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The Effect of Methyl *n*-Butyl Ketone on Response Rates of Rats Performing on a Multiple Schedule of Reinforcement

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Six rats were tested daily on a multiple fixed ratio 5 fixed interval 3-minute schedule of reinforcement. Saline (control) oral administrations were given during each week of testing except for 1 day per week on which methyl *n*-butyl ketone (MBK), an industrial solvent, was administered orally in concentrations of 68, 135, 270, or 406 mg/kg (representing 3, 5, 10, and 16%, respectively, of the oral LD_{50}). Each rat received each MBK concentration on three to six occasions over the course of the study. When compared to the preceding control (saline) day, fixed interval performance (response rate) after MBK administration was reduced by a mean of 20% at 68 mg/kg, 30% at 135 mg/kg, 40% at 270 mg/kg, and 57% at 406 mg/kg. All changes were statistically significant at $P \leq 0.03$.

INTRODUCTION

Methyl *n*-butyl ketone (MBK), or 2-hexanone, is used extensively in lacquers, adhesives, and various operations requiring solvents. In 1973, a number of workers in Columbus, Ohio, developed tingling, stiffness and reduced control of extremities, foot and wrist drop, paresthesias in hands or feet, and weight loss. Electrodiagnostic abnormalities included positive waves and fibrillations and reduced motor unit potentials in distal muscles. Ulnar, peroneal, tibial, and supral nerves had nerve conduction velocity considered abnormally slow. The symptoms were demonstrated to result from what has variously been called peripheral or distal polyneuropathy, or just peripheral neuropathy. Investigations showed that 22% of the workers in the industrial department where MBK had recently been introduced gave evidence of neurologic symptoms, but only 3% of the workers in all other departments of the company (where there was little or no MBK) had such symptoms (Allen *et al.*, 1975).

A number of experimental studies have reported on the neurological effects of MBK exposure. Peripheral neuropathy developed in chickens, rats, and cats within 3 months of exposure to 200–600 ppm of MBK for 24 hours a day, 7 days a week (Mendell *et al.*, 1974). Rats exposed to 1300 ppm of MBK for 4 months, 6 hours per day, 5 days per week also developed peripheral neuropathy (Spencer *et al.*, 1975). Spencer and Schaumburg (1975) produced a similar neuropathy with 2,5-hexanedione, a major metabolite of MBK. The possibility of 2,5-hexanedione as a common mechanism for MBK and *n*-hexane neuropathy has been suggested

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by Spencer *et al.* (1975). Common to the experimental neuropathies was giant axonal swelling (Spencer and Schaumburg, 1975).

Johnson *et al.* (in press) reported a reduced response rate in rats working on a multiple fixed ratio, fixed interval schedule of reinforcement within 2 weeks of exposure to 1000–1500 ppm of MBK for 6 hours per day, 5 days per week. Further, they reported that monkeys exposed to 100 ppm of MBK developed statistically significant decreases in mean conduction velocity in the sciatic–peroneal nerve after 8 months of 6 hours per day, 5 days per week exposure.

The LD_{50} for intraperitoneal (ip) and oral administrations of MBK in rats were 914 and 2590 mg/kg, respectively (Smyth *et al.*, 1954). Raleigh *et al.* (1975) reported that both cats and dogs survived twice-a-day subcutaneous injections, 5 days a week, of 150 mg/kg of MBK for 2 months. Rats receiving once daily injections of 340 mg/kg of 2,5-hexanedione also survived for 19 weeks. Animals in these studies developed peripheral neuropathy. However, there is no literature on the effects of short duration exposures to concentrations of MBK and only one study (Johnson *et al.*, in press) which utilized behavioral tests of laboratory exposed animals. The present study was designed to examine the effects of short duration exposures to low level concentrations of MBK on a behavioral test.

METHOD

Subjects. Six male Sprague–Dawley cesarean-derived albino rats obtained from Charles River Laboratories served as subjects. The rats were approximately 3 months old at the outset and 14 months old at the termination of the study. They were maintained at a weight of 300 (±10) g throughout the study. One rat died during the course of the study, resulting in an $N = 5$ at one concentration; the death could not be linked with an MBK administration, and overt clinical symptoms, such as foot dragging, were not observed in this animal prior to death.

Apparatus. Six standard operant test enclosures, approximately 33 × 35 × 30 cm, were used. A fan exhausted air from the chambers at approximately 1–2 air changes per minute, drawing in air from the laboratory. This fan and the amplified output of a Grason–Stadler[2] 455C noise generator provided 73 db white noise which served to mask external stimuli. The test enclosure included a panel on which a Gerbrands rat bar and a 45-mg feeder were mounted. Noyes 45-mg rat pellets were delivered as reinforcements into a food cup to the right of the bar. Two 28-V dome-shaped lamps, one red and one yellow, were mounted side by side, 8 cm above the bar. The center-to-center distance between the two 1.2-cm-diameter lamps was 9 cm. A PDP-12 (Digital Equipment Corporation) computer using SKED software (State Systems) served to control the schedules and record inter-response times (IRTs). Technical Grade MBK (97% pure by chromatographic analysis) was obtained from the Eastman Kodak Company and introduced into the stomach of the rats through an 18-gauge, 8-cm-long feeding needle attached to a 0.25-ml glass Luer-Lok syringe. The syringe could be read with an accuracy estimated to be ±5% at the lowest concentration.

Procedure. The rats were trained on a multiple fixed ratio 5 fixed interval 3-minute (mult FR5 FI3′) schedule of reinforcement (Ferster and Skinner, 1957).

[2] Mention of a company or product name does not constitute endorsement by NIOSH.

In a mult FR5 FI3′ schedule, there are two totally separate component schedules which occur successively during the test session. Under the FR5 schedule, the subject received a reinforcement when he had made five responses on the manipulandum (bar). In the FI3′ schedule, the subject received a reinforcement following the first response made after 3 minutes had elapsed (responses made prior to the end of the 3 minutes had no effect). The two schedules alternated; a red light was associated with the FR component of the schedule and a yellow light (in a different location) was associated with the FI. IRTs were recorded from each component separately. Daily test sessions lasted for approximately 1 hour. The rats were weighed and fed immediately after each test session so that all food was consumed at least 20 hours prior to the test session on the following day.

After shaping the bar press response, the rats were given a period of 3 months of 5 days per week testing to allow full stabilization of the response rate on the two schedules with their associated stimuli (the lamps). Following the 3-month period, the rats were placed in a chamber with 1000 ppm of MBK for 6 hours on four separate occasions, several days apart. These airborne exposures were terminated when another experiment concluded and thus precluded further work with this mode of administration; it is mentioned here only in order to present the entire exposure history of the rats in the experiment. One additional month of stabilization on the schedule was then given. During the subsequent 6 months, four concentrations of MBK were administered orally through the feeding needle. The four concentrations of MBK were 406, 270, 135, and 68 mg/kg. The oral LD_{50} of 3.19 ml/kg (2590 mg/kg) is equivalent to 0.96 ml for 300-g rats. Thus, the MBK concentrations in the present study represented 16, 10, 5, and 2.5% of the oral LD_{50} for rats.

Design. The experimental design in this study was a baseline reversal (Sidman, 1960) in which each subject was used as his own control.[3] At approximately 15 minutes before the scheduled test run, each rat received one administration of either MBK (1 day of the week) or an equal quantity of saline (3 days of the week); on the other day, Monday, the rats did not receive any administration. MBK was given on either Wednesday or Thursday according to a randomized procedure in order to reduce the likelihood of a conditioned response developing; the day preceding MBK (on which saline had been administered) was used as the comparison (control) day against which the MBK administration was analyzed. Thus, each rat's individual performance on the day of MBK administration was compared to his own performance on the preceding day of saline administration. This may be distinguished from comparing performance of independent groups, each exposed to a different concentration level. Each animal received all four MBK concentrations from three to six times (replications) per concentration over the 6 months during which MBK was administered.[4]

[3] The baseline reversal design and its many variations have been developed, tested, and evaluated most extensively in the *Journal of the Experimental Analysis of Behavior* since 1958. This design is typical of designs used in the pharmacologic evaluation of drugs against behavioral baselines as can be seen over the past 10 years in the *Journal of Pharmacology and Experimental Therapeutics*. The design is not often seen in the evaluation of toxic chemicals, although it is seen on occasion [e.g., Evans *et al.* (1975), reporting methylmercury research].

[4] The order of concentrations, chosen pragmatically, was as follows: 0.05, 0.10, 0.10, 0.10, 0.10, 0.15, 0.05, 0.05, 0.15, 0.10, 0.10, (one animal dies), 0.05, 0.025, 0.05, 0.025, 0.15, 0.025, 0.025.

RESULTS AND DISCUSSION

There were no permanent, overt clinical signs of weakness or foot drop in any of the rats during the course of the study. After 406 mg/kg doses, some rats displayed sluggish movements and unsteadiness when placed in the test apparatus. These changes disappeared by the next day. Thus, the behavioral effects reported in the subsequent paragraphs occurred prior to the development of any permanent, overt clinical symptoms.

Response rate from the FI component of a multiple schedule is a sensitive indicator of chemical effects (Thompson and Boren, 1977). The nature of the FI schedule is such that large increases in rate can occur (i.e., faster responding), as can sizeable decreases in rate (i.e., slower responding). In the present study, the overall number of responses for each session was converted into responses per minute (the response rate). The response rate in the session in which MBK was administered was then compared to the response rate on the preceding (saline) session. Over the course of the study, the frequency of a decrease in rate after MBK administrations (compared to the control day) was 67% after 68 and 135 mg/kg, 87% after 270 mg/kg, and 86% after 406 mg/kg.

The mean response rate at each MBK concentration (i.e., mean of three to six sessions) and the mean rate from the three to six prior-day control sessions for each rat, from each level of MBK, were determined and treated as an overall estimate of each rat's performance at each concentration, with its respective control. The mean MBK response rate of every rat was below the respective mean control rate at all four MBK concentrations; the differences were compared by the Walsh test (Siegel, 1956). The difference in rates was significant by a one-tail test at $P = 0.03$ for 68 mg/kg ($N=5$ rats) and at $P = 0.02$ ($N=6$) for 135, 270, and 406 mg/kg concentrations. The probability levels of 0.02 and 0.03 are basically limited by the small N of 5 (68 mg/kg) and 6 (other three dose levels). These data do establish that single administrations of MBK as low as 2.5% of the LD_{50} can cause behavioral changes in rats.

The mean decrease in rate at each concentration was converted to percentage change from control and then summed across all animals. The mean percentage reduction in response rate was 20% at 68 mg/kg of MBK, 30% at 135 mg/kg, 40% at 270 mg/kg, and 57% at 406 mg/kg. This can be seen in Fig. 1. The percentage rate change for each individual animal is shown in Fig. 2. Three animals, rats 0, 1, and 2, show the general trend presented in Fig. 1; two animals, rats 3 and 4, show consistent, but slight, effects at concentrations below 406 mg/kg and then show an increase at 406 mg/kg. The point for rat 5 at the 68 mg/kg concentration is substantially affected by one session of very slow performance, which helps to explain the shape of the curve. It is important to note the individual differences in reactivity to the various levels of the MBK. At the larger concentrations, some animals were mildly affected (rats 3 and 5), while other animals were nearly incapacitated (rats 0 and 2). At the highest MBK level, the least affected was rat 3, which had a 15% reduction, while rat 0 stopped responding entirely. The statistically significant results noted in the preceding paragraph should not mask the fact of large individual differences in reactivity to MBK.

After a long series of MBK administrations, as in this experiment, it is possible that the MBK effects could accumulate and cause a general deterioration of per-

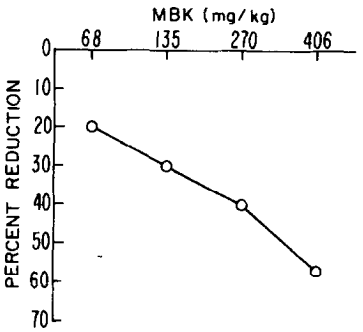

FIG. 1. Mean percentage reduction in FI responses per minute of all rats at each MBK concentration (milligrams per kilogram).

formance over time. It is also possible that a progressive sensitization to the chemical could develop, or quite the reverse, a gradual tolerance to the chemical could occur, as is often seen with drugs. In the present experiment, there was no general deterioration of performance over the 6-month course of the administrations; the performance remained stable on saline days over the course of the study. The data from the experiment do, however, tend to support the possibility of a gradual development of tolerance. A comparison was made of the percentage decrease in response rate on the first administration at a given MBK concentration as compared to the final administration at that concentration. In the case of 406 and 270 mg/kg, these administrations were 3 months apart, and in the case of 135 mg/kg, the administrations were 4 months apart. The 68 mg/kg concentration was not included in the evaluations because the first and last administrations were only 1 month apart and occurred toward the end of the study. At the 406 mg/kg concentration, four rats had a reduced (percentage) decrement on the final administration, one rat had a greater decrement, and one rat showed virtually no change. At

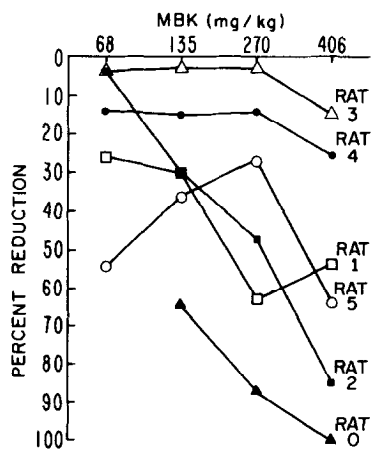

FIG. 2. Individual mean percentage reduction in FI responses per minute at each MBK concentration (milligrams per kilogram) for each rat.

270 mg/kg, two rats had a reduced effect, three rats had an increased effect, and one rat showed no change. At 135 mg/kg, all six rats had a reduced decrement on the final administration. Thus, over the three concentrations there were 13 reductions in effect, 7 increases, and 2 no changes. This suggests a development of tolerance. Since the present experiment was not specifically designed to evaluate the question of tolerance, the conclusion is only speculative.

The median IRTs in the FI component of the multiple schedule were compared. Median IRTs were longer on the day of MBK administration in all six rats at 406 and 270 mg/kg ($P = 0.02$ by one-tail Walsh test), in all five rats at 68 mg/kg ($P = 0.03$ by one-tail Walsh test), and in five of six rats at 135 mg/kg ($P = 0.03$ by one-tail Walsh test). There were three to six IRT medians at each MBK level for each rat. The mean of the medians is presented in Fig. 3 for each MBK concentration, for each rat. The dashed line on each section is the mean of all control IRT medians for that animal. The unusual shifts seen in overall rate in rat 5 at 68 mg/kg and rat 1 at 406 mg/kg were also present in the FI IRTs. These findings are consistent with the data on overall rate of response in FI presented above.

The median IRTs in the FR component of the multiple schedule were compared. There was an increase after MBK, as compared to the previous saline day, in all six animals at 406 and 270 mg/kg ($P = 0.02$ by one-tail Walsh test) and in five of the six animals at 135 mg/kg ($P = 0.03$ by one-tail Walsh test), but in only three of five

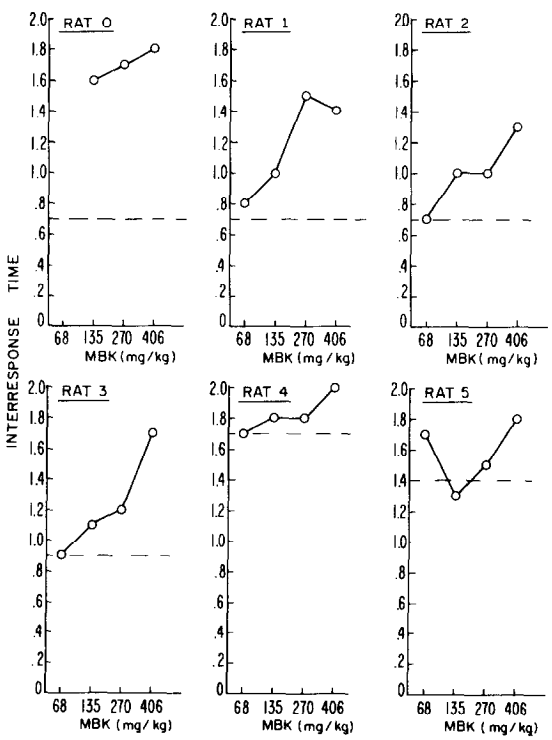

FIG. 3. Individual FI inter-response times of each rat at each MBK concentration (milligrams per kilogram). The dashed line represents the mean inter-response times from all control sessions.

animals at 68 mg/kg. The direction of these findings is consistent with the FI results; the unusual shifts in FI performance of rat 5 at 68 mg/kg and rat 1 at 406 mg/kg were also present in the FR results. The control performance in the FR component was relatively stable in all but rat 4.

A FR schedule invariably generates a higher overall rate of response than a FI schedule. On a multiple schedule, different stimuli are associated with the different component schedules and often, as in the present experiment, a set pattern of alternation of the schedules gives the animal additional cues as to which schedule is in force. Therefore, each animal responds consistently at different rates on the different schedules. Such was the case in the present experiment. A chemical may disrupt the discrimination between the two schedules, and the animals may respond at the same rate regardless of which schedule is in force. The rats in the present study responded at a higher rate under the FR component of the multiple schedule than under the FI component on 85% of the MBK days (excluding days on which a total cessation of responding occurred) as compared to 87% of the saline days. This suggests strongly that the MBK did not cause a major disruption in the rats' ability to distinguish between the schedules and respond appropriately.

Recent work by DiVincenzo *et al.* (1976) on the metabolism of MBK provides an approximate estimate of the MBK body burden in the present study. DiVincenzo *et al.* administered 450 mg/kg of MBK (0.56 ml/kg which approximated the highest dose in the present study of 0.50 ml/kg) intraperitoneally to nonfasted guinea pigs. The concentration of MBK in serum at 1 hour postinjection was 175 mg/liter and the half-life of MBK in serum was 78 minutes (rats in the present study were tested for 1 hour, from 15 to 75 minutes after the injection). This suggests that nearly half of the MBK could have been eliminated from the serum by the end of the daily test session in the present experiment.

In conclusion, response rate on an FI schedule demonstrated a statistically significant decrease of 20% at 68 mg/kg, 30% at 135 mg/kg, 40% at 270 mg/kg, and 57% at 406 mg/kg. All animals had decreases at every concentration, but there were considerable individual differences in the severity of the deficit. Both FI and FR IRTs were increased as a function of MBK concentration. All results occurred in animals which did not develop clinical signs of neuropathy during the course of the experiment.

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