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PROGRESS REPORT

EVALUATION OF PERFORMANCE IMPAIRMENT BY SPACECRAFT CONTAMINANTS

(Period Covered July 7, 1976 - February 28, 1977)

Prepared For:

National Aeronautics and Space Administration

Lyndon B. Johnson Space Center

Houston, Texas 77050

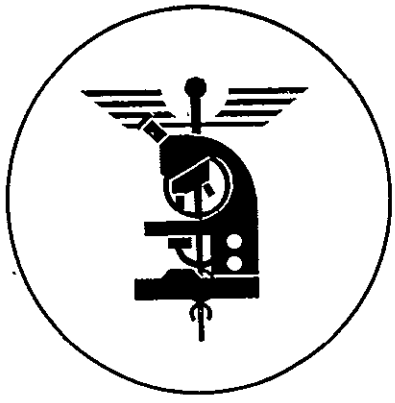
Contract No. NAS 9-14743

Prepared By:

Irving Geller

Roy J. Hartmann, Jr.

Victor M. Mendez



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This research was concerned with the evaluation of environmental contaminants (isolated as off-gases in Skylab and Apollo missions). Specifically, six contaminants, selected by the Project Monitor and the Principal Investigator, were evaluated for their effects on the behavior of juvenile baboons. The concentrations of contaminants, used in the baboon studies, were determined through preliminary range-finding studies with laboratory rats. The contaminants evaluated were acetone, methyl ethyl ketone (MEK), methyl isobutyl ketone (MIBK), trichloroethylene (TCE), heptane and Freon 21. When the studies of the individual gases were completed, the baboons were also exposed to a mixture of MEK and TCE.

The data obtained in these studies revealed alterations in the behavior of baboons exposed to relatively low levels of the contaminants. These findings were presented at the First International Symposium on Voluntary Inhalation of Industrial Solvents in Mexico City, June 21-24, 1976. Proceedings of these meetings will be published. A preprint of the publication is enclosed as an addendum to this report.

In addition to the behavior work conducted at Southwest Foundation, the contractor has provided personnel to establish an in-house behavioral-testing capability at NASA. Dr. Dane Russo, on TDY from SFRE, has set up and programmed operant conditioning equipment furnished by Johnson Space Center. He is training personnel in the use of the equipment and the conduct of the experiments. Using operant training procedures, he has established baseline behaviors in laboratory rats and is currently evaluating single and combined doses of amphetamine and scopolamine, drugs which have been used by astronauts on previous space missions.

MATERIALS AND METHODS

A. Rat Studies.

Two large stainless steel and glass chambers, provided by the National Aeronautics and Space Administration (NASA), were used for the preliminary range-finding studies with rats (Figure 1). A Scientific Prototype Skinner box, designed specifically for gas exposure experiments was kept in each NASA chamber. Both behavioral training of rodents and testing during contaminant exposures were conducted in the Skinner boxes.

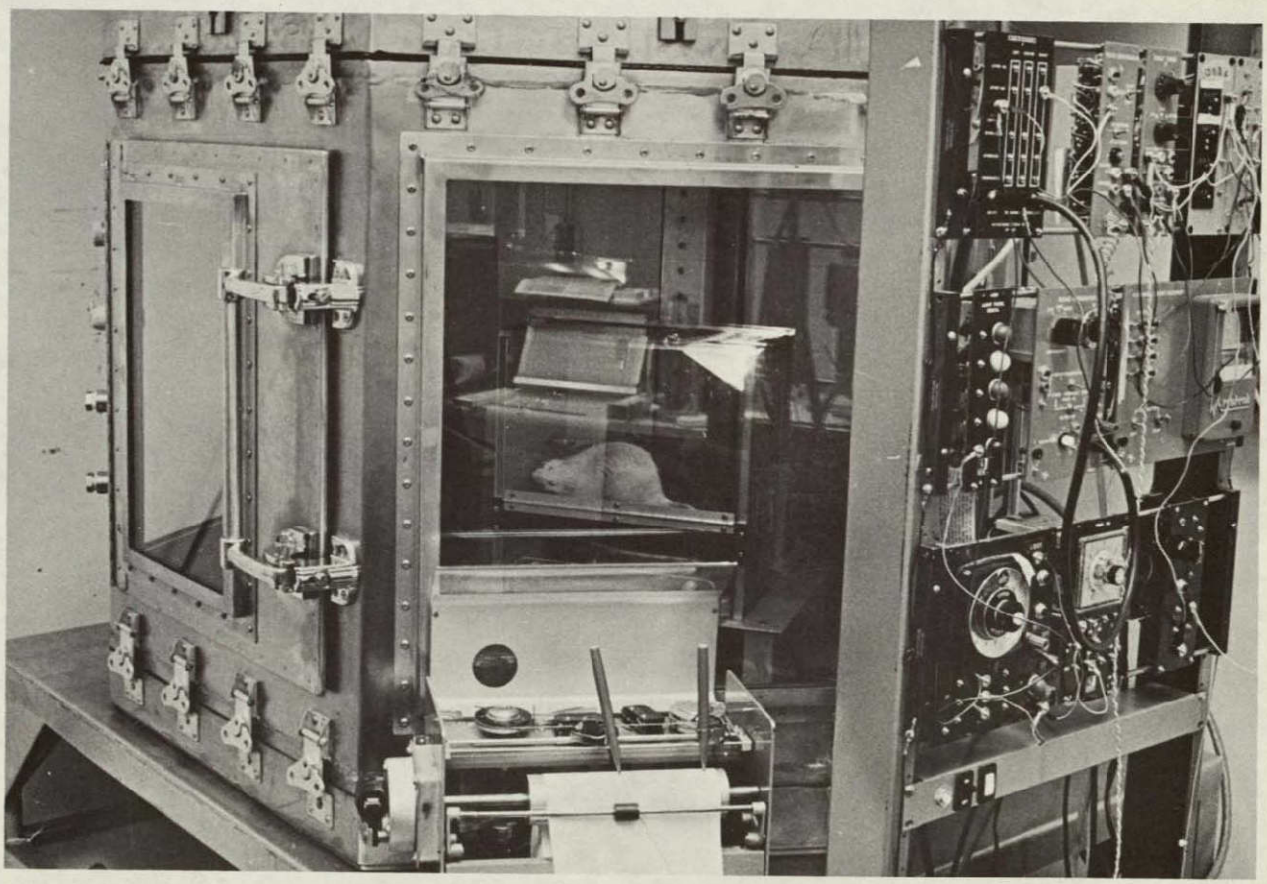

Figure 1. NASA Rat Exposure Chamber.

The subjects were Holtzman, Sprague-Dawley male rats approximately 90-120 days old at the start of the experiment. The rats were gradually reduced to 80% of their normal body weight and then trained to press a lever for a liquid food reward on a 2-minute variable-interval schedule of reinforcement. On this procedure, rats were made hungry and first trained to press a lever in order to obtain food on a continuous reinforcement basis (crf); that is to say that every lever response activates the feeder to produce a food reward. The schedule is then changed to a 2-minute variable-interval schedule (2-min VI) in which rewards are obtainable at random intervals on the average of once every two minutes.

Behavior typically obtained on this schedule is a steady output of lever responses with relatively little variation from day-to-day. The behavior is extremely sensitive to drug effects insofar as changes in response rate may be demonstrated with a dose at which behavioral changes may not be evident through direct observation of an animal.

When the behavioral baselines for the VI rats became relatively stable, preliminary range-finding studies were conducted to determine concentrations of contaminants for use in the baboon discrimination studies.

B. Baboon Studies.

For these studies, two large stainless steel exposure chambers provided with an air lock were used to expose the trained animals to the gases. Each of the two identical chambers housed two trained animals in home cages. Animals in one chamber were exposed chronically over a 7-day period to a contaminant at the concentration determined in the preliminary range-finding studies with rats. Animals in the other chamber served as controls and were exposed to clean air during the same time period. Thus, not only did we have other animals as controls, but

each animal was able to serve as its own control, in that exposure data could be compared with data obtained pre- and post-exposure. The match-to-sample task was conducted during a 5-day period on Monday to Friday. Data obtained under exposure conditions were compared with data obtained on the same days under pre-exposure control conditions.

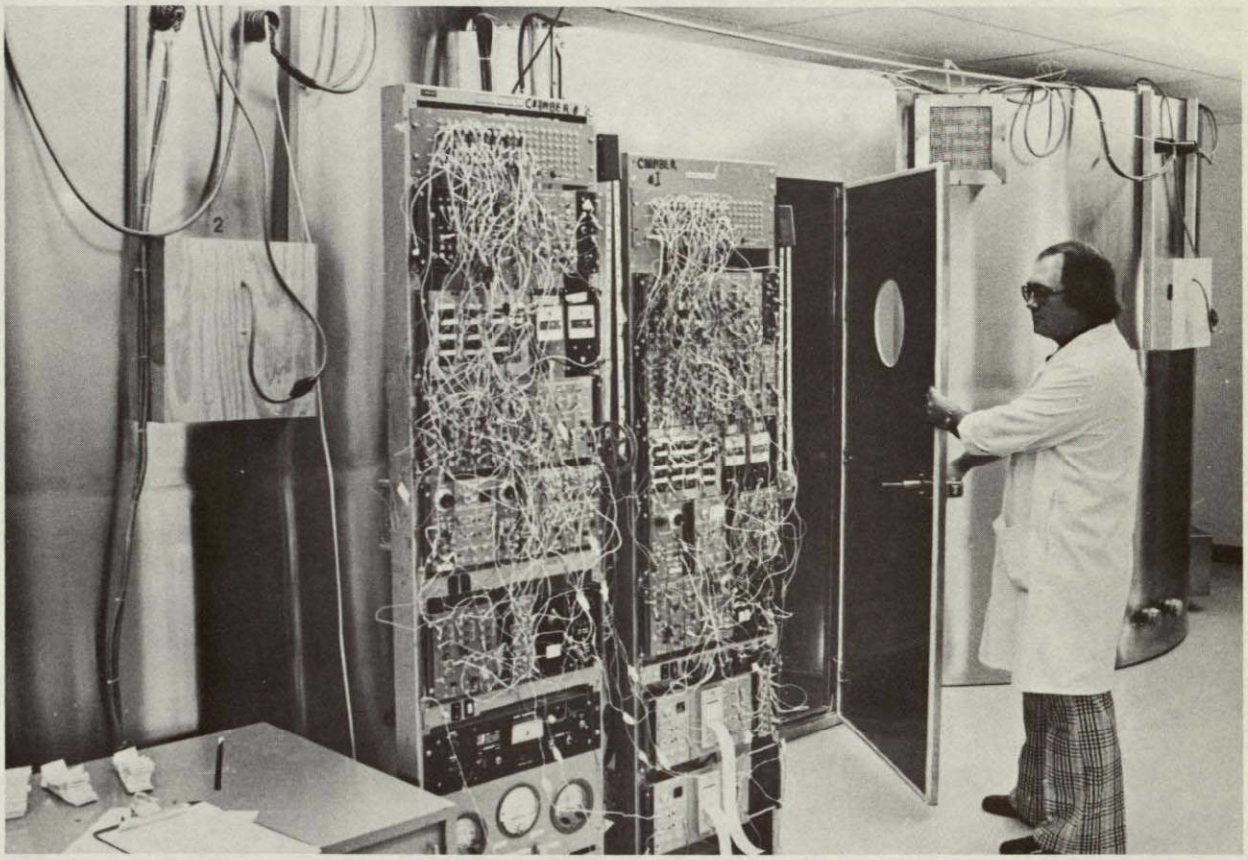

Figure 2. Baboon Exposure Chambers with Air Lock.

Figure 2 shows the exposure chambers which are approximately 9 feet high and 9 feet in diameter. The young baboons lived in cages within the exposure chambers. The cages were designed so that an intelligence panel could be slipped down between

the outside wall of the cage and the baboon. The panel was instrumented with a row of three, round, transluscent discs in one wall. Under the appropriate experimental conditions, pressing either end disc produced a food reward in the form of a pellet.

The lowest effective dose of the contaminant as determined by preliminary range-finding studies with rats was used as the starting dose for chronic 7-day exposure studies in juvenile baboons. The baboons were trained on an operant behavior procedure which measured perceptual acuity and discrimination performance. This has been referred to as a match-to-sample task. Activation of the session timer set a 2-minute variable-interval (VI) programming tape in motion. The tape programmed the occurrence of center stimuli on the average of once every two minutes. The VI tape was inoperative during each trial which began with the illumination of one of the stimuli on the center key (probe stimulus). This stimulus was terminated at the end of a 30-second period or by a response on the key. Termination of the stimulus activated a timer for 120 seconds (delay interval). At the end of the delay interval, stimuli appeared on either key adjacent to the center key. The correct matching stimulus was varied between these two keys in a mixed order. A response on the correct key (stimulus matches center key stimulus) terminated the stimuli, activated the feeder, and produced a banana pellet reward. Responses on the incorrect key simply terminated the stimuli and again set the VI tape in motion.

Daily experimental sessions of two hours duration were conducted for each animal on Monday through Friday of each week. Readings were taken every 15 minutes during the 2-hour experimental session. Table 1 is a reproduction of the format used in recording the data for each session. A record was kept of: the number of probe stimuli presented during each 15 minute segment, the number of

correct matching responses on the left and right keys, and the number of incorrect responses on these keys. A record was also kept of any extra responses that might occur on the three keys when the stimuli were not activated (pre-neutral responses) or during the delay interval (post-neutral responses). The time it took the subject to respond with a key press after a stimulus was activated was also measured (reaction time).

TABLE 1: FORMAT USED FOR RECORDING DATA FROM CHRONIC EXPOSURE STUDIES

PROCEDURE: Matching to Sample Discrimination

Date _____ Page _____ Subject _____

Ctr. Sample								
Ctr. Response								
Total Correct								
Total Wrong								
Correct Right								
Wrong Right								
Correct Left								
Wrong Left								
Pre-Neut. Left								
Pre-Neut. Ctr.								
Pre-Neut. Right								
Post-Neut. Left								
Post-Neut. Ctr.								
Post-Neut. Right								
RTM Ctr.								
Mean Time								
RTM Lt. & Rt.								
Mean Time								
% Resp. Ctr. Samp.	%	%	%	%	%	%	%	%
% Resp. Correct	%	%	%	%	%	%	%	%
% Resp. Wrong	%	%	%	%	%	%	%	%
% Resp. Cor. Rt.	%	%	%	%	%	%	%	%
% Resp. Wrng. Rt.	%	%	%	%	%	%	%	%
% Resp. Cor. Lt.	%	%	%	%	%	%	%	%
% Resp. Wrng. Lt.	%	%	%	%	%	%	%	%
Readings Taken								

C. Exposures and Monitoring of Gases.

The method of generation of atmospheres of organic volatiles was dependent on the physical state of the compounds at room temperature. For liquids, the vapor saturation technique (1) was used. A different method was used for the production of atmospheres of Freon 21 (a gas at room temperature). The basic components used in the vapor saturation method are shown in Figure 3 below.

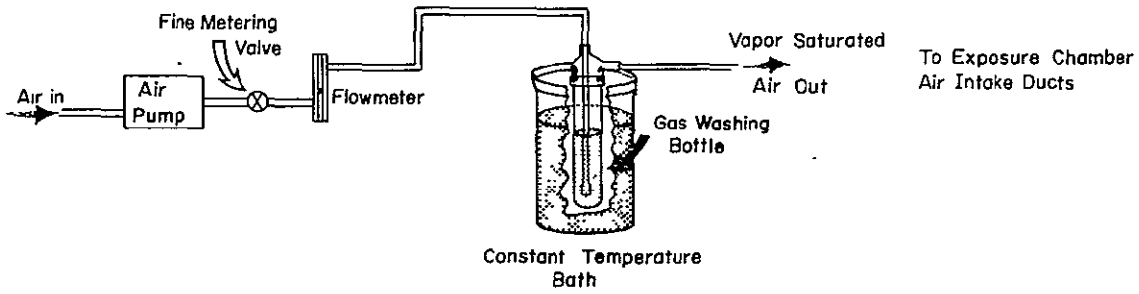

Figure 3. Schematic Diagram of Vapor Saturation Method.

In this method, air is merely bubbled through a gas washing bottle containing the liquid to be vaporized. In passing through the liquid, the air becomes saturated with vapor which is then directed to the air intake ducts of the exposure chamber. Changing the flowrate, by means of the fine metering valve, or changing the temperature of the constant temperature bath allows a large range of pollutant concentrations to be produced in the exposure chamber. The technique is simple and works well for liquids with relatively low boiling points.

For Freon 21, a slightly different procedure was employed. In this case, the container of Freon 21 was placed in the constant temperature bath. At a temperature of 30°C, a pressure of 20 PSI was obtained within the container. A valve and a flowmeter were inserted in the line connecting the container of Freon 21 to the air intake ducts of the exposure chambers. As in the vapor saturation method, the concentration within the chamber was controlled by changing the flow-rate of gas to the chamber or changing the temperature of the constant temperature bath. Satisfactory results were obtained using this method.

The methods described above worked quite well for short-term exposures (up to six hours) in the small chambers used for obtaining range-finding data. However, the limited volume of the gas washing bottles presented a problem when the vapor saturation method was attempted on long-term (7-9 days) exposures in the large chambers used for baboon exposures. The use of two systems using larger gas washing bottles (500 ml) alleviated the problem somewhat, but for the exposures requiring the higher concentrations of volatile organics, frequent refilling of the bottles was necessary. Since the concentration was maintained 24 hrs a day, frequent trips at odd hours were required to insure that an adequate supply of liquid was maintained in the gas washing bottles. A simple system was developed to overcome this problem. The approach was to add a reservoir having a very large volume from which liquid could be siphoned to replenish the amount of pollutant being vaporized in the gas washing bottle. The reservoir was a common 1 gallon reagent bottle with a cap modified to accept a septum. The top of the gas washing bottle was similarly modified. Two lengths of 1/8" Teflon tubing were then inserted through holes drilled in the septa connecting the reservoir and the gas washing bottle. One tube, inserted deeply into both the gas washing bottle and the solvent reservoir was used as the siphon. The other

tube, terminated a short distance past the septa, was used to equalize the pressure in both containers. As the liquid vaporized from the vapor saturator, a new supply of fresh liquid was automatically siphoned from the reservoir. Placing the reservoir on a lab jack allowed the level of liquid in the gas washing bottle to be adjusted. As the level of liquid in the vapor saturator dropped, the jack was raised, thereby causing liquid to flow from the reservoir to the vapor saturator until the level of liquid in both containers was the same. The entire contents of the reservoir were transferred, as needed, to the vapor saturator using this method. In practice, this modification allowed unattended operation of the vapor saturation method for the production of pollutant atmospheres for periods of up to 16 hours.

Flame ionization gas chromatography was used to monitor the concentration of atmospheres or organic volatiles used in animal exposure studies. For the range-finding studies, a Varian Aerograph, Model 400D instrument was used. The atmospheres used in the baboon exposure studies were monitored using an especially designed monitoring system utilizing a Hewlett-Packard, Model 5720A gas chromatograph as the main component.

For the short-term exposures, concentrations of volatile organic vapors were determined by a direct comparison of air samples from the exposure chamber with standard samples. The standard samples were prepared in the following manner. Using the ideal gas laws, the amount of substance necessary to produce a desired concentration in a container whose volume had been accurately determined was calculated. The proper amount was injected into the container by means of a Hamilton μℓ syringe. After sufficient time was allowed for complete vaporization of the liquid, the syringe needle was withdrawn from the septum. At least two such standards were prepared on the day prior to and again on the day of an animal exposure

experiment. Enough standards were prepared to obtain a linear relationship between peak height and concentration. The concentration of chamber samples was determined by comparison of their peak height with those of the standard samples. Standard samples were injected during exposure experiments.

For the large chambers, because of the special requirements of long-term, 24 hour per day exposures, a more elaborate system for monitoring was employed. The main component of the system, as has already been mentioned, was a Hewlett-Packard gas chromatograph which was modified to accept a Valco SSA-V6-HPA six port gas sampling valve. The valve was activated by an air-operated valve actuator controlled by a Valco VSP 300 programmer. The programmer allowed the selection of three time intervals necessary for proper sampling and subsequent recording of exposure concentration data. During the first period, an air sampling pump was turned on, withdrawing a sample of chamber air from the center of the chamber, filling the 3 ml sampling loop attached to the gas sampling valve. The recorder chart drive was also turned on during this period. At the end of the first period and the beginning of the next period, the programmer activated the valve actuator injecting the 3 ml sample of chamber air onto the gas chromatographic column. The length of this period was selected so as to allow sufficient time for recording of the GC peak obtained. At the end of the second period the gas sampling valve was returned to the sampling position and the recorder chart drive was turned off. At the end of this period, the entire cycle was repeated. Using this system completely unattended, sampling and recording of gas chromatographic data proportional to the concentration of organic volatiles in the exposure chamber was possible during non-working hours. The concentration in parts per million (ppm) was determined by comparison with standard samples.

Mention should be made of the safeguards present which insured prompt attentio

should the concentration within the chamber exceed set limits. This was especially important during non-working hours. An electronic sensing device, designed and built by the Department of Mechanical Sciences, Southwest Research Institute, monitored the output of the gas chromatograph. The device was used in conjunction with the VSP 300 programmer so that the sensing device was in operation only during the recording time interval. The device compared the maximum voltage output of the gas chromatograph during this period with two standard voltages corresponding to the upper and lower limits of pollutant concentration desired. If either the lower limit was not met or the upper limit exceeded, the device triggered an automatic telephone dialer which played a prerecorded message to SFRE security personnel, who in turn contacted technically competent personnel who determined the cause of the problem and took action to rectify the situation.

Using the methods described, concentrations of the various volatile organic solvents studied were maintained at levels which varied less than $\pm 10\%$ of the desired value throughout the exposure periods for the majority of the experiments. Typical examples for short-term exposures are shown in Figure 4 and for long-term exposures, in Figure 5.

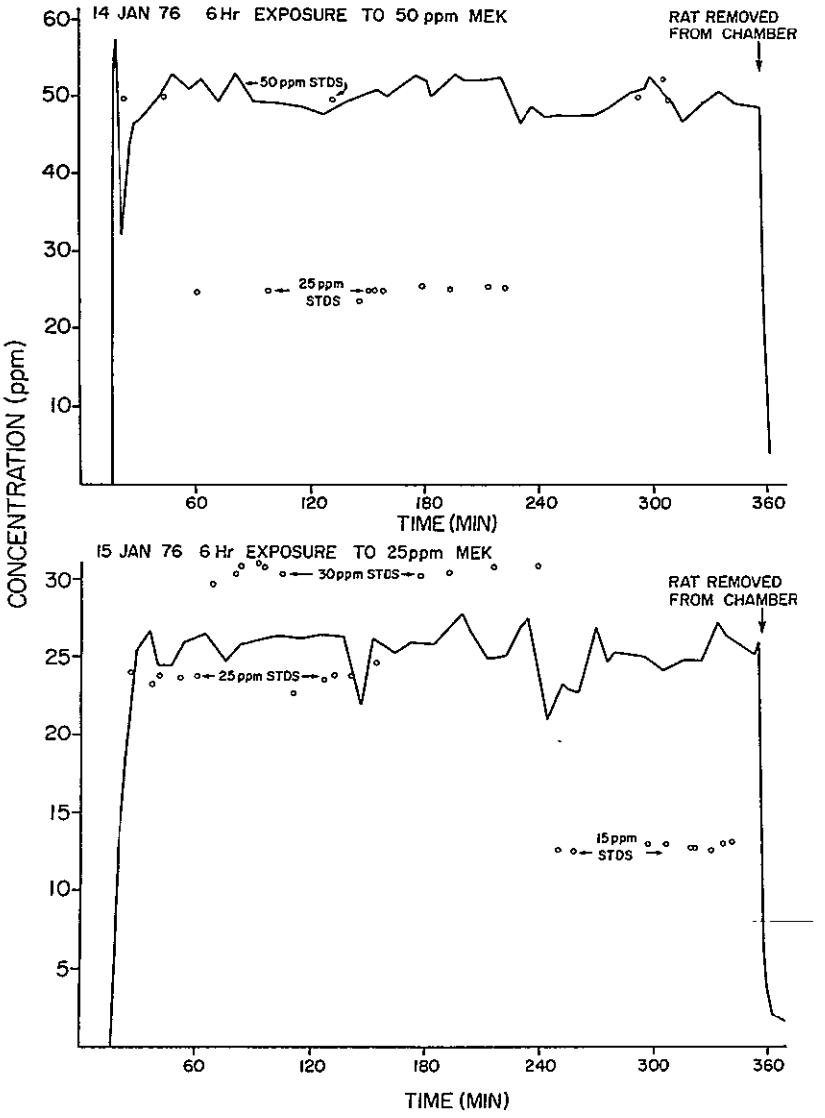

Figure 4. Gas Chromatograph Data for Short-Term (6 hr) Exposure.

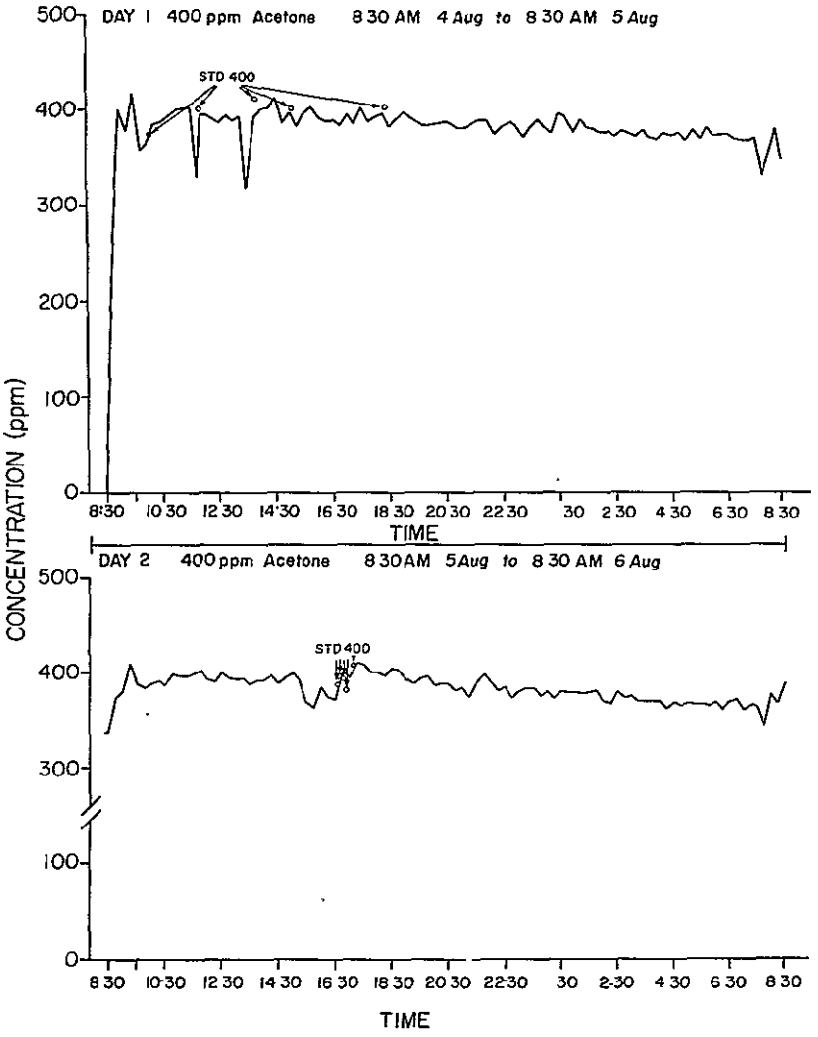

Figure 5. Gas Chromatographic Data for Long-Term (24 hr) Exposure.

RESULTS

MEK - RATS

Figure 6 contains cumulative records which illustrate the effects of 25 ppm MEK in a single rat. These records represent the 3rd hour of an experimental

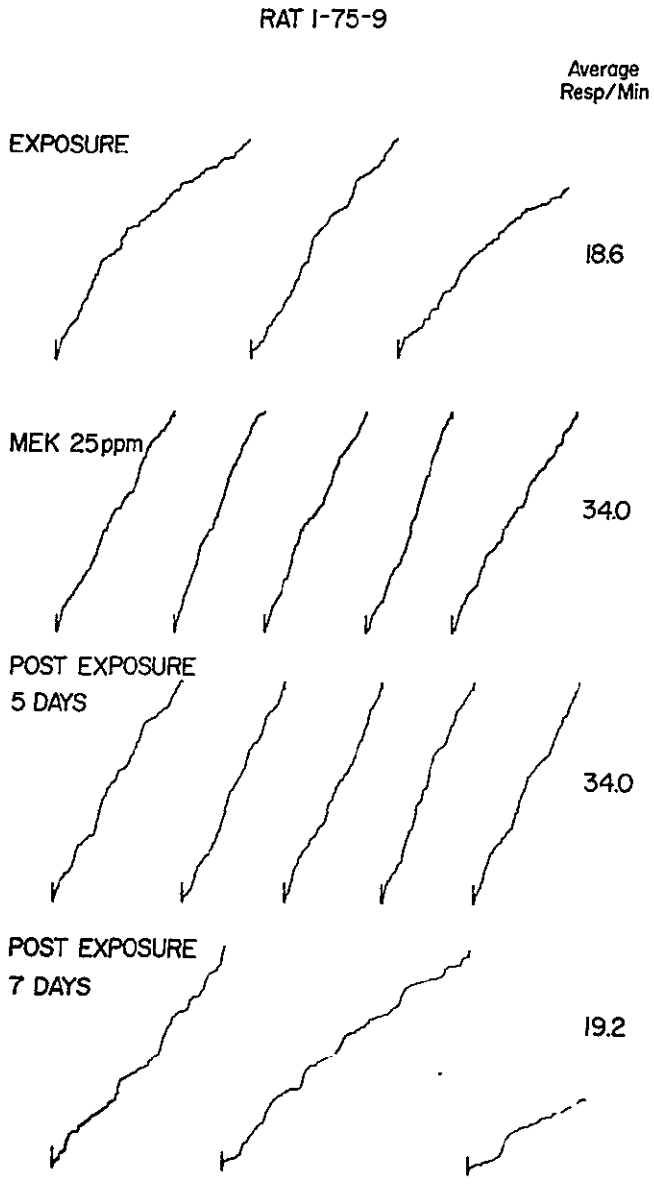

Figure 6. Effects of 25 ppm Methyl Ethyl Ketone (MEK) on Variable-Interval Rate of Rat 1-75-9.

session. Lever responding increased almost two-fold under MEK. The average response rate of 18.6 responses/minute during control increased to 34 responses/minute under MEK. Five days post-exposure, average response rate was still 34 responses/minute, while seven days post-exposure, the response rate was 19.2 responses/minute or almost back to the pre-exposure control level. It is of interest to note that toxicity studies of MEK required doses of 500-3500 ppm to produce narcosis in chickens, cats or rats (2).

Similar data for the other rats are shown in Table 2. The pre-exposure sessions preceded the MEK sessions by two days. Variable-interval response rates increased in all animals exposed to 25 ppm MEK during a 6-hour period. The effect was greatest for rat 11 where the average response rate increased from 12.17 per minute to 61.1 responses/minute under MEK. For this animal, the VI response rate remained high for eleven days post-exposure. On the sixteenth post-exposure day, the rat decreased to below the pre-exposure control level. For rats 4, 9 and 10, average response rates approximated pre-exposure control levels on the second, seventh and sixth post-exposure sessions, respectively.

TABLE 2: EFFECT OF 25 PPM METHYL ETHYL KETONE (MEK) ON VARIABLE-INTERVAL RESPONSE RATES DURING SIX HOUR EXPERIMENTAL SESSIONS

Average Responses/Minute

Rats	Pre-Exposure	MEK	Days Post-Exposure						
			2	3	5	6	7	11	16
4	18.04	25.45	18.71						
9	18.60	34.14			34.43		19.18		
10	13.07	18.33		18.47		13.82			
11	12.17	61.1			17.47		27.96	31.96	8.49

MEK - BABOONS

Chronic 7-day exposures conducted with MEK at 20 and 40 ppm revealed no impairment of the discrimination task at either of these concentrations. However, a change was observed in the extra responses made by baboon 382 during the delay interval as well as in the animal's reaction time.

In Figure 7 are shown the average responses per minute during the delay interval for baboons 382 and 529. Each bar represents extra responses averaged for five, 2-hour sessions. A rather dramatic reduction in extra responses occurred during the delay interval when baboon 382 was exposed to MEK at 20 ppm.

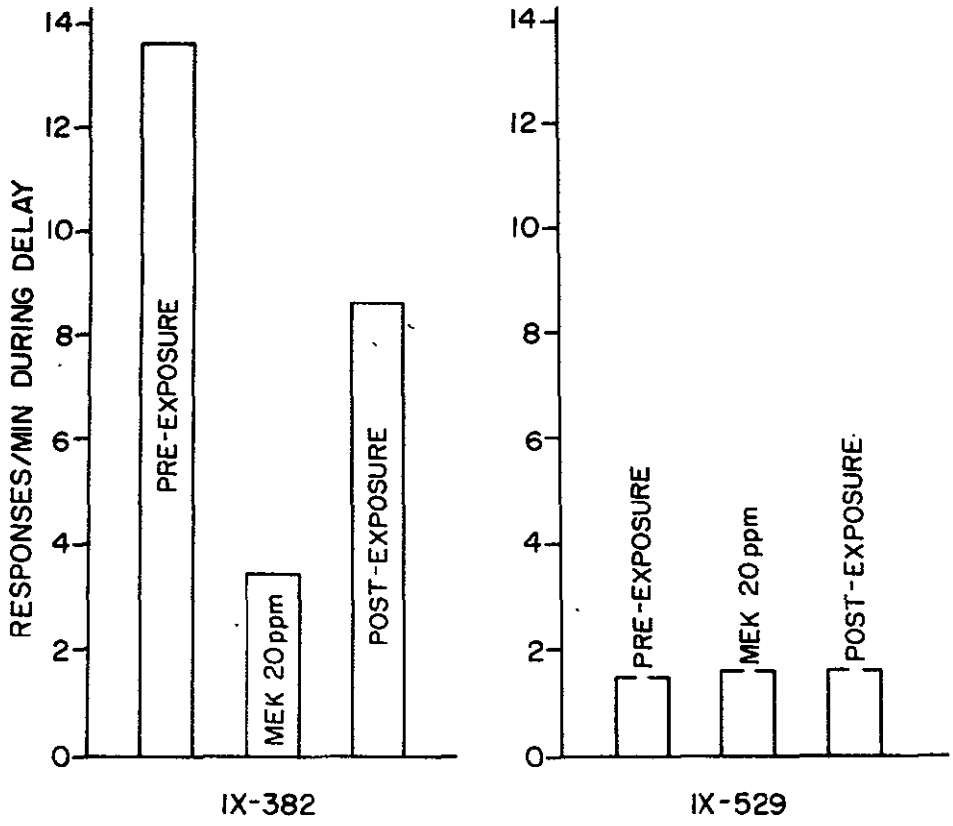

Figure 7. Average Responses/Minute During Delay Intervals for Baboons 382 and 529. (Each bar represents extra responses averaged for five, 2-hr sessions.)

No change in extra responses was observed for baboon 529 who was relatively calm and made few extra responses during the pre-and post-exposure control periods. The reduction of extra responses for 382 might suggest a reduction of "anxiety" level for this animal.

Prior to MEK this animal found it difficult to refrain from responding during the 2-minute delay interval and made many extra responses. In a previous study with rats in a simple discrimination paradigm, we reported a reduction of extra responses and a concomitant improvement of performance following administration of anti-anxiety agents (3). These findings lend support to the speculation that the effects of MEK on 382 may reflect, in part, reduced "anxiety".

Table 3 shows that mean reaction times on the left and right response keys increased for all animals under MEK. The effect was significant for baboon 382 ($P<.05$ - Student t test).

TABLE 3: EFFECTS OF METHYL ETHYL KETONE (MEK) ON REACTION TIME IN THE BABOON

		Average Reaction Time in Seconds		
Baboon	MEK PPM	Control	Exposure	
382	20	2.438	3.305	$P < .05$
529	20	1.873	1.914	N.S.
380	40	1.383	1.666	N.S.
531	40	1.383	1.675	N.S.

MIBK - RATS

Figure 8 contains cumulative records which illustrate the effect of 25 ppm MIBK on the variable-interval response rate of a rat. Each of these records represents the 3rd hour of the experimental session. The average response rate under MIBK was 45.2/min, a 58% increase over the pre-exposure control of 26.5. Seven days post-exposure, the response rate had not returned to control levels.

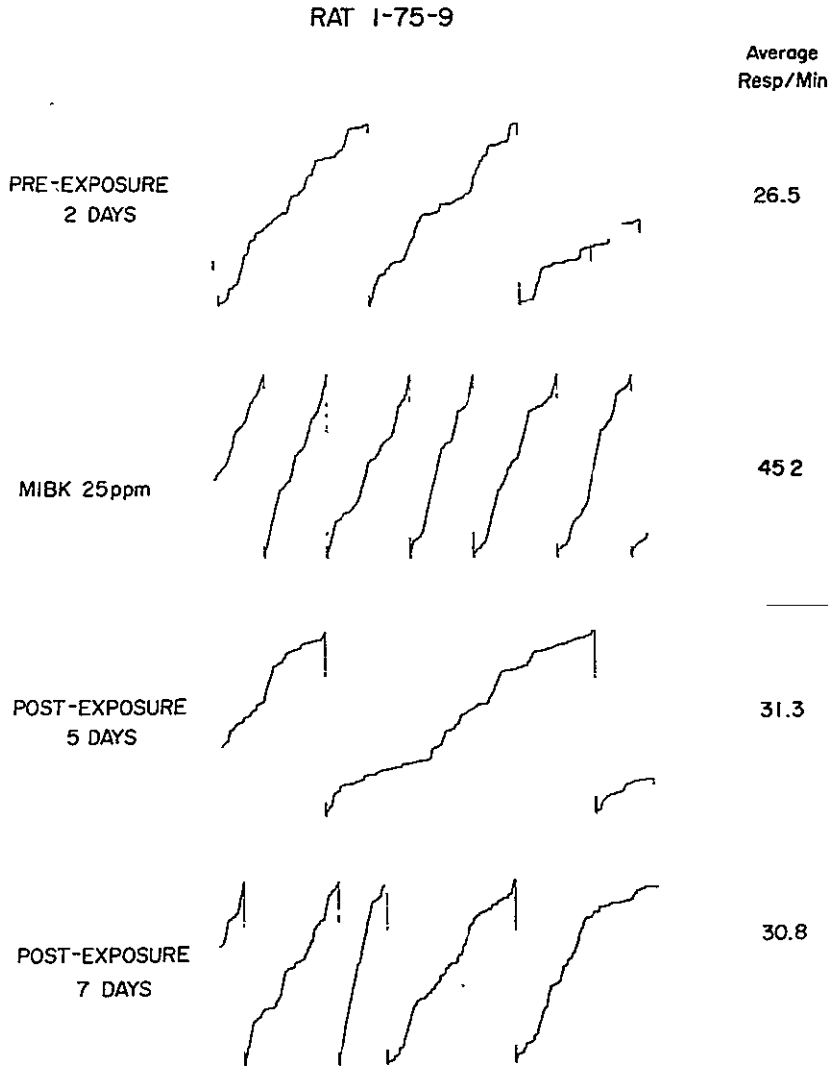

Figure 8. Effect of 25 ppm Methyl Isobutyl Ketone (MIBK) on Variable-Interval Response Rate of the Rat.

MIBK exposure data, for other rats, are shown in Table 4. Rat 6 was exposed to MIBK at 50 ppm; all other MIBK exposures were at 25 ppm. All pre-exposure sessions preceded the MIBK sessions by two days. The greatest increase in response rate occurred for rats 9 and 11. A decrease in response rate was seen with rats 10 and 12.

TABLE 4: EFFECT OF METHYL ISOBUTYL KETONE (MIBK) ON VARIABLE–INTERVAL RESPONSE RATES DURING THREE HOUR EXPERIMENTAL SESSIONS

	MIBK	Average Responses/Minute							
	Concentration	Pre-Exposure	Exposure	Days Post-Exposure					
Rat	(PPM)	2 Days	MIBK	2	5	6	7	8	12
4	25	12.6	12.2	10.2	11.8		10.0		
5	25	14.1	18.6		29.9		19.5		
7	25	16.4	19.9						
9	25	26.5	45.3		31.4		47.5		36.4
10	25	42.4	35.4	36.3		28.9		49.2	
11	25	22.5	47.3		35.9		24.3		22.9
12	25	12.9	8.9	10.1		10.9		12.0	
6	50	20.6	20.6	13.0	17.6		23.5		

MIBK - BABOONS

Chronic exposure of baboons to MIBK at 25, 35, 50 and 75 ppm did not impair the match-to-sample discrimination task. However, a change in extra responses during delay interval was observed.

In Figure 9 are shown the effects of 50 ppm of MIBK over a 7-day period. The extra responses have been compartmentalized into ten second intervals so that one may determine where responses occurred during the 2-minute delay

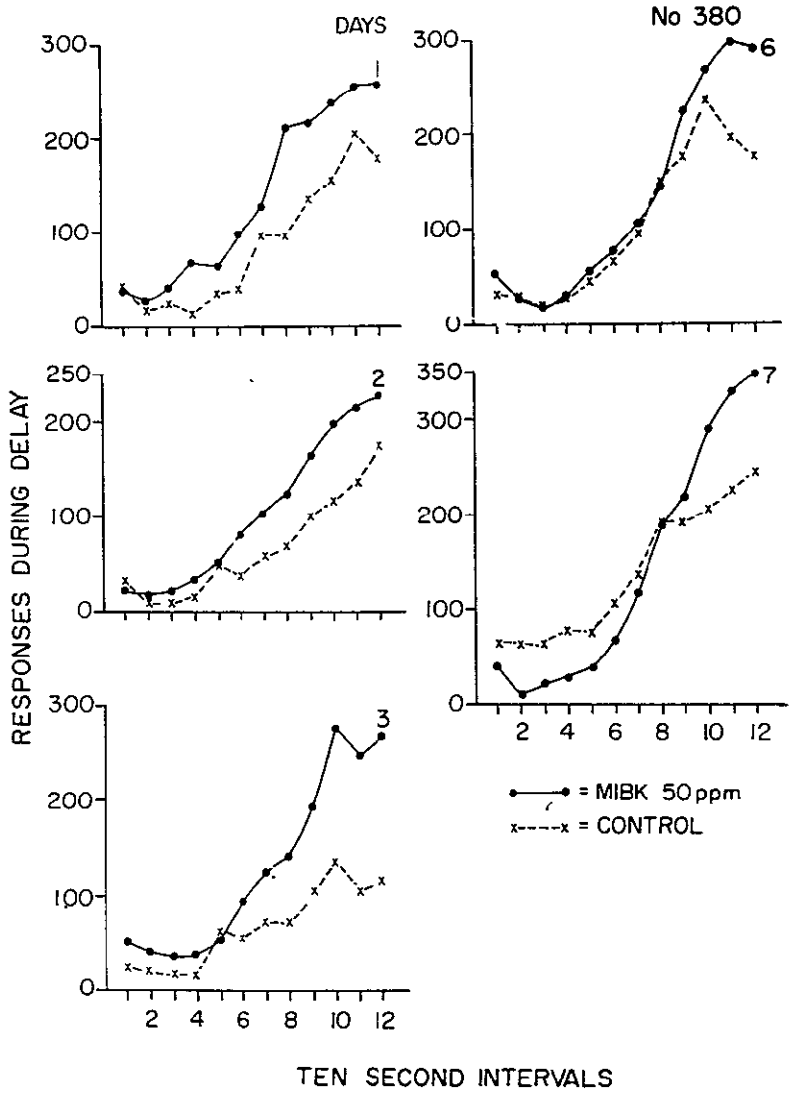

Figure 9. Effect of 50 ppm Methyl Isobutyl Ketone (MIBK) over a 7-Day Period in the Baboon. (Solid lines represent MIBK data and broken lines, control data obtained on the same day one week before exposure. Each point on the graph represents the total number of extra responses made for all delay periods during the indicated 10 sec interval.)

interval. The solid lines represent the MIBK exposure data and the broken lines show control data which were obtained on the same day, one week before exposure. Each point on the graph represents the total number of extra responses made for all delay intervals for the indicated ten second interval. Under MIBK this baboon found it more difficult to wait during the delay interval. Extra responses increased on all of the days that the operant tests were conducted. An increase in the concentration of MIBK to 75 ppm for an additional 48 hours did not increase the effect further.

In Table 5 are responses during delay intervals averaged over days for the chronic exposure periods. The control averages were based upon the same number of days during the pre-exposure period. At 25 ppm of MIBK responses increased significantly for animal 382 ($P<.05$) but were unchanged for animal 529. At 50 ppm of MIBK responses increased significantly for animal 380 ($P<.01$) and also increased for animal 531, although not significantly.

TABLE 5: EFFECTS OF METHYL ISOBUTYL KETONE (MIBK) ON AVERAGE RESPONSES DURING DELAY INTERVAL

Baboon	MIBK PPM	Average Responses During Delay Interval		
		Control	Exposure	
382	25	223	448	$P < .05$
529	25	147	137	N.S.
380	50	906	1492	$P < .01$
531	50	1151	1329	N.S.

The results of the MEK and MIBK studies show that with operant techniques one may observe alterations in behavior at relatively low concentrations of volatile substances. MEK and MIBK at the doses studied did not impair a baboon's ability to discriminate or remember stimuli. However, extra responses during the delay interval increased or decreased during chronic exposure to the ketones. MEK at 20 ppm reduced extra responses in baboon 382 while MIBK at 50 ppm increased extra responses in baboon 380. Increases or decreases in extra responses might be an indicator of alterations in the animal's anxiety levels.

ACETONE - RATS

Figure 10 contains cumulative records which illustrate the effect of acetone

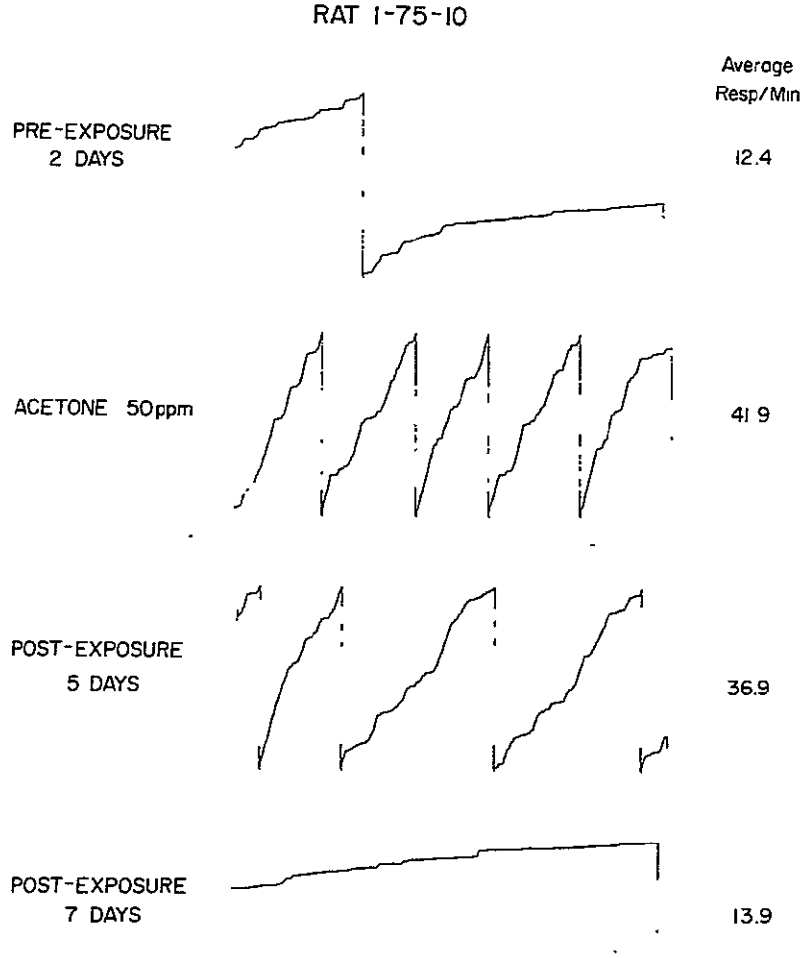

Figure 10. Effect of 50 ppm Acetone on Variable-Interval Response Rate of the Rat.

at 50 ppm in rat 10. Each record is taken from the 3rd hour of the experimental session. The average rate of 41.9 responses/minute during acetone exposure represents more than a three-fold increase over the average response rate of 12.4 responses/minute during pre-exposure control. This effect was still present five days post-exposure. By the seventh day post-exposure, the subject's response rate had returned to the pre-exposure control level.

Acetone data for all concentrations tested are listed in Table 6. Differential effects on response rates may be seen at concentrations of 35 to 100 ppm. A decrease in response rate occurred for rat 1-75-11 at 50 ppm and returned to pre-exposure control levels by the seventh day post-exposure.

TABLE 6: EFFECT OF ACETONE ON VARIABLE–INTERVAL RESPONSE RATES DURING THREE HOUR EXPERIMENTAL SESSIONS

Rat	Acetone Concentration (PPM)	Average Responses/Minute							
		Pre-Exposure 2 Days	Exposure Acetone	Days Post-Exposure					
				2	3	5	6	7	8
2-75-6	25	7.5	8.3	8.3		12.1		10.2	
1-75-9	35	22.4	28.3			31.6		27.5	
1-75-11		12.7	20.8				19.0		29.1
2-75-12		12.8	16.0		13.5	14.0		12.7	
1-75-12		7.8	8.4				11.1		10.3
1-75-10	50	12.4	41.9			36.9		13.9	
1-75-11		36.2	18.1			26.3		42.7	
1-75-12		7.9	7.8			9.3		9.8	
2-75-6		11.3	12.3		11.9	12.9		13.1	
2-75-12		8.5	15.5	16.8		16.7		16.3	
2-75-18	75	45.5	54.6	50.9		42.3		49.0	
2-75-26		37.4	40.9	39.8		32.7		37.2	
2-75-18	100	49.8	69.9				61.1		75.1
2-75-26		36.9	38.8	43.5			38.4		37.8

ACETONE - BABOONS

Since the starting concentration of 100 ppm had very little effect on the behavior of two baboons during the first three days of chronic exposure, concentrations were increased to 400 ppm during the next 4-day period. Very little difference in behavior was observed between exposure and pre-exposure control

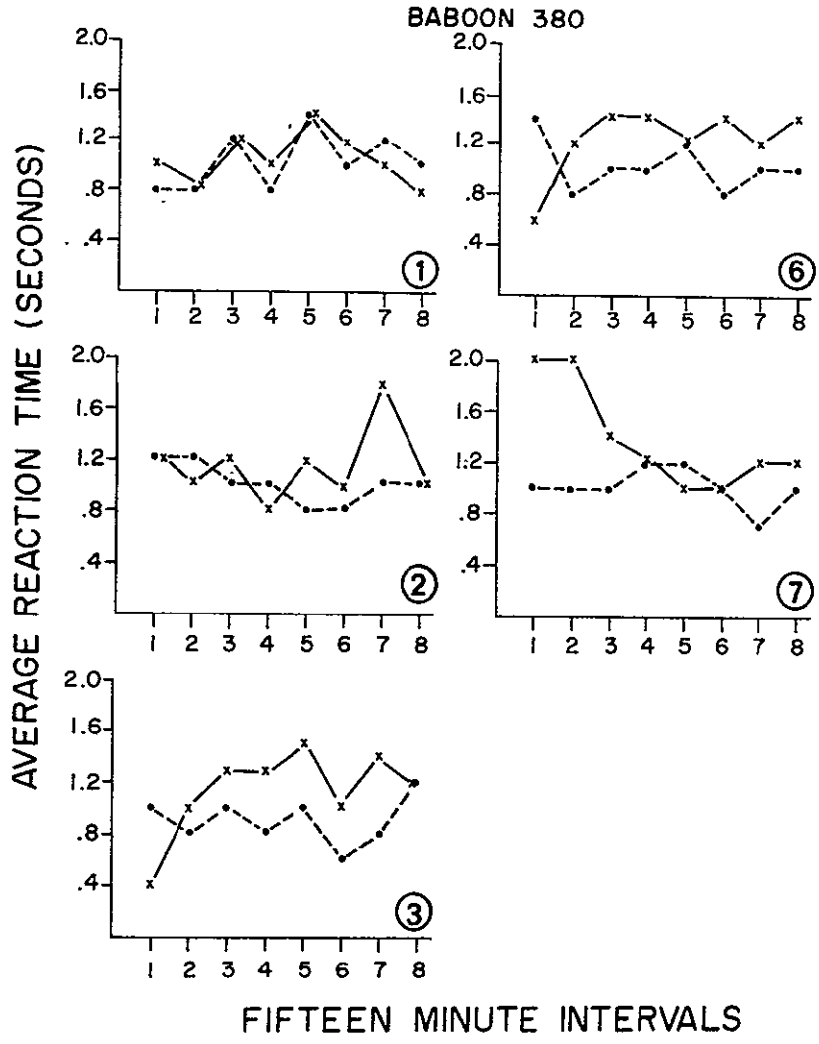

Figure 11. Effect of 400 ppm Acetone on Average Reaction Time in the Baboon. (Solid lines represent Acetone data and broken lines, control data obtained on the same days one week before exposure. The circled numbers indicate the day of exposure.)

periods. Exposure of the other two baboons to 400 ppm during a 7-day period produced a slowing of the baboon's reaction time. This is illustrated by the data in Figure 11. The ordinate shows average reaction time which was calculated by dividing the total time required to respond to all left and right stimuli by the total number of trials during each 15 minute segment of a 2-hour session. The solid lines represent exposure data and the broken line, control data obtained one week earlier. The circled numbers indicate day of exposure. On days one and two, reaction times were similar to controls. On days three, six and seven, a slowing of reaction time occurred. This reached a maximum of 2 seconds during the first 30 minutes of the seventh day experimental session.

TRICHLOROETHYLENE - RATS

Figure 12 contains cumulative records illustrating the effects of trichloroethylene in a single rat. The records represent the 3rd hour of an experimental session. Lever responding increased from an average of 30/minute during control to 35.3/minute under the 200 ppm of trichloroethylene. For this animal, lever responding approximated control value five days post-exposure.

Table 7 shows data for a number of rats exposed to trichloroethylene in doses ranging from 50 to 600 ppm. Although no systematic changes in response rate occurred, rate enhancement effects were evident under 50, 200 and 300 ppm trichloroethylene. It is of interest to note that at 400 and 600 ppm the maximum increases in VI response rate occurred on the fifth and seventh post-exposure days.

TRICHLOROETHYLENE - BABOONS

Chronic exposure of the baboon to trichloroethylene was discontinued after three days because changes in behavior were observed at both the 200 and 400 ppm concentrations. For the animals exposed to 200 ppm TCE, responses during the

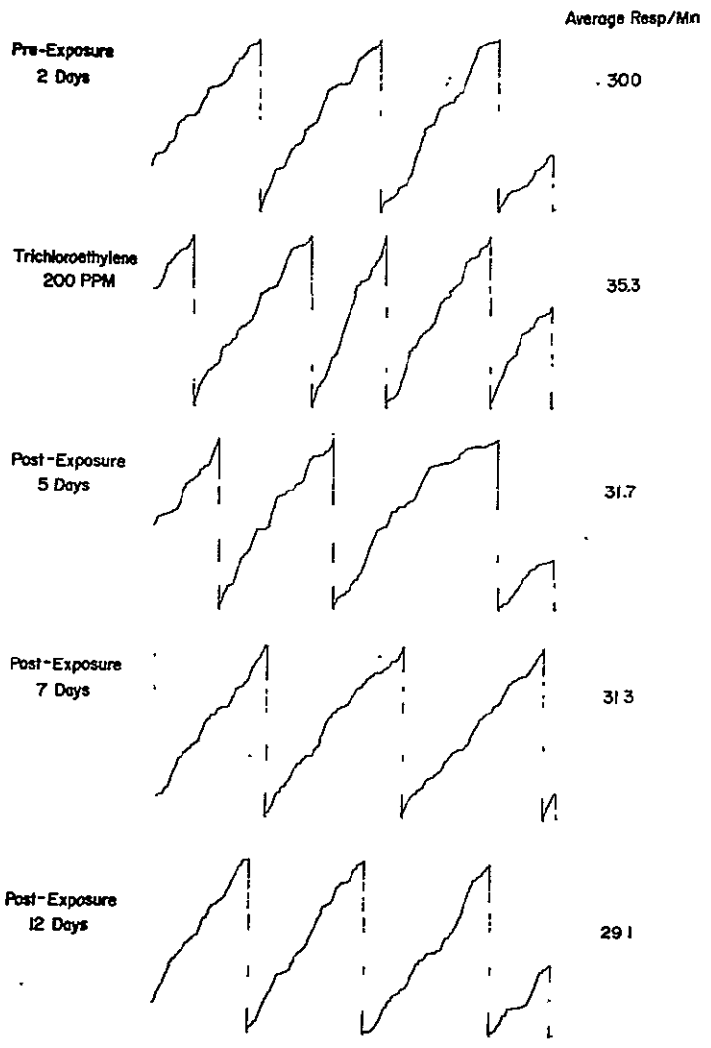

Figure 12. Effect of 200 ppm Trichloroethylene (TCE) on Variable-Interval Response Rate of the Rat.

TABLE 7: EFFECTS OF TRICHLOROETHYLENE ON VARIABLE–INTERVAL RESPONSE RATES DURING THREE HOUR EXPERIMENTAL SESSIONS

RAT	TRICHLOROETHYLENE CONCENTRATION (PPM)	Average Responses/Minute					
		Pre-Exposure	Exposure	Days Post-Exposure			
		2 Days	Trichloroethylene	2	5	7	12
2-75-12	50	14.5	19.0	18.4	17.6	15.6	
1-75-9	75	38.3	36.5		29.7	28.1	32.8
2-75-6	100	10.7	9.6	12.1	8.0	9.9	
1-75-10	200	30.0	35.3		31.7	31.3	29.1
2-75-18	225	61.0	57.0	48.8	63.2	38.3	
2-75-26	300	21.5	24.4	25.7	22.6	28.7	
1-75-11	400	13.1	14.0		18.6	17.2	12.2
1-75-12	600	10.1	12.2		17.8	17.7	13.8

delay period increased. The effect was greater on days one and two for baboon 380 and was maximal on day three for baboon 531 (Figure 13). This increased responding was accompanied by a lowering of reaction times although not to a significant degree. For the other two baboons, the higher concentration of TCE (400 ppm) produced little change in responses during the delay period but reaction times for both animals increased. This effect was significant for baboon 382 ($P<.05$).

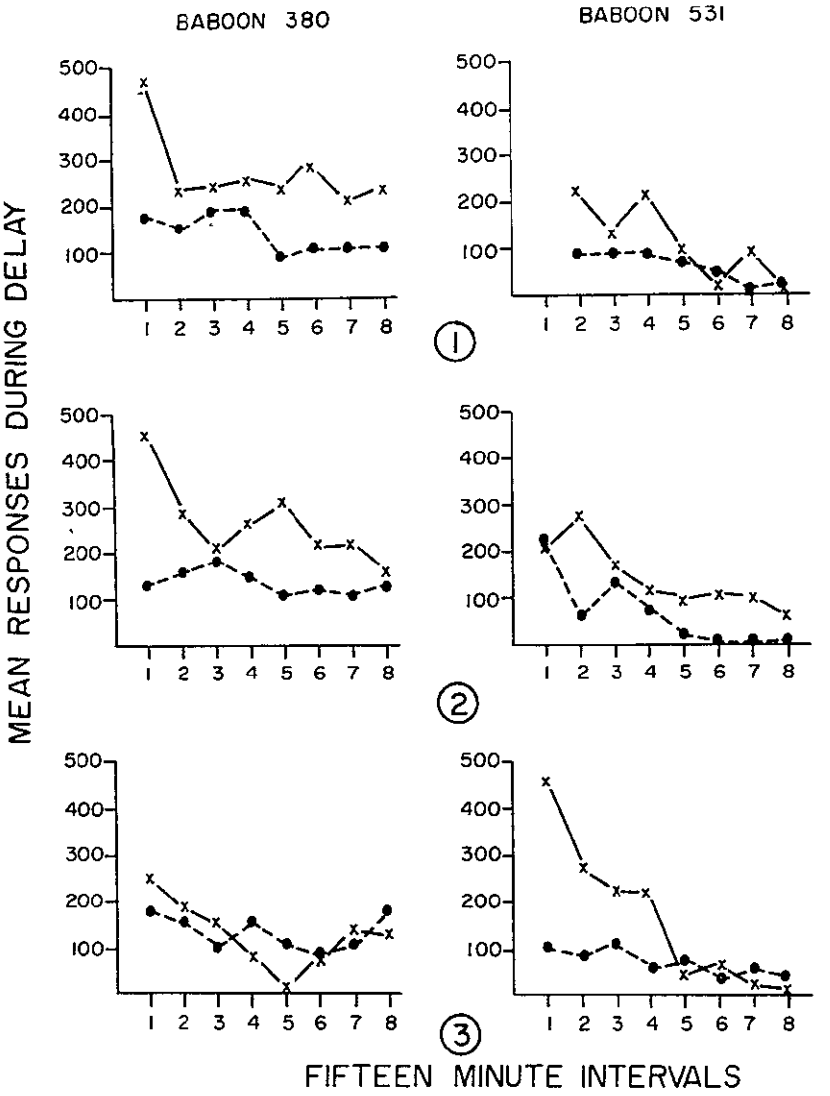

Figure 13. Effect of 200 ppm Trichloroethylene (TCE) on Responses During Delay Periods in the Baboon. (Solid lines represent TCE data and broken lines, control data obtained on the same days one week before exposure. The circled numbers show the day of exposure.)

FREON 21 - RATS

Table 8 shows the changes in VI rates produced by exposure of rats to Freon 21 in concentrations ranging from 200-800 ppm. The 200 ppm concentration produced a slight increase in responding of two rats. Higher concentrations of Freon 21 resulted in either a reduction or no change in response rates.

TABLE 8: EFFECTS OF FREON 21 ON VARIABLE-INTERVAL RESPONSE RATES DURING THREE HOUR EXPERIMENTAL SESSIONS

RAT	FREON 21 CONCENTRATION (PPM)	Average Responses/Minute					
		Pre-Exposure	Exposure	Days Post-Exposure			
		2 Days	Freon 21	2	5	7	12
2-75-6	200	6.6	7.7	6.1	5.8	5.8	
1-75-9	200	28.8	31.5		24.3	25.0	29.0
1-75-10	400	34.4	33.7		33.0	38.0	41.0
2-75-12	400	16.8	12.5	17.9	15.5	18.3	
2-75-18	600	54.7	55.8	59.9	50.1	49.7	
2-75-26	600	23.6	23.6	20.9	23.8	21.3	
1-75-11	800	22.0	14.7		25.2		26.6
1-75-12	800	9.7	10.9		17.6	17.9	8.9

FREON 21 - BABOONS

Two baboons were exposed to 200 ppm of Freon 21 for six days and to 400 ppm for an additional three days. Data for one of these animals (529) are shown in Figure 14. There was an increase in responses during the delay interval for baboon 529, an effect which dropped out on days three and six and seven. Based on these findings it was decided to increase the concentration for the other

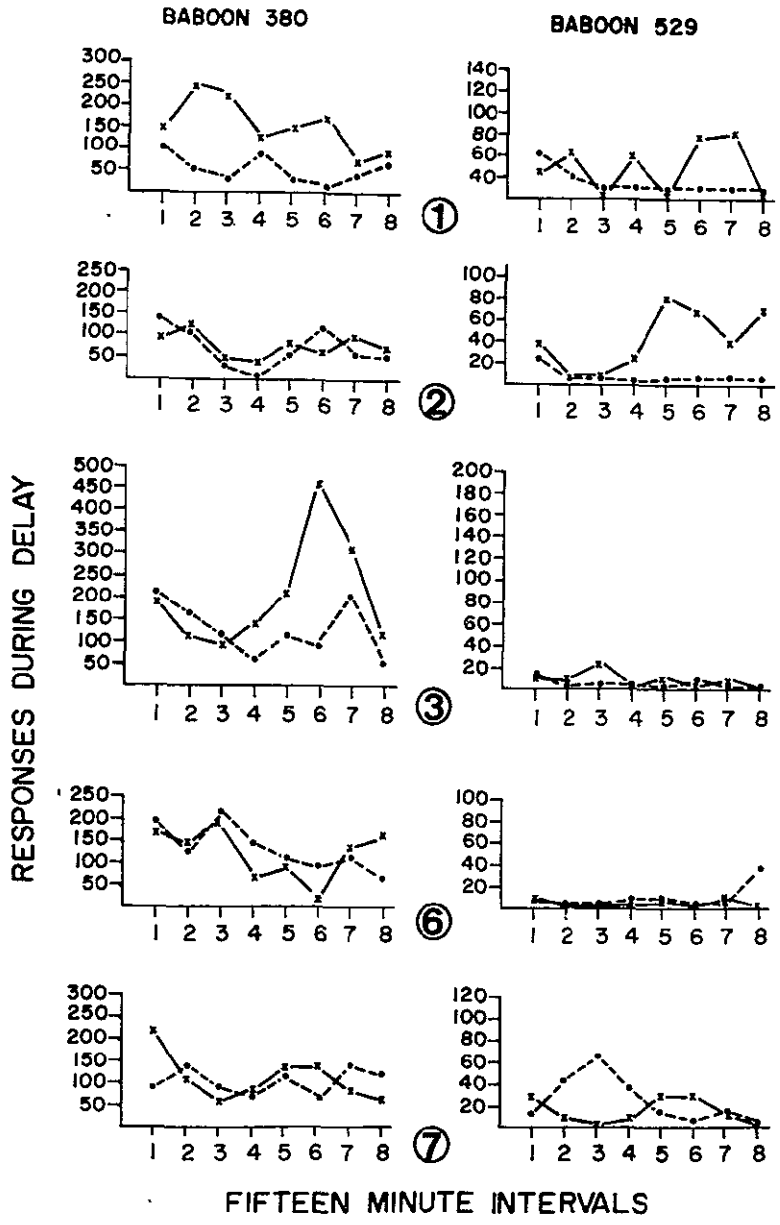

Figure 14. Effect of Freon 21 on Responses During Delay Periods in the Baboon. (Solid lines represent Freon 21 data and broken lines, control data obtained on the same day one week before exposure. The circled numbers show the day of exposure. Animal 529 was exposed to 200 ppm Freon 21 for 6 days and 400 ppm for 3 days. Animal 380 was exposed to 600 ppm for 8 hr a day during a 9-day period and 300 ppm for 16 hr a day during the same time period.)

baboons. Since one batch of Freon 21 was determined to be contaminated and delivery of the replacement material was not according to schedule, it was necessary to reduce the exposure concentration for these animals to 300 ppm during the night hours. Therefore, baboons 380 and 531 were exposed to 600 ppm Freon 21 during an 8-hour period and to 300 ppm for sixteen hours of the day. Effects observed under these conditions were as under 200 ppm for baboon 529. Data for one of these animals (baboon 380) show that responses during the delay interval were increased only on days one and three. During the first 24 hours of exposure to 600 ppm of Freon 21, baboons 380 and 531 developed a severe diarrhea which disappeared during the second day of exposure.

HEPTANE - RATS

An illustration of heptane effects on variable-interval response rate of the rat is shown in the cumulative records of Figure 15. On the pre-exposure day, the average response rate for rat 27 was 19.4. Heptane at 400 ppm reduced the average response rate to 13.7. On the first post-exposure day, the average response rate was almost back to the pre-exposure control level while on the sixth post-exposure day, the rat was above the control level. Data shown in Table 9 are for rats 25, 27 and 28 exposed to heptane at 200, 400 and 600 ppm, respectively. For rat 25, the low rate responder, heptane at 200 ppm reduced the average response rate from 4.8 to 3.8 resp/min. During the first three post-exposure sessions, the average response rate was above the pre-exposure control level but on the sixth post-exposure day, it approximated the control. For rat 27, the pre-exposure average control rate was reduced under heptane 200 ppm from 19.4 to 13.7/minute. For this rat, the data obtained on the sixth post-exposure day also approximated the pre-exposure control response rate. The 600 ppm concentration of heptane

produced a precipitous drop in response rate for rat 28. The response rate on the sixth post-exposure day still had not returned to the control level.

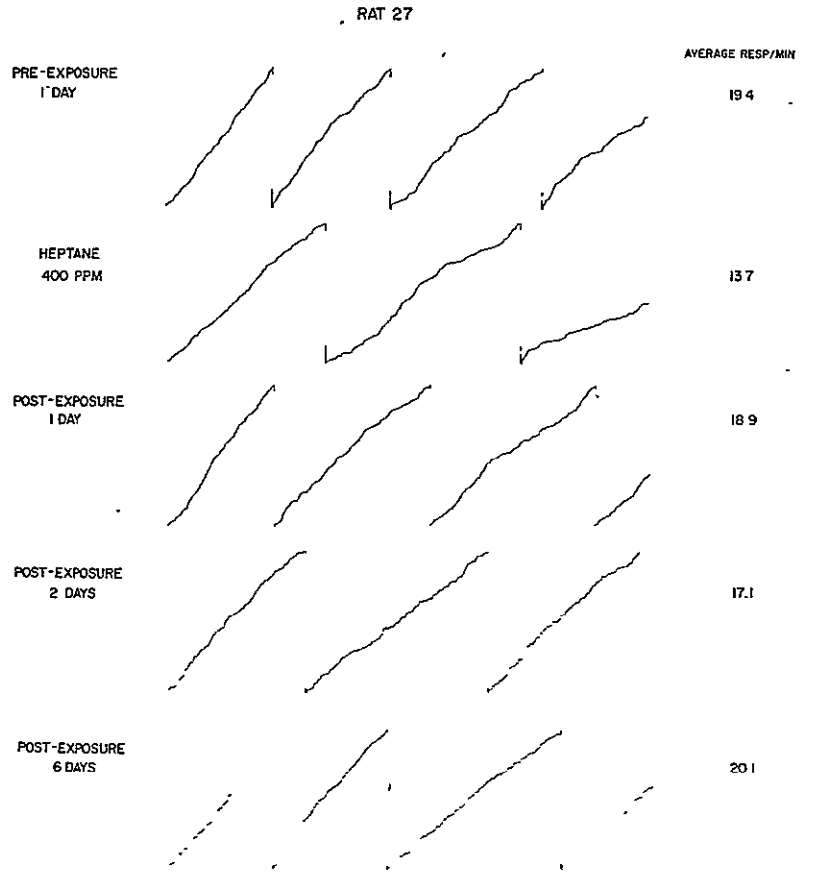

Figure 15. Effect of 400 ppm Heptane on Variable-Interval Response Rate of the Rat.

TABLE 9: EFFECTS OF HEPTANE ON VARIABLE-INTERVAL RESPONSE RATES

RAT	HEPTANE CONCENTRATION (PPM)	AVERAGE RESPONSES/MINUTE					
		PRE-EXPOSURE CONTROL	EXPOSURE HEPTANE	DAYS POST-EXPOSURE 1	2	3	6
25	200	4.8	3.8	5.6	5.6	5.3	4.4
27	400	19.4	13.7	18.9	17.1	12.9	20.1
28	600	22.6	10.1	14.5	14.9	13.6	17.0

HEPTANE - BABOONS

Two baboons were exposed to heptane at 100 ppm on the first exposure day and 200 ppm during the next six days. No systematic changes were observed for response rates during the delay intervals. However, reaction times increased for both animals. These changes are shown in Figure 16. For baboon 529 an increased reaction time is evident on days three, six and seven. For baboon 382 this effect can be seen on days six and seven. Qualitatively similar effects were observed for 380 and 531, the baboons exposed to 400 ppm heptane. These data are shown in Figure 17.

In Table 10 are reaction times in seconds averaged over days for the chronic exposure periods. The control averages were based upon the same number of days during the pre-exposure period. Statistical analyses of these data revealed a significant increase in reaction time for three of the four baboons exposed to heptane. Reaction time increased for baboon 529 although not significantly.

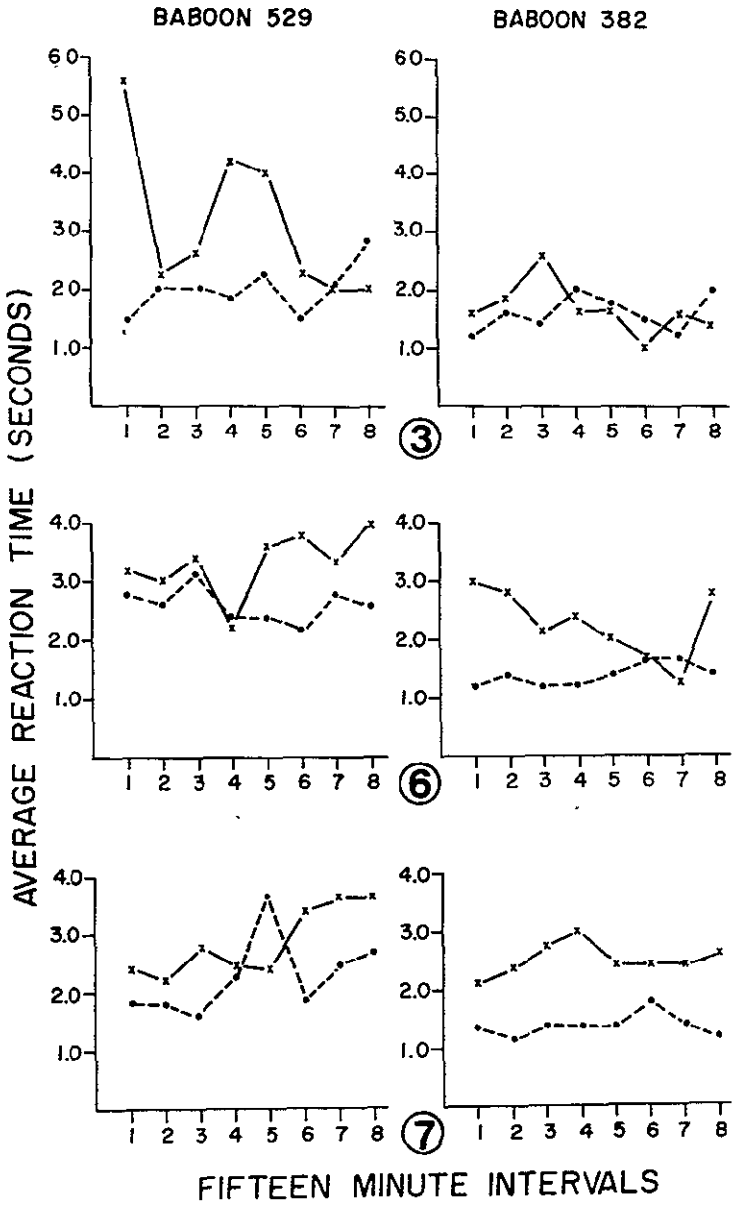

Figure 16. Effect of Heptane on Average Reaction Time in the Baboon. (Solid lines represent Heptane exposures and broken lines show control data obtained on the same days one week before exposure. Circled numbers show exposure days.)

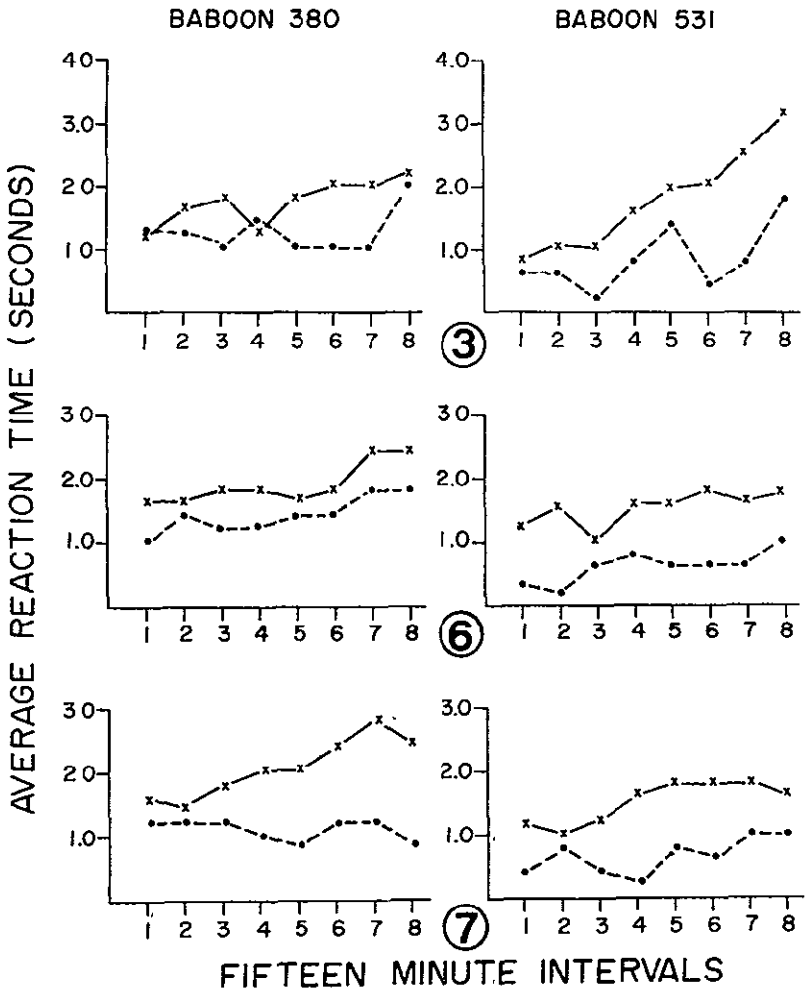

Figure 17. Effect of 400 ppm Heptane on Average Reaction Time in the Baboon. (Solid lines represent exposure data and broken lines, control data obtained on the same day one week before exposure. Circled numbers show exposure days.)

TABLE 10: EFFECTS OF HEPTANE ON REACTION TIME IN THE BABOON

		Average Reaction Time in Seconds		
Baboon	Heptane PPM	Control	Exposure	
382	100 - 200	1.465	1.97	$P < .05$
529	100 - 200	2.515	2.87	N.S.
380	400	1.27	1.725	$P < .01$
531	400	.705	1.28	$P < .05$

BABOON EXPOSURES TO COMBINATION OF MEK & TCE

Figure 18 shows a decrease in responses during the delay interval under a combination of 40 ppm MEK and 200 ppm TCE. For this animal, 200 ppm TCE alone produced an increase in responses during the delay interval while 40 ppm MEK alone had little effect. Figure 19 shows the changes in average reaction times that occurred under the individual gases and the combined gases. Under 40 ppm MEK reaction times were increased slightly above control levels on four of the exposure days. Under 200 ppm TCE, reaction times were decreased on the first two exposure days. Exposure of the animals to the same concentrations of MEK and TCE in combination produced increased reaction times which reached a maximum of 2 seconds on the third exposure day and remained at this level for the 7-day period.

Data obtained for baboon 531 under the same treatment conditions were similar but not as striking as for baboon 380. Responses during the delay interval were unchanged under 40 ppm MEK, increased under 200 ppm TCE and decreased under 40 ppm MEK and 200 ppm TCE during one exposure day. On four of the exposure days,

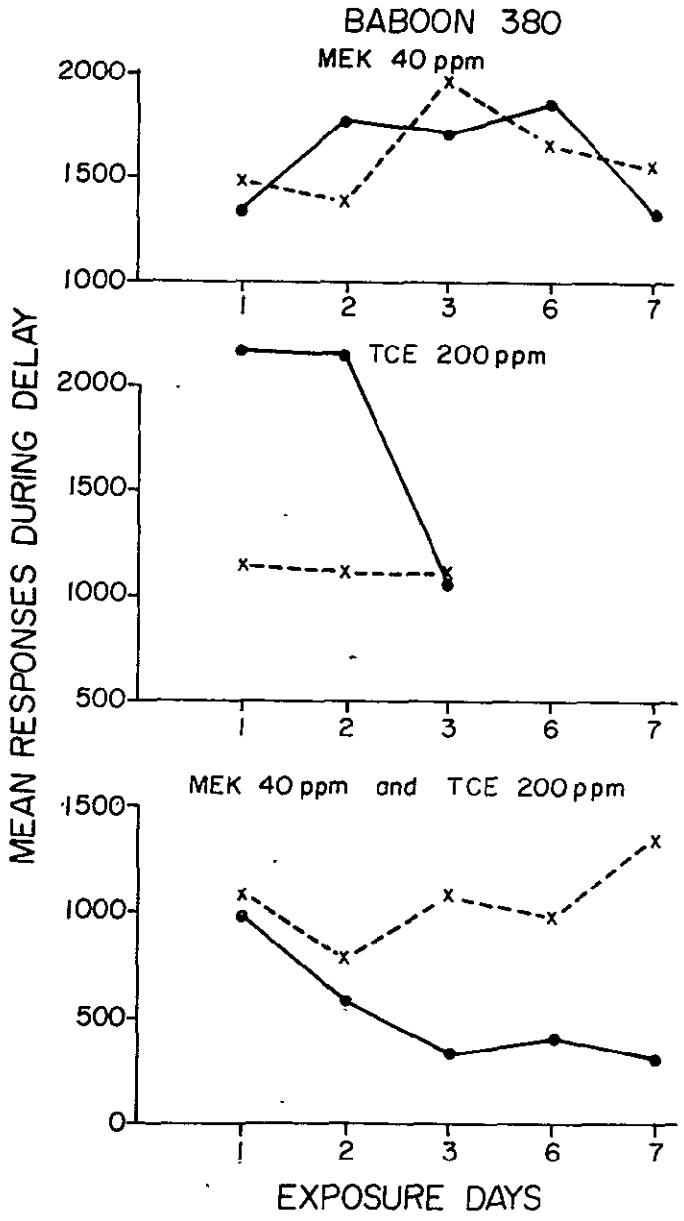

Figure 18. Effect of 40 ppm Methyl Ethyl Ketone (MEK) and 200 ppm Trichloroethylene (TCE) Alone and in Combination on Responses During Delay Interval in the Baboon. (Solid lines show exposure data and broken lines, control data obtained one week before exposure.)

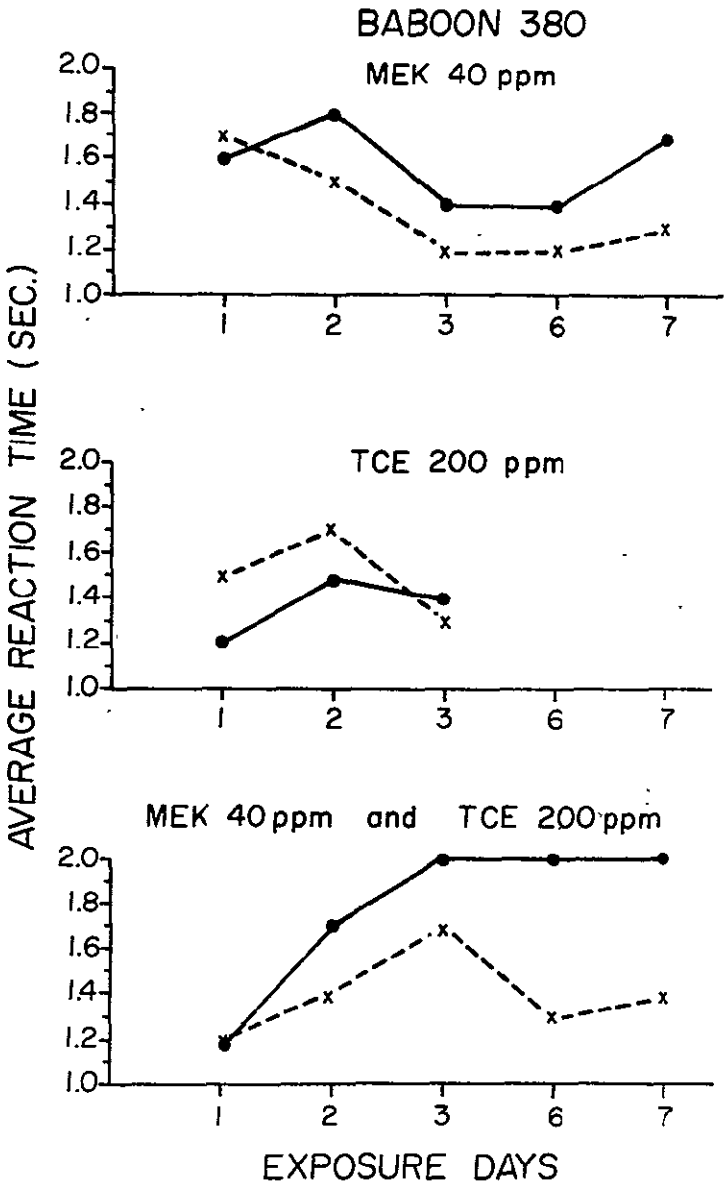

Figure 19. Effect of 40 ppm Methyl Ethyl Ketone (MEK) and 200 ppm Trichloroethylene (TCE) Alone and in Combination on Average Reaction Time in the Baboon. (Solid lines show exposure data and broken lines, control data obtained one week before exposure.)

responses during the delay intervals approximated that of the control sessions. Increased reaction times under the combination of gases also occurred on but one of the exposure days.

DISCUSSION

METHYL ETHYL KETONE (MEK)

Based on their review of the MEK literature, Zakhari *et al.* (4) suggest that values derived from animal experiments may be too high to serve as estimates in man. Doses of MEK required to produce effects in humans, were lower than reported for most animal studies. Nakaaki (5) noted deficiencies in perception of time duration during exposure of humans to 90-270 ppm MEK over a 4-hour period. In the present experiments MEK produced an enhancement of response rates in rats exposed to 25 ppm and an increase in extra responses of baboons exposed to 20 to 40 ppm. It should be noted that these values are far below the Threshold Limit Value (TLV) of 200 ppm. These observations indicate that the use of operant techniques to study the effects of contaminants on the behavior of juvenile baboons should allow the accumulation of data having greater implications for estimation of MEK effects in humans.

METHYL ISOBUTYL KETONE (MIBK)

Alterations of rat lever-pressing behavior, described in this report, occurred at 25 ppm MIBK or at ¼ of the TLV. An increase in extra responses occurred in baboons at ½ the TLV or at 50 ppm. The concentrations are considerably below those previously reported in human studies on which the TLV of 100 ppm was based in part. Subjects evaluated with a sensory response test estimated 100 ppm of MIBK to be the maximum concentration they could tolerate over an 8-hour period (6). In another study, exposure of workers to 100 ppm MIBK resulted in complaints of headaches, nausea and irritation of the respiratory tract (7). Our data with MIBK also demonstrate the sensitivity of the operant methods and the value of the juvenile baboon as the subject of choice for such studies.

ACETONE

Acetone which has a TLV of 1000 ppm is believed to be less toxic than MEK or MIBK (8). This TLV is ten times the value acceptable in the Soviet Union. The demonstrated alteration of rat lever-pressing behavior at 1/20 of the current TLV and the slowing of baboon reaction time produced by 400 ppm acetone on the third, sixth and seventh exposure days, strongly suggest that the TLV of 1000 ppm may be too high.

FREON 21

A recent communication from the DuPont Company indicates that the TLV of 1000 ppm for Freon 21 is inappropriate as the gas is substantially more toxic than other Freon refrigerants (9). Chronic exposure of rats to 1000 and 5000 ppm showed evidence of liver damage after 45 days of exposure. Rats showed significant mortality, loss of hair and liver cirrhosis after 90 days exposure at the .1% and .5% exposure levels.

In the present experiments, exposure of rats or baboons to Freon 21 at dose levels ranging from 200 to 800 ppm produced alterations in behavior. The increase in number of responses made by the baboon may be a reflection of an increased "nervousness or jitteryness". Changes in behavior of laboratory animals occurred at dose levels below the 1000 ppm TLV level.

TRICHLOROETHYLENE (TCE)

In human behavior studies concentrations of TCE have ranged from a minimum of 100 ppm to as much as 1000 ppm. In one study (10) which used psychomotor and visual performance tests, a subject was exposed two times for 8-hour periods to each of four concentrations of TCE. Although 100 ppm of TCE produced no significant effect on motor performance, there was a progressive decline in performance

with 200, 300 and 500 ppm TCE.

In another study (11) in which eight subjects were exposed for 2-hour periods to concentrations of 100-1000 ppm TCE, adverse effects were observed on performance tests at the 1000 ppm concentration. An enhancement of the adverse effects was seen after ingestion of alcohol.

Another investigator found no effects in two subjects exposed to 100 ppm TCE for four hours (12). However, five subjects exposed to 200 ppm for one to seven hours over a period of days, reported sleepiness and fatigue during the fourth and fifth exposure days.

Behavioral studies with animals have been limited generally to laboratory rodents. In investigations (13,14) which used three testing paradigms, rats were exposed daily over a 44 week period to concentrations of 20-800 ppm TCE. Food-rewarded rope climbing was not affected by the exposure, although spontaneous rope climbing increased. Alternating left to right turning behavior was increased at 400-600 ppm while swimming speed was reduced at 400 ppm TCE.

Most of the TCE animal studies required a concentration of at least 400 ppm in order to produce a behavioral change. However, Horvath and Formanek (15) reported a disturbance of cortical activity after 72-121 days in rats exposed to 46 ppm TCE.

Most of the human behavior studies required a minimum of 200 ppm to produce an effect. In the present experiments, rate enhancement effects were observed in rats exposed to 50, 200 and 300 ppm TCE. In the baboon studies spontaneous or 'extra' responses increased at 200 ppm TCE. This observation is in agreement with the above cited rat studies (13,14) in which spontaneous rope climbing increased in rats exposed to 20-800 ppm. A slowing of reaction time occurred in the baboon during the 400 ppm TCE exposure. Although this

concentration is four times the current TLV, the exposure duration required, was only three days.

HEPTANE

The TLV of 500 ppm for heptane may be too high. It is based on studies which required much greater concentrations to demonstrate measurable effects. To produce narcosis in mice (16) within 30 to 60 minutes, a concentration of 10,000 to 15,000 ppm was required. Higher concentrations produced convulsions and death. Slight dizziness has been reported in man after six minutes of exposure to 1000 ppm (16), while higher concentrations produced vertigo and incoordination. CNS involvement occurred without mucous membrane irritation and brief exposures of four minutes to 5000 ppm caused nausea and loss of appetite.

In the present studies behavioral alterations were evident at concentrations below the TLV. For three of the four baboons, reaction times were affected significantly under heptane at 200 or 400 ppm. At these concentrations, the animals' reaction times increased from a minimum of one second to as much as four and six seconds.

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APPENDIX

EFFECTS OF KETONES ON OPERANT BEHAVIOR OF LABORATORY ANIMALS*

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Substances which are abused through inhalation for their stimulating effect and euphoria-producing qualities include ketones such as acetone, methyl ethyl ketone (MEK) or methyl isobutyl ketone (MIBK). Laboratory studies of such compounds have, with a few noted exceptions, been limited to studies of extremes in toxicity (Shirabe *et al.*, 1974; Bass, 1970).

This research represents the application of operant techniques for the evaluation of the central nervous system effects of ketones. Operant conditioning has been demonstrated to be invaluable in behavioral pharmacology. Tests have been devised for the successful preclinical evaluation of a class of compounds referred to as anti-anxiety agents or minor tranquilizers (Geller and Seifter, 1960). Similar techniques have been used to demonstrate toxicity of pheniprazine, which had been reported to produce red-green color blindness in humans. Hanson *et al.* (1964) showed that pheniprazine, a monoamine oxidase (MAO) inhibitor, disrupted a visual red-green color discrimination in pigeons. The effect was shown to be reversible on withdrawal of the drug. It is somewhat surprising that the extensive literature on drug abuse is lacking in basic behavior studies of volatile substances. Although the reason for this deficiency is not immediately obvious, it may be due in part

*This research was supported by NASA Contract: NAS 9-14743.

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to the technical problems inherent in the handling of volatile substances. Weiss and Laties (1969) have reviewed a few industrial toxicology studies which show that operant conditioning techniques can be useful in studying the effects of gases on behavior.

Armstrong *et al.* (1963) investigated the effects of mercury vapor on the behavior of pigeons trained on a multiple schedule of reinforcement and produced reversible changes in behavior when neither gross changes nor overt pathology were detectable. Beard and Wertheim (1967), who studied the effects of carbon monoxide upon time discrimination in humans, were able to detect the effects of 50 ppm with a 90-minute exposure time. They also were able to detect effects of low concentrations of CO on the performance of rats working on a spaced response drug schedule. The concentration of CO required to produce a change in performance was a function of the pause between responses required by the schedule. When the pause between responses had to be 30 seconds, a 10-minute exposure to 100 ppm significantly decreased response rate. With a 10-second pause requirement, 40 minutes of exposure were required to produce the same effect. These studies demonstrate that operant methods permit the demonstration of subtle effects of volatile substances at doses and exposure times far below those that would be required to demonstrate effects with gross observational techniques.

Low concentrations of a number of chemical agents have been identified in spacecraft atmospheres as a result of off-gasing from non-metallic materials. Since astronauts will be required to spend long periods of time in Spacelab, it is most important to determine the effects on the human of chronic exposure to low doses of the contaminants. These presentations deal with the effects of MEK, MIBK and acetone on lever-pressing behavior of laboratory rats and the effects of MEK and MIBK on the behavior of young baboons trained on a match-to-sample discrimination task.

(INSERT FIGURE 1 HERE)

Figure 1 shows one of two large, steel and glass chambers provided by National Aeronautics and Space Administration (NASA) which were used for the rat exposure studies. Concentration levels of gases in the chambers were monitored with a Hewlett-Packard gas chromatograph.

The subjects were Holtzmann, Sprague-Dawley male rats approximately 90-120 days old at the start of the experiment. They were trained in Skinner boxes within the gas exposure chambers. The rats were gradually reduced to 80% of their normal body weight and then trained to press a lever for a liquid food reward on a 2-minute variable interval schedule of reinforcement. On this procedure, rats are made hungry and first trained to press a lever in order to obtain food on a continuous reinforcement basis (crf); that is to say that every lever response activates the feeder to produce a food reward. The schedule is then changed to a 2-minute variable interval schedule (2-min VI) in which rewards are obtained at random intervals on the average of once every two minutes.

Behavior typically obtained on this schedule is a steady output of lever responses with relatively little variation from day to day. The behavior is extremely sensitive to drug effects insofar as changes in response rate may be demonstrated with a dose at which behavioral changes may not be evident through direct observation of an animal.

In preliminary range-finding studies, six rats trained on the VI schedule were exposed to different concentrations of methyl ethyl ketone (25 to 800 ppm). Experimental sessions were of two hour duration and exposures spaced at least one week apart. MEK produced an increase in lever-pressing rates at many of the doses tested. In order to clearly demonstrate this effect over a longer time period, experimental sessions were increased to six hour duration and four

animals were exposed to 25 ppm MEK. Because of the session length, it was not practical to run animals more frequently than every other day.

(INSERT FIGURE 2 HERE)

Figure 2 contains cumulative records which illustrate the effect of MEK in a single rat. These records represent the 3rd hour of an experimental session. Lever responding increased almost two-fold under MEK. The average response rate of 18.6 responses/minute during control increased to 34 responses/minute under MEK. Five days post-exposure, average response rate was still 34 responses/minute, while seven days post-exposure, the response rate was 19.2 responses/minute or almost back to the pre-exposure control level. It is of interest to note that toxicity studies of MEK required doses of 500-3500 ppm to produce narcosis in chickens, cats or rats (Abdel-Rahman *et al.*, 1976).

Similar data for the other rats are shown in table 1.

(INSERT TABLE 1)

The pre-exposure sessions preceded the MEK sessons by two days. Variable-interval response rates increased in all animals exposed to 25 ppm MEK during a six hour period. The effect was greatest for rat 11 where the average response rate increased from 12.17 per minute to 61.1 responses/minute under MEK. For this animal, the VI response rate remained high for eleven days post-exposure. On the sixteenth post-exposure day, the rate decreased to below the pre-exposure control level. For rats 4, 9, and 10, average response rates approximated pre-exposure control levels on the second, seventh and sixth post-exposure sessions, respectively.

(INSERT FIGURE 3 HERE)

Figure 3 contains cumulative records illustrating the effect of 25 ppm MIBK on the variable-interval response rate of a rat. Each of these records represents the 3rd hour of the experimental session. The average response rate under MIBK was 45.2/min., a 58% increase over the pre-exposure control of 26.5. Seven days post-exposure, the response rate had not returned to control levels.

Acetone studies were conducted with rats at concentrations between 25 and 100 ppm.

(INSERT FIGURE 4 HERE)

Figure 4 contains cumulative records which illustrate the effect of acetone at 50 ppm in rat 1-75-10. Each record was taken from the 3rd hour of the experimental session. The average rate of 41.9 responses/minute during acetone exposure represents more than a three-fold increase over the average response rate of 12.4 responses/minute during pre-exposure control. This effect was still present 5 days post-exposure. By the 7th day post-exposure, the subject's response rate had returned to the pre-exposure control level.

The data obtained from these preliminary range-finding studies with rats provided the necessary dose-response information for use in the baboon discrimination studies.

For these studies, two large stainless steel exposure chambers provided with an air lock were used to expose the trained animals to the gases. Each of the two identical chambers housed two trained animals in home cages. Animals in one chamber were exposed chronically over a seven day period to a contaminant at the concentration determined in preliminary range-finding

studies with rats. Animals in the other chamber served as controls and were exposed to clean air during the same time period. Thus, not only did we have other animals as controls, but each animal was able to serve as its own control in that exposure data could be compared with data obtained pre and post-exposure.

(INSERT FIGURE 5 HERE)

Figure 5 shows the exposure chambers which are approximately 9 feet high and 9 feet in diameter. The young baboons lived in cages within the exposure chambers. The cages were designed so that an intelligence panel could be slipped down between the outside wall of the cage and the baboon. The panel was instrumented with a row of three, round transluscent discs in one wall. Under the appropriate experimental conditions, pressing either end disc produced a food reward in the form of a pellet.

The lowest effective dose (LED) of the contaminant as determined by preliminary range-finding studies with rats was used as the starting dose for chronic 7-day exposure studies in juvenile baboons. The baboons were trained on an operant behavior procedure which measured perceptual acuity and discrimination performance. This has been referred to as a match-to-sample task. Activation of the session timer set a two-minute variable interval programming tape in motion. The tape programmed the occurrence of center stimuli on the average of once every two minutes. The VI tape was inoperative during each trial. Each trial began with the illumination of one of the stimuli on the center key (probe stimulus). This stimulus was terminated at the end of a 30-second period or by a response on the key. Termination of the stimulus activated a timer for 120 seconds (delay interval). At the end of the delay interval, stimuli appeared on either

key adjacent to the center key. The correct matching stimulus was varied between these two keys in a mixed order. A response on the correct key (stimulus matches center key stimulus) terminated the stimuli, activated the feeder and produced a banana pellet reward. Responses on the incorrect key simply terminated the stimuli and again set the VI tape in motion.

A record was kept of: the number of probe stimuli presented during each 15 minute segment, the number of correct matching responses on the left and right keys and the number of incorrect responses on these keys. A record was also kept of any extra responses that may have occurred on the 3 keys when the stimuli were not activated (pre-neutral responses) or during the delay interval (post-neutral responses). The time it took the subject to respond with a key press after a stimulus was activated was also measured (reaction time).

Chronic seven day exposures were conducted with MEK at 20 and 40 ppm. No impairment of the discrimination task occurred at either of these concentrations. However, a change was observed in the extra responses made by baboon 382 during the delay interval.

(INSERT FIGURE 6 HERE)

In figure 6 are shown the average responses per minute during the delay interval for baboons IX-382 and IX-529. Each bar represents extra responses averaged for five, two hour sessions. A rather dramatic reduction in extra responses occurred during the delay interval when baboon 382 was exposed to MEK at 20 ppm. No change in extra responses was observed for baboon 529 who was relatively calm and made few extra responses during the pre and post-exposure control periods. The reduction of extra responses for IX-382 might suggest a reduction of "anxiety" level for this

animal. Prior to MEK this animal found it difficult to refrain from responding during the 2 minute delay interval and made many extra responses. In a previous study with rats in a simple discrimination paradigm, we reported a reduction of extra responses and a concomitant improvement of performance following administration of anti-anxiety agents (Geller, Hartmann & Blum, 1971). These findings lend support to the speculation that the effects of MEK on IX-382 may reflect, in part, reduced "anxiety".

Chronic exposure of animals to MIBK at 25, 35, 50 and 75 ppm did not impair the match-to-sample discrimination task. However, a change in extra responses during the delay interval was observed.

(INSERT FIGURE 7 HERE)

In figure 7 are shown the effects of 50 ppm of MIBK over a seven day period. The extra responses have been compartmentalized into ten second intervals so that one might determine where responses occurred during the 2 minute delay interval. The solid lines represent the MIBK exposure data and the broken lines show control data which were obtained on the same day one week before exposure. Each point on the graph represents the total number of extra responses made for all delay intervals for the indicated ten second interval. Under MIBK this baboon found it more difficult to wait during the delay interval. Extra responses increased on all of the days that the operant tests were conducted. An increase in the concentration of MIBK to 75 ppm for an additional 48 hours did not increase the effect further.

The results of these studies show that with operant techniques one may observe alterations in behavior with relatively low doses of volatile substances. MEK and MIBK at the doses studied did not impair a baboon's

ability to discriminate or remember stimuli. However, extra responses during the delay interval increased or decreased during chronic exposure to the ketones. MEK at 20 ppm reduced extra responses in baboon 382 while MIBK at 50 ppm increased extra responses in baboon 380. Increases or decreases in extra responses might be an indicator of alterations in the animal's anxiety levels.

It should be stressed that these limited data, although extremely interesting, are very preliminary. Therefore, any definite conclusions must await further in depth studies of more animals over a more extensive dose range of the contaminants.

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LEGEND OF FIGURES

Figure 1: NASA Rat Exposure Chamber.

Figure 2: Effect of 25 ppm MEK on Variable-Interval Response Rate of Rat 1-75-9.

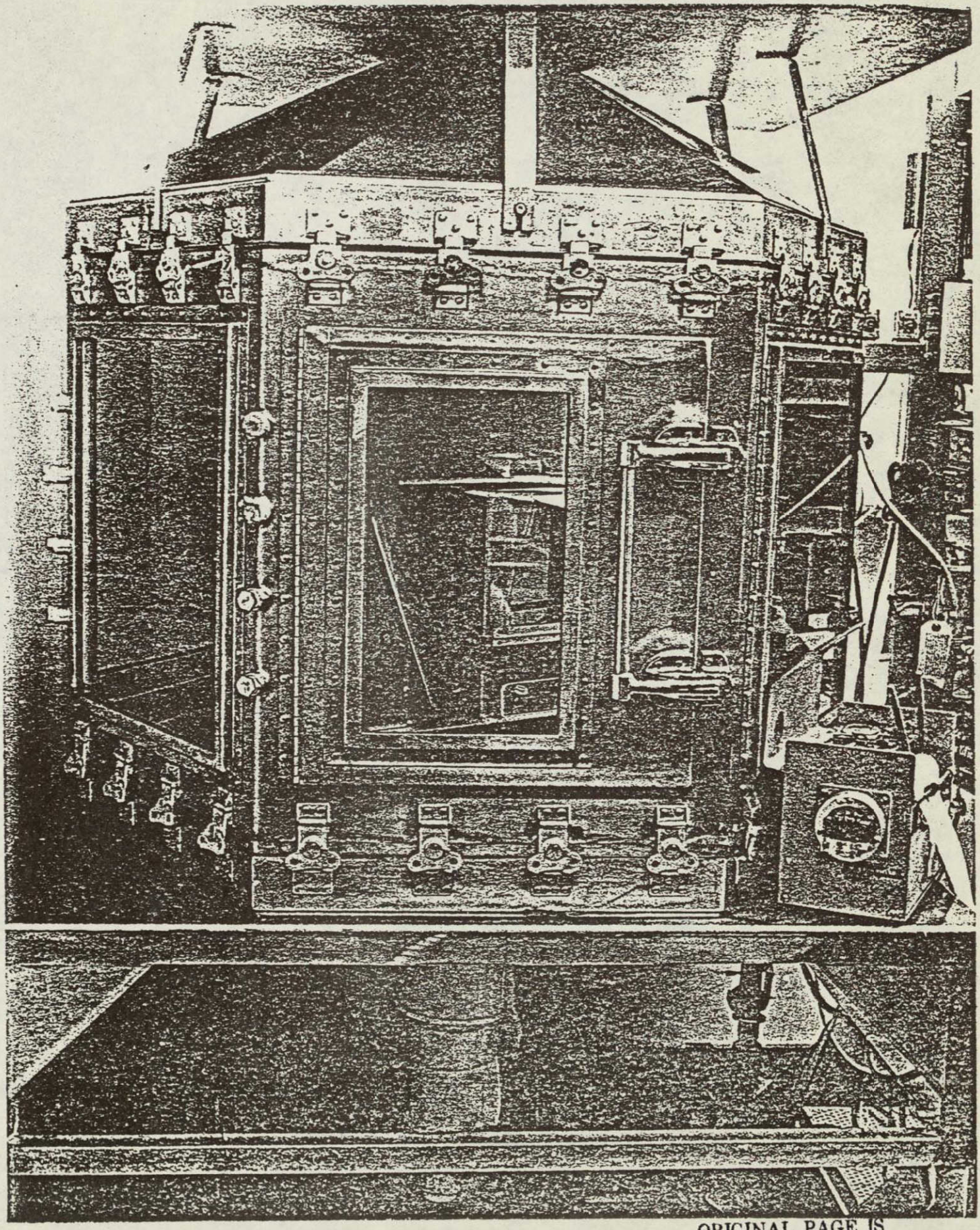
Figure 3: Effect of 25 ppm MIBK on Variable-Interval Response Rate of Rat 1-75-9.

Figure 4: Effect of 50 ppm Acetone on Variable-Interval Response Rate of Rat 1-75-10.

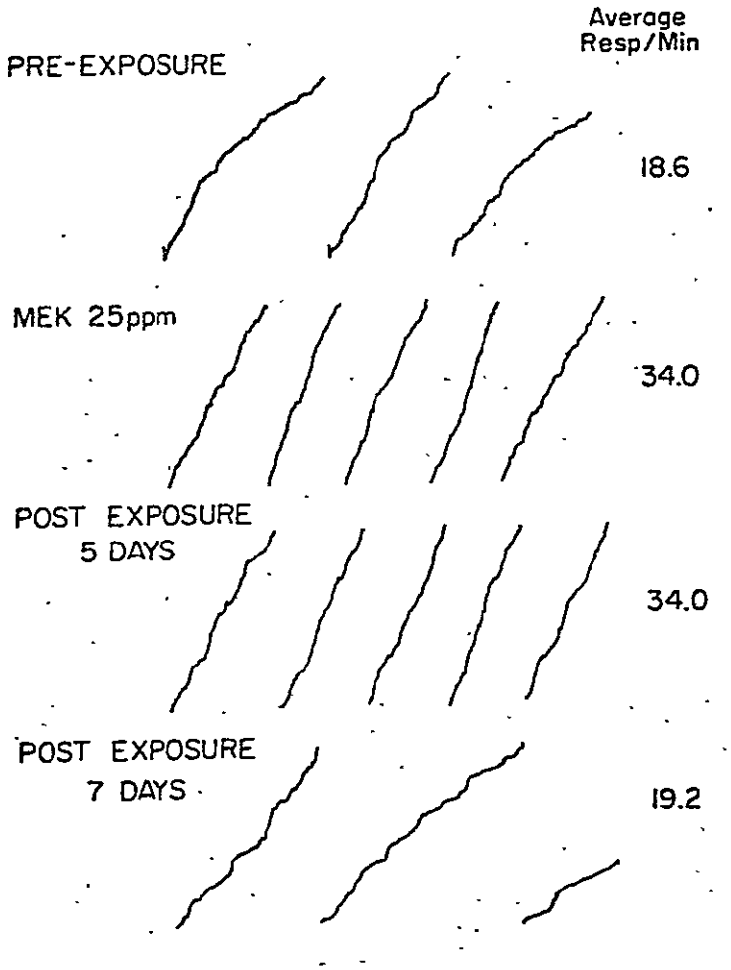
Figure 5: Baboon Exposure Chambers with Air Lock.

Figure 6: Average Responses/Minute During Delay Intervals for Baboons IX-382 and IX-529. (Each bar represents extra responses averaged for five, two hour sessions.)

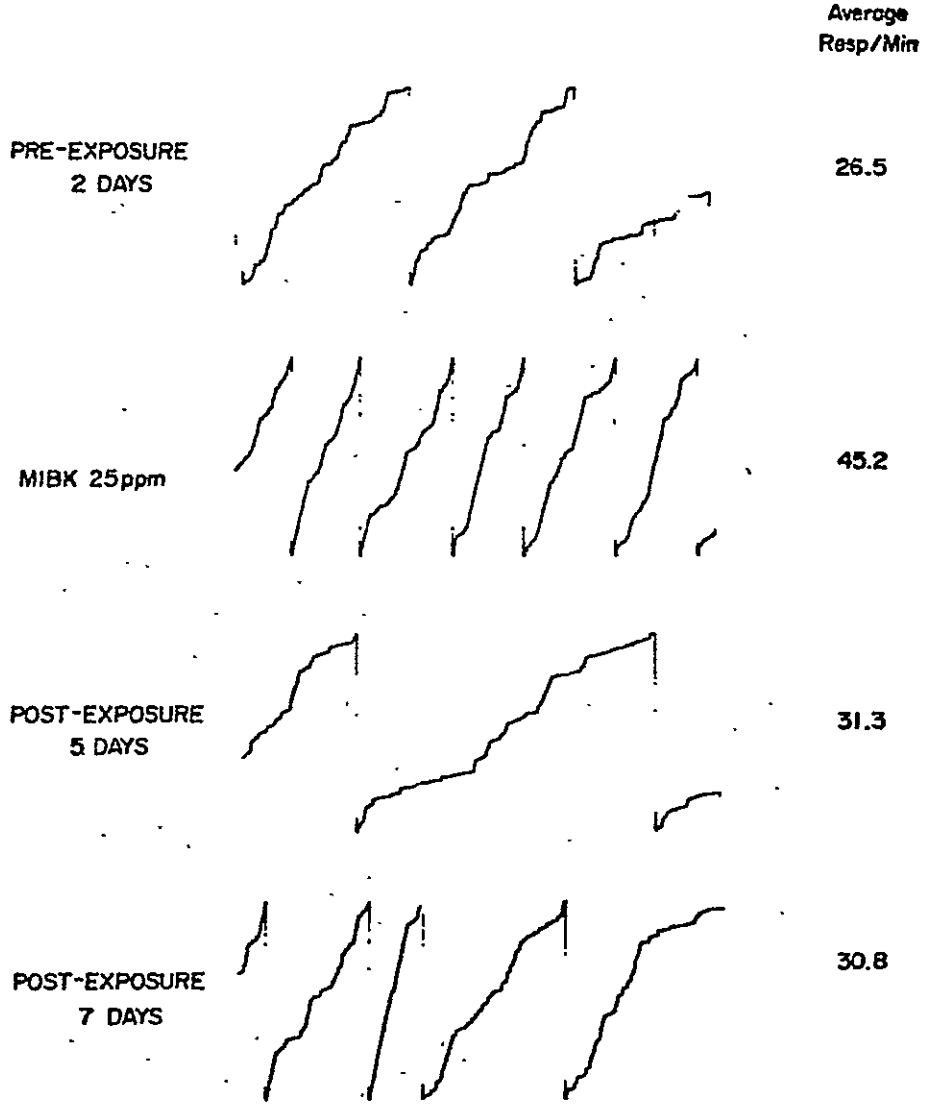
Figure 7: Effect of 50 ppm MIBK Over a 7 Day Period in the Baboon. (Solid lines represent MIBK data and broken lines control data obtained on the same day one week before exposure. Each point on the graph represents the total number of extra responses made for all delay periods during the indicated 10 second interval.)



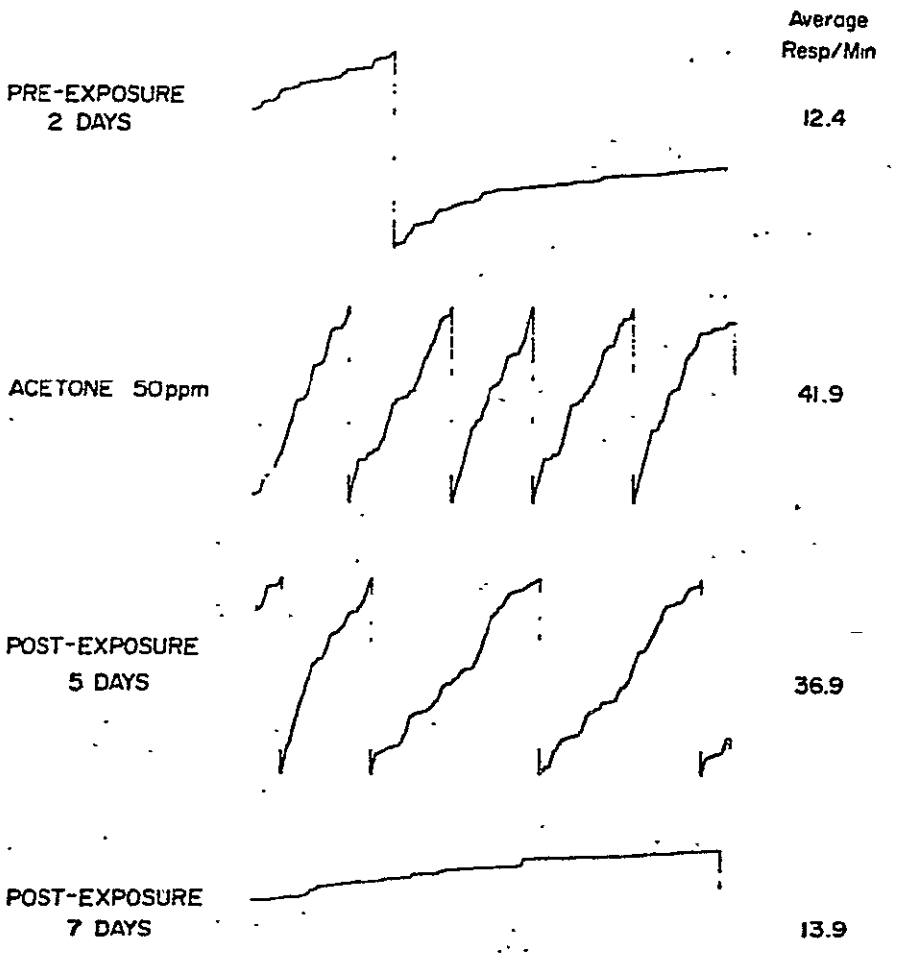
RAT 1-75-9


Average
Resp/Min
PRE-EXPOSURE
18.6
MEK 25ppm
34.0
POST EXPOSURE
5 DAYS
34.0
POST EXPOSURE
7 DAYS
19.2

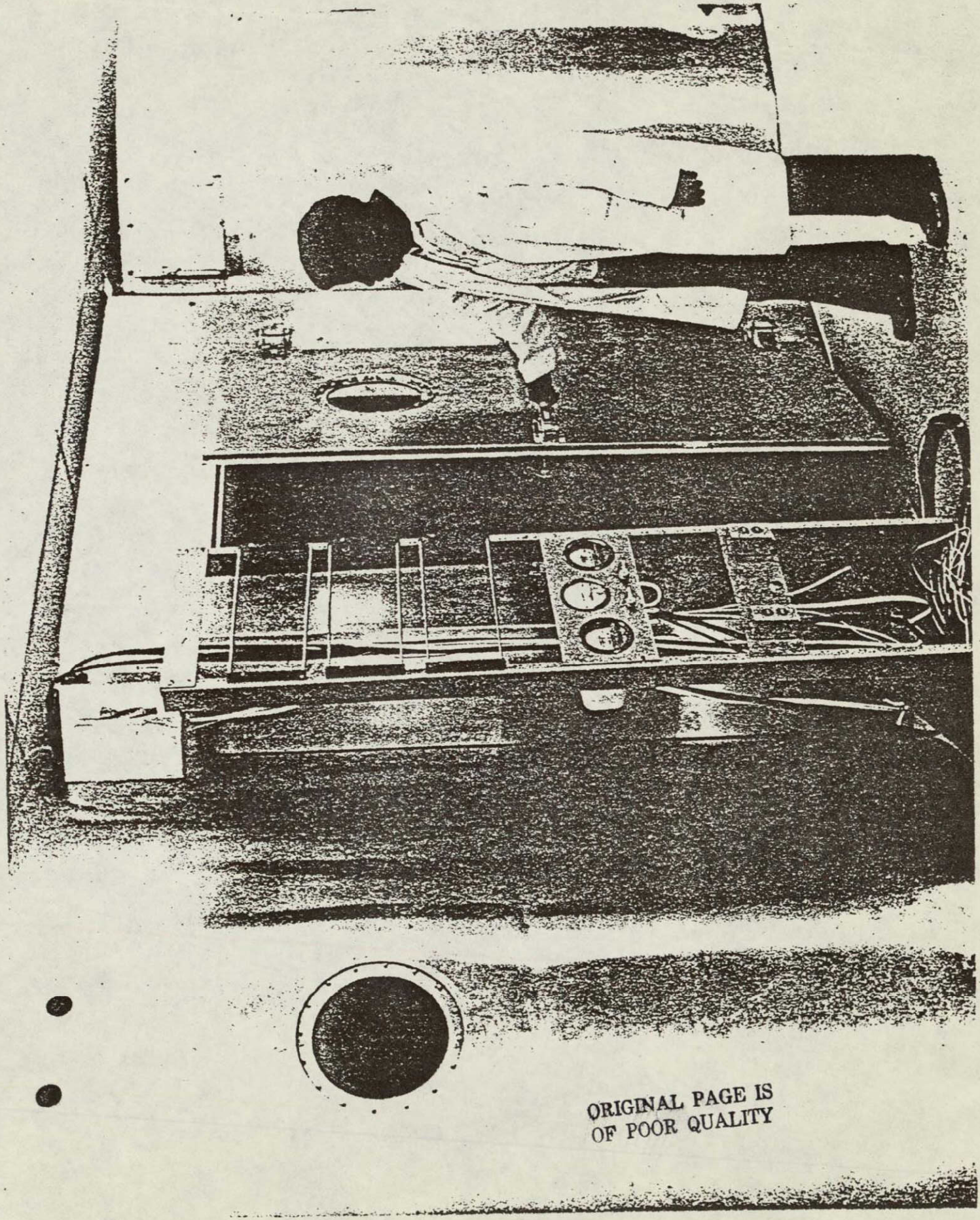
RAT I-75-9

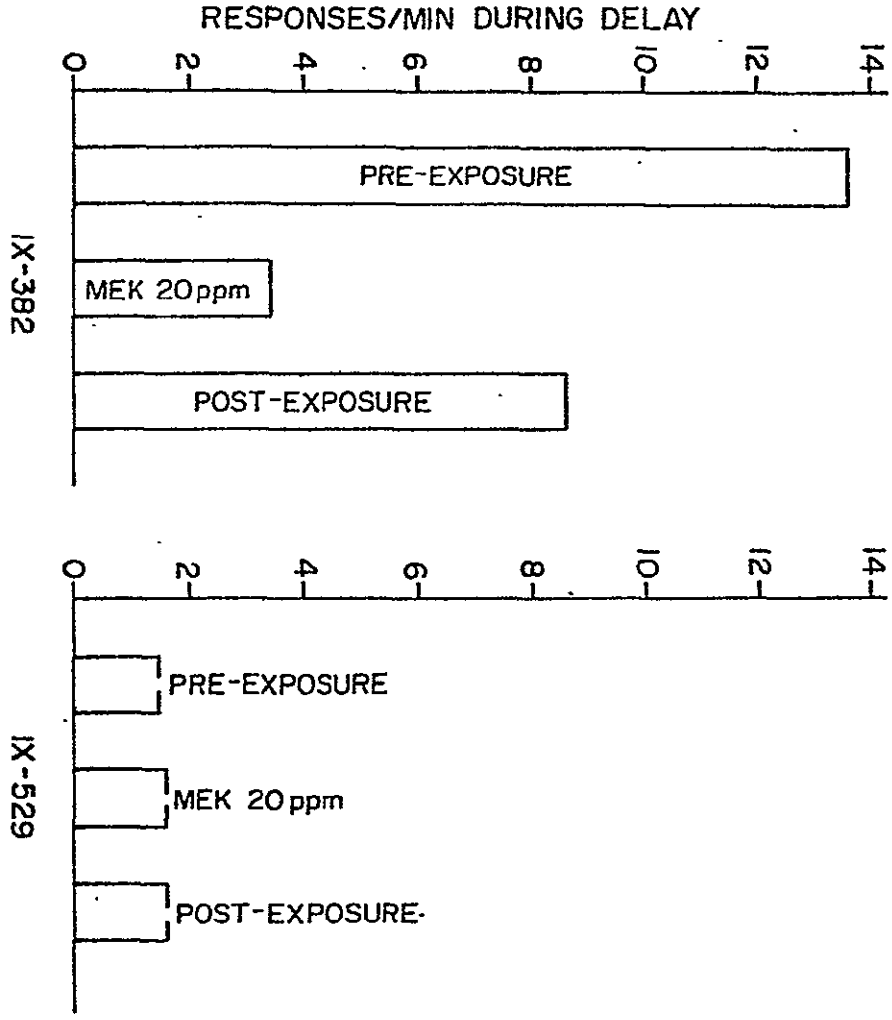


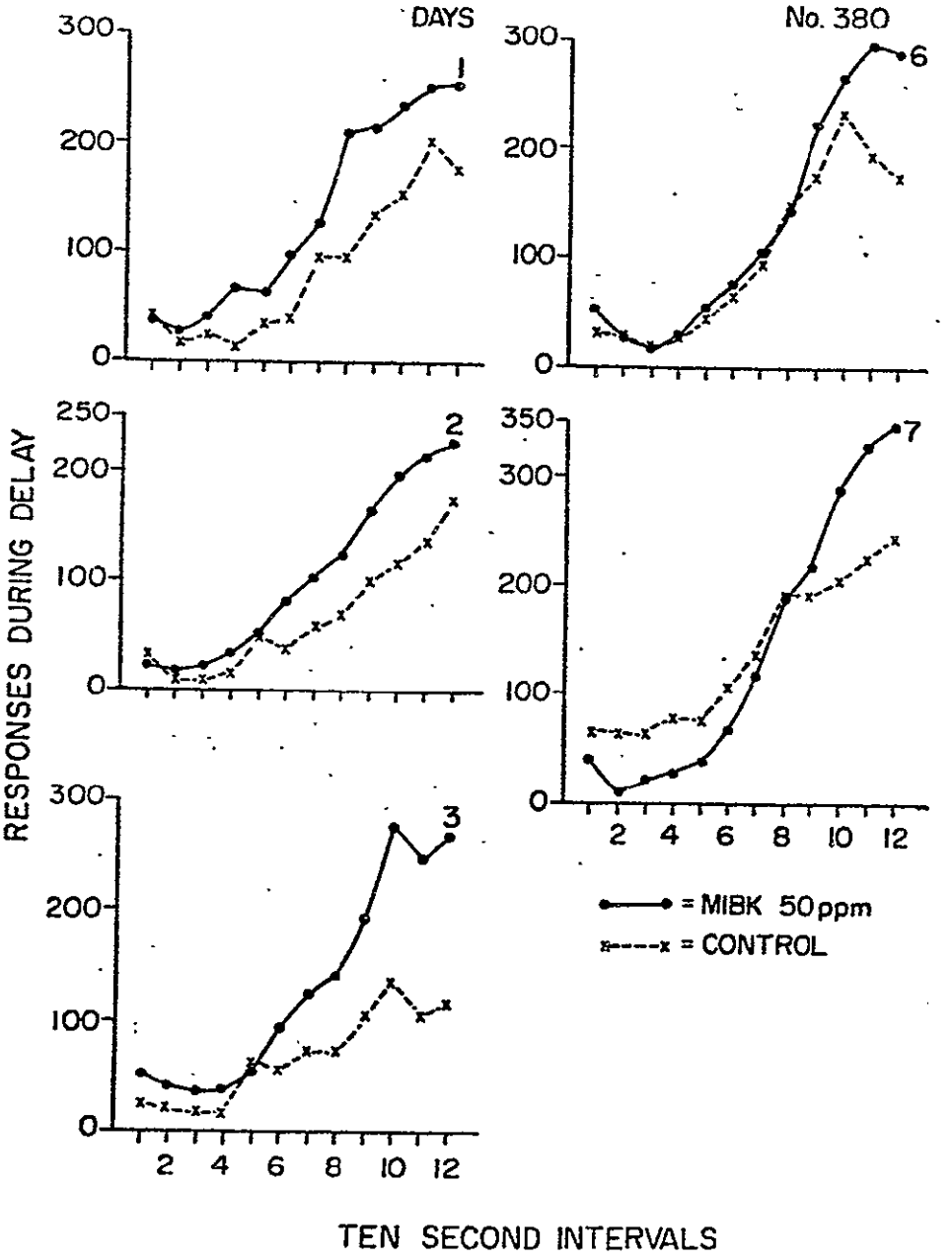
Effect of 25ppm MIBK on Variable-Interval Response Rate of the Rat



Effect of 50ppm Acetone on Variable-Interval Response Rate of the Rat




RESPONSES/MIN DURING DELAY
0
2
4
6
8
10
12
14
PRE-EXPOSURE
MEK 20ppm
POST-EXPOSURE
IX-382
0
2
4
6
8
10
12
14
PRE-EXPOSURE
MEK 20ppm
POST-EXPOSURE
IX-529


DAYS
No. 380
1
2
3
6
7
300
200
100
0
250
350
RESPONSES DURING DELAY
2 4 6 8 10 12
TEN SECOND INTERVALS
= MIBK 50ppm
= CONTROL

Effect of 25 ppm Methyl Ethyl Ketone on Variable Interval Response Rates

During Six Hour Experimental Session

AVERAGE RESPONSES/MINUTE

RATS	Pre-Exposure	MEK	Days Post-Exposure						
			2	3	5	6	7	11	16
4	18.04	25.45	18.71						
9	18.60	34.14			34.43		19.18		
10	13.07	18.33		18.47		13.82			
11	12.17	61.1			17.47		27.96	31.96	8.49