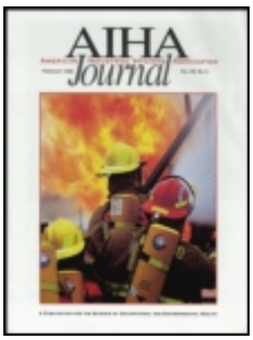

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An electrodiagnostic study of the neurotoxicity of methyl n-amyl ketone

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The neurotoxicity of methyl n-amyl ketone was investigated in a chronic inhalation study lasting 9 months. Rats and monkeys were exposed 6 hrs/day, 5 days/week, to mean MAK levels of 0, 131, and 1025 ppm. Electrodiagnostic measures of nervous system function revealed no neurotoxic impairment at either MAK exposure. Body weights were similarly unaffected. Gross and histopathology also indicated no adverse effects of MAK. It was concluded that MAK does not possess neurotoxic properties similar to those possessed by methyl n-butyl ketone.

An electrodiagnostic study of the neurotoxicity of methyl n-amyl ketone

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introduction

The ketone family of chemicals has found widespread use in such industrial applications as paint and varnish formulation, printing operations, and in the manufacture of vinyl and acrylic coatings and adhesives. The principal toxicological effects of the ketone solvents were summarized[1] as being irritation of the mucous membranes of the eyes and nasal passages, narcosis at sufficiently high exposure levels, loss of auditory and corneal reflexes, and depression of body temperature, respiration rate and heart rate. The primary route of toxic exposure is by inhalation of vapors,[2] though, skin contact with ketones can dissolve protective oily layers, leading to drying and fissuring of the affected areas. The result is reduced protection against bacterial infection.[2]

Although the narcotic properties of the ketones at high concentrations and the resulting depression of the central nervous system were recognized,[1] no investigations concerned with possible neurotoxic properties of the ketones at low exposure levels were reported until an industrial incident occurred in 1973 which involved methyl n-butyl ketone (2-hexanone) exposure. This episode led to an outbreak of distal polyneuropathy in workers exposed to methyl n-butyl ketone (MBK) while engaged in the manufacture of printed fabrics.[3,4] Although the affected workers were also exposed to two other ketone solvents, methyl ethyl ketone (2-butanone) and methyl isobutyl ketone (4-methyl-2-pentanone), subsequent laboratory studies utilizing experimental animals have shown that MBK is neurotoxic to laboratory animals.[5–7] Experimental investigations have shown the pathological consequences to the peripheral nervous system of MBK exposure to be nerve fiber degeneration in the distal regions of motor and sensory fibers located in the hindlimbs and to a lesser extent in the forelimbs.[7] Pathologic changes in the spinal cord, medulla oblongata, and cerebellum have also been reported.[7] The pattern of peripheral and central nerve fiber degeneration is characterized by multifocal swellings of the axon. Associated with this swelling are localized changes in the myelin sheath covering the axon, apparently caused by slippage away from the swollen regions of the axon.[6,7]

Electrophysiological studies[8] indicated that MBK exposures of 976 parts per million (ppm) (6 hrs per day, 5 days per week) decreased the maximum motor nerve conduction velocity (NCV) of sciatic-tibial and ulnar nerves in both monkeys and rats, decreased the amplitude of evoked muscle action potentials, increased the latency of visually evoked brain potentials in monkeys, and impaired performance of rats on a multiple schedule of reinforcement of bar

pressing behavior. Decrements in the motor NCV of the sciatic-tibial nerve was observed at a MBK level as low as 98 ppm (6 hrs/day, 5 days/week) following 9 months of exposure for monkeys and 7 months for rats.

Since MBK has clearly been identified as possessing neurotoxic properties, there is concern that other ketones may also possess similar unsuspected toxic properties. A major metabolite of MBK, 2,5-hexanedione, was identified[9] as causing peripheral neuropathy in experimental animals of several species. Based on this report, it was suggested[10] that two other ketones, methyl n-amyl ketone (2-heptanone) and ethyl n-butyl ketone (3-heptanone), might also be expected to oxidize to a 2,5-diketone, specifically, 2,5-heptanedione. Since the potential neurotoxicity of 2,5-heptanedione had not been studied, controlled inhalation toxicology studies were recommended[10] to investigate the possible neurotoxic effects of methyl n-amyl ketone (MAK) and ethyl n-butyl ketone.

A review of the literature revealed only two reports concerned with the possible neurotoxic properties of MAK. The first published study[1] that examined MAK toxicity was concerned with effects on guinea pigs of single high concentration (1500-4800 ppm) exposure. The results indicated that 1500 ppm MAK was irritating to the mucous membranes, 2000 ppm was strongly narcotic, and that 4800 ppm caused narcosis and death after 4 to 8 hours of exposure. Prolonged exposure to MAK caused varying degrees of irritation to the mucous membranes and depression of rectal temperature, respiratory rate, and heart rate.

A recent report[11] contains a brief description of an inhalation toxicology investigation using rats. The study was performed for the purpose of trying to identify the neurotoxic agent present in a commercial lacquer thinner that was responsible for inducing severe neurologic dysfunction in 7 persons who were "huffing" the thinner.[11,12] MAK was a new ingredient in the lacquer thinner thought to have induced the neuropathies; previous formulations by the same manufacturer apparently had not contained MAK.[11] Though details of experimental design are not given, it is stated that exposure of 10 rats for 2300 hours to 1300 mg/m^3 (278 ppm) MAK resulted in no clinical signs of neurologic impairment, nor were histologic changes noted in peripheral nerves.[11]

The purpose of the study described in this report was to investigate the possible neurotoxic properties of MAK through use of electrophysiological methods shown previously to be sensitive to the neurotoxic properties of MBK.[8]

methods and materials

exposure conditions

The two MAK target exposure levels chosen for animal exposure were 100 ppm and 1000 ppm. The 100 ppm level was selected to test the adequacy of the current Federal occupational exposure limit for MAK, which is 100 ppm. The 1000 ppm exposure was chosen in anticipation of yielding a demonstrable neuropathy if MAK was found to be neurotoxic. All exposures were 6 hours per day, 5 days per week, and lasted 9 months.

Technical grade MAK was vaporized, mixed with conditioned room air, and passed into 2 stainless steel 6.37 m^3 chambers. Gas chromatography showed the MAK to be 97 percent pure. The following impurities in the MAK supply were identified by GC/MS analysis: 4-methyl-2-pentanone, 2-hexanone, 5-methyl-2-hexanone, 2-nonanone, and 2-ethyl-1-hexanol, each of which represented 0.5 percent or less of the MAK supply. Technical grade MAK was chosen for animal exposure, rather than analytical grade, because worker exposure occurs predominantly with the former.

Each exposure chamber housed 8 monkeys and 50 rats. Control animals were housed in cages identical to those used in the 2 MAK-exposed groups. All cages possessed wire mesh floors in order to provide proper distribution of MAK vapor patterns within the exposure chambers. Chamber MAK concentrations were measured by gas chromatography using 5 liter or 10 liter samples for the 1000 ppm and 100 ppm exposure chambers, respectively, 2-3 times each day with adjustments made to maintain the chambers at planned levels. The average value for the 2-3 samples acquired each day was used to characterize that day's MAK exposure. The temperature and relative humidity of the exposure chambers ranged from 23-26°C and 40-50 percent, respectively, for the duration of the study.

experimental animals

Thirty albino male rats (Sprague-Dawley, Charles River Laboratories) and 24 male monkeys (Macaca fascicularis) were randomly assigned prior to MAK exposure to groups consisting of 10 rats and 8 monkeys for each exposure condition. The mean pre-exposure body weights for the three groups of rats were 272 gms (SD = 18), 259 gms (SD = 6), and 269 gms (SD = 14) for the 0, 100, and 1000 ppm MAK exposure levels, respectively. Corresponding pre-exposure mean body weights for the three groups of monkeys were 5.08 kg (SD = 1.20), 4.63 kg (SD = 1.31), and 4.65 kg (SD = 1.30).

tests of neurologic function

Animals to be neurologically tested were removed at monthly intervals according to a random schedule from the exposure chambers at the end of a day's MAK exposure and placed in a holding area for testing the following day. Testing was routinely conducted such that a minimum of 3 days of MAK exposure had occurred prior to neurologic examination. The typical elapsed time between removal from MAK exposure and neurological testing was 16 hours. All electrophysiological testing of the experimental animals was performed with the senior investigator, who conducted the testing, blind to the exposure history of each animal. The following measures were obtained from both rats and monkeys: maximum motor nerve conduction velocity (NCV) of the sciatic-tibial nerve, NCV of the ulnar nerve, and amplitude of evoked muscle action potentials (MAP) recorded from muscles in response to electrical stimulation of the sciatic and ulnar nerves. In addition, electroencephalograms and visually evoked potentials were recorded from monkeys. The details of the tests of neurologic function are identical to those previously reported.[8] All animals were administered an anesthetic prior to neurologic testing. Rats were given IP injections of 35 mg/kg sodium pentobarbital plus 160 mg/kg of chloral hydrate. Monkeys received 15 mg/kg ketamine hydrochloride intramuscularly.

statistical protocol

The basic configuration of this study falls into a class of statistical models known as "repeated measurements" designs. Each response variable (e.g., rat ulnar NCV) was measured on each animal at ten different equally spaced points in time, at one month intervals. The first measurement on each response variable was made after the animals had acclimated to the exposure chambers, but prior to commencement of MAK exposures. These pre-exposure measurements were used as covariates in the subsequent statistical analyses. The other nine measurements obtained at monthly intervals were treated as a multivariate response vector for each animal.

The alternative to this multivariate approach is to consider "time" to be a factor having nine levels and treat the problem as a univariate two-way analysis of covariance. Such an approach, however, makes it necessary to assume that the nine observations for a given animal over time are independent, or at least have a constant covariance. Therefore, the multivariate analysis of covariance (ANCOVA) was considered to be the more realistic approach. The Multivariate General Linear Model Macro (GLMM) addition to an existing Statistical Analysis System[13] procedure was used to perform the analysis. The use of this computer program made it possible to test for differences among treatment groups with respect to the vector of mean responses at the nine times. In addition, it was possible to test for differences due to the nine time periods, including a check for linear, quadratic, and cubic trends. A test was then made to determine if the time trends were similar for each treatment group (treatment by time interaction). In addition to the baseline covariate, the weight of the animals was also used as a covariate. Means were adjusted accordingly before the above tests were made whenever a covariate was significant. Using analysis of covariance, the treatment means were adjusted for a statistically significant covariate by mathematically removing any effect that weight gain or initial response had upon subsequent responses. This enabled treatment groups to be compared on an equal basis, regardless of possible differences in initial response or growth patterns in the animals. However, in order to gain nine degrees of freedom in the error term, the weight covariate was removed from the model whenever it was statistically nonsignificant.

TABLE I
ANCOVA Results for Neurologic Data

Measure	Species	F-value[a]	Degrees of Freedom	Prob.
1. Sciatic-tibial NCV	Monkey	1.43	2,20	0.26
2. Ulnar NCV	Monkey	0.15	2,21	0.86
3. MAP amplitude (sciatic stimulation)	Monkey	1.33	2,20	0.29
4. MAP amplitude (ulnar stimulation)	Monkey	1.68	2,20	0.21
5. Visually Evoked Potentials	Monkey			
a) RMS value		1.20	2,19	0.34
b) Latency of P_1		1.13	2,18	0.35
c) Latency of N_1		1.61	2,17	0.23
6. Electroencephalograms	Monkey			
a) Alpha activity		0.84	2,17	0.44
b) Beta activity		1.21	2,17	0.32
c) Delta activity		1.26	2,17	0.30
d) Theta activity		1.43	2,17	0.27
7. Sciatic-tibial NCV	Rat	0.31	2,26	0.74
8. Ulnar NCV	Rat	0.89	2,27	0.42
9. MAP amplitude (sciatic stimulation)	Rat	1.36	2,27	0.27
10. MAP amplitude (ulnar stimulation)	Rat	2.11	2,18	0.15

[a]F-value for group treatment effect.

results

Both rats and monkeys in the two MAK exposure groups were exposed for 9 months. The mean daily MAK levels for this period were calculated to be 131 ppm (SD = 30) and 1025 ppm (SD = 118) for the two exposure groups.

Findings from the ANCOVA analysis of all 15 neurologic measures are given in Table I, which lists each dependent variable, the F-value for group treatment effect, degrees of freedom, and probability level. A review of Table I shows that no dependent variable approached statistical

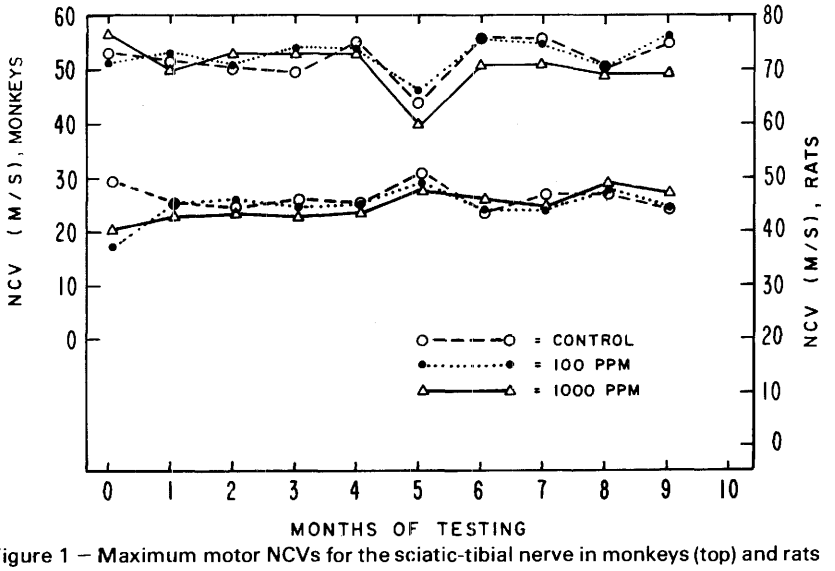

Figure 1 – Maximum motor NCVs for the sciatic-tibial nerve in monkeys (top) and rats (bottom) according to MAK exposure group.

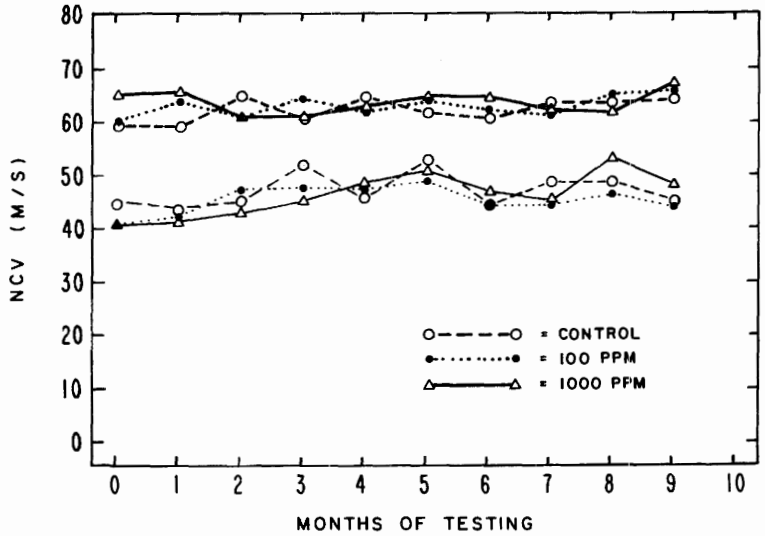

Figure 2 – Maximum motor NCVs for the ulnar nerve in monkeys (top) and rats (bottom) according to MAK exposure group.

significance ($p < 0.05$). In other words, neurologic data from the two MAK exposure groups did not significantly differ from the control group data for either monkeys or rats.

Figures 1 and 2 show the mean NCV data for the sciatic-tibial nerves and the ulnar nerve for both monkeys and rats. It should be mentioned that the mean values plotted in Figures 1 and 2 have been adjusted by the two covariates (i.e., pre-exposure value and body weight) used in the ANCOVA. Review of Figures 1 and 2 shows no evidence of MAK-induced decrements in NCV.

No animals showed any clinical signs of illness during the course of the study. No impairments in locomotion, grip, or gait were observed. As shown in Figure 3, body weights were normal and were unrelated to MAK exposure.

At the termination of the study all animals were sacrificed and both gross and histopathology examinations were performed on the

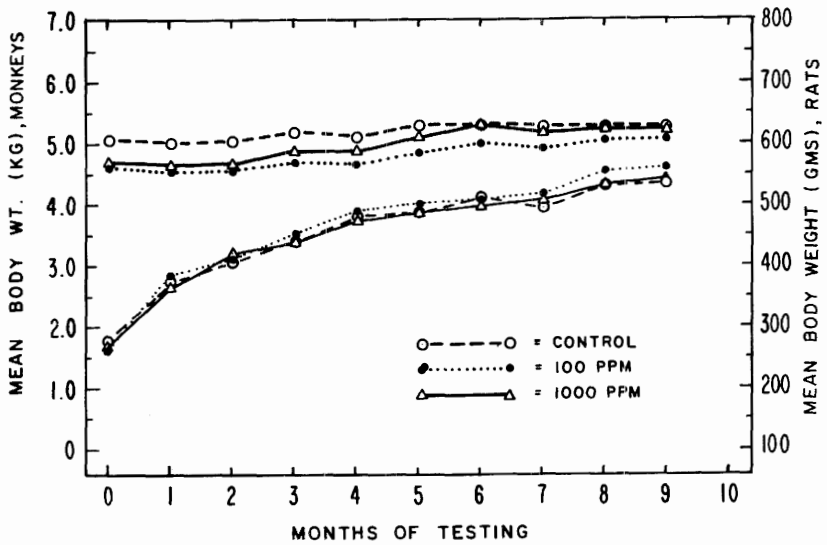

Figure 3 – Mean body weights for monkeys (top) and rats (bottom) according to MAK exposure group.

lungs, heart, liver, spleen, kidneys, adrenals, pancreas, testes, and brain. All tissues were unremarkable and no tissue damage related to MAK exposure was observed.

discussion

The evidence from this study shows that 9 months of MAK exposure at mean daily concentrations of 131 and 1025 ppm, using electrodiagnostic methods shown previously to be sensitive to the neurotoxic effects of methyl n-butyl ketone, induced no measurable signs of neurotoxicity in laboratory animals. As previously reported,[8] both rats and monkeys showed a statistically significant decrease in sciatic-tibial motor nerve conduction velocity after 4 months of exposure to approximately 1000 ppm of MBK. No similar effect, however, was found for MAK after 9 months of exposure.

A previous investigation[1] concerning the acute toxicity of MAK utilized short duration (5-25 hrs), high concentration (1500-4800 ppm) exposures. Evidence was found of central nervous system depression in the form of decreases in heart rate, rectal temperature, and respiration rate. However, examination of these data (Figs. 19, 20, 21)[1] shows that guinea pigs exposed to 1500 ppm MAK showed an initial CNS depression, but showed signs of returning to pre-exposure values after about 15 hours of exposure. It is difficult to compare our findings with this previous report, since their study involved acute, high concentration exposures, whereas the present study was concerned with long-term MAK exposures at lower concentrations. However, our electroencephalographic data do not show CNS depression as might have been indicated by extrapolation of results from this earlier study.

Our failure to find neurotoxic effects attributable to MAK agrees with the results from a recent report[11] that failed to observe clinical and histopathologic signs of neurotoxicity in rats exposed to 278 ppm for 2300 hours.

Failure to observe any MAK-induced neurotoxicity in this investigation provides an interesting observation on the comparative toxicities of MAK and MBK. As mentioned, an earlier study[1] found that MAK, which contains 7 carbon atoms in its molecule, was more toxic in terms of narcotic effectiveness than was MBK, which is a 6-carbon molecule. A subsequent report[2] also noted that if single dose LD_{50} values are used to compare the relative toxicities of the ketones, then toxicity was predicted to increase with increased molecular size. On this latter basis, MAK would be more toxic than MBK by a ratio of 2.59/1.67. However, the findings from the present study suggest that the relative neurotoxicity of the ketones may not necessarily increase with increased chain length of a ketone's molecule.

Finally, though MAK by itself appears to be relatively nontoxic by our tests, it remains to be proved whether or not MAK (and other similar ketones) are capable of exerting a synergistic action on other, more toxic solvents. It is known,[14] for example, that methyl ethyl ketone (MEK) will potentiate the toxicity of methyl n-butyl ketone. Whether or not MAK possesses a similar property is unknown.

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