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Inhalation Toxicity of Methyl *n*-Amyl Ketone (2-Heptanone) in Rats and Monkeys

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Inhalation Toxicity of Methyl *n*-Amyl Ketone (2-Heptanone) in Rats and Monkeys. LYNCH, D. W., LEWIS, T. R., MOORMAN, W. J., PLOTNICK, H. B., SCHULER, R. L., SMALLWOOD, A. W., AND KOMMINENI, C., (1981). *Toxicol. Appl. Pharmacol.* **58**, 341–352. A subchronic inhalation study was conducted in which male rats and monkeys were exposed at 0 (controls), 131, or 1025 ppm methyl *n*-amyl ketone (MAK) for 10 months (6 hr/day, 5 days/week). Comprehensive cardiopulmonary, clinical chemistry, metabolism, and tissue distribution studies were performed. No statistically significant alterations of pulmonary function, electrocardiographic, or clinical chemistry parameters were observed. Methyl *n*-amyl ketone and methyl *n*-amyl alcohol were detected in urine and serum from monkeys exposed to MAK for 10 months. Inhaled MAK did not induce rat liver microsomal enzymes. Results of tissue distribution studies of [^{14}C]MAK in rats comparing ip and inhalation routes of exposure were similar, and preexposure to unlabeled MAK did not alter the tissue distribution compared to animals without prior exposure to MAK. Liver tissue had the highest level of radioactivity regardless of the route of administration; however, no liver pathology was observed. Urinary excretion accounted for 25% of the administered dose after 12 hr. In general, a lack of toxicity was noted in both species exposed to MAK.

Methyl *n*-amyl ketone (2-heptanone, MAK) is used as an industrial solvent for nitrocellulose, as an inert reaction medium, in lacquers, and as a constituent of artificial carnation oils in perfumery (Hawley, 1974; Windholz, 1976). Few inhalation toxicological data on MAK are present in the literature (USDHEW, 1978). Smyth *et al.* (1962) determined the acute lethality of MAK and found no deaths among six rats exposed at 2000 ppm for 4 hr while six out of six rats died at 4000 ppm for 4 hr. Specht *et al.* (1940) studied the acute inhalation toxicity of MAK in guinea pigs. They exposed female guinea pigs at either 1500, 2000, or 4800 ppm MAK continuously for up to 15 hr and recorded rectal temperature, respiration rate, pulse, and behavioral effects. The major effects seen at all three concentrations were strong mucous membrane irritation and a dose-related depression of physiologic parameters. Two deaths occurred at 4800 ppm after 120 and 280 min, respectively. Prockop and Couri (1977) exposed 10 rats to MAK vapor at a concentration of 1300 mg/m^3 (278 ppm) for 2300 hr and reported no clinical or histological signs of neurotoxicity. Duration of exposure and other details of this investigation are lacking. Regarding the metabolic fate of

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MAK, Kamil *et al.* (1953) reported that 41% of orally administered MAK (0.95 g/kg) in rabbits was excreted as 2-heptyl glucuronide, along with small amounts of unchanged ketone in the urine.

Recent epidemiological evidence (Billmaier *et al.*, 1974; Allen *et al.*, 1975) has linked methyl *n*-butyl ketone (MBK, 2-hexanone) to the occurrence of peripheral neuropathy in workers exposed to this solvent in a coated fabrics plant. This discovery was followed by a number of laboratory studies which verified that MBK was the etiologic agent (Duckett *et al.*, 1974; Mendell *et al.*, 1974; Spencer *et al.*, 1975; Johnson *et al.*, 1977, 1979). Due to the close structural similarity between MBK and MAK and the possible common metabolic biotransformation of these ketones as suggested by Nielson (1975), a 10-month inhalation study was conducted to ascertain whether exposure to MAK might produce peripheral neuropathy in experimental animals as well as to provide data on the effects of long-term inhalation exposures to MAK.

The neurophysiological portion of the study has been reported by Johnson *et al.* (1978, 1979), who found no change in nerve conduction velocities in rats or monkeys exposed to MAK at 131 or 1025 ppm (6 hr/day, 5 days/week) for up to 10 months. Those findings did not support the neurotoxicity of MAK as suggested by observations of polyneuropathy in young men who were "huffing" a commercial solvent containing MAK (Prockop *et al.*, 1974; Prockop and Couri, 1977).

The present paper reports the results on studies of cardiopulmonary function, clinical chemistry, metabolism, and tissue distribution of MAK in rats and monkeys exposed to MAK vapor for up to 10 months.

METHODS

Atmospheric generation and analysis. The experimental animals were exposed under dynamic conditions to nominal MAK concentrations of 0, 100, and 1000 ppm (6 hr/day, 5 days/week). Technical grade MAK (Ashland Chemical Co., Division of Ashland Oil Co., Columbus, OH) was utilized in these studies. Analysis by gas chromatography/mass spectroscopy (gc/ms) indicated that the MAK was 97% pure with the following impurities identified (each less than 0.5%): 4-methyl-2-pentanone, 2-hexanone, 5-methyl-2-hexanone, 2-nonanone, and 2-ethyl-1-hexanol. MAK vapor concentrations were generated by heating liquid MAK in temperature-controlled flasks and using known air flows to carry the vapor into 6.37-m^3 exposure chambers. Exposures were conducted under dynamic flow conditions with tangential airfeed manifolds maintained at 40 liters per minute with a pressure of -0.25 cm H_2O. Chamber MAK concentrations were monitored at least twice daily by pulling 10 and 5 liters of air (from the 100 and 1000 ppm chambers, respectively) through charcoal tubes (USDHEW, 1977). Charcoal was desorbed with carbon disulfide and aliquots were injected into a gas chromatograph. Internal standardization was used with MBK as the internal standard. A Carle Model 8000 thermal conductivity gas chromatograph (Carle Instruments Inc., Fullerton, CA) equipped with a stainless-steel 10% FFAP column (185 × 0.32 cm), was used for analyses. Chamber concentrations were verified on a Perkin-Elmer Model 9000 gas chromatograph (Perkin-Elmer Corp., Norwalk, CT) with a flame ionization detector prior to the start of animal exposures. Adjustments were made during each exposure day to maintain the exposure concentrations at planned levels. The mean value for two to three samples obtained each day was used to characterize that day's MAK exposure. Because of the potential toxic hazard of the exposures, these studies were conducted in chambers equipped with an airlock previously described by Wagner *et al.* (1969).

Animals. Male, cesarean-derived, Sprague–Dawley rats (Laboratory Supply Co., Inc., Indianapolis, IN)[1] and male Cynomolgus monkeys (*Macaca fasicularis* —Primate Imports Corp., Long Island, NY) were employed in this study. Groups of 50 rats and eight monkeys were randomly assigned to each of the three treatment groups. Monkeys weighed 4.81 ± 1.26 kg ($\bar{x}$ ± SD) and rats 258 ± 25 g at the start of the study. Two separate groups of rats were employed for the tissue distribution studies. Rats in the ip tissue distribution study weighed 229 ± 15 g while rats in the inhalation study weighed 532 ± 52 g. The latter rats were part of the original 150 rats allocated to the study. The inhalation tissue distribution studies were conducted approximately 6 months after the start of the 10-month inhalation study and the body weights reflect the age of the animals at that point in time. Rats and monkeys

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

were quarantined for 2 weeks and 1 month, respectively, before initiation of this study. Stainless- and galvanized-steel open-wire cages with wire mesh floors were used as housing cages to provide adequate distribution of solvent vapors within the exposure chambers. Monkeys were individually housed on one side of the chambers while the rats were housed in groups of two to four, one row above the other, opposite the monkeys. Control animals were housed in similar cages. Both exposure groups of animals were housed in their respective chambers 24 hr/day, with cage locations rotated at monthly intervals. Control animals were housed in separate animal quarters and exposed to filtered air 24 hr/day. Rats were sacrificed by pentobarbital overdoses (Nembutal Sodium, Abbott Laboratories, North Chicago, IL) on the day following completion of 1, 3, 6, and 10 months of exposure. Monkeys were sacrificed by pentobarbital overdose after 10 months of exposure. Gross necropsies were performed on all animals at each scheduled sacrifice. Lungs, liver, heart, spleen, kidneys, adrenals, pancreas, testes, and brain were evaluated histopathologically. The tibial branch of the sciatic nerve from both species was prepared for neuropathological examination according to the method of Spencer *et al.* (1975). Rats and monkeys were fed standard laboratory pellet diets (Rodent Laboratory Chow and Monkey Chow—Jumbo, Ralston Purina Co., St. Louis, MO). Monkeys were given fresh fruit (oranges, apples, or bananas) two times per week. Tap water was available *ad libitum* and food was available to the rats except during the exposure period. Monkeys were fed once daily following cessation of that day's exposure.

Cardiopulmonary studies. Cardiopulmonary evaluations including mechanical properties (compliance and resistance), all lung volumes, flow-volume dynamics, distribution of ventilation, diffusion, and gas exchange were conducted on all monkeys prior to the start of exposures (baseline) and after 6 months exposure to MAK. Monkeys were randomly selected and tested on the day following an MAK exposure. Monkeys from each treatment group (including controls) were tested on each testing day. Testing of all monkeys in the study was completed over a 3-day period. Standard six-lead electrocardiographic examinations (ECGs) were conducted on all monkeys at the same time intervals as the pulmonary function testing.

Immediately before testing, the monkeys were immobilized with 0.2 ml of Sernylan (phencyclidine hydrochloride—Bio-Centic Labs, ST. Joseph, MO) and anesthetized with pentobarbital sodium (35 mg/kg). Atrophine sulfate (0.34 mg/kg—W.A. Butler Co., Columbus, OH) was also administered to counteract the excessive secretary response noted as a side effect of Sernylan and to decrease peristalsis.

Following the induction of anesthesia, an esophageal baloon was placed in the lower third of the esophagus and an 18–22f endotracheal tube was inserted into the trachea with the aid of a laryngoscope. The cuff of the endotracheal tube was inflated and excessive length of the distal end trimmed even with the end of the mouth. The animal was then placed into the plethysmographic chamber, ventral side up. Pulmonary mechanics were obtained from simultaneous volume, flow, and transpulmonary pressure tracings displayed on a 12-channel photographic recorder (Model DR-12, Electronics for Medicine, White Plains, NY). Airflow through the pneumotach was measured with the differential transducer and electrically integrated to produce a volume trace. Dynamic pulmonary compliance was calculated from simultaneous volume and transpulmonary pressure tracings at points of zero flow (Mead and Whittenberger, 1953). Total expiratory resistance was calculated from the inverse slope of the pressure flow loop, after electrical subtraction of a voltage proportional to volume from the pressure signal (Mead and Whittenberger, 1953). For the purpose of making uniform comparisons of flow resistance among experimental subjects, the values were measured at a flow rate of 200 ml per sec and at moderate respiratory rates (12–18 breaths per min). All mechanics were measured while the animal was breathing spontaneously through the pneumotach only. Initially and periodically throughout the testing the animal was inflated for 10 sec to expand atelectactic areas (Mead and Collier, 1959). A static compliance curve was obtained from resting and expiratory position by serial inflations of 10 ml through a unidirectional check valve. The pulmonary function testing requiring breathing maneuvers (lung volumes, maximum expiratory flow-volume curve, diffusing capacity, nitrogen washout, and closing volume) were performed using a variable pressure plethysmograph chamber as previously described by Moorman *et al.* (1975). The basic method employed is similar to that used in an external tank respirator; however, a hydraulic control system enables the operator to bring about inspiration, expiration, breath holding, and breathing rate within the anatomical and physiological limits of the animal. Both flow and volume are controlled secondarily by changes in the pressure surrounding the animal. Inspiratory and expiratory air flow can be controlled from very low rates to the maximum within each subject. Likewise, volume can be controlled for both maximum inspiration and expiration. Inspiratory capacity is obtained by rapid depressurization to -70 cm H_2O from resting tidal position. Prior to the flow-volume testing it was determined that plethysmograph pressures of $+70$ cm H_2O would be used to produce maximal expirations. Inspection of flow-volume curves at increasing driving pressures showed that flow limitation characteristics had been reached at volumes above 50% total lung capacity when the plethysmograph pressure was greater than 70 cm H_2O. Therefore flow maxima at

50 and 40% of total lung capacity were values taken at an effort-independent zone of the flow volume curve. The curves were highly reproducible and demonstrated a low coefficient of variation (2–2.5%) in the effort-independent zone.

To ensure that sufficient intrathoracic driving pressure was developed, esophageal pressure was recorded during forced expirations. A pressure gradient was observed across the chest wall, however, intrapleural pressures of 30–35 cm H_2O, which are sufficient to produce flow maxima, were achieved. A volume error, as a result of thoracic gas compression, was calculated to be approximately 3% at 50% TLC with the intrapleural pressure of 30–35 cm H_2O. This error was considered irrelevant because the results were compared in animals tested at the same driving pressures.

Breathing manipulations can be performed in anesthetized animals because of the apnea produced on inflation as a result of the inflation reflex documented by Hering and Breuer (1968). This inspiratory inhibition has been demonstrated by recording action potentials from the phrenic nerve.

Inspiratory capacity and forced vital capacity were recorded during a maximum inspiration. Flow and volume were recorded during a maximum inspiration, followed by a maximum expiration. Flow and volume tracings were recorded, which provided the essential data points for calculating forced expiratory flows and volumes and peak expiratory flow. This procedure of maximum inspiration followed by maximum expiration was performed initially and therefore for all test maneuvers, ensuring equal volume and flow histories.

The methods of Brashear *et al.* (1966) and Mitchell and Renzetti (1968) were combined to obtain values for single breath diffusing capacity and total lung capacity. The calculations for single breath diffusing capacity were performed according to the method described by Wagner *et al.* (1971). Gas analyses were done using a respiratory mass spectrometer (Model MGA 1100, Perkin-Elmer Corp., Norwalk, CT).

Combined values for nitrogen washout and closing volume were obtained according to the method of Buist and Ross (1973).

Clinical chemistry studies. Blood was obtained from monkeys using femoral vein punctures at preexposure and on the day following 1, 3, and 6 months of exposure. The following tests were conducted on serum using a Technicon SMA 12-60 (Technicon Instruments Corp., Tarrytown, NY): glutamate-oxaloacetate transaminase, lactic dehydrogenase, alkaline phosphatase, total bilirubin, albumin, total protein, cholesterol, uric acid, blood urea nitrogen, glucose, inorganic phosphate, and calcium. In addition, creatine phosphokinase, red cell cholinesterase, lactic acid, triglycerides, and blood glutathione were also measured. Creatine phosphokinase was determined on a centrifugal analyzer (Centrifichem, Union Carbide Corp., Rye, NY) using Worthington Statzyme reagents (Worthington Biochemical Corp., Freehold, NJ). The method was an adaptation of the Rosalki (1967) procedure. Red cell cholinesterase was determined using a modified Michel (1949) procedure. Lactic acid was determined using a "BMC Lactate Kit" based on the measurement of NADH produced by the substrate lactic acid in the presence of added lactic dehydrogenase (Boehringer Manheim Corp., New York, NY). Reduced glutathione in red blood cells was determined on a Technicon Auto-Analyzer using the method of Jocelyn (1962). Triglycerides were determined fluorometrically on a Technicon Auto-Analyzer using the method of Leon *et al.* (1970).

Metabolic studies. Blood and urine samples were collected from rats and monkeys exposed to MAK for 10 months. Blood samples from rats to be analyzed for MAK and its metabolites were obtained by cardiac puncture within 1 hr after termination of exposure. Urine samples were collected overnight (on ice) from fasted rats housed individually in stainless-steel metabolism cages. Monkey urine and serum samples were collected at necropsy approximately 18 hr after the last MAK exposure. Blood samples from both species were immediately centrifuged to separate serum samples. Serum samples were put into 3-ml reaction vials, sealed with Parafilm, and frozen. Samples were analyzed for high boiling point metabolites by direct injection of serum or urine into a gas chromatograph using the method of DiVincenzo *et al.* (1976). Serum MAK and methyl *n*-amyl alcohol (MAA, 2-heptanol) were determined using a headspace technique. Samples were equilibrated at 60°C in septum vials for 30 min and 2.0 ml of headspace was injected into a Perkin-Elmer Model 3920B gas chromatograph. Operating conditions were as follows: column (305 × 0.32-cm stainless-steel packed with 10% carbowax 20 M with terephthalic acid on 80/100 mesh Supelcoport-Supelco Inc., Bellefonte, PA), carrier (nitrogen) at 30 ml/min, temperatures (injector 120°C, column 100°C, detector interface 150°C), detector (flame ionization).

In addition, the liver microsomal enzyme induction potential of MAK was evaluated by injecting pentobarbital sodium (25 mg/kg) ip into rats that inhaled MAK at 0, 100, and 1000 ppm MAK for 10 months and comparing sleeping times. Rats were injected about 18 hr after cessation of the preceeding day's MAK exposure.

Tissue distribution studies. Studies were undertaken to determine the tissue distribution of ^{14}C-labeled MAK in rats following ip or inhalation exposures. [^{14}C]MAK (U-amyl) with a specific activity of 2.5 mCi/mmol was used for the ip studies (American Radiochemical Corp., Sanford, FL). The labeled compound was diluted with "unlabeled" MAK (98%, Tennessee Eastman Co., Kingsport, TN) and mixed with

corn oil to yield a concentration of 2.5 mg MAK/ml of treatment solution. The treatment solution had a specific activity of 4763 dpm/μg MAK, and was administered at a dosage of 10 mg MAK/kg (4 ml/kg). Sample preparation and counting were performed by the method of Plotnick and Conner (1976). Tissues, urine, and feces were collected 2, 4, 8, 12, 24, 48, and 72 hr after dosing (three rats per time interval). In the inhalation studies, four groups of rats (three rats per group) were exposed under dynamic conditions to [^{14}C]MAK at 100 ppm for 6 hr in individual glass chambers (Model CR-350, Nuclear Associates, Inc., Westbury, NY). Two groups of rats (Groups I and II) had been exposed to unlabeled MAK at 100 ppm for 6 months prior to the tissue distribution study. Groups III and IV had no prior exposure to MAK. Groups I and III were sacrificed immediately after the 6 hr exposure to [^{14}C]MAK. The remaining two groups were sacrificed 4 hr after the completion of their exposures to [^{14}C]MAK. These studies were conducted to determine if prior MAK exposure would result in alterations in the tissue distribution of the compound. [^{14}C]MAK was diluted with "unlabeled" MAK as in the ip studies. The mixture used to generate the MAK vapor had a specific activity of 225 dpm/μg. Vapor concentrations were determined with charcoal tubes as described previously. These studies were conducted in a laboratory fume hood to prevent contamination of the working environment. After transit through the inhalation chambers the [^{14}C]MAK was trapped in ethanol and was disposed of as radioactive waste according to standard regulations.

Statistical analysis. Each of the cardiopulmonary variables was analyzed with a one-way multivariate analysis of variance (MANOVA) using the MGLM program developed by Starmer and Grizzle (1968). Control and exposure groups were compared at each time point and at all time points simultaneously. These data were also analyzed using a multivariate extension of the Kruskal–Wallis test (1952) as described in Puri and Sen (1966). Clinical chemistry parameters were evaluated by use of Student's t test and $p < 0.05$ was considered significant.

RESULTS

Atmospheric Generation and Analysis

The mean chamber concentrations of MAK for the targeted 100 and 1000 ppm chambers were 131 ± 30 and 1025 ± 136 ppm ($\bar{x}$ ± SD) for 218 exposure days. The mean concentration of 131 ppm times 6 hr gives a concentration times time (C × T) of 786 which corresponds to an 8-hr time weighted average of 98 ppm versus a C × T of 800 for the current federal occupational exposure limit (8 hr × 100 ppm). Concentration values obtained from the 100 ppm chamber were corrected for a charcoal desorption efficiency of 86%. Temperatures and relative humidity of the exposure chambers ranged from 23 to 26°C and 40 to 50%, respectively, for the duration of the study.

Animal Observations

Both species tolerated the exposures without developing overt signs of toxicity. There were no statistically significant differences in body weights in either species, as previously reported by Johnson *et al.* (1978, 1979). One monkey death and several rat deaths occurred during the course of the study, but they were not related to MAK exposure. Rat deaths were attributed to chronic murine pneumonia and the monkey death to an overdose of anesthetic. As previously reported by Johnson *et al.* (1978, 1979) there were no gross or microscopic changes in organs and tissues from either species related to MAK exposure, nor was there any evidence of neuropathollogy in the tibial branch of the sciatic nerves.

Cardiopulmonary Studies

Data generated in this study (Table 1) were categorized according to effect. In the first category, dynamic compliance, inspiratory capacity, expiratory flow maximum at 90% of vital capacity, expiratory flow maximum at 90% of vital capacity over vital capacity, and diffusion capacity over total lung capacity all showed no significant differences within or between the control and the two exposure groups after 6 months of MAK exposure.

TABLE 1

PULMONARY FUNCTION PARAMETERS MEASURED IN MONKEYS FOLLOWING 6 MONTHS OF INHALATION EXPOSURE TO METHYL *n*-AMYL KETONE

Parameter	Units	Controls	131 ppm	1025 ppm
Dynamic compliance	ml/cm H_2O	13.77 ± 8.69[a] (5.80–34.20)[b]	20.50 ± 19.54 (5.10–63.8)	11.80 ± 5.40 (5.90–21.33)
Inspiratory capacity	ml	236.50 ± 64.72 (135.00–312.00)	189.75 ± 36.28 (139.00–245.00)	246.29 ± 59.58 (143.00–329.00)
Expiratory flow maximum at 90% of vital capacity	liter/min	8.43 ± 2.71 (4.39–13.20)	15.75 ± 7.91 (4.46–27.30)	13.80 ± 7.12 (5.95–28.10)
Expiratory flow maximum at 90% of vital capacity over vital capacity	liter/min/ml	0.37 ± 0.15 (0.16–0.60)	0.75 ± 0.41 (0.30–1.46)	0.56 ± 0.26 (0.22–1.00)
Diffusion capacity over total lung capacity	%	1.48 ± 0.18 (1.21–1.66)	1.69 ± 0.19 (1.40–1.91)	1.38 ± 0.15 (1.17–1.67)
Static compliance	ml/cm H_2O	21.74 ± 14.40 (10.53–50.00)	18.37 ± 5.81 (9.28–28.15)	21.53 ± 9.25 (11.34–40.82)
Specific static compliance	cm H_2O^{-1}	8.62 ± 5.42 (4.71–18.28)	6.80 ± 2.10 (3.80–9.61)	9.12 ± 4.19 (4.59–16.48)
Expiratory reserve volume	ml	197.00 ± 60.05 (106.00–276.00)	179.50 ± 67.52 (79.00–255.00)	171.43 ± 32.21 (129.00–218.00)
Total lung capacity	ml	509.13 ± 110.13 (376.00–657.00)	442.25 ± 109.83 (304.00–574.00)	501.71 ± 86.22 (383.00–628.00)
Residual volume	ml	74.13 ± 17.67 (41.00–96.00)	72.50 ± 28.35 (31.00–120.00)	77.14 ± 32.33 (31.00–138.00)
Expiratory flow maximum at 75% of vital capacity	liter/min/ml	1.31 ± 0.48 (0.81–2.11)	2.09 ± 0.99 (1.05–3.93)	1.61 ± 0.48 (0.97–2.29)
Diffusion capacity	ml/min/mm Hg	7.45 ± 1.37 (5.84–10.09)	7.53 ± 2.15 (4.86–10.17)	6.84 ± 1.82 (4.89–10.49)
Closing volume	ml	36.75 ± 13.79 (15.00–63.00)	57.50 ± 34.55 (15.00–120.00)	38.43 ± 22.04 (12.00–81.00)
Specific dynamic compliance	cm H_2O^{-1}	5.35 ± 3.03 (3.10–12.45)	7.50 ± 5.48 (2.62–19.57)	4.84 ± 2.03 (2.31–8.65)
Nitrogen washout	% N_2 rise	0.49 ± 0.28 (0.13–0.88)	0.72 ± 0.39 (0.25–1.18)	0.62 ± 0.33 (0.25–1.30)
Average flow resistance	ml H_2O/liter/sec	14.64 ± 5.15 (8.40–21.40)	26.11 ± 13.33[c] (8.30–41.70)	10.12 ± 3.54 (6.00–14.40)
Total expiratory flow resistance	ml H_2O/liter/sec	18.94 ± 8.06 (7.15–31.60)	37.54 ± 20.50 (7.80–72.60)	38.47 ± 16.98 (11.40–57.23)
Forced expiratory volume in 1 sec/vital capacity	%	91.24 ± 3.73 (85.37–96.00)	96.64 ± 2.57 (92.45–100.00)	96.18 ± 2.90 (90.43–99.03)
Expiratory flow maximum at 75% of vital capacity/vital capacity	liter/min/ml	1.31 ± 0.48 (0.81–2.11)	2.09 ± 0.99 (1.05–3.93)	1.61 ± 0.48 (0.97–2.29)
Expiratory flow maximum at 90% of vital capacity/vital capacity	liter/min/ml	0.37 ± 0.15 (0.16–0.60)	0.7 ± 0.41 (0.30–1.46)	0.56 ± 0.26 (0.22–1.00)
Closing volume	%	8.89 ± 3.43 (2.87–14.20)	15.51 ± 8.80 (4.98–34.60)	9.01 ± 4.69 (4.42–18.10)

[a] Values are the mean ± 1 SD of eight monkeys.
[b] Range of measured values.
[c] Statistically significant ($p < 0.05$) compared to control.

TABLE 2

METHYL *n*-AMYL KETONE (MAK) AND *n*-AMYL ALCOHOL (MAA) IN SERUM FROM RATS AND MONKEYS EXPOSED TO MAK FOR 10 MONTHS

Species	Exposure group	n^a	MAK[b] (range)	MAA[b] (range)	Post-MAK[c] peaks
Monkey	Control	2	ND[d]	ND	0/2[e]
	131 ppm	3	7.4[f] (ND–7.4)	ND	1/3
	1025 ppm	4	15.6 ± 6.5 (3.5–27.2)	2.0 ± 1.0[g] (ND–3.0)	2/4
Rat	Control	5	ND	ND	0/5
	131 ppm	6	11.6 ± 2.9 (4.9–24.2)	ND	1/6
	1025 ppm	4	27.0 ± 3.6 (18.5–35.8)	ND	4/4

[a] Number of samples.
[b] Mean ± SE of detectable concentrations expressed as μg/ml.
[c] Higher boiling point peaks which could not be further identified.
[d] None detected (less than 1.0 μg/ml).
[e] Number of serum samples with post-MAK peaks/number of samples analyzed.
[f] Only one sample had detectable MAK.
[g] Only two samples had detectable MAA.

The second category includes parameters where the observed trends were similar and increasing (compared to baseline values) among both exposed and control monkeys. These data included: static compliance, specific static compliance, expiratory reserve volume, total lung capacity, residual volume, expiratory flow maximum at 75% of vital capacity, diffusion capacity, and closing volume. These increasing trends in all three treatment groups probably reflect natural growth processes in the monkeys.

The measurements for specific dynamic compliance and nitrogen washout showed equivalent and decreasing trends over the three test groups. The reason why all three groups would experience declines in these parameters is unclear. Mean average flow resistance data were increased ($p < 0.05$) in the 131 ppm group relative to the controls while the 1025 ppm group exhibited decreased average flow resistance compared to the controls but this difference was not statistically significant.

For the following tests, data from both exposed groups were consistently higher than control values: total expiratory flow resistance, forced expiratory volume in 1 sec over vital capacity, expiratory flow maximum at 75% of vital capacity over vital capacity, expiratory flow maximum at 90% of vital capacity over vital capacity, and closing volume (phase IV over vital capacity).

There were no changes in ECGs which could be attributed to MAK exposure.

Analysis of the data indicates that MAK exposures for 6 months under the conditions of this study did not result in any significant changes in the overall cardiopulmonary status of the test animals. The high degree of variability in the cardiopulmonary parameters due to individual variations of the monkeys made the detection of subtle changes difficult.

Clinical Chemistry Studies

A few individual parameters, e.g., serum inorganic phosphate, were statistically different from controls in only one group of exposed animals and only at one sampling period. Overall there were no dose-related alterations in the clinical chemistry profile of the test monkeys which could be related to MAK exposure.

Metabolic Studies

MAK was detected and quantified in both serum and urine from rats and monkeys exposed to both concentrations of MAK, while methyl *n*-amyl alcohol was detected only in the serum and urine from monkeys exposed to 1025 ppm MAK (Tables 2 and 3). The column "Post-MAK Peaks" refers to the presence of high boiling peaks in the urine or serum samples. Further identification of these peaks was attempted but was unsuccessful, as the suspected metabolites (2,6-heptanedione and 2-keto-6-hydroxyheptane) were not available commercially and access to a gc/ms was not possible during this study.

TABLE 3

METHYL *n*-AMYL KETONE (MAK) AND *n*-AMYL ALCOHOL (MAA) IN URINE FROM RATS AND MONKEYS EXPOSED TO MAK FOR 10 MONTHS

Species	Exposure group	*n*[a]	MAK[b] (range)	MAA[b] (range)	Post-MAK[c] peaks
Monkey	Control	2	ND[d]	ND	0/2[e]
	131 ppm	1	15.3[f]	ND	1/1
	1025 ppm	3	3.5 ± 0.9[g] (ND–4.4)	5.1 ± 0.2[g] (ND–5.3)	2/3
Rat	Control	3	ND	ND	0/3
	131 ppm	4	7.7 ± 3.7 (1.0–18.1)	ND	1/4
	1025 ppm	2	6.0 ± 3.2 (2.8–9.2)	ND	2/2

[a] Number of samples.
[b] Mean ± SE of detectable concentrations expressed as μg/ml.
[c] Higher boiling point peaks which could not be further identified.
[d] None detected (less than 1.0 μg/ml).
[e] Number of urine samples with post-MAK peaks/number of samples analyzed.
[f] Only one sample was analyzed.
[g] Only two samples had detectable concentrations.

Ten of 12 urine and serum samples from rats and monkeys exposed to MAK at 1025 ppm possessed significant post-MAK peaks in their chromatograms versus 4 of 14 samples from animals exposed to 131 ppm MAK. The variability of these data reflects the differing times of sample collection and the limited sample size. These peaks had retention volumes located in an area where one would expect the dione and the 2-keto-6-hydroxyheptane. Chromatograms from control animals were void of peaks in these areas. In addition, there were no significant differences in pentobarbital sleeping times.

Tissue Distribution Studies

Results of the tissue distribution studies with ^{14}C-labeled MAK by both ip and inhalation routes of exposure are summarized in Tables 4 and 5. The mean air concentration for the 6 hr inhalation exposures was 132 ± 6.2 ppm. At all time intervals studied and regardless of the route of administra-

TABLE 4

DISTRIBUTION OF ^{14}C-METHYL *n*-AMYL KETONE IN SELECTED TISSUES OF MALE RATS AT VARIOUS TIME INTERVALS FOLLOWING A 10 MG/KG INTRAPERITONEAL DOSE

Tissue	Time (hr) 2	4	8	12	24	48	72
Lungs	4.12 ± 0.37[a]	3.54 ± 0.20	2.61 ± 0.14	2.52 ± 0.11	1.66 ± 0.14	1.18 ± 0.01	1.01 ± 0.09
Heart	3.90 ± 0.40	2.59 ± 0.22	1.58 ± 0.17	1.08 ± 0.09	0.89 ± 0.07	0.76 ± 0.03	0.66 ± 0.03
Liver	15.95 ± 0.26	10.81 ± 0.16	9.55 ± 2.62	6.48 ± 0.32	3.93 ± 0.31	2.18 ± 0.13	1.86 ± 0.13
Spleen	3.88 ± 0.40	3.51 ± 0.23	3.34 ± 0.38	2.39 ± 0.20	1.64 ± 0.11	1.42 ± 0.10	1.26 ± 0.26
Pancreas	9.09 ± 0.55	4.98 ± 0.31	3.17 ± 0.66	1.98 ± 0.36	1.86 ± 0.58	1.02 ± 0.05	1.01 ± 0.07
Adrenals	3.79 ± 0.71	3.60 ± 0.53	3.20 ± 0.28	2.76 ± 0.49	2.22 ± 0.32	1.81 ± 0.23	1.75 ± 0.46
Kidneys	7.95 ± 0.36	5.92 ± 0.54	4.20 ± 0.22	3.31 ± 0.11	2.27 ± 0.08	1.68 ± 0.08	1.36 ± 0.16
Testes	2.74 ± 0.10	2.01 ± 0.31	1.18 ± 0.05	1.15 ± 0.25	0.80 ± 0.21	0.57 ± 0.04	0.44 ± 0.03
Brain[b]	2.18 ± 0.09	1.50 ± 0.13	0.75 ± 0.06	0.54 ± 0.03	0.34 ± 0.02	0.33 ± 0.02	0.32 ± 0.01
Medulla	1.61 ± 0.21	BLD[c]	BLD	BLD	BLD	BLD	BLD
Blood	4.00 ± 0.37	3.08 ± 0.17	1.72 ± 0.14	1.44 ± 0.03	0.85 ± 0.03	0.67 ± 0.03	0.54 ± 0.04
Muscle[d]	2.32 ± 0.17	1.66 ± 0.12	0.93 ± 0.01	0.68 ± 0.06	0.53 ± 0.04	0.39 ± 0.09	0.48 ± 0.06
Spinal cord	1.47 ± 0.32	BLD	BLD	BLD	BLD	BLD	BLD
Fat[e]	2.94 ± 0.95	1.44 ± 0.39	1.13 ± 0.28	2.64 ± 0.93	1.17 ± 0.38	0.56 ± 0.27	1.28 ± 0.13

[a] Mean ± SE in μg/g of tissue or /ml of fluid (expressed as parent compound) based on duplicate determinations on three animals at each time interval.
[b] With medulla removed.
[c] Below the limit of detection (2 times background).
[d] Left gastrocnemius.
[e] Suprarenal fat.

tion, the liver had the highest level of radioactivity followed, in general, by kidney, pancreas, and lung. The tissue distribution of MAK did not correspond to any observed gross or histopathological damage. Suspected target tissues, e.g., brain, had low levels of radioactivity, and portions of sciatic nerves counted were below the limit of detection at all time intervals studied. Urinary and fecal excretion data for the ip study are presented in Table 6. Urinary excretion peaked at 12 hr and remained relatively constant through 48 hr. The reason for the lack of continuity between the 72 hr data and the other timed samples is puzzling and could not be explained by water deprivation nor lost urine volume. The individual values at 72 hr were 4.5, 13.7, and 33.3% of the administered dose. Fecal excretion through 72 hr after dosing represented less than 2% of the administered dose. There were no significant differences in the relative tissue distribution due to the route of exposure. Prior exposure to "unlabeled" MAK did not alter the pattern of tissue distribution of inhaled [^{14}C]MAK when compared with rats with no previous MAK exposure (Table 5).

TABLE 5

DISTRIBUTION OF ^{14}C-METHYL *n*-AMYL KETONE IN SELECTED TISSUES OF MALE RATS AT VARIOUS TIME INTERVALS FOLLOWING A 6 HR INHALATION EXPOSURE TO MAK AT 132 PPM

Tissue	Group I[a]	Group III[b]	Group II[c]	Group IV[d]
Lungs	35.71 ± 2.07[e]	33.39 ± 4.88	21.65 ± 1.60	24.09 ± 1.93
Heart	15.99 ± 1.25	16.91 ± 1.05	12.17 ± 3.09	9.42 ± 1.33
Liver	67.55 ± 9.90	58.12 ± 4.58	41.99 ± 5.68	32.50 ± 0.44
Spleen	19.93 ± 2.48	17.27 ± 2.58	16.66 ± 3.89	13.22 ± 2.60
Pancreas	39.02 ± 7.37	41.96 ± 6.57	28.96 ± 5.19	25.85 ± 1.04
Adrenals	22.52 ± 4.74	19.35[f]	20.44 ± 7.20	13.12 ± 1.81
Kidneys	55.26 ± 1.74	49.32 ± 5.80	41.25 ± 12.61	27.29 ± 0.72
Testes	15.77 ± 1.70	15.11 ± 1.86	10.47 ± 2.34	7.78 ± 0.74
Brain[g]	12.20 ± 1.61	11.81 ± 1.20	7.23 ± 1.44	5.76 ± 0.42
Blood	20.26 ± 2.48	19.74 ± 2.50	16.26 ± 5.54	9.72 ± 0.91
Muscle[h]	10.00 ± 1.47	9.39 ± 1.23	5.89 ± 1.32	4.50 ± 0.14
Fat[i]	19.72 ± 1.30	14.77 ± 2.95	4.26[f]	3.03 ± 0.41

[a] Rats exposed to unlabeled MAK at 100 ppm for 6 months followed by a 6 hr exposure to labeled MAK and sacrificed immediately.

[b] Rats exposed only to labeled MAK for 6 hr and sacrificed immediately.

[c] Rats exposed to unlabeled MAK at 100 ppm for 6 months followed by a 6 hr exposure to labeled MAK and sacrificed 4 hr after termination of exposure.

[d] Rats exposed only to labeled MAK for 6 hr and sacrificed 4 hr after termination of exposure.

[e] Mean ± SE expressed as μg of tissue or /ml of fluid (expressed as parent compound) based on duplicate determinations on three animals at each time interval.

[f] Mean of samples from two animals.

[g] With medulla removed.

[h] Left gastrocnemius.

[i] Suprarenal fat.

TABLE 6

PERCENTAGE OF DOSE OF TOTAL RADIOACTIVITY DERIVED FROM ^{14}C-METHYL *n*-AMYL KETONE RECOVERED IN URINE AND FECES AT VARIOUS TIME INTERVALS AFTER DOSING[a]

Time interval (hr)[b]	Urine	Feces
2	9.99 ± 1.44	0.25 ± 0.06
4	17.96 ± 5.66	0.18 ± 0.04
8	20.27 ± 2.08	0.21 ± 0.02
12	25.46 ± 1.14	0.29 ± 0.07
24	25.51 ± 1.31	0.70 ± 0.13
48	25.79 ± 0.47	0.81 ± 0.05
72	17.17 ± 8.48	1.08 ± 0.22

[a] [^{14}C]MAK(U-amyl) was diluted with carrier, mixed with corn oil, and administered as a single ip dose (10 mg MAK/kg). Rats were housed individually in stainless-steel metabolism cages and urine was collected in ice baths. At the conclusion of the experiment rats were killed by an ip injection of pentobarbital. Values represent mean ± SE of duplicate determinations on three animals at each time interval.

[b] Elapsed time between dosing and sacrifice.

DISCUSSION

Data generated in this study indicate that 10-month inhalation exposures to MAK at either 131 or 1025 ppm (6 hr/day, 5 days/week) did not produce demonstrable changes in the cardiopulmonary and clinical chemical indices evaluated. Although some statistically significant changes occurred in a few of these parameters, no dose-related changes were observed and no pulmonary function or clinical chemistry changes were indicative of decreased or altered organ function.

The metabolism of MAK was evaluated and the parent compound as well as *n*-amyl

alcohol were identified and quantified. Peaks for other higher boiling metabolites were found, although further identification of these compounds was not possible. 3-Heptanone, 6-hydroxy-3-heptanone, and 2,5-heptanedione have been detected in the serum of rats exposed by inhalation to 3-heptanone (ethyl *n*-butyl ketone; Katz *et al.*, 1980). 3-Heptanol, however, was not detected in these animals. 2-Hexanol has been reported as a blood and urinary metabolite in rats following oral (DiVincenzo *et al.*, 1977; Eben *et al.*, 1979) and ip administration (Abdel-Rahman *et al.*, 1976) of MBK. However, due to the lack of positive identification of other potential MAK metabolites, additional comparisons between the metabolism of six and seven carbon aliphatic ketones must await further investigation.

The lack of peripheral neuropathy in both rats and monkeys in this study (previously reported by Johnson *et al.*, 1978, 1979) would indicate that MAK at the two concentrations tested and the metabolites of MAK at the concentrations found in the animals are not neurotoxic. Spencer *et al.* (1978) also reported negative neuropathological findings in rats administered 0.5% MAK in drinking water for up to 12 weeks.

Patterns of tissue distribution studies comparing ip and inhalation routes of exposure were essentially identical. A principal route of excretion of ^{14}C-labeled MAK administered ip was via the kidneys, with approximately 25% of the administered dose appearing in the urine within 12 hr. Due to the vapor pressure of the compound, a significant amount of the dose was probably eliminated in expired air. By comparison, DiVincenzo *et al.* (1977) reported that urinary radioactivity accounted for 50% of a po dose of ^{14}C-labeled MBK. They also reported that respiratory $^{14}CO_2$ accounted for 38% of the measured radioactivity. Inhaled MAK did not induce liver microsomal enzymes based on the failure of MAK to alter rat pentobarbital sleeping times. Couri *et al.* (1977) reported that inhaled MBK (225 ppm continuously for a 7 day period) did not alter hexobarbital sleeping time measurements in Wistar rats.

This study supplements the acute inhalation studies of Specht *et al.* (1940) and Smyth *et al.* (1952) and complements the behavioral and neurophysiological evaluations of Anger *et al.* (1979) and Johnson *et al.* (1978, 1979).

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