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Effects of Acetone, Methyl Ethyl Ketone and Methyl Isobutyl Ketone on a Match-to-Sample Task in the Baboon[1,2]

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GELLER, I., E. GAUSE, H. KAPLAN AND R. J. HARTMANN. *Effects of acetone, methyl ethyl ketone and methyl isobutyl ketone on a match-to-sample task in the baboon.* PHARMAC. BIOCHEM. BEHAV. 11(4) 401–406, 1979.—Acetone, methyl ethyl ketone (MEK) and methyl isobutyl ketone (MIBK) were evaluated for effects on a delayed match-to-sample discrimination task in the juvenile baboon. The animals were exposed to 1/2 the threshold limit value (TLV) of each gas for 24 hr per day during a 7-day period. They were also exposed to a combination of MEK and MIBK at the same exposure concentrations. Each exposure condition affected accuracy of performance minimally but resulted in increased and decreased extra responses during the delay intervals. Response times were slowed under acetone, MEK or MIBK. In contrast to the effects of the individual gases, exposure to a combination of the same doses of MEK and MIBK produced a consistent increase in extra responses during delay and a concomitant decrease in response times. Changes in tissue uptake and metabolism are suggested as possible mechanisms to explain this observation.

Ketones Acetone Methyl ethyl ketone Methyl isobutyl ketone
Delayed match-to-sample task Juvenile baboon

INHALATION of high concentrations of organic solvent vapors produces a state of euphoria, a property which has led to the widespread, abusive inhalation of any mixture of solvents easily available (glue-sniffing, paint-sniffing). This practice frequently has tragic consequences for both the individuals involved and for society at large due to the mental and emotional derangement produced.

Solvents abused through inhalation include acetone, methyl ethyl ketone (MEK) and methyl isobutyl ketone (MIBK), members of the ketone family. These vapors have also been found to be contaminants of spacecraft atmospheres [3]. Laboratory studies of such compounds have generally been limited to traditional studies of extremes in toxicity [2,11]. Zakhari *et al.* [15], in their review of the MEK literature, suggested that values derived from animal experiments might be too high to serve as estimates for effects in man since the doses required to produce effects in animals were higher than those required for human studies.

The literature reviewed by these investigators did not include studies which employed operant technology. Operant behavior techniques are extremely sensitive and in many instances one may detect behavioral toxic effects that may not be evident through gross observational methods. Operant discrimination tasks are useful in that they lend themselves to the simultaneous measurement of a number of CNS mediated functions. For example, the delayed match-to-sample discrimination task measures associative learning, visual reproduction (short-term memory), similarities or dissimilarities in stimuli, psychomotor function and response or reaction time. In the present study a match-to-sample operant discrimination task was used to compare the central nervous system (CNS) effects of acetone, MEK and MIBK when baboons were exposed to 1/2 the threshold limit value (TLV) [14]. Animals were also exposed to a combination of two ketones (MEK and MIBK) at the same concentrations in order to delineate possible additive, more than or less than additive effects.

METHOD

Two large stainless steel chambers equipped with a walk-in air lock were used to conduct the exposures. Four juvenile male baboons, approximately 2 years old, were housed in behavioral test chambers which were maintained in the large exposure chambers. The behavioral test chambers were designed so that an intelligence panel could be slipped down between the outside wall of the cage and the baboon. The intelligence panel was equipped with a row of three translu-

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TABLE 1

FIVE DAY AVERAGES OF PERCENT CORRECT RESPONSES OBTAINED PRIOR TO (C) AND DURING (E) 7-DAY EXPOSURES

Ketones	100 PPM MEK		50 PPM MIBK		500 PPM Acetone		100 PPM MEK/ 50 PPM MIBK	
Baboon	C	E	C	E	C	E	C	E
529	100	96.0	100	98.5	99	98.4	100.0	99.5
382	100	99.0	100	100.0	100	96.0	100.0	99.5
380	100	99.0	100	96.5	100	97.5	99.0	97.5
531	100	98.5	100	94.0	100	98.0	99.5	100.0

cent discs which served as levers. Under the appropriate experimental conditions, pressing either disc produced a banana pellet reward. Experimental sessions of 2 hr duration were conducted on Monday through Friday of each week.

When the session timer was activated, a variable interval (VI) programming tape was set in motion. The tape programmed the occurrence of a stimulus on the center lever on the average of one every 3 min. The VI tape was inoperative during each trial which began with the illumination of one of the stimuli on the center lever or probe stimulus. This stimulus was terminated at the end of a 30-sec period or by a response on the lever. Termination of the stimulus activated a timer for 2 min, called the delay interval. At the end of the delay interval, stimuli appeared on both levers adjacent to the center lever. The correct matching stimulus was varied between these two levers in a mixed order. A response on the correct lever, when the stimulus matched the center lever stimulus, terminated the stimuli, activated the feeder and produced a banana pellet reward. Responses on the incorrect lever 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-min segment of a 2-hr session, the number of correct matching responses on the left and right levers and the number of incorrect responses on these levers. A record was also kept of any extra responses that may have occurred on the three levers when the stimuli were not activated or during the delay interval. The time it took the subject to respond with a lever press after a stimulus was activated was also measured (response time). After the baboons were trained to 90–100% efficiency on the discrimination task, they were exposed to acetone, methyl ethyl ketone (MEK) or methyl isobutyl ketone (MIBK). Exposure was by means of the vapor saturation technique [4]. For the vapor saturation method, air is 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 with the fine metering valve or changing the temperature of the constant temperature bath allows one to produce a range of pollutant concentrations in the exposure chamber. The technique is simple and works well for substances that are liquids at room temperature. A Hewlett-Packard gas chromatograph, modified for automatic sampling, was employed. This allowed for automatic sampling, quantitation and recording of pollutant concentrations in the exposure chamber.

Exposures were of 7 days duration. Animals were exposed to 100 ppm MEK, 50 ppm MIBK, 500 ppm acetone and a combination of 100 ppm MEK and 50 ppm MIBK. At least 1 month elapsed between each of the above exposures. While two subjects in one of the chambers were being exposed to a contaminant atmosphere, the animals in the other chamber served as controls and were exposed to clean air during the same period. Thus, not only did other animals serve as controls, but each animal was able to serve as its own control, in that exposure data could be compared with data obtained from pre- and postexposure time. Behavioral testing was conducted for a 2-hr period in the morning for one animal and in the afternoon for one animal.

RESULTS

Table 1 shows average percent correct responses for 5 work days of each 7-day exposure (E) and for 5 work days of the control week (C) prior to the 7-day exposure week. Practically no errors occurred during control sessions while at most, only one or two errors occurred during gas exposures. The ketones produced minimal effects on the accuracy of the discrimination.

Figure 1 shows the number of extra responses made by each animal during the delay intervals as a function of days of exposure to ketone vapors. The data expressed as percent of control (C) were derived by comparing daily averages during exposure periods with a weekly average of five daily sham-exposure (control) sessions conducted during the week immediately preceding each ketone exposure. Exposures were initiated on Wednesdays and were continuous through the next Tuesday; behavioral test sessions were conducted on Wednesday, Thursday and Friday and the following Monday and Tuesday. During exposure to 500 ppm acetone, two of the four baboons (529 and 380) showed marked changes in the number of extra responses made during exposure compared to their own baseline values; for baboon 380, extra responses increased by some 200–300% on all five exposure test sessions, while 529 showed an increase in extra responses above control levels for the first 2 days of exposure and a consistent decrease below control range for the 3rd, 6th and 7th days. For the other two animals, one (382) exhibited a decrease below control ranges for three of the five exposure test sessions, while for the other 2 days, extra responses were within the low extremes of control ranges; extra responses made by baboon 531 were outside control ranges for only one test session, the 7th day of exposure.

Exposure to 100 ppm MEK had no effect on the number of extra responses made by Baboon 531 on any of the 5 test

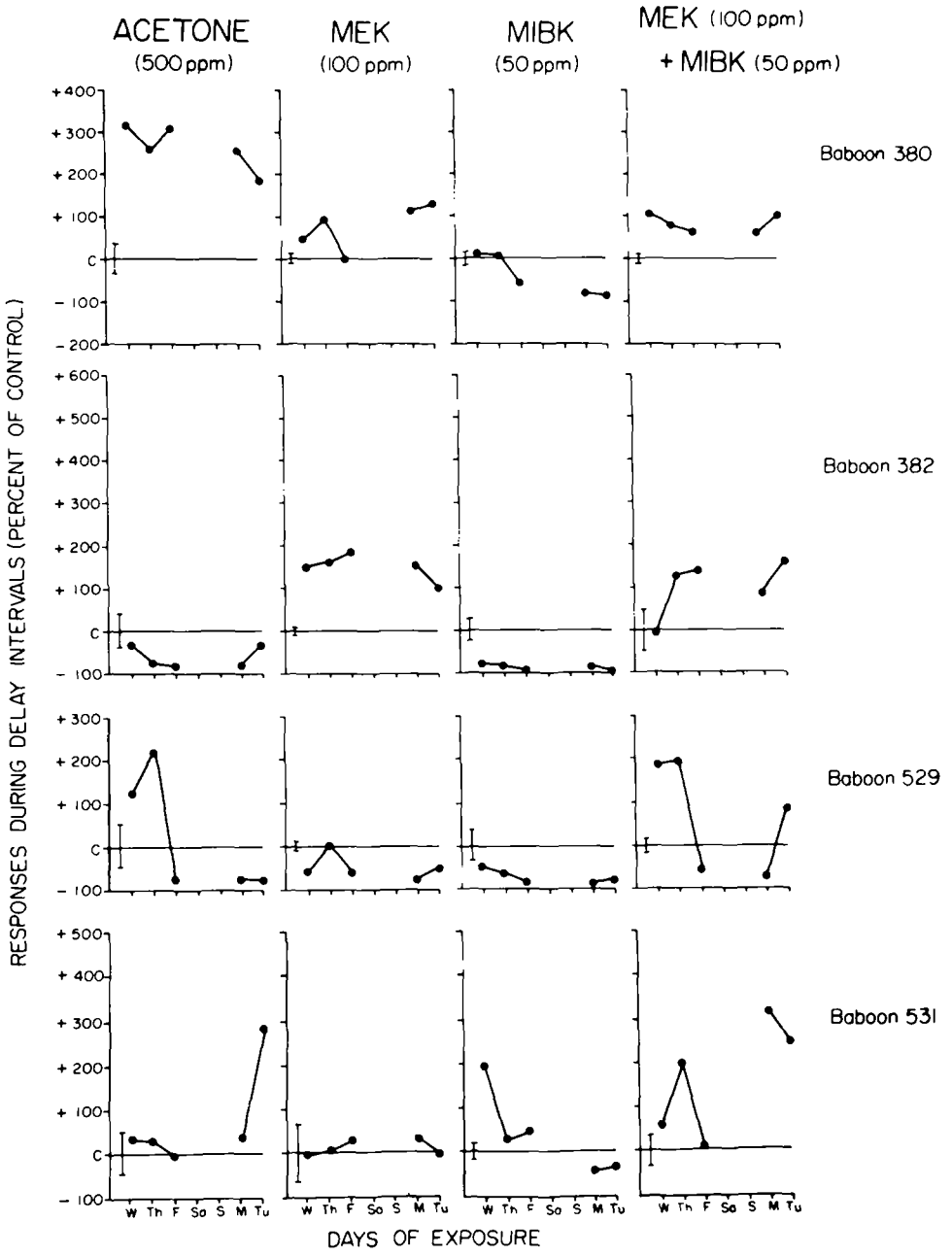

FIG. 1. Extra responses during delay interval as a function of 7 days of exposure to ketone vapors. Data are expressed as percent of control. [Control lines represent averages of 5 daily sham-exposure (control) sessions conducted during the week immediately preceding the ketone exposure. Brackets indicate ± SE.]

days; Baboon 382, on the other hand, showed marked increases in the number of extra responses on all 5 test days, and Baboon 380 on 4 of 5 test days; in contrast, Baboon 529 showed significant decreases in number of extra responses on 4 of the 5 test days. Under an atmosphere of 50 ppm MIBK, one animal (531) made an increased number of extra responses for the first 3 days of exposure and a decreased number for the last 2 days; two animals (382 and 529) had a decreased number of extra responses for all 5 test days, while the other baboon (380) exhibited extra responses within control range for the first 2 test days and a significant decrease in number of extra responses for the last 3 test days.

When the exposure atmosphere was MEK (100 ppm) combined with MIBK (50 ppm), the numbers of extra responses for two animals (380 and 529) were significantly different from control ranges on all five exposure test days, being increased for all five sessions for 380 and increased for

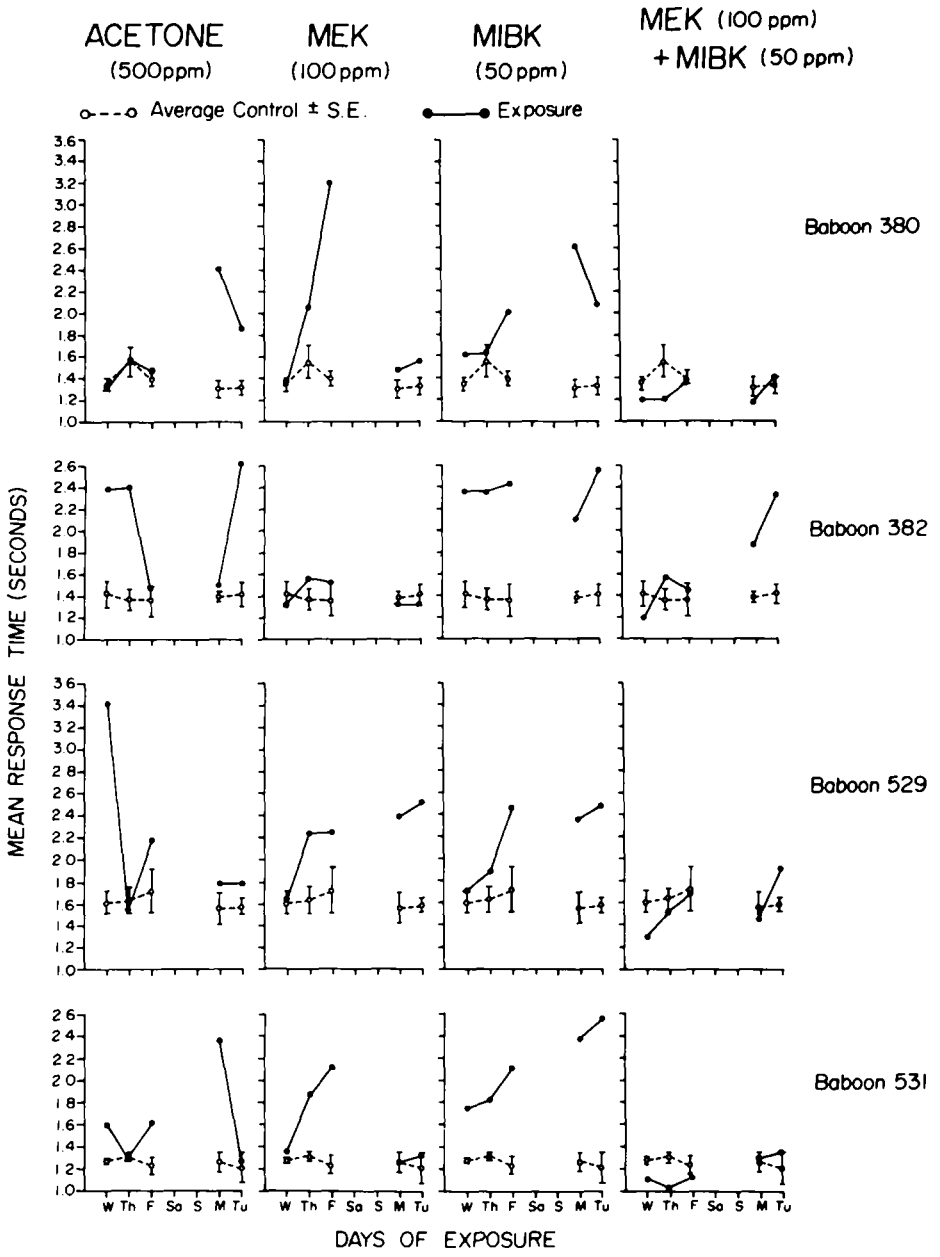

FIG. 2. Mean exposure times as a function of 7 days of exposure to ketone vapors. (Broken lines show control response times in seconds obtained by averaging data of control sessions conducted one week prior to each of 4 exposure sessions. Brackets indicate ± SE.)

three and decreased for two sessions for Baboon 529. The other two animals (531 and 382) had one test session each for which the extra responses were within control limits, but showed increased extra responses for the other four test sessions. All four baboons showed a significant increase in the number of extra responses for either all or the majority of their test sessions when exposed to the mixture of the two vapors.

Figure 2 shows the mean response time under each exposure condition. The broken line represents average control response time obtained by averaging the data of the control sessions conducted 1 week prior to each of four exposure sessions. The brackets represent standard errors. Each single ketone exposure produced an increase in response times. The maximal effect occurred for Baboon 529 on the 1st day of exposure to 500 ppm acetone and for Baboon 380 on the 3rd day of exposure to 100 ppm MEK. MIBK increased response time on every behavioral test day for all animals. MEK increased respone time on almost every exposure day except Day 6 and 7 for Baboons 531 and 382 and, except for Day 1, for Baboons 529 and 382.

Exposure to the combinations of 100 ppm MEK and 50

ppm MIBK yielded response times in control range on all but Days 6 and 7 of exposure for Baboon 382. Thus, the combined effects of MEK and MIBK were much less than additive.

DISCUSSION

Both MEK and MIBK have been considered to be relatively safe, i.e., they do not produce peripheral neuropathy as does methyl n-butyl ketone (MnBK) [13]. MEK is widely used as an industrial solvent for which the TLV concentration is 200 ppm; primary effects in this concentration range are considered to be irritation of nose, throat, and eyes and an objectionable odor [8] although low-grade intoxication has been reported to occur at concentrations of 300–600 ppm [12]. MIBK is also employed as an industrial solvent; it too has an objectionable odor even at its TLV concentration of 100 ppm, while twice the TLV, or 200 ppm, has been reported to cause eye and nose irritation, headache, dizziness and nausea [8]. Since both of these ketones are narcotic in higher concentration, we have undertaken to determine if exposure to atmospheric concentrations of one-half the TLV might be associated with low-level effects upon CNS function. For detection of acute, functional CNS effects, we have employed operant conditioning behavior in a primate (baboon). The operant behavior selected was a match-to-sample discrimination task and the experimental protocol allowed the performance of each baboon during inhalation exposure to be compared to his performances during a clean-air sham exposure in the same chamber immediately prior to each exposure.

The results of these studies have indicated that while one-half the TLV concentration of MEK or MIBK produced no significant effect upon accuracy of performance of the task, these low concentrations did produce consistent effects upon two other behavioral parameters: mean response time and response during delay. Both MEK (100 ppm) and MIBK (50 ppm) when given singly caused a slowing of response times for all four baboons on most or all of their exposure test sessions. This effect could be an early manifestation of the incoordination and narcosis which are observed at much higher concentrations [10]. This effect could also be related to the deficiencies in perception of time duration as a consequence of exposure to 90–270 ppm MEK for 4 hr [9]. In the experiments described herein, the effect upon response time occurred during the 1st day of exposure for all four animals exposed to MIBK (although one animal, Baboon 529, exhibited a value barely outside control range), but the slowing of response time was not observed until the second day of continuous exposure to MEK. This immediate effect of MIBK and the delay in effect of MEK could be associated with the differences in solubility of the two ketones; MEK is apparently soluble in water in all proportions while MIBK has limited solubility in water (1.91%) [15]. This difference in properties suggests that MIBK would reach a steady-state level in the blood rapidly and begin to accumulate in tissues while MEK concentrations in blood and other tissues would attain maximal steady-state levels much more slowly [1]

Of particular interest is the observation that although MEK or MIBK alone consistently produced a slowing of response time, when the same concentrations of the two vapors are combined, there was no effect upon response time (except for the last 2 days of exposure for one animal, Baboon 382). In fact, there is a suggestion of a negative effect or a shortening of response time. This combination of the two vapors also caused a consistent increase in the extra responses during delay for all four animals, although there was not a clear-cut pattern of effects upon this parameter produced by the single vapors. The increase in extra responses during the delay periods might be indicative of an activation effect which is compatible with the observed decrease in response times even beyond control levels. Increased responses during the delay periods probably reflect an alerting response. (The animals are getting ready for the matching stimuli and respond more rapidly when the stimuli are activated.) The mechanism for the disappearance of effect upon response time when the two vapors were combined is not known at this time; possible mechanisms include alterations in tissue uptake and in metabolism. For example, tissue uptake of either single agent may be markedly affected by one compound acting as a co-solvent for the other so that partition between hydrophilic and lipophilic compartments is altered; or the binding of either compound to specific receptor sites *in vivo* may be inhibited by the presence of the other compound. A special case of competition for receptor sites could involve the active site of drug-metabolizing enzymes such as microsomal monooxygenases or associated enzymes, leading to significant effects upon the character and quantity of metabolites produced; a related mechanism would involve induction, or de novo enzyme synthesis, by one compound. This last mechanism, alteration of metabolism to produce greater quantities of a neurotoxic metabolite, appears to be involved in the potentiation of MnBK peripheral neurotoxicity by simultaneous inhalation of MEK. Couri *et al.* [5] found that MEK caused increased metabolism of hexobarbital in rats (measured as sleep times), whether given alone or in combination with MnBK, but that MnBK itself did not affect barbiturate metabolism. The activities of certain hepatic drug-metabolizing enzymes, including aniline hydroxylase, aminopyrine demethylase, neoprontosil reductase and p-nitrobenzoate reductase, were also found to be elevated 2- to 3-fold by inhalation of MnBK-MEK mixtures. MnBK is metabolized to three compounds, one of which (2,5-hexanedione) is the neurotoxic species; and another of which (2-hexanol) is metabolically recycled to MnBK to be metabolized to more 2,5-hexanedione; enhanced metabolism in this case probably results in the production of more of the neuroactive 2,5-hexanedione before clearance through excretion [6,7].

The co-solvency mechanism mentioned above could be particularly significant for nervous system tissue where the barriers between nerve cells and the circulation—i.e., myelin and the blood-brain barrier—are highly lipophilic, causing preferential uptake of hydrophobic molecules, whereas penetration into the cytoplasm of nerve cell bodies would be facilitated by greater hydrophilic character of the molecule, or in the case of co-solvents, two different molecules forming a single phase.

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