AMRL-TR-71-65

Full as — AD-730291

EFFECT OF 90-DAY CONTINUOUS EXPOSURE TO METHYLISOBUTYLKETONE ON DOGS, MONKEYS AND RATS

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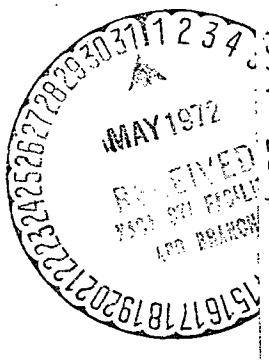
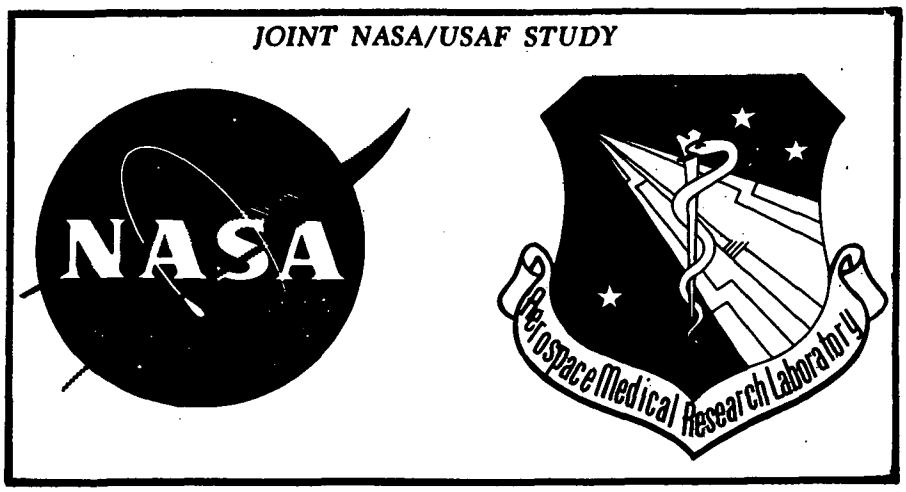


JUNE 1971

JOINT NASA/USAF STUDY



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

AIR FORCE: 20-8-71/100

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)	2a. REPORT SECURITY CLASSIFICATION
SysteMed Corporation Corporate Headquarters Suite 472 4500 Campus Drive, Newport Beach, Calif. 92660	UNCLASSIFIED
	2b. GROUP N/A

3. REPORT TITLE

EFFECT OF 90-DAY CONTINUOUS EXPOSURE TO METHYLISOBUTYLKETONE ON DOGS, MONKEYS AND RATS

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

Final Report, June 1970 - September 1970

5. AUTHOR(S) (First name, middle initial, last name)

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6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
June 1971	23	4

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
F33615-70-C-1046	SysteMed Corporation Report Number W-71003
b. PROJECT NO. 6302	
c. Task No. 630201	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d. Work Unit No. 630201008	AMRL-TR-71-65

10. DISTRIBUTION STATEMENT

Approved for public release; distribution unlimited

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	Aerospace Medical Research Laboratory, Aerospace Medical Division, Air Force Systems Command, Wright-Patterson AFB, OH 45433

13. ABSTRACT

Continuous exposure of rats, dogs and monkeys to 410 mg/M^3 methylisobutylketone vapor (MIBK) was conducted to evaluate the provisional spacecraft exposure limit of 20 ppm established by the Space Science Board, NAS/NRC in 1968. The exposure conducted in a simulated space cabin environment did not produce any measurable changes in dogs or monkeys. Rats developed hyaline droplet nephrosis within 2 weeks of exposure which was reversible upon removal from the MIBK even after 90 days. The data obtained indicated that the 60-minute emergency exposure limit of 100 ppm and the 90- and 1000-day provisional limits as established by the Space Science Board contain a wide margin of safety.

Key Words:

Methylisobutylketone
Toxicology
Space cabin limits
Dogs
Monkeys
Rats

DD FORM 1 NOV 65 1473

Security Classification

FOREWORD

This is one of a series of technical reports describing results of the experimental laboratory program being conducted in the Toxic Hazards Research Unit. This report is concerned with chronic inhalation toxicity of methylisobutylketone (MIBK), a solvent used in the manufacture of plastic and a common spacecraft contaminant. The research was sponsored by the National Aeronautics and Space Administration under Amendment No. 4 to NASA Purchase Request T-64130(G), funds applied to Air Force Contract F33615-70-C-1046. Work was performed by SysteMed Corporation personnel located at Wright-Patterson Air Force Base. K. C. Back, PhD, Chief of the Toxicology Branch, was the technical contract monitor for the Aerospace Medical Research Laboratory.

J. D. MacEwen, PhD, was the principal investigator for the SysteMed Corporation. Acknowledgement is made to P. M. Chikos, Capt., MC, for the encephalographic studies described and to R. L. Patrick, MD, of the Laboratory for Experimental Biology, St. Louis, Missouri for histopathological studies.

This report is designated as SysteMed Corporation Report No. W-71003.

This technical report has been reviewed and approved.

CLINTON L. HOLT, *Colonel, USAF, MC*
Commander
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SECTION I

INTRODUCTION

Methylisobutylketone (MIBK) is used as a solvent in a plastic formulation which, in the pliable manufactured end product form, is relatively noncombustible in an oxygen-rich environment. This quality in the finished plastic product makes it a highly useful material for space cabin equipment such as seat padding.

The manufactured product contains, entrapped in the plastic, some residual MIBK which will outgas under reduced pressure conditions and may appear as a contaminant in the spacecraft environment.

Industrial experience with MIBK has not shown any adverse physiological effects on man other than headache or nausea at or around the industrial Threshold Limit Value (TLV) of 100 ppm. Elkins (reference 1) reported that exposed workers developed some tolerance to MIBK during the working week but lost this tolerance over the weekend. Silverman et al. (reference 2) found that a 100-ppm exposure to MIBK was acceptable to 12 human volunteers for a 15-minute period but that 200 ppm was objectionable due to odor intensity. Because high air concentrations of MIBK have a narcotic action which would affect human performance, further information about prolonged or continuous exposure to this chemical was desired. Two-week range-finding experiments were conducted at 100 ppm and 200 ppm MIBK under continuous exposure conditions to establish criteria for a subsequent longer term study. This 90-day study was designed and performed to evaluate the continuous inhalation toxicity of MIBK under space cabin conditions.

SECTION II

MATERIALS AND METHODS

ANIMAL EXPOSURE FACILITIES

Animal exposure facilities (references 3 and 4) of the Aerospace Medical Research Laboratory were used for both the 2-week and the 90-day continuous experiments. The altitude chambers (Thomas Domes) are capable of operating at pressures between 5 psia (258 mm Hg) and 14.7 psia (760 mm Hg). They are equipped to operate with either 100% oxygen, ambient air, or mixtures of these gases over a range of 20% to 100% oxygen in the atmosphere of the chamber. The chambers are automated to control temperatures at 72 ± 5 F and relative humidity at 50 ± 10%. Gas flow through a chamber may be varied from 0 to 125 cfm. A flow rate of 40 cfm maintains the carbon dioxide concentration below 0.5% (usual range of 0.05-0.10%) when the chamber has a full load of animals.

EXPERIMENTAL CONDITIONS

The 2-week range-finding experiments were conducted in the altitude chambers operating in the ambient pressure mode. Air flow was maintained at 40 cfm and chamber temperature at 72 F. The absolute pressure was held at 725 mm Hg to seal the chamber and prevent contamination of the surrounding laboratory environment with MIBK vapor. After completion of the 2-week studies a 90-day continuous exposure study was performed at 260 mm Hg pressure, mixed-gas conditions. During the latter experiment, the gas flow was maintained at 40 cfm and temperature was held in the 72 ± 5 F range. The gas mixture used was 68% O_2 - 32% N_2 at 5 psia pressure.

CONTAMINANT GENERATION AND MONITORING OF CHAMBER CONCENTRATIONS

The MIBK used in these studies was purchased from the Matheson Company, Incorporated, East Rutherford, New Jersey. The liquid was introduced into an all glass vaporizing unit by means of a dual syringe pump from a large reservoir. Dry air flowing through the heated vaporizer carried the MIBK vapor through a flowmeter and metering valve system into the chamber air supply duct. The stainless steel tubing between the vaporizer and metering valve was heated to prevent recondensation of the MIBK. Heating was not necessary after dilution in the chamber air supply duct.

A gas chromatographic procedure was developed for contaminant monitoring on a semi-continuous basis. Air samples were taken from a position in the chamber just above the breathing zone of the dogs and continuously pumped to the analyzer system where an automatic sampling valve took samples every 5 minutes. The samples were introduced directly into the gas chromatograph sample inlet.

The MIBK in the gas sample was separated on a 10-inch column of Porapak Q operated at 190 C and detected with a flame ionization detector. The retention time of MIBK in this system was 1.5 minutes which allowed convenient sampling at 5 minute intervals. MIBK vapor calibration standards made up in Mylar® bags were used daily and a variation in detector response of $\pm$ 5% was found. The variation from one bag to another when run the same day was approximately 2%.

SECTION III

EXPERIMENTAL RESULTS

RESULTS OF RANGE-FINDING STUDIES

Rats, mice, dogs and monkeys were continuously exposed to a mean concentration of 100 ppm MIBK for 2 weeks at ambient conditions in the Thomas Domes. Test animals included 4 monkeys, 8 dogs, 40 mice, and 50 rats. As controls, 3 monkeys, 4 dogs, 20 mice, and 25 rats were placed in another Thomas Dome under the same conditions with the exception of contaminant. One monkey in each group had cortical electrodes implanted for evaluation of central nervous system (CNS) effects.

Test programs were designed to evaluate the inhalation effects of the MIBK exposure as outlined below:

Preexposure Tests

1. Body Weight
2. Clinical Chemistry
3. Hematology
4. EEG

During Exposure Tests

1. Spontaneous Activity Measurement
2. Symptomatology
3. Mortality Response

Postexposure Tests

1. Body Weight
2. Organ to Body Weight Ratios
3. EEG
4. Clinical Chemistry
5. Hematology
6. Pathology
7. Blood pH and Gases

There were no signs of toxic response during exposure. At the end of the 2-week exposure period there was no difference in cortical activity between the exposed and control monkey nor were any significant differences observed in hematologic or clinical chemistry measurements for either dogs or monkeys. Gross pathologic examination

of tissues from both exposed and control animals failed to reveal any apparent differences. Blood gas measurements made on dogs did not show any effects attributable to MIBK exposure. Tissue specimens were collected at necropsy and examined microscopically.

Organ weight and organ to body weight ratios were evaluated and the kidneys found significantly heavier in the rats exposed to MIBK (see table I).

TABLE I

Effect of 2-Week Exposure to Inhaled Methylisobutylketone (100 ppm) on Organ Weights of Albino Rats

	Heart	Lung	Liver	Spleen	Kidneys
			EXPOSED		
N	50	48	50	50	50
$\bar{x}$ Organ Wt.[1]	1.0	1.2	8.6	0.8	1.7**
$\bar{x}$ Ratios[2]	0.416	0.547	3.756	0.353	0.729**
			CONTROLS		
N	25	23	24	25	25
$\bar{x}$ Organ Wt.[1]	0.9	1.3	8.4	0.8	1.5
$\bar{x}$ Ratios[2]	0.417	0.569	3.753	0.346	0.670

1 grams
2 grams/100 grams body weight
** significantly different from controls at 0.01 level

Since the only preliminary indication of a toxic response to the 2-week inhalation exposure of 100 ppm MIBK was its effect on rat kidney weight and a slight indication of depressed growth in rats, a second 2-week exposure was conducted at an atmospheric concentration of 200 ppm MIBK, and the same biological measurements and examinations were performed.

The animals exposed to 200 ppm MIBK showed no outward toxic effects that could be attributed to the 2-week exposure. Again the only effect observed was on rats in which both the liver and kidney weights were statistically different from those in the control group as shown in table II.

TABLE II

Effect of 2-Week Exposure to Inhaled Methylisobutylketone (200 ppm) on Organ Weights of Albino Rats

EXPOSED

	Heart	Lung	Liver	Spleen	Kidneys
N	50	46	50	50	50
$\bar{x}$ Organ Wt.[1]	0.9	1.3	9.0**	0.8	1.8**
$\bar{x}$ Ratios[2]	0.357*	0.499	3.445**	0.291	0.694**

CONTROLS

N	50	42	50	50	50
$\bar{x}$ Organ Wt.[1]	0.9	1.3	8.2	0.8	1.5
$\bar{x}$ Ratios[2]	0.343	0.510	3.198	0.303	0.582

1 grams
2 grams/100 grams body weight
* significant at the 0.05 level only
** significant at the 0.01 level

From the data obtained in this experiment and observations made from the previous MIBK inhalation study, the kidney appeared to be the organ primarily affected by the exposure to MIBK. Physical properties of MIBK (bp 117 C) suggest that the kidney may be the major route of excretion. The persistent finding of significant changes in kidney weights and kidney to body weight ratios in the rat indicated that MIBK exposure caused kidney changes. Histopathologic examination of the rat kidney revealed toxic nephrosis in the proximal tubules of MIBK exposed rats at both exposure levels.

Based on these results, the 100 ppm MIBK exposure level was selected for the continuous 90-day study under simulated space cabin conditions.

90-DAY CONTINUOUS EXPOSURES

The experimental animal species selected for prolonged continuous exposure included dogs, rats and monkeys. All animals used were males. The numbers of animals used in the test and control groups were as follows:

Albino Rats	100	- Wistar Strain
Dogs	8	- Beagle Variety
Monkeys	2	- Macaca mulatta

Two additional monkeys were placed in each group for special studies by other laboratories. These monkeys were surgically prepared with exteriorized kidneys for repetitive punch biopsy. The biopsy material was prepared for electron microscopic studies and submitted to the Albany Medical Center at the request of the National Aeronautics and Space Administration (NASA). The results of these examinations were not available when this report was prepared.

Experimental Conditions

The test animals were exposed to 410 mg/M^3 MIBK vapor (100 millimole/25 M^3) for a period of 90 days in an altitude chamber operated at 5 psia pressure and a 68% O_2 - 32% N_2 gas mixture. The control group of animals was maintained in a separate altitude chamber, under identical environmental conditions, except that no MIBK was present.

Clinical Tests of Dogs and Monkeys

All dogs were examined biweekly, including the month prior to initiation of the experiment. At the time of each examination, the dogs were weighed and blood samples were taken for hematology and a battery of clinical chemistry tests.

Liver function tests (bromsulphalein [BSP] dye retention) were performed preexposure and immediately postexposure. Serum acid phosphatase and serum glucuronide determinations were done preexposure and at 30 and 60 days.

At termination, two dogs from each group were transferred to the postexposure holding room for 60 days to determine reversibility of effects should any lesions be found. The remaining six dogs in each group were killed, examined grossly, and samples of liver, brain, kidney, heart, lung, spleen, and endocrine glands were taken for histological evaluation.

Tests on Rats

Rats were weighed preexposure and biweekly during the exposure period to determine growth rate. Two rats from each group were necropsied at weekly intervals for 3 weeks and then at biweekly intervals thereafter. After 2 weeks exposure, 10 rats were removed from each chamber and necropsied in groups of 2 at biweekly intervals to determine reversibility of the kidney lesion as seen in the preliminary experiments. At

termination of the experiment, 10 rats from each group were removed and saved for serial sacrifice for reversibility studies, 10 were submitted to histopathology, and the remaining rats were necropsied and the visceral organs weighed for determination of organ to body weight ratios.

RESULTS OF LARGE ANIMAL EXPOSURES

Clinical Measurements

The results of clinical chemistry and hematology tests on dogs and monkeys did not reveal any biologically significant differences between the exposed animals and their controls. The biweekly mean values of these determinations are shown in table III for dogs and in table IV for monkeys.

Serum acid phosphatase levels determined on dogs and monkeys before exposure and at various intervals during exposure are shown in table V. Although some increase over baseline level was seen in the animals during exposure, the values were not different from those of control animals.

The results of serum glucuronidase activity measurements are shown in table VI. The difference between the exposed and control monkeys is relatively large but remains fairly constant throughout the experiment and is not related to the MIBK exposure.

Liver function tests were performed on dogs before and after 90-day exposure to MIBK. Bromsulphalein (4 mg/kg) was given intravenously and the dye concentration measured in blood 15 minutes later. There were no significant differences between the exposed and control animals as shown in table VII.

Histopathology:

Dogs

Tissue sections from heart, lung, brain, liver, spleen, kidney, adrenals and pituitary glands were examined in both exposed and control animals. No differences were observed between the two groups. Since the range-finding experiments had shown the kidney to be the target organ, the routine hematoxylin and eosin stained sections were supplemented with thin sections (1 μ) stained with toluidine blue, methylene blue and oil red-O. In the thin sections, hyaline droplets were noted in one exposed and in one control dog while the oil-red O showed fat

TABLE III

Clinical Laboratory Results on MIBK
Exposed and Control Dogs

Weeks Exposed	HCT (vol %)		HGB (grams %)		RBC (millions)	
	Exposed	Control	Exposed	Control	Exposed	Control
-1	45	44	15.3	14.8	6.8	6.8
1	43	46	14.6	14.9	6.4	6.5
3	47	44	15.6	15.5	6.6	5.9
5	44	43	15.2	14.9	6.7	6.5
7	47	45	15.6	14.7	6.8	6.5
9	47	48	15.5	16.0	5.3	7.1
11	47	46	15.5	14.7	6.8	6.7
13	47	47	15.4	15.4	5.7	5.8

Weeks Exposed	WBC (thousands)		Sodium (meq/liter)		Potassium (meq/liter)	
	Exposed	Control	Exposed	Control	Exposed	Control
-1	10.1	10.4	149	148	4.7	4.5
1	16.0	13.7	144	146	4.5	4.4
3	13.9	16.2	149	149	4.8	4.5
5	11.7	13.6	150	146	4.9	4.4
7	11.8	13.2	146	145	4.8	4.5
9	10.9	11.3	151	147	4.4	4.4
11	9.6	13.4	148	150	4.6	4.5
13	12.5	13.2	152	151	5.0	4.6

TABLE III -- CONTINUED

Weeks Exposed	Cholesterol (mg %)		Calcium (mg %)		Total Phosphorus (mg %)	
	Exposed	Control	Exposed	Control	Exposed	Control
-1	159	130	10.3	10.2	4.6	4.8
1	---	---	10.5	10.3	4.8	4.6
3	151	131	10.3	10.3	4.8	4.6
5	153	144	10.6	10.2	4.8	5.3
7	159	148	10.6	10.3	5.0	4.9
9	153	151	10.5	10.4	4.5	4.7
11	149	154	10.5	10.3	3.9	4.5
13	149	144	10.6	10.6	5.0	4.9

Weeks Exposed	Total Bilirubin (mg %)		Albumin (grams %)		Total Protein (grams %)	
	Exposed	Control	Exposed	Control	Exposed	Control
-1	0.2	0.3	3.6	3.6	5.8	5.9
1	0.2	0.2	3.6	3.8	6.1	6.4
3	0.3	0.3	3.5	3.4	6.2	6.1
5	0.2	0.2	3.6	3.6	6.2	6.2
7	0.2	0.2	3.6	3.7	6.2	6.2
9	0.2	0.2	3.6	3.7	6.3	6.4
11	0.3	0.3	3.7	3.8	6.3	6.3
13	0.1	0.1	3.8	3.6	6.3	6.4

TABLE III -- CONCLUDED

Weeks Exposed	Uric Acid (mg %)		BUN (mg %)		Glucose (mg %)	
	Exposed	Control	Exposed	Control	Exposed	Control
-1	0.7	0.6	13	13	103	104
1	0.7	0.6	14	12	86	91
3	0.6	0.7	12	12	96	96
5	0.5	0.5	13	13	104	101
7	0.6	0.6	12	14	102	106
9	0.4	0.4	11	14	101	114
11	0.5	0.6	13	13	101	101
13	0.4	0.4	10	12	98	108

Weeks Exposed	Alkaline Phosphatase (KA units)		Creatinine (mg %)		Chloride (meq/liter)	
	Exposed	Control	Exposed	Control	Exposed	Control
-1	22	14	0.8	0.8	115	114
1	22	19	0.6	0.7	115	115
3	13	14	0.6	0.6	116	112
5	13	12	0.7	0.6	114	114
7	10	10	0.7	0.8	112	112
9	10	10	0.6	0.7	118	117
11	11	11	0.7	0.7	117	116
13	11	12	0.6	0.6	116	112

TABLE IV

Clinical Laboratory Results on MIBK
Exposed and Control Monkeys

Weeks Exposed	HCT (vol %)		HGB (grams %)		RBC (millions)	
	Exposed	Control	Exposed	Control	Exposed	Control
-1	39	37	12.1	12.2	5.4	5.4
1	40	41	12.0	12.2	5.4	5.4
3	40	36	12.3	12.5	5.2	5.4
5	35	39	11.4	12.1	5.0	5.3
7	38	38	11.8	11.9	4.9	5.4
9	36	38	11.4	12.3	4.7	5.3
11	36	39	11.3	11.4	4.9	5.4
13	37	40	11.1	12.0	4.8	5.0

Weeks Exposed	WBC (thousands)		Sodium (meq/liter)		Potassium (meq/liter)	
	Exposed	Control	Exposed	Control	Exposed	Control
-1	9.4	8.6	151	151	4.3	3.9
1	6.8	5.2	148	150	4.2	4.6
3	8.0	4.8	153	151	4.5	4.1
5	7.2	4.8	149	146	4.3	4.1
7	5.8	5.4	150	148	5.1	4.5
9	6.2	5.1	152	151	4.5	4.5
11	5.6	5.1	147	154	4.6	3.8
13	6.7	5.3	155	151	4.8	4.4

TABLE IV -- CONTINUED

Weeks Exposed	Cholesterol (mg %)		Calcium (mg %)		Total Phosphorus (mg %)	
	Exposed	Control	Exposed	Control	Exposed	Control
-1	198	168	10.9	10.8	5.9	4.2
1	---	---	10.8	11.0	7.6	7.5
3	225	188	10.4	10.6	8.1	6.8
5	210	205	10.2	10.6	7.7	7.4
7	198	188	10.6	10.4	8.4	8.0
9	218	195	10.2	10.8	7.4	6.4
11	188	215	10.2	10.6	6.1	7.6
13	208	188	10.8	10.7	7.0	6.0

Weeks Exposed	Total Bilirubin (mg %)		Albumin (grams %)		Total Protein (grams %)	
	Exposed	Control	Exposed	Control	Exposed	Control
-1	0.2	0.2	4.7	4.7	7.9	7.6
1	0.3	0.4	5.1	5.1	8.1	7.9
3	0.3	0.4	5.0	5.1	8.1	7.9
5	0.4	0.3	4.7	5.1	7.8	7.8
7	0.3	0.4	4.8	5.0	7.7	7.6
9	0.3	0.4	5.1	5.0	7.8	7.7
11	0.3	0.4	5.1	5.2	7.5	7.7
13	0.1	0.1	5.3	5.0	8.0	7.7

TABLE IV -- CONCLUDED

Weeks Exposed	Uric Acid (mg %)		BUN (mg %)		Glucose (mg %)	
	Exposed	Control	Exposed	Control	Exposed	Control
-1	0.5	0.5	22	18	90	98
1	0.6	0.5	15	17	85	65
3	0.6	0.5	17	18	93	80
5	0.4	0.5	20	17	95	80
7	0.5	0.4	18	22	80	75
9	0.3	0.4	18	14	85	83
11	0.3	0.4	18	22	70	78
13	0.4	0.3	16	13	78	88

Weeks Exposed	Alkaline Phosphatase (KA units)		Creatinine (mg %)		Chloride (meq/liter)	
	Exposed	Control	Exposed	Control	Exposed	Control
-1	64	47	1.2	1.3	115	116
1	53	50	0.9	1.1	113	110
3	50	44	0.9	1.0	112	112
5	54	45	1.3	1.4	111	110
7	54	49	1.2	1.3	110	110
9	56	45	1.1	1.2	115	116
11	51	48	1.1	1.2	108	111
13	47	45	1.0	0.9	114	111

TABLE V

Serum Acid Phosphatase Levels* in Animals Continuously Exposed to 410 mg/M^3 MIBK

DOGS

	Baseline	30 Days	60 Days
Controls (8)			
Total	5.4 (3.1-8.2)	5.0 (3.8-6.5)	5.8 (4.2-8.3)
Prostatic	2.1 (1.3-2.7)	2.2 (1.4-3.2)	2.3 (1.9-3.1)
Residual	3.4 (1.8-5.5)	2.9 (2.0-3.8)	3.4 (1.6-5.3)
Exposed (8)			
Total	4.4 (3.6-5.1)	5.3 (3.7-9.0)	5.3 (4.1-6.9)
Prostatic	1.9 (1.5-2.3)	2.2 (1.7-3.0)	2.3 (1.8-3.0)
Residual	2.5 (1.8-3.1)	3.2 (1.4-6.6)	2.9 (2.0-4.5)

MONKEYS

Controls (2)			
Total	20.8 (20.5, 21.2)	20.9 (19.2, 22.7)	20.2 (19.5, 21.0)
Prostatic	5.5 (5.4, 5.6)	5.5 (3.9, 7.2)	5.3 (5.0, 5.7)
Residual	15.3 (14.9, 15.8)	15.4 (15.3, 15.5)	15.0 (14.5, 15.3)
Exposed (2)			
Total	16.1 (15.6, 16.6)	19.4 (17.8, 21.0)	19.6 (16.2, 23.0)
Prostatic	4.4 (3.7, 5.2)	5.5 (5.4, 5.7)	5.6 (5.3, 5.9)
Residual	11.7 (11.4, 11.9)	13.8 (12.4, 25.3)	14.0 (10.9, 17.1)

*The enzyme activity is measured in International Units (uM p-nitrophenol formed per minute per liter serum @ 37 C, pH 4.8).

TABLE VI

Serum Glucuronidase Activity* in Animals Continuously Exposed to 410 mg/M^3 MIBK

DOGS

	Baseline	30 Days	60 Days	90 Days
Controls (8)	18.8 (12.8-27.7)	20.3 (10.6-29.2)	18.7 (13.1-24.6)	18.9 (13.0-25.5)
Exposed (8)	18.6 (13.8-23.9)	18.2 (11.5-26.6)	17.7 (12.5-22.6)	15.1 (11.6-18.6)

MONKEYS

Controls (2)	2.9 (2.5, 3.3)	4.9 (5.3, 5.4)	4.5 (3.8, 5.1)	4.6 (3.8, 5.3)
Exposed (2)	8.0 (7.6, 8.3)	10.1 (8.6, 11.5)	8.9 (6.8, 11.0)	8.5 (6.8, 10.1)

*Units - Micrograms of phenolphthalein liberated per hour per ml of serum at 38 C, pH 4.5.

TABLE VII

Results of BSP Liver Function Tests on Animals Continuously Exposed to 410 mg/M^3 MIBK for 90 Days

	Percent Dye Retention	
Control Dog Number	0 Day	90 Day
M-48	6.5	4.5
M-64	8.0	8.0
N-10	9.0	5.0
M-76	5.5	10.0
N-08	8.5	0.5
N-06	8.0	10.0
M-42	8.5	5.5
M-14	10.0	11.5
Exposed Dog Number		
N-14	11.0	11.0
M-66	8.0	7.5
M-70	7.0	7.0
M-44	11.0	12.5
M-26	8.0	3.2
M-34	7.0	6.0
M-30	7.5	7.0
M-32	8.5	8.0

scattered in a few tubules at the corticomedullary junction in exposed dogs. The latter finding is commonly seen in untreated dogs. The methylene blue stains showed no differences between the exposed and control animals.

Monkeys

The two monkeys in each group without translocated kidneys were examined histologically. The same tissues as examined in dogs were screened with H and E stains. One of the two exposed monkeys exhibited focal chronic inflammation of the kidney. No other pathological changes were noted.

RESULTS OF RAT EXPOSURES

Growth

The growth rates (measured biweekly) of both the exposed and control groups of rats are shown in figure 1. There was no significant effect upon the growth rate as a result of continuous exposure to MIBK for 90 days.

Organ Weight

The effect of exposure to MIBK on albino rat organ weights and organ to body weight ratios is shown in table VIII. There is a statistically significant difference in the liver and kidney weights of the exposed animals with a corresponding increase in organ to body weight ratios for these tissues.

Histopathology

No significant pathologic changes were seen in any of the organs examined from control rats. All exposed rats, however, showed hyaline droplet degeneration of the proximal tubules with occasional foci of tubular necrosis upon completion of the 90-day exposure to 410 mg MIBK/M^3. Rats removed from exposure after 15, 22, 28, 71 and 85 days also showed the same changes in kidney tubules. Thus, as few as 15 days of exposure to the experimental condition produced some pathologic change in kidney tissue. A trend toward a linear progression of hyaline droplet degeneration over the time period of exposure was observed, although individual animals did not all follow this pattern. Also of note, hyaline droplets tended to be larger with time, possibly as a result of coalescence of the smaller droplets.

Figure 1

Growth Rate of Albino Rats Continuously
Exposed to MIBK for 90 Days

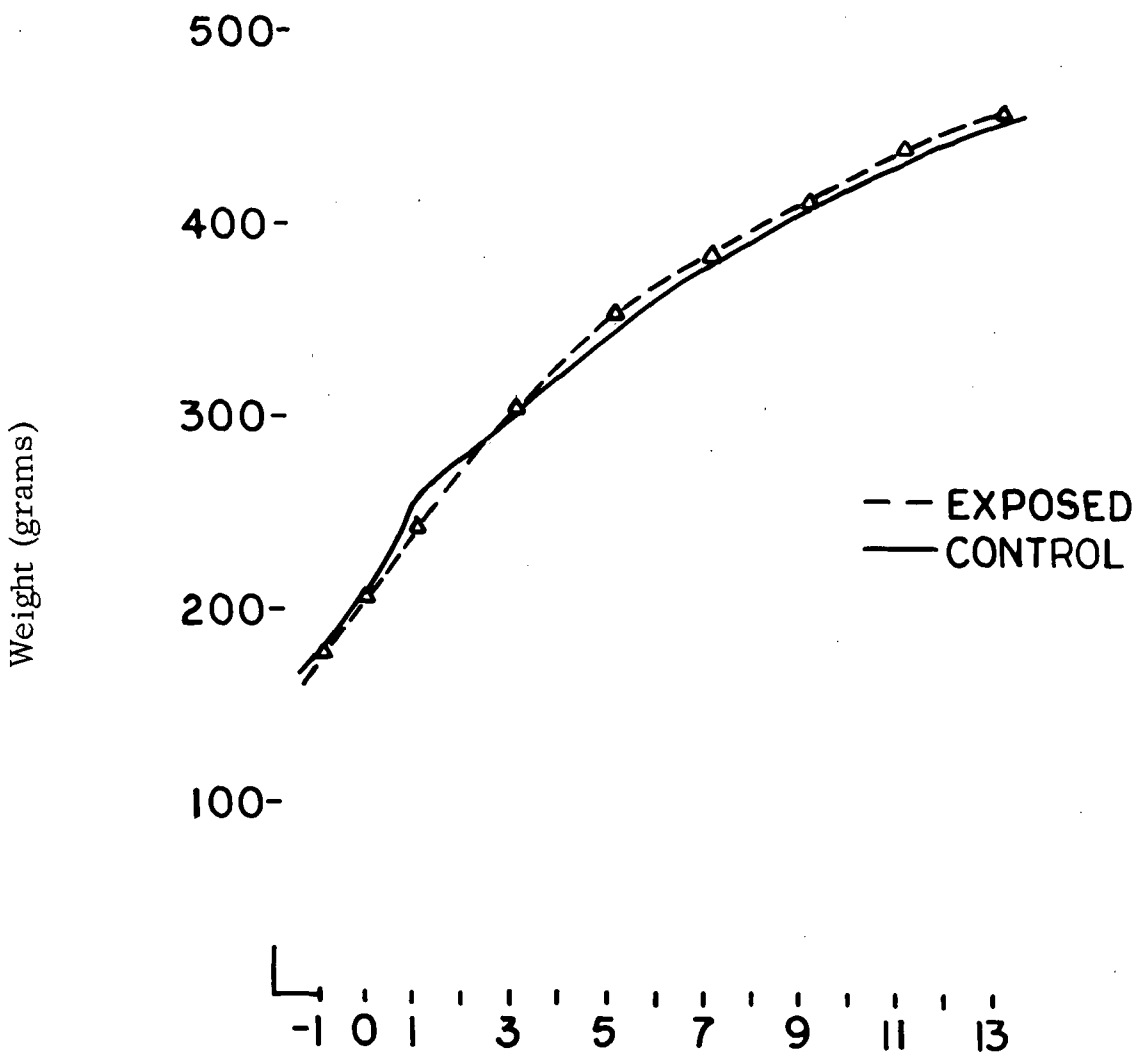

TABLE VIII

Effect of 90-Day Continuous Exposure to MIBK on Organ and Organ to Body Weight Ratios in Albino Rats

	Organ Weight (grams)		Ratios (gram/100 grams body weight)	
	Test	Control	Test	Control
	N = 56	N = 55	N = 56	N = 56
Heart	1.3	1.3	0.302	0.306
Lung	1.5	1.5	0.352	0.359
Liver	10.8**	9.9	2.477**	2.305
Spleen	0.7	0.7	0.159	0.160
Kidney	3.1**	2.6	0.713**	0.604

** significant at the 0.01 level

Although rat liver weights and liver to body weight ratios showed a statistically significant increase in exposed animals, there were no pathologic changes associated with this increase.

Rats removed for reversibility studies after 15-day exposure to MIBK revealed a gradual reversion of kidney tubular damage with time which appeared to be completely reversed in those rats held for 60 days postexposure. The rats retained and serially killed for reversibility studies after 90-day exposure also exhibited recovery from the MIBK induced lesion but not as rapidly as those exposed for a shorter period.

SECTION IV

CONCLUSIONS

Animals exposed continuously to 410 mg/M^3 MIBK for 90 days under space cabin environmental conditions were not adversely affected, with the exception of albino rats. Hyaline droplet tubular nephrosis developed in albino rats under the experimental conditions used but did not result in debilitation or death. The lesions developed within 2 weeks of exposure and were reversible upon removal from the MIBK environment even after 90-day exposure. Based on the results of these experiments in space cabin environments, concentrations of MIBK up to 410 mg/M^3 (100 ppm equivalent) should be tolerable for man for the time period investigated. Furthermore, these data indicate that the 60-minute emergency limit of 100 ppm and the 90- and 1000-day provisional limit of 20 ppm as established by the Space Science Board, NAS/NRC, in 1968 contain a wide margin of safety.

REFERENCES

1. Elkins, H. B., The Chemistry of Industrial Toxicology, 2nd ed. Wiley, New York, 1959.

2. Silverman, L., H. Schulte and M. First, "Further Studies on Sensory Response to Certain Industrial Solvent Vapors," J. Ind. Hyg. Toxicol., 28, 262, 1946.

3. MacEwen, J. D., Toxic Hazards Research Unit Design and Construction Phase, AMRL-TR-65-125, Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio, September, 1965, AD 624473.

4. Thomas, A. A., "Low Ambient Pressure Environments and Toxicity," A. M. A. Arch. Environ. Health, 11, 316, 1968.

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