

C. Step 3, Separation of Tar Bases - C Fractions

1. HCl Precipitation

Raffinate II was titrated to acid with an ethereal-HCl solution (approximately 20% gaseous HCl in ethyl ether). Extreme care with constant agitation are required to prevent formation of a black resinous material. The fine gray brown powder, recovered by filtration is benzene washed (3 times) and is designated as Raffinate III.

2. Aqueous H₂SO₄ Extraction

A counter current double extraction of Raffinate III with cold 10% H₂SO₄ (2:1) results again in a 3-phase system (aqueous, benzene and a precipitate).

The precipitate after both benzene and water wash is designated as C₂. The aqueous and benzene phases were separated then washed. The aqueous phase was neutralized with 10% NaOH to a pH greater than 8. A thick oil-like material was released on neutralization and recovery methods are similar to tar acid recovery process. The pooled subfractions are designated C₃. The benzene phase is designated Raffinate IV.

D. Step 4, Neutral Oil Fraction - D Fraction

The benzene fraction, Raffinate IV, contains the neutral oil portion of the coal tar. The benzene was removed by distillation using a Snyder fractionating column. The vat temperature at completion was kept below 150 C and the tower temperature approached 95 C. Fraction A and D received no further treatment before submission for the carcinogenic study.

E. Step 5, Neutralization and Recombination of Subfractions

In order to minimize the number of initial fractions for the carcinogenic study, the subfractions of tar acids and bases were combined to make a single tar acid group (B) and tar base group (C).

1. B Fraction

(a) Neutralization

The B₁ fraction on initial separation was of heavy tarry consistency and strongly alkaline. Partial neutralization was accomplished by a process of "cutting in" dilute sulfuric acid. In time, the material became more friable but still on standing returned to alkaline. B₁ was stored in this condition until the separation process was completed. It was then air dried, ground to a fine powder and suspended in distilled water. Final neutralization was accomplished by titration with dilute sulfuric acid to a pH of less than 7 followed by filtration and water washing to neutrality. At this point, the material on air drying is a very fine powder.

The B₂ fraction 2a + 2b were released from solution by neutralization so needed no further neutralization. B_{2a} had the consistency of a thick tarry oil and B_{2b} of a light oil.

The B₃ fraction was a fine powder having an alkaline reaction in water. It was ground fine and treated as B₁. The resulting material was on air drying similar to neutralized B₁ and probably represents compounds that had initially escaped separation as B₁ due to particle size. They were quantitatively added together at this point.

(b) Recombination

Mixing of B_2 subfractions gave a heavy oil-like material which thinned on heating. Then the mixed $B_1 + B_3$ material was slowly added with constant stirring. The resultant mixture on solution of $B_1 + B_3$ became a thick flowing tar, which is designated as Fraction B, the combined tar acid fractions.

2. C Fraction

(a) Neutralization of C_1 was accomplished by mixing it with hot 2% NaOH and extracting with benzene. Due to limited solubility of the neutralized material, repeated extractions were found necessary, along with almost one third as many changes of NaOH solution. Degree of completeness of neutralization was determined by following the decrease of Cl^- in the water phase along with the diminishing benzene solubility of the remaining C_1 . Two phases resulted C_{1a} , the benzene extract, and C_{1b} , the remaining precipitate. The extract C_{1a} was concentrated to less than 25% benzene.

Neutralization of C_2 proceeded on identical lines except much less material was needed and completeness of extraction was determined only by the significant reduction of benzene solubility. A $C_{2a} + C_{2b}$ resulted.

It was not necessary to neutralize C_{3a} or C_{3b} since they were obtained by neutralization of an aqueous phase during the separation.

(b) Recombination

The C fractions were recombined by mixing the liquid portions together (C_{1a}, C_{2a}, C_{3a}, and C_{3b}) with added heat, then carefully stirring in C_{1b} and C_{2b}. Fraction C probably contains from 10-20% benzene. This was left in as the distillate from the Snyder column developed a tar base odor during final stage of concentration.

Column Separation of Neutral Oil Fraction

Solvent dilution adsorption chromatography was applied to the neutral oil fraction. Dry packed alumina (Woelm, neutral activity - Grade 1) columns 1" x 16" both activated and 2% deactivated, were studied. N-pentane, paradioxane and methanol were used as eluents, and in that order. Activation of the alumina was accomplished by heating at 200 C until constant weight was attained, usually overnight. Partial deactivation was achieved by stirring the activated material at ambient humidity until it gained 2% of its dry weight. The material was then stored in sealed jars.

Results

The separation of crude coal tar by the method described above gave 9 recovered fractions. Table 59 shows the percentage and weight of each collected fraction from Figure 22 in terms of recovered material.

The separation of the neutral oil fraction by absorption chromatography was performed on activated and 2% deactivated alumina. The resulting fractions were observed using IR spectroscopy. The two systems differed basically in

TABLE 59. APPROXIMATE AMOUNTS OF COAL TAR FRACTIONS

	<u>Fraction (grams)</u>	<u>Subfraction (grams)</u>	<u>% of Total Fraction-Subfraction</u>
Fraction A	1000		11
Fraction B	837		9
B ₁		300	3
B _{2a}		257	3
B _{2b}		210	2
B ₃		70	1
Fraction C	1415		15
C ₁		1200	13
C ₂		65	1
C ₃		150	2
Fraction D	6000		65

the pentane fraction. On partially deactivated alumina evidence is found for aliphatics and aromatics in the pentane fraction whereas on activated alumina the pentane fraction shows only saturated aliphatics. According to Snyder (1968) the remaining separation is due almost entirely to number of aromatic carbon atoms in the molecules. Table 60 shows the separations by percent of neutral oil in each fraction.

Thin layer chromatography was performed on the eluant fractions of the neutral oil for comparison of fractions and for possible separation to individual compounds. Fluorescence spot observation was the only means of visualization used successfully. In one separation, over 36 spots were noted following two dimensional developments. Since it was not our purpose to determine specific compounds, this technique was not pursued further.

Blood Cyanide ($\text{CN}^{\ominus}$) Analysis

The extensive study performed for the Federal Aviation Agency (FAA) last year (MacEwen and Vernot, 1973), (Darmer and Smith, 1972) on the inhalation toxicity of HCN demonstrated a large variability in blood $\text{CN}^{\ominus}$ values in animals exposed to a single concentration. As a result of this, experiments were designed to investigate the causes of this variability and to determine whether concentration of blood $\text{CN}^{\ominus}$, possibly including its metabolite, thiocyanate ($\text{CNS}^{\ominus}$), could be related to lethality during or after exposures to HCN.

TABLE 60. RESULTS OF ELUTION ADSORPTION CHROMATOGRAPHY ON
NEUTRAL OIL FRACTION OF COAL TAR

<u>Fraction</u>	<u>2% Deactivated Alumina</u>	<u>Activated Alumina</u>
n-pentane	10%	2%
P-dioxane	85%	95%
methanol	3.5%	1%
residue	1.5%	2%

The initial procedure used for the determination of blood $\text{CN}^\ominus$, diffusion of HCN from acidified blood into a strongly basic aqueous solution and measurement of $\text{CN}^\ominus$ with an ion specific electrode, had been calibrated with additions of known amounts of $\text{CN}^\ominus$ to blood. Although this should have accounted for any loss of $\text{CN}^\ominus$ in blood, there were two possible areas of loss - volatilization from the blood being sampled from the exposed animal and reaction of $\text{CN}^\ominus$ in the living animal. In order to estimate the significance of the loss from blood during sampling, 4 ml portions of rat blood containing known amounts of $\text{CN}^\ominus$ ion were placed in sealed test tubes of 15 ml total volume. After one hour, the entire headspace was collected in a syringe containing 1.0 ml of 0.1 N sodium hydroxide, by displacement with water. After shaking for 3 minutes, the concentration of $\text{CN}^\ominus$ was measured using the specific ion electrode. No $\text{CN}^\ominus$ was detected in solutions derived from blood samples containing 5 to 10 $\mu\text{g}/\text{ml}$ $\text{CN}^\ominus$ demonstrating that the losses were not caused by volatilization.

In order to investigate the loss of $\text{CN}^\ominus$ in living animals, rats weighing 180 grams were infused over a period of 1-minute with 1 ml of physiological saline solution containing 28.5 μg $\text{CN}^\ominus$ /ml. The estimated volume of blood in a 180 gram rat was 10.4 ml giving a $\text{CN}^\ominus$ dose of 2.5 $\mu\text{g}/\text{ml}$ blood. Blood samples were taken by cardiac puncture after 1 minute's circulation, and $\text{CN}^\ominus$ concentration measured by the diffusion technique. The concentrations of $\text{CN}^\ominus$ found ranged from 0.003 to 0.130 $\mu\text{g}/\text{ml}$. These results demonstrated that there are mechanisms operating in the live animal to decrease blood $\text{CN}^\ominus$ values markedly and in an extremely variable manner.

A new method for blood $\text{CN}^{\ominus}$ was developed to determine whether results obtained were independent of methodology. The technique utilizes 2 cylindrical flasks, a 35 ml reaction flask and 20 ml absorber flask containing 2 ml 0.1 N NaOH. The reaction flask contains 2 ml blood, 2 ml trichloroacetic acid solution (15 g/100 ml) and 0.2 ml dilute silicone antifoam. The 2 flasks are connected by glass tubing so that when suction is applied to the absorber flask, air is bubbled through both flasks in the system and cyanide is vaporized from the reaction flask into the absorber flask where the $\text{CN}^{\ominus}$ concentration is measured using a specific ion electrode. Air flow of 240 ml/min for 20 minutes provides 95% recovery of $\text{CN}^{\ominus}$ added in vitro to blood at concentrations ranging from 1 to 8 $\mu\text{g/ml}$.

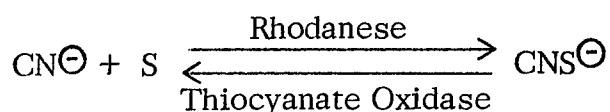
Rats weighing about 400 g each were infused with 58 μg $\text{CN}^{\ominus}$ in saline over a minute's time. The blood was then sampled for $\text{CN}^{\ominus}$ analysis after about 2 minutes. Table 61 lists the results obtained on the same samples by the new distillation technique and the Conway diffusion technique used previously.

TABLE 61. CYANIDE ION CONCENTRATION IN THE BLOOD OF RATS INFUSED WITH 58 μg $\text{CN}^{\ominus}$

<u>Rat No.</u>	<u>Distillation</u>	<u>Diffusion</u>
1	0.8 $\mu\text{g/ml}$	0.7 $\mu\text{g/ml}$
2	0.7 $\mu\text{g/ml}$	0.7 $\mu\text{g/ml}$
3	Sample Lost	0.6 $\mu\text{g/ml}$
4	0.6 $\mu\text{g/ml}$	0.6 $\mu\text{g/ml}$
5	0.7 $\mu\text{g/ml}$	0.6 $\mu\text{g/ml}$

The values indicate that the new technique gives results very close to the diffusion, but without requiring a 3-hour diffusion period.

The analysis of $\text{CN}^\ominus$ in blood is complicated by equilibrium with thiocyanate ($\text{CNS}^\ominus$) catalyzed in blood by two enzyme systems and also influenced by the presence of available sulfur which appears to be quite variable.



A large amount of rhodanese is available in the liver for detoxification of the $\text{CN}^\ominus$ by conversion to thiocyanate, but a limitation may be set by the availability of sulfur. Thiosulphate has been used as a cyanide antidote by providing an additional sulfur source. The natural sulfur sources for this reaction appear to include bound cystine and β -mercaptopyruvic acid. In searching for a blood analysis which might correlate with lethality, a method was developed for determination of $\text{CNS}^\ominus$.

The method made use of the fact that $\text{CNS}^\ominus$ was not associated with the red cells. The red blood cells were removed by centrifuging. Trichloroacetic acid was added to precipitate protein which was also removed by centrifugation. A standard concentration of ferric (Fe^{+3}) ion, made up by dissolving pure iron wire in a mixture of nitric and hydrochloric acid, was added and the absorbance measured at 480 nm. Although the absorbance is

not linear with $\text{CNS}^{\ominus}$ concentrations because different complexes are formed with different $\text{Fe}^{+3}/\text{CNS}^{\ominus}$ ratios, the reproducibility is excellent. This procedure is a variation of one developed by Rosenberg and Riber (1972).

The in vivo fate of injected $\text{CN}^{\ominus}$ or $\text{CNS}^{\ominus}$ was then followed using these analyses. Young male rats were anesthetized and injected intravenously (inferior vena cava) with cyanide or thiocyanate in saline solution. One hundred μg $\text{CN}^{\ominus}$ was injected over a 100 second period to prevent high localized concentrations which would kill the rat. These rats were held for various time periods, bled once and the blood analyzed for cyanide and thiocyanate as shown in Table 62.

TABLE 62. RAT BLOOD CONCENTRATIONS OF $\text{CN}^{\ominus}$ AND $\text{CNS}^{\ominus}$ AFTER INTRAVENOUS INJECTION OF 100 μg $\text{CN}^{\ominus}$

<u>Time Post Injection</u>	<u>$\text{CN}^{\ominus(1)}$ $\mu\text{g}/\text{ml}$</u>	<u>$\text{CNS}^{\ominus(1)}$ $\mu\text{g}/\text{ml}$</u>
Control	0.00	0.0
Control	0.00	0.0
2.3 min.	1.30	0.0
6 min.	0.81	0.0
20 min.	0.22	---
20 min.	0.32	0.0
40 min.	0.16	0.0

⁽¹⁾ Value found minus control level.

The rapid $\text{CN}^{\ominus}$ loss and failure of $\text{CNS}^{\ominus}$ to build up in the blood indicated total cyanide exposure could not be easily determined by blood cyanide and/or thiocyanate.

In the same manner as for $\text{CN}^{\ominus}$, 500 μg $\text{CNS}^{\ominus}$ was injected i. v. over a 30-second period into rats. The rats were held and each bled once at different times post injection. Blood was then analyzed for cyanide and thiocyanate with results shown in Table 63.

TABLE 63. RAT BLOOD CONCENTRATIONS OF $\text{CN}^{\ominus}$ AND $\text{CNS}^{\ominus}$ AFTER INTRAVENOUS INJECTION OF 500 μg $\text{CNS}^{\ominus}$

<u>Time Post Injection</u>	<u>$\text{CN}^{\ominus(1)}$ $\mu\text{g/ml}$</u>	<u>$\text{CNS}^{\ominus(1)}$ $\mu\text{g/ml}$</u>
1.2 min.	0.00	10.7
2.5 min.	0.00	6.6
5 min.	0.13	5.6
10 min.	0.17	3.9
10 min.	0.31	4.9
20 min.	0.27	5.0
40 min.	0.27	3.9

⁽¹⁾Value found minus control level.

Table 63 demonstrates that significant levels of $\text{CN}^{\ominus}$ are built up after injection of $\text{CNS}^{\ominus}$. However, $\text{CNS}^{\ominus}$ concentration does not decrease as rapidly as $\text{CN}^{\ominus}$ after injection, since 60% of the 2.5 minute level is present after 40 minutes whereas only 12% of the 2.3 minute $\text{CN}^{\ominus}$ level is present (Table 62).

This work developed good analyses for cyanide and thiocyanate in whole blood. Using these analyses, cyanide and thiocyanate were found to separate in blood, the cyanide associating with the blood cells and the thiocyanate with the plasma. In vivo work showed rapid decreases of both cyanide and thiocyanate when injected into the bloodstream. The loss of cyanide did not cause a corresponding increase in thiocyanate in the blood so total dose could not be determined using both analyses.

Engineering Programs

The Facility Engineering Department objectives during the past report period have continued to be concentrated on supporting laboratory requirements. Considerable efforts have been extended on renovating all systems in operation at the facility. Preventive maintenance programs are routinely evaluated and modified according to the needs of the system. A major project completed was the removal of equipment located in Domes 2 and 3 and equipping those domes similarly to the remaining domes. Several modifications that had been made in other domes during the past 2 years were incorporated into the aforementioned domes.

Additional Animal Holding Facilities

As a result of the increased long term postexposure holding of animals for evaluation of carcinogenic effects of coal tar aerosols and other chemicals. Building 429 was modified to serve these requirements. The existing round preexposure chambers and their associated utilities were removed. The chambers had been installed on raised concrete slabs which were extended to approximately 10 feet by 12 feet to provide a level working service area. Modular racks were fabricated similar to the wall-mounted racks installed in the Building 79 preexposure and postexposure rooms. The rack provides 4 columns of 18" shelves, 8 shelves high and 24 feet long. The total shelf space available for rodent cages in Building 429 is now 768 feet. This will accommodate approximately 800 cages of the type currently in use at the facility. Modular construction allows rearranging of racks to accommodate any of the various shapes and sizes currently being used. Shelf construction is composed of four and one half inch round aluminum rods spaced six inches apart, allowing ease in cleaning. Drinking water is provided by removable plastic bottles attached to each cage. Two automatic water dispensers were installed on each row of shelves for filling bottles, and hose outlets were provided for cleaning purposes.

Relative Humidity Monitoring and Control System - Facility A

Relative humidity measurements in Facility A were achieved using thermocouple probes as the basic temperature transducer. A basically identical system was designed at the time of construction of Facility B with the exception of replacing the thermocouple transducers with a thermistor type. Use of thermistor transducers provides less drift, easier calibration and more reliable service. To standardize calibration procedures in the facility and achieve the advantages of a thermistor system the relative humidity system in Facility A was modified. Concurrently, the panel housing system components was relocated from the basement parts room to the first floor of Facility A (Figure 23). This move increases available storage space and provides better accessibility to the panel for maintenance and calibration requirements. A 24" high rack panel was mounted on top of the original panel to house the thermistor probe readout units. The combined assembly was located adjacent to the O₂ control panel in Facility A.

Contaminant Vent System - Facility A

A positive discharge contaminant vent system was installed in Facility A similar to the system in Facility B. This system provides positive venting of contaminants from each of the contaminant generating stations adjacent to Domes 1 through 4. In addition, the new system permits venting of contaminants during emergency conditions through a bypass activated with the opening of solenoid valves operated by the emergency power system.



Figure 23. Relative humidity control panel.

The system consists of a centrifugal blower located in the equipment mezzanine connected to the contaminant vent piping located in the basement of Facility A. A schematic diagram of the system is shown in Figure 24. A solenoid valve is incorporated at the inlet of the system and activated by a negative pressure switch. This switch prevents discharge of contaminants in the contaminant vent valves in case of blower failure. The system is also interconnected with the Main Alarm System to annunciate emergency conditions in case of blower failure.

Renovation of Facilities

A general renovation of the ambient laboratory area was undertaken during the past year. Complete repair and maintenance of all items in the ambient laboratory was accomplished. Walls were patched where needed and all loose and damaged ceiling tiles were removed and replaced. Obsolete electrical conduit was removed and piping was supported where needed. Power panels located in the ambient laboratory were repainted and relabeled and utility lines were color coded for use. Two Thomas Domes, previously dedicated to special use in materials testing and primate performance testing, were stripped and refitted for standard inhalation toxicity testing programs.

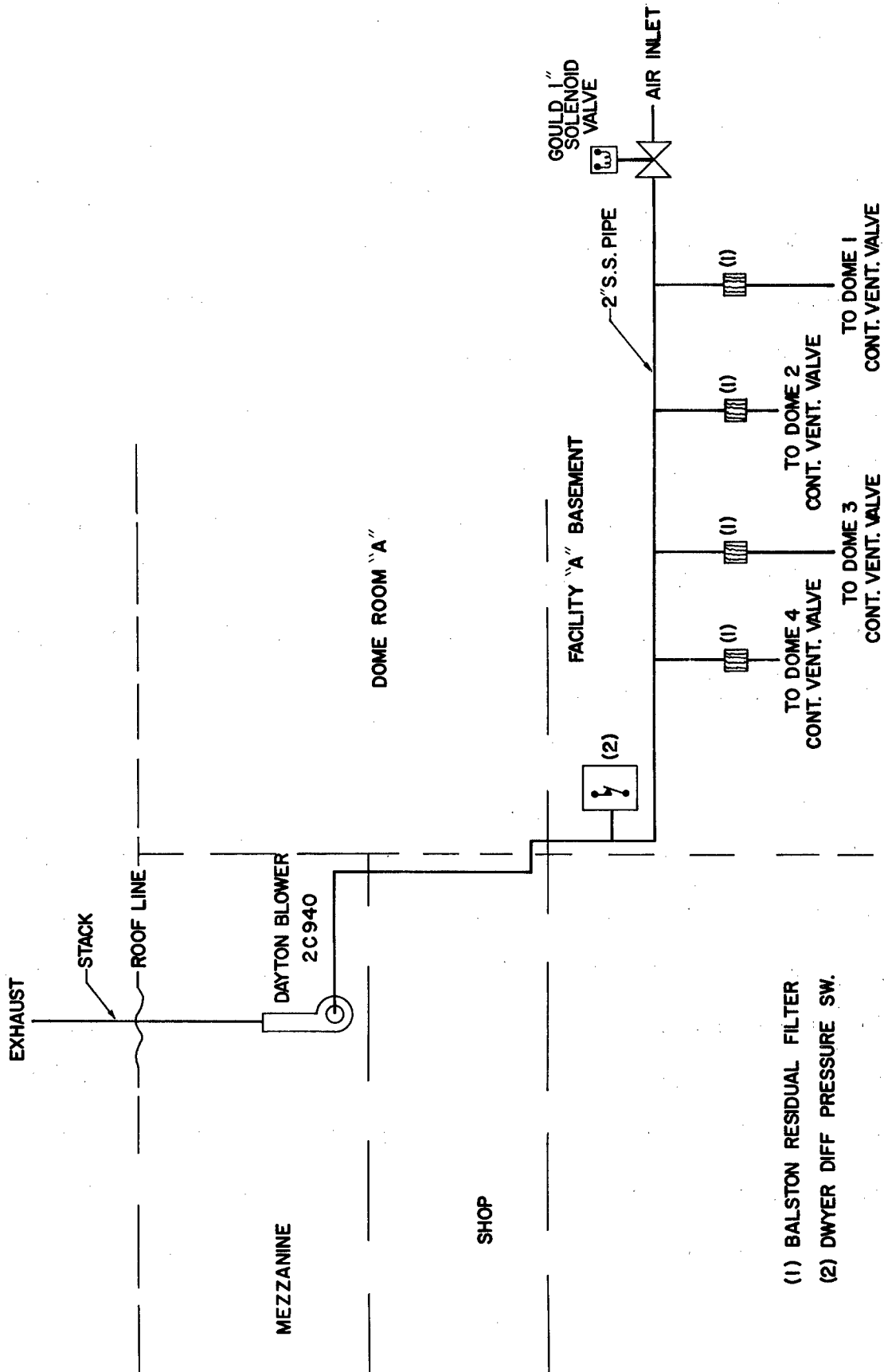


Figure 24. A schematic view of the contaminant venting system.

Airlock Drains

The airlock drains of the Thomas Dome have presented a frequent problem of operation and represented a high frequency corrective maintenance problem. The manual operating valve for the airlocks was located in a very difficult area to reach. A system was designed utilizing an electrically operated ball valve to provide remote actuation of the airlock drain system. This valve was located near the drain outlet of the airlock and the operating controls placed near the other airlock controls. The control consists of an electrical switch having two positions, open or close, and three indicating lights. The light indications are green, open; yellow, operating; and red, closed. This provides a clear indication of valve condition and will also indicate if foreign objects in the valve prevent full closure.

Coal Tar Studies

Modifications are in process on Domes 2 and 3 for long term inhalation experiments to be conducted using coal tar aerosols. The animal loading will be significantly different than typical dome experiments. The exposure group will consist of 14 monkeys, 18 rabbits, 80 weanling rats and approximately 225 mice. The 18 rabbits and 225 mice are to be exposed in Dome 1 and the 14 monkeys and 80 rats in Dome 3. Ten additional monkey cages were mounted on a common rack with the existing monkey cages in Dome 3. Some of the monkey cages required minor modification to be used for coal tar studies

(Figure 25). Cage requirements for the rabbits are being satisfied by fabricating six cages to house three rabbits each. The rabbit cages, shown in Figure 26, are of stainless steel mesh and sheet construction. The floor area of each cage is 12 square feet which meets all requirements for good animal care.

Several modifications were required to adapt dome operation to coal tar exposures. Inlet piping to the dome was removed and a removable plastic pipe insert fabricated. Attached to the end of the pipe is a flexible hose which leads to the coal tar generation apparatus. Use of the pipe insert allows easy removal of the inlet line for cleaning after exposure. Three of the existing one inch glass windows were replaced with plastic panels of the same thickness. Holes were drilled in each panel for sampling probes. To prevent equipment utilized in the exhaust system from being damaged by coal tar the filter boxes were modified to accept an absolute filter identical to the ones used in the ambient chamber coal tar studies. This will remove all traces of coal tar from the exhaust systems.

Materials Screening System

Renovation of the system used in the cabin materials screening studies is currently in progress. This system when placed in operation will be utilized for perfluoropentane studies. The system had been taken out of operation and mothballed. Before renovation of the system an

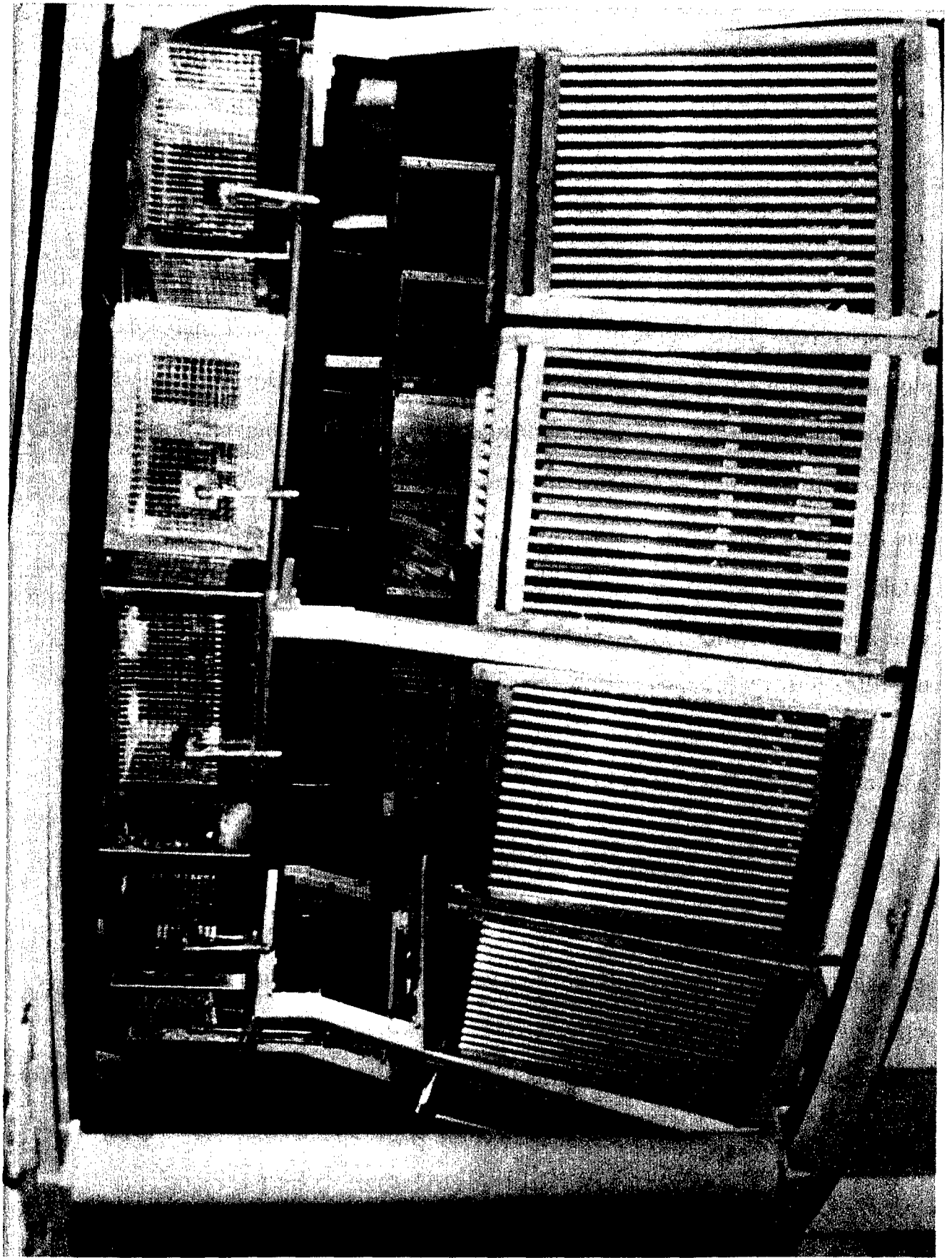


Figure 25. Monkey cages - coal tar study.

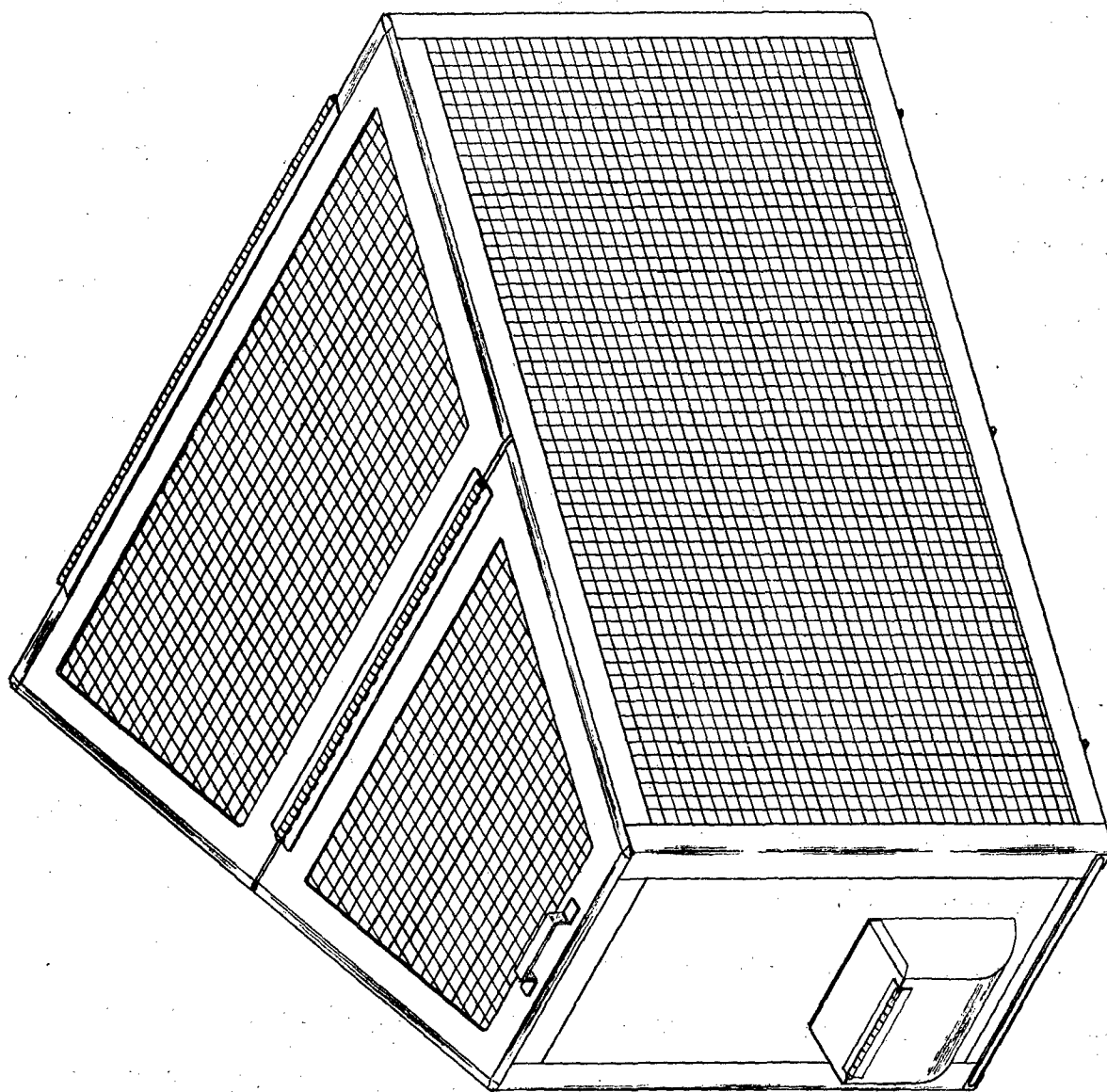


Figure 26. Rabbit cages - coal tar study.

exhaustive analysis was conducted of the system operation and also the requirements of the perfluoropentane study. The existing system had undergone several modifications previous to being mothballed and the determination was made to rectify various operating problems of the system.

One of the major problems encountered previously was system leaks. Original chamber construction was of stainless steel and glass. New chambers shown in Figure 27, were designed to be constructed from clear plastic sheets. Chamber volume was increased slightly to allow for waste collection separation from the animal area. Plastic panels placed on a thirty degree angle lead to the drain outlet. The chambers are divided into three separate areas. Each area will be serviced by a circular door. A door is also included for service and cleaning requirements of the waste collection area. Water flush pipes for waste removal were installed on each side of the chambers. The chamber racks were completely stripped of existing plumbing. The plumbing was replaced with all copper construction. The use of disconnects was held to a minimum and flexible hoses were used only where absolutely necessary. The LiOH canisters were modified to improve ease of changing canisters. The top plate was modified by adding a guide ring of the same diameter as the LiOH canisters. The system will have the capability of operating either as a dynamic flow system or as a closed recirculating system. The flow diagram of the system is shown in Figure 28. The three chambers were located in the inter-connecting airlock. Due to space availability, the operating console and the sample pumps and dryers are located on the first floor adjacent to the basement stairwell. A schematic layout of the system is shown in Figure 29.

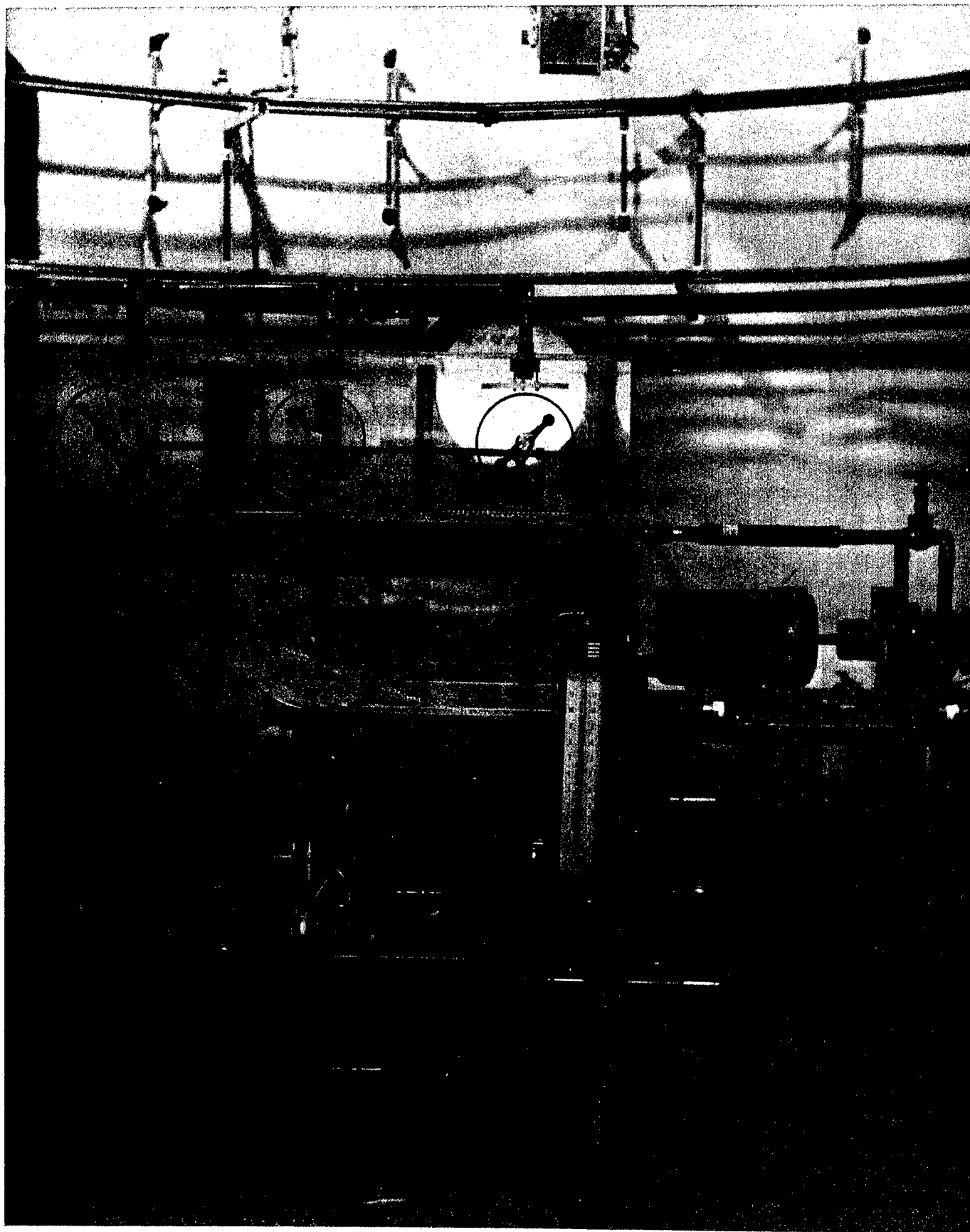


Figure 27. Materials screening exposure chamber.

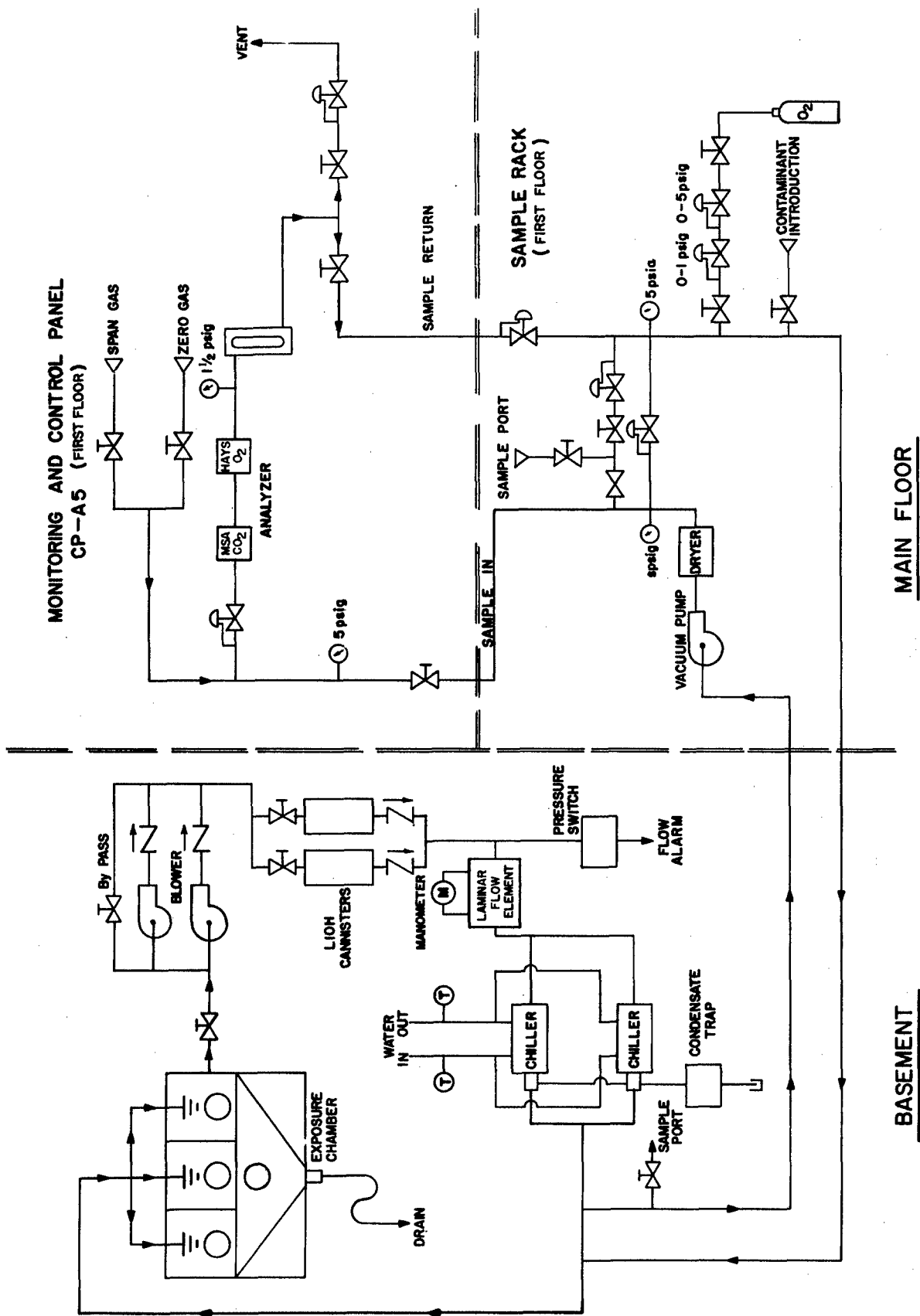


Figure 28. Flow diagram - materials screening system.

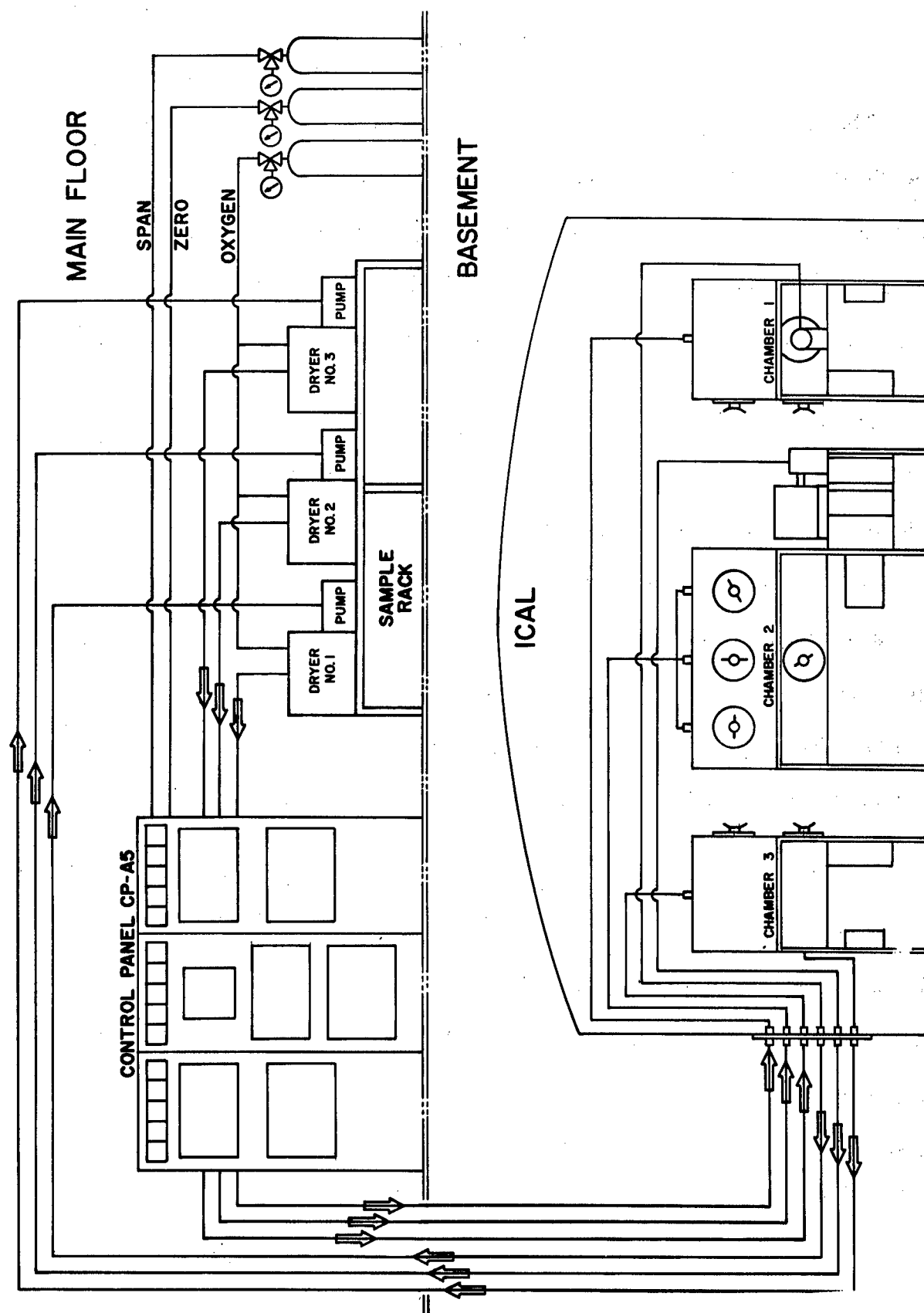


Figure 29. Schematic layout - materials screening system.

Training Programs

The Laboratory Operations and Animal Care Training Programs described in the 1971, 1972 and 1973 annual reports were continued this year as programmed. The Standard Operating Procedures (SOP) Manual was rewritten so that there is now a clearly defined and separate set of procedures for ambient as well as altitude dome inhalation studies.

Phase I and Phase II formal training cycles were scheduled for all new technicians. These training cycles consist of equipment operation and failures of such equipment as well as emergency procedures involving personnel responsible for the operation of the Thomas Domes and the exposure laboratory area. Six technicians completed Phase I and Phase II training during the past 12 months. One returning technician completed a condensed refresher course of Phase I and Phase II training. All experienced technicians participated in the on-the-job training of the new technicians.

Monthly Emergency Training Procedures are deliberate equipment failures or simulated emergencies involving personnel responsible for the Thomas Domes and the exposure laboratory area. These procedures provide a refresher training as well as insuring that the technician will react properly in the event of an actual emergency. The technicians involved in these training procedures are monitored by their supervisor to insure that

the SOP is adhered to. The following list details the emergency training procedures covered during the past 12 months:

<u>Date</u>	<u>Procedure</u>	<u>Personnel Participation*</u>
June 1973	Air Compressor Failure	A
July 1973	Rescue of an Incapacitated Dome Entrant	A, B, D
August 1973	Vacuum Pump Failure	A
September 1973	Fire in the Airlock with Entrant	A, B, D
October 1973	Ambient Blower Failure	A
November 1973	Air Compressor Failure	A
December 1973	Complete Power Failure	A
January 1974	Rescue of an Incapacitated Dome Entrant from Airlock	A, B, D
February 1974	Complete Power Failure (actual)	A
March 1974	Fire in the Dome with Entrant	A, B, D
April 1974	Vacuum Pump Failure	A
May 1974	Fire in the Airlock with Entrant	A, B, D

*A - Shift Operator

B - Safety Observer B

C - Safety Observer C (used only during altitude experiments)

D - Dome Entrant

Purina Animal Care Course

All incoming technicians are required to complete the Ralston Purina Animal Care Course. This course is primarily a self study course which lays a foundation for further study in the field of Laboratory Animal Science. Eight technicians successfully completed this course during this reporting period.

American Association for Laboratory Animal Science (AALAS) Certification Program

Since last year's annual report, one technician has been certified at the second level of certification (laboratory animal technician). There are several technicians who desire to become certified in the AALAS program, but must fulfill the one year time and experience requirement before taking the examination for the first level of certification (assistant animal technician). It is expected that at least four technicians will become certified as assistant animal technicians, one as animal technician and one as animal technologist by December of 1974. The Veterinary Medicine Branch of AMRL has written, distributed and is preparing to conduct formal classroom training as an aid to all technicians at the THRU and particularly those technicians desiring to take the AALAS certification examinations. The course consists of 41 hours of lecture and related laboratory procedures in the field of laboratory animal science.

COURSE OUTLINE FOR LABORATORY ANIMAL TECHNICIAN TRAINING

1. Medical Terminology (1 hour)
2. Classification of Animals (1 hour)
3. Genetics (1 hour)
4. Types, Physiological Characteristics and Uses of the Most Frequently Used Laboratory Animals (2 hours)
5. Anatomy and Physiology (4 hours)
6. Public Law (1 hour)
7. Animal Housing and Caging (2 hours)
8. Colony Management (2 hours)
9. Pharmacology (1 hour)
10. Animal Handling (2 hours)
11. Surgery (2 hours)
12. Clinical Laboratory (6 hours)
13. Nutrition (1 hour)
14. Quarantine and Standardization of Laboratory Animals (1 hour)
15. Animal Diseases (10 hours)
16. Unusual Species Used in Research (2 hours)
17. Necropsy and Tissue Sampling (2 hours)

The need for a formal course in laboratory animal science was evident after conducting four coal tar aerosol experiments over a 2-year period which began in 1972. All animals in these aerosol experiments are held for their lifetime after the exposure phase of the experiment. The THRU

technicians must now be able to identify various animal diseases as well as skin tumors (a result of the coal tar aerosol) in our colony of approximately 3000 animals currently being housed at the THRU. Current trends in the conduct of chronic inhalation studies suggest the need for long-term postexposure observation and testing of experimental animals. Therefore, education of the entire group of technicians in the field of laboratory animal science is of great concern particularly as it relates to animal care and maintenance.

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