CHRONIC TOXICITY OF JP-4 JET FUEL

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Major technical areas discussed included ecosystem modeling, water pollution, water reuse, toxic hazards evaluation of fire extinguishants, environmental carcinogenesis, and cellular toxicology.

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PAPER NO. 11

CHRONIC TOXICITY OF JP-4 JET FUEL

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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 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 either to 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. Those constituents detailed in the military specification are shown in Table 1. Obviously, these constituents represent only a fraction of the total content of JP-4 jet fuel, the remainder consisted completely of unspecified hydrocarbon compounds.

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

Constituent	Maximum % or Concentration	
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

*Now affiliated with the National Environmental Research Center, Research Triangle Park, North Carolina.

The American Conference of Government Industrial Hygienists (ACGIH) guidelines indicate that a single TLV for gasoline and/or petroleum distillates is not applicable but that 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 which provide 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. Aksoy et al. (1972) also report an increase in the red blood cell osmotic fragility in humans subjected to long-term benzene exposure.

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

Increased	Decreased	Other
Lymphocytes	Hemoglobin	Bone Marrow:
Eosinophiles	Red Blood Cells	Erythroid Hyperplasia
Monocytes	White Blood Cells	and Maturation Arrest
Nucleated Red Blood Cells	Platelets	in Erythrocytic and
Reticulocytes	RBC Life Span	Granulocytic Series
Serum Bilirubin	Serum Alkaline Phosphatase	
Serum Lactic Dehydrogenase		

Several acute toxicity tests with JP-4 jet fuel were performed in this laboratory as preliminary to a chronic toxicity investigation. Single oral doses of JP-4 diluted in corn oil administered at 8000 mg/kg produced no deaths in rats. Although sporadic mouse deaths occurred, total mortality could not be achieved at the highest attainable dosage of 1000 mg/kg. A subsequent saturated vapor inhalation test of 6-hours duration to an estimated JP-4 concentration of 38 mg/liter resulted in poor coordination and convulsions in several of the rats, but no mortality.

Gas chromatographic analysis of liquid JP-4 indicated the measured concentration of benzene to be 0.3% by weight. Because of its variable composition, the average molecular weight of JP-4 is unknown. JP-4 concentrations, therefore, are expressed as a fraction of their total hydrocarbon content and measured as mg/liter. Utilizing a Beckman hydrocarbon analyzer, it was determined that the JP-4 vapor concentration which produced 25 ppm benzene contained 5.0 mg/liter total hydrocarbons.

The study, designed to properly assess the inhalation hazard associated with chronic exposure to JP-4 vapors, included four species of laboratory animals exposed to two total hydrocarbon concentrations of the fuel, 5.0 mg/liter (25 ppm benzene), and 2.5 mg/liter (12.5 ppm benzene). Additionally, a positive control group exposed to 25 ppm benzene and an air exposed control group were also maintained. All exposures were intermittent industrial-type exposures of 6 hours per day duration repeated for 5 days per week. The exposure conditions are outlined in Table 3. Test and control groups consisted 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 was housed in a separate Thomas Dome operated at 40 CFM airflow and 710 mm Hg pressure to avoid leakage of the fuel vapors.

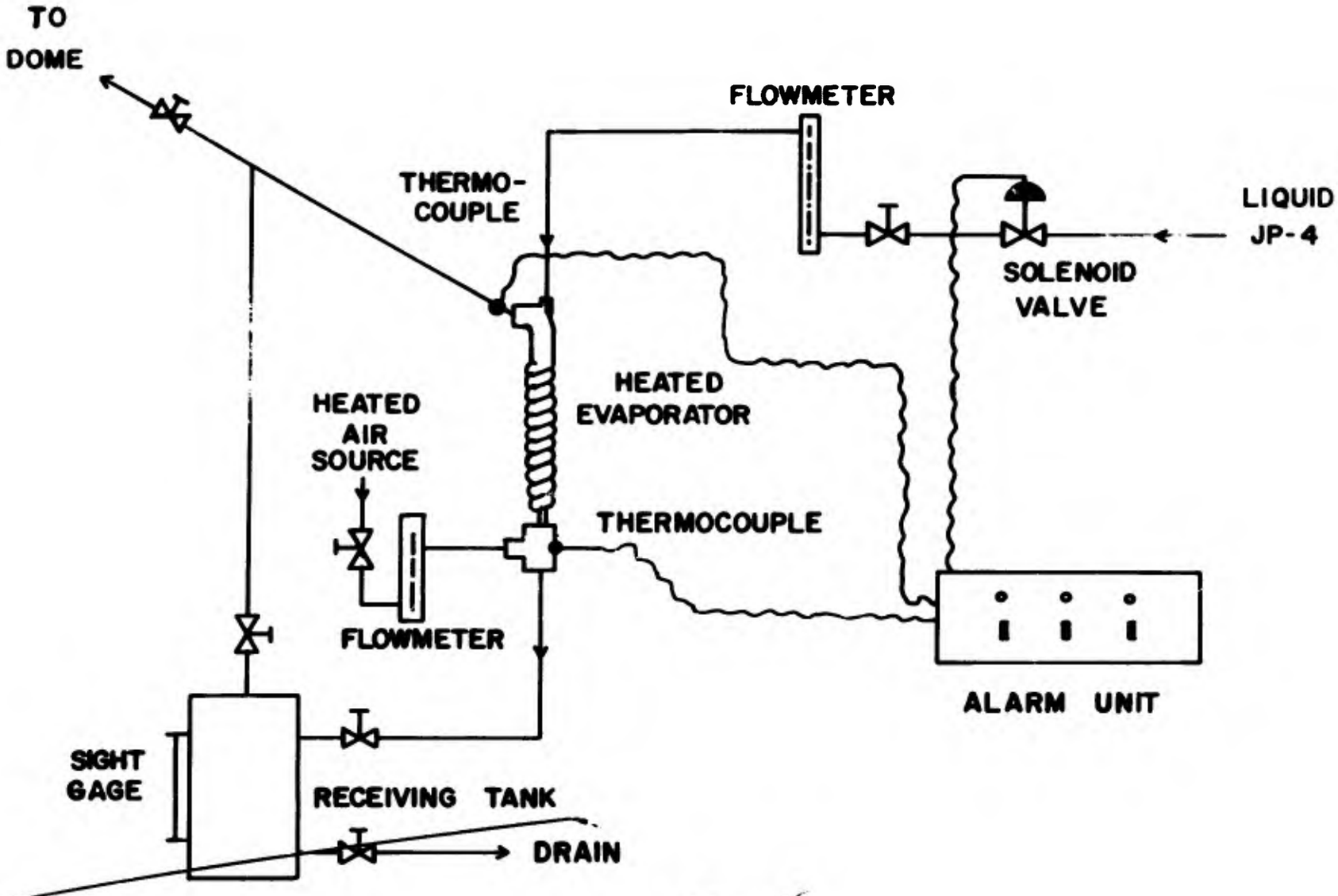
TABLE 3. EXPOSURE CONDITIONS

Dome	Exposure Level
1	UNEXPOSED CONTROLS
2	25 ppm BENZENE (POSITIVE CONTROL)
5	2.5 mg/liter TOTAL HYDROCARBON
6	5 mg/liter TOTAL HYDROCARBON

The JP-4 used in this study was supplied by the Air Force in 55 gallon steel barrels. All fuel received by our laboratory was representative of that found in actual use situations and conformed to the previously listed military specification.

The system used to introduce JP-4 vapors into Thomas Domes is shown in Figure 1. Liquid JP-4 was introduced under pressure from a 55 gallon supply drum. It passed through a glass flowmeter to a heated glass evaporator through which an air supply carried the JP-4 vapors to the exposure dome. Excess JP-4 not vaporized in the evaporator was routed to a receiving tank where it was collected. Thermocouples were placed at the top and bottom of the glass evaporator to sense any increase in temperature indicative of combustion and activate an alarm and solenoid valve system to cut off fuel supply.

Benzene generation for the positive control exposure was achieved simply by use of an infusion pump and glass syringe. Liquid benzene was metered through a "T" fitting to a copper line. Air flowing through the line vaporized the benzene which was then metered through a glass flowmeter to the dome.


TO
DOME
FLOWMETER
THERMO-
COUPLE
LIQUID
JP-4
SOLENOID
VALVE
HEATED
AIR
SOURCE
HEATED
EVAPORATOR
THERMOCOUPLE
FLOWMETER
ALARM UNIT
SIGHT
GAGE
RECEIVING TANK
DRAIN

F.1 48

Figure 1. Introduction system for JP-4.

Continuous analyses of JP-4 chember concentrations were achieved by pumping samples from the dome exhausts into total hydrocarbon analyzers calibrated using known concentrations of propane with the same detection sensitivity as JP-4 vapor. Quantitation of benzene content in JP-4 domes was by gas chromatographic analysis. Benzene vapors from the positive control dome were continuously analyzed using a total hydrocarbon analyzer calibrated with known benzene standards.

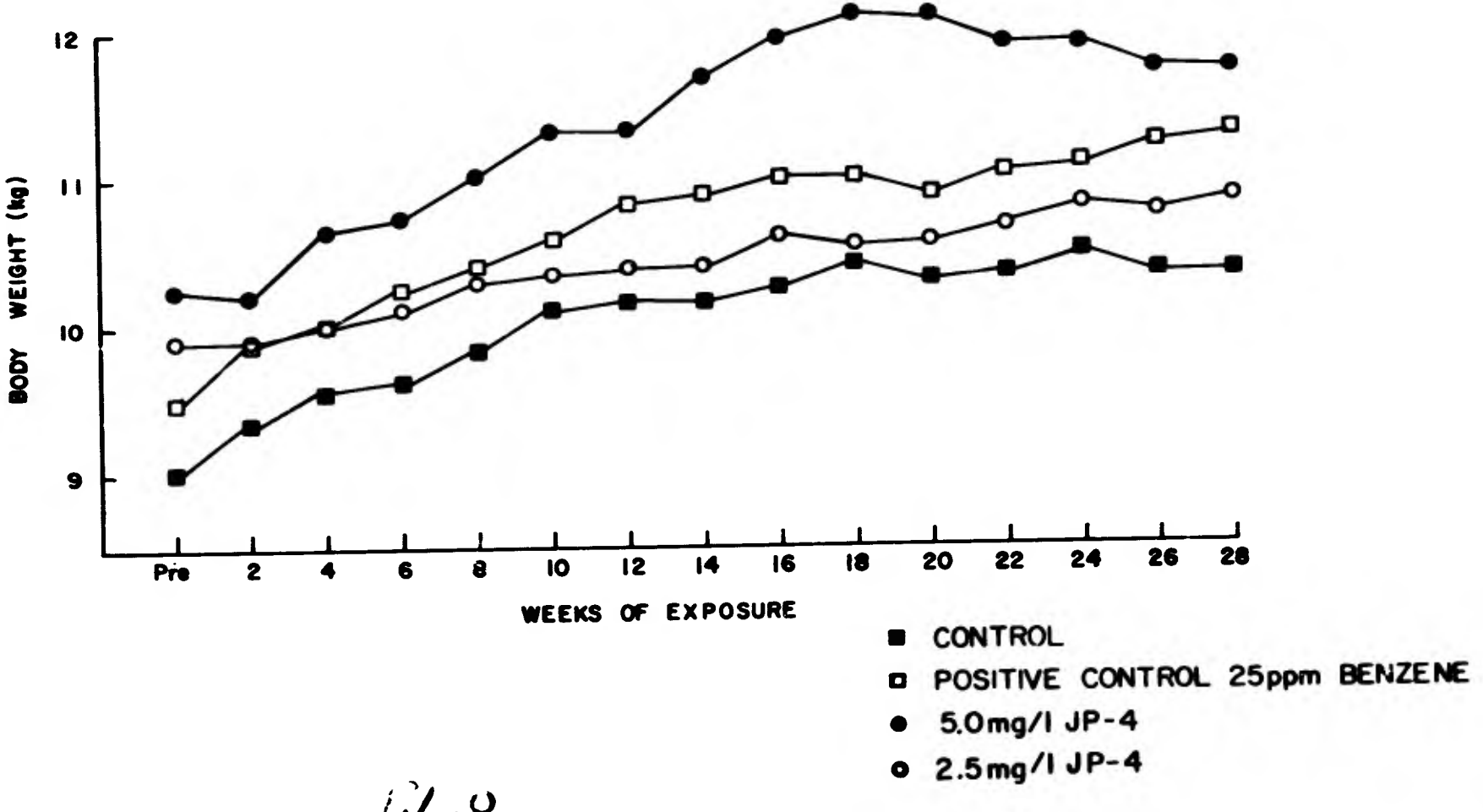
The parameters selected to measure the chronic toxicity of JP-4 vapors and effects of benzene at the industrial TLV included biweekly clinical hematology and chemistry tests on dogs and monkeys and biweekly body weights on dogs, monkeys, and rats. The dog and monkey clinical chemistry regimen utilized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

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

Hematology	Chemistry
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	

Activity depression in dogs during the initial three weeks of the exposure was one of the two toxic signs noted 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 high level 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 was not observed again throughout the remainder of the study.

There were no dog or monkey deaths in either the exposed or control groups throughout the exposure. There was one rat and two mouse deaths in both the 25 ppm benzene and the 5.0 mg/liter JP-4 exposed groups. The JP-4 exposed rat and one JP-4 exposed mouse died after 4 months of exposure. The benzene exposed 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/liter JP-4 exposed mouse that died after six months exposure indicated a left lung abcess, 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/liter JP-4 vapors.


BODY WEIGHT (kg)
12
11
10
9
Pre
2
4
6
8
10
12
14
16
18
20
22
24
26
28
WEEKS OF EXPOSURE
CONTROL
POSITIVE CONTROL 25ppm BENZENE
5.0mg/l JP-4
2.5mg/l JP-4

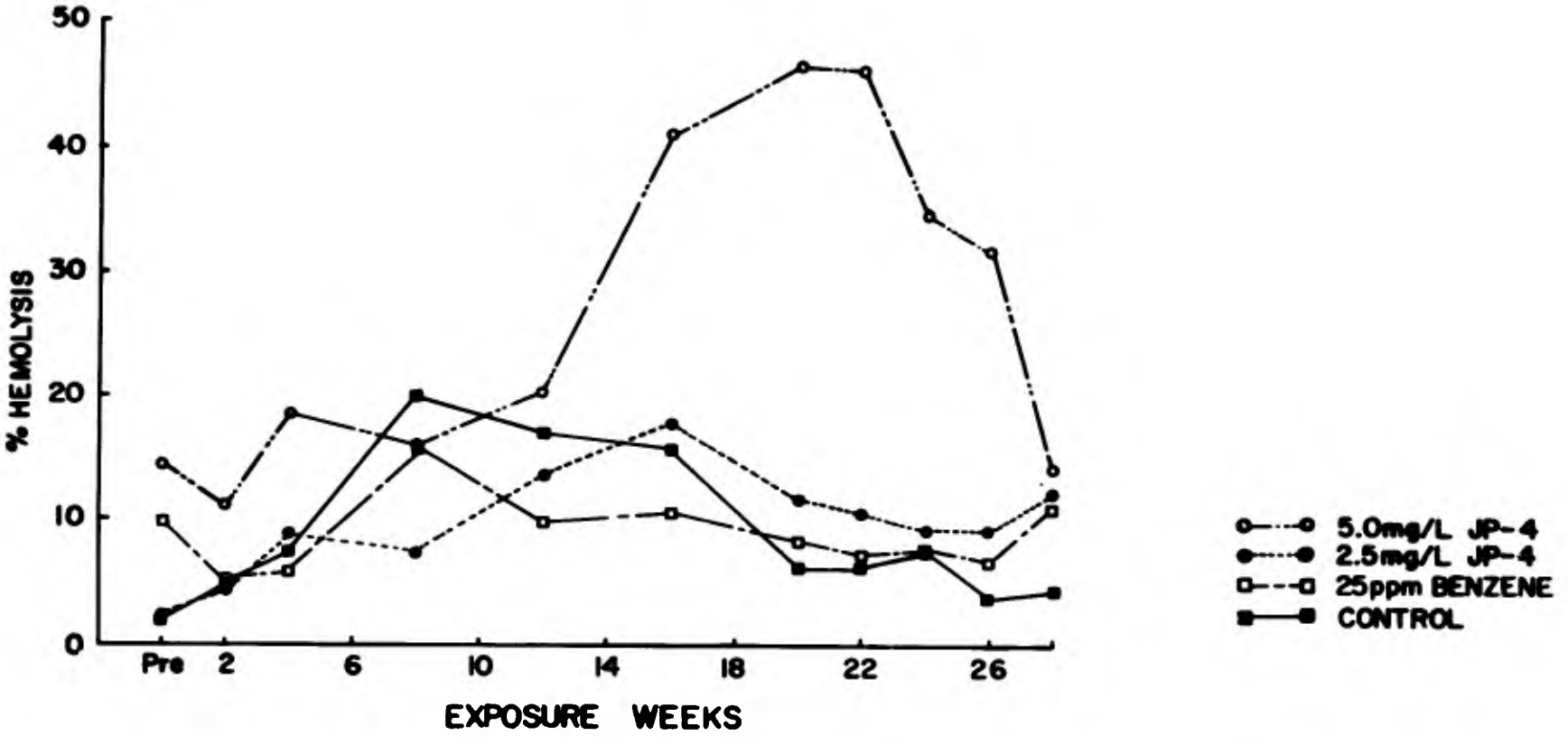
1210 1200

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/liter exposed dogs was observed and continued until the eighteenth week of exposure (Figure 2). There were no significant differences between the growth rates of any of the exposed monkeys when compared to their control group. Exposed rats demonstrated completely normal growth rates for all groups in the study.

Figure 2. Effect of exposure to JP-4 or benzene on dogs.

Variable sex dependent hematologic effects from chronic benzene exposure have been reported 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, however, 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 none of these differences was indicative of any trends that could 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.


50
40
30
20
10
0
% HEMOLYSIS
Pre
2
6
10
14
18
22
26
EXPOSURE WEEKS
5.0mg/L JP-4
2.5mg/L JP-4
25ppm BENZENE
CONTROL

R. . 1

7200

Biweekly RBC osmotic fragility testing was performed using a modification of the Davidsohn et al. (1969) method. 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/liter JP-4 was noted between the twelfth and twenty-second weeks of exposure (Figure 3). The fragility increase occurred between the 0.5 and 0.4% saline solutions used in the fragility assay. There was no evidence of hemolytic effects noted in any of the hematologic parameters tested at the same time the RBC fragility measurements were made Increased fragility in the 5.0 mg/liter JP-4 female dogs diminished after 22 weeks of exposure and returned to normal by 28 weeks of exposure.

Figure 3. Effect of 5.0 mg/liter JP-4 on ♀ dog RBC fragility at 0.45% saline concentration.

Beginning at 22 weeks of exposure, all beagles were sampled for RBC fragility measurements on a biweekly basis. Table 5 shows the average RBC fragilities for males in each of the three test and control groups, as measured at 0.45% saline concentration, from 22 weeks through 28 weeks exposure. Although the increase in fragility in the 5.0 mg/liter JP-4 female dogs reflected the greatest change from control, there may possibly have been an effect on the male beagles exposed to both concentrations of JP-4 vapor. The males exhibited an increase in fragility over control values; however, there does not appear to be a dose response relationship, i. e. increased fragility in 5.0 mg/liter JP-4 exposed male dogs was not greater than that measured in the 2.5 mg/liter JP-4 exposed male animals. Because data on the male RBC fragility values do not exist before 22 weeks of exposure, it is impossible to assess the fragility effects before this time.

TABLE 5. RBC FRAGILITIES FOR MALE BEAGLES AT 0.45% SALINE

	% Hemolysis			
	Control	25 ppm Benzene	5.0 mg/liter JP-4	2.5 mg/liter JP-4
22 Weeks	4.4	8.8	16.9	18.4
24 Weeks	6.3	12.6	13.7	19.8
26 Weeks	5.6	8.6	6.2	14.7
28 Weeks	4.5	6.9	3.7	9.6

Hematology measurements including hematocrit, hemoglobin, and RBC determinations, done on 5 rats sacrificed from each of the domes at 8 and 16 weeks, failed to show any statistically significant differences 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 any monkey values which could be attributed to exposure conditions. Dog serum glucose values, however, reflected statistically higher than controls values at 12, 16 and 18 weeks of exposure for the animals exposed to 5.0 mg/liter JP-4 vapors. This rise in serum glucose levels occurred concurrently with the apparent increase in growth rate manifested by the 5.0 mg/liter JP-4 dogs mentioned previously. 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 facilitate the initiation of additional blood studies which would aid in the elucidation of mechanisms accountable for the limited hematologic 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/liter JP-4 exposed dogs, bone marrow studies on iliac crest samples of control and 5.0 mg/liter JP-4 exposed dogs, and red blood cell density distribution determinations on all exposed and control dogs.

The red blood cell density distribution studies and methemoglobin measurements taken on all control and 5.0 mg/liter JP-4 exposed dogs at 26 weeks of exposure failed to demonstrate any difference in test values versus those of the controls. Also, the bone marrow taken from the iliac crest showed no differences between exposed and control values.

Exposure to JP-4 jet fuel vapors has produced increased RBC fragility in female dogs exposed to 5.0 mg/liter 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/liter JP-4 vapors or 25 ppm benzene at the same time periods. Although the increase in fragility does not appear to be a sex specific effect, occurring only in the females, there is a possibility that a similar but reduced effect was also occurring in the males. A conclusive argument for a sex specific effect is precluded by the lack of sufficient data samples for the RBC fragility information. It is our opinion that, although this effect was found in vitro, it apparently had no effect upon the erythrocyte function of the dogs in vivo.

The study was terminated after 33 weeks of exposure. With the exception of 20 rats, 20 mice, and 3 dogs per concentration group, all animals were sacrificed at this time. Gross pathological examination of all species revealed no lesions which could be attributed to exposure.

Organ and organ to body weight ratios for rats have been analyzed statistically and are shown in Table 6. Significant differences from control values were found in organ weights and organ to body weight ratios in the 5.0 mg/liter JP-4 rats. An increase in weight was found in the lung, liver, spleen, and kidney. Micropathological examination of these tissues failed to reveal any dose related effects which could be attributed to this increase in organ weights. I would like to state at this time that we received the pathology report only yesterday and have not had time to examine everything in detail.

TABLE 6. EFFECT OF EXPOSURE TO 5.0 MG/LITER JP-4 ON RAT ORGAN AND ORGAN/BODY WEIGHT RATIOS

	Control		JP-4, 5.0 mg/liter	
Organ	Weight	Ratio	Weight	Ratio
Lung	2.23	0.466	2.38	0.493*
Liver	14.31	2.98	15.75*	3.27**
Spleen	0.90	0.187	1.02*	0.213*
Kidney	3.36	0.700	3.76*	0.782**

*Significant at 0.05 level.
**Significant at 0.01 level.

What appears to be a chemical related effect is the finding of two pulmonary adenomas in 19 mice exposed to 5.0 mg/liter JP-4 vapor. In addition, a mouse was found to have lymphosarcoma in the lungs, spleen, and lymph nodes with metastases to other organs. This mouse was also from the 5.0 mg/liter JP-4 exposed group. No similar lesions were found in the mice from any of the other groups.

As mentioned previously, we have kept rats and mice postexposure from all levels. These animals will be watched carefully and examined at the time of death for pulmonary pathology.

Based on these data, it is suggested that workmen should not be allowed to inhale more than 2.5 mg/liter JP-4 vapors for extended periods of time, i.e., eight hours a day, 5 days per week. It must be emphasized that this standard recommendation is only an estimate based on the available experimental data and is subject to modification following a more detailed examination of the histopathological results.

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