Safety Assessment of MIBK (Methyl Isobutyl Ketone)[1]

MIBK (Methyl Isobutyl Ketone) is an aliphatic ketone that functions as both a denaturant and solvent in cosmetic products. Current use in cosmetic products is very limited, but MIBK is reported to be used in one nail correction pen (volume = 3 ml) at a concentration of 21%. The maximum percutaneous absorption rate in guinea pigs is 1.1 μmol/min/cm^2 at 10 to 45 min. Metabolites include 4-hydroxy-4-methyl-2-pentanone (oxidation product) and 4-methyl-2-pentanol (4-MPOL) (reduction product). Values for the serum half-life and total clearance time of MIBK in animals were 66 min and 6 h, respectively. In clinical tests, most of the absorbed MIBK had been eliminated from the body 90 min post exposure. MIBK was not toxic via the oral or dermal route of exposure in acute, short-term, or subchronic animal studies, except that nephrotoxicity was observed in rats dosed with 1 g/kg in a short-term study. MIBK was an ocular and skin irritant in animal tests. Ocular irritation was noted in 12 volunteers exposed to 200 ppm MIBK for 15 min in a clinical test. A depression of the vestibulo-oculomotor reflex was seen with intravenous infusion of MIBK (in an emulsion) at 30 μM/kg/min in female rats. The no-observed-effect level in rats exposed orally to MIBK was 50 mg/kg. Both gross and microscopic evidence of lung damage were reported in acute inhalation toxicity studies in animals. Short-term and subchronic inhalation exposures (as low as 100 ppm) produced effects in the kidney and liver that were species and sex dependent. Dermal doses of 300 or 600 mg/kg for 4 months in rats produced reduced mitotic activity in hair follicles, increased thickness of horny and granular cell layers of the epidermis, a decrease in the number of reactive centers in follicles (spleen), an increase in the number of iron-containing pigments in the area of the red pulp (spleen), and a reduction in the lipid content of the cortical layer of the adrenal glands. Neuropathological changes in the most distal portions of the tibial and ulnar nerves were observed in young adult rats which inhaled 1500 ppm MIBK for up to 5 months. No adverse effects were seen in any other neurological end point by any route of exposure in other studies using rats or other animal species. Clinical tests demonstrated a threshold for MIBK-induced irritation of the lungs at 0.03 to 0.1 mg/L after 1 min of respiration. MIBK was not mutagenic in the Ames test or in a mitotic gene-conversion assay in bacteria. Mammalian mutagenicity test results were also negative in the following assays: mouse lymphoma, unscheduled DNA synthesis, micronucleus, cell transformation, and chromosome damage. MIBK did not induce any treatment-related increases in embryotoxicity or fetal malformations in pregnant Fischer 344 rats or CD-1 mice that inhaled MIBK at concentrations of 300, 1000, or 3000 ppm. There was evidence of treatment-related maternal toxicity only at the highest concentration tested. MIBK applied to the tail of rats daily at doses of 300 or 600 mg/kg for 4 months produced changes in the testes, including a reduction in the number of spermatocytes, spermatids, and spermatozoa. An ongoing carcinogenicity study of MIBK being conducted by the National Toxicology Program will be considered when the results are available. On the basis of the information that is currently available, MIBK is considered safe as used in nail polish removers and as an alcohol denaturant in cosmetic products.

INTRODUCTION

This safety assessment focuses on the use of MIBK (Methyl Isobutyl Ketone) in cosmetic products. MIBK functions as both an alcohol denaturant and solvent, but most current cosmetic uses are solvent uses.

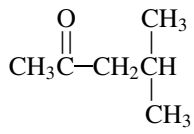
The European Chemical Industry Ecology and Toxicology Centre (ECETOC) prepared an earlier review of the toxicity of MIBK (ECETOC 1987). ECETOC concluded that the relatively high volatility of MIBK, its rapid atmospheric phototransformation, ready biodegradability, and low mammalian and aquatic toxicity indicate that the environmental hazards of MIBK are negligible.

In a more recent review, the World Health Organization (WHO) reached a similar conclusion (WHO 1990). The WHO concluded that the relatively high volatility, rapid atmospheric phototransformation, ready biodegradability, and low mammalian and aquatic toxicity of MIBK indicate that adverse environmental effects of this substance are only likely to occur after accidental spills or from uncontrolled industrial effluents.

CHEMISTRY

Chemical and Physical Properties

MIBK (CAS no. 108-10-1) is the aliphatic ketone that conforms to the following formula (Wenninger, Canterbery, and McEwen 2000):



MIBK has also been described as a branched chain hydrocarbon that is photochemically reactive (Billmaier et al. 1974). Other names for this chemical are as follows: Methyl Isobutyl Ketone; Isopropylacetone; 4-Methyl-2-Pentanone; and 2-Pentanone, 4-Methyl- (Wenninger, Canterbery, and McEwen

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TABLE 1
Chemical and physical properties of MIBK

Property	Value	References
Octanol/water partition coefficient (Log P)	1.966	Barrat 1997
	1.38	WHO 1990
	1.31	Tanii, Tsuji, and Hashimoto 1986
Molecular weight	100.16	WHO 1990
Physical form	Liquid	Verschueren 1983
Color	Colorless	Verschueren 1983
Odor	Faint, ketonic, and camphor	Budavari 1989
Taste	Sweet	Verschueren 1983
Solubility in water	17 g/L (20°C)	Verschueren 1983
	2.04% by weight (28°C)	EPA 1979
Boiling point	116.2°C (116°C to 119°C) at 101 kPa	EPA 1979
Freezing point	−80.26°C (range: −80°C to −85°C)	EPA 1979
Melting point	−84.7°C	EPA 1979
Flashpoint	14°C (closed cup)	Verschueren 1983
Autoignition temperature	460°C	Verschueren 1983
Explosion limits in air	1.4 to 7.5% vol at 101 kPa	Verschueren 1983
Specific gravity	0.8017 at 20°C/4°C	Verschueren 1983
	Not more than 0.799	Committee of Revision of the United States Pharmacopeial Convention 2000
Refractive index (n_D^{20})	1.395 to 1.397	Verschueren 1983
Viscosity	0.58 to 0.61 mPa at 20°C	Verschueren 1983
Vapor density (air = 1)	3.45	Verschueren 1983
Vapor pressure	1.99 kPa (20°C)	Verschueren 1983
	15 mm Hg (20°C)	EPA 1979
Concentration in saturated air	27 g/m^3 at 20°C and 101 kPa	Verschueren 1983

2000), and MIK; 2-Methyl-4-Pentanone; Hexanone; Hexone; Isopropyl-Acetone; 4-Methyl Pentan-2-One; 4-methyl-2-Oxopentane; 2-Methyl Propyl Methyl Ketone; and Isobutyl-methyl ketone (WHO 1990).

The chemical and physical properties of MIBK are summarized in Table 1.

Methods of Production

The commercial production of MIBK involves acetone condensation, followed by catalytic hydrogenation (Environmental Protection Agency [EPA] 1976; Chemical Manufacturers Association [CMA] 1999b).

According to Zakhari et al. (1977), acetone is dimerized to diacetone alcohol by a liquid phase reaction at 0°C to 20°C over a fixed-bed, alkaline catalyst. Diacetone alcohol is then dehydrated at 100°C to 120°C to 4-methyl-3-penten-2-one (aka mesityl oxide) in the presence of a weak acid. Finally, mesityl oxide is hydrogenated over nickel or copper at temperatures from 120°C to 165°C.

The CMA (1981) confirmed this process, noting that MIBK is typically manufactured via the aldol condensation of acetone to form diacetone alcohol. This is described as an enclosed, continuous process. Diacetone alcohol is then dehydrated to form mesityl oxide, site-limited intermediate, which is hydrogenated to MIBK. The crude MIBK is purified by continuous distillation.

Impurities

MIBK is 99% pure (by mass) and may contain the following impurities: <0.3% dimethyl heptane, <0.1% water, <0.06% methyl isobutyl carbinol, <0.03% mesityloxide, <0.002% acetic acid, and <0.002% nonvolatiles (WHO 1990). Another source indicates that MIBK is >98% pure and contains 0.9% methyl *n*-butyl ketone and trace amounts of 4-methyl-2-hydroxypentane (Eastman Kodak Company 1992).

Spencer et al. (1975) reported a 3% concentration of the contaminant, methyl *n*-butyl ketone (3.0%) in commercial MIBK. In 1999, however, MIBK producers indicated that methyl *n*-butyl ketone was either no longer found in MIBK or was found in trace amounts (typically 0.01% to 0.06% and always less than 0.1%) (CMA 1999a). Other impurities in MIBK include: methyl amyl alcohol, acetone, and 3-methyl-2-butanone (CMA 1999b).

Reactivity

MIBK does not undergo hydrolysis. However, because it is a branched-chain ketone, it may be photochemically active. The

half-life for the evaporation of MIBK is 33 h (Mackay and Wolkoff 1973).

MIBK has been described as dangerous when exposed to heat, flame (moderate explosion hazard), or oxidizers (Lewis 2000). Some of the oxidizing agents that MIBK may react violently with include peroxides, nitrates, and perchlorates. Additionally, when heated, MIBK may form peroxides by auto-oxidation; the peroxides may explode spontaneously (WHO 1990). MIBK ignites on contact with potassium *tert*-butoxide, and can react vigorously with reducing materials (Lewis 2000).

Analytical Methods

MIBK has been analyzed by gas chromatography (DiVincenzo, Kaplan, and Dedinas 1976; Raccio and Widomski 1981; Fernandes 1985; Cobb and Braman 1991), gas chromatography with flame ionization detection (EPA 1973; Moshlakova and Indina 1986), gas chromatography with mass spectroscopy (Zlatkis and Liebich 1971; Bellanca et al. 1982; Weller and Wolf 1989), high-resolution capillary gas chromatography (Clair, Tua, and Simian 1991), and infrared spectroscopy (Committee of Revision of the United States Pharmacopeial Convention 2000).

USE

Purpose in Cosmetics

MIBK functions as a denaturant and solvent in cosmetic products (Wenninger, Canterbery, and McEwen 2000). Frequency of use data provided by the Food and Drug Administration (FDA) in 1998 indicated that MIBK is used in 2 out of a total of 34 products in the nail polish and enamel remover category (FDA 1998). Data submitted to CTFA in 2000 indicate that MIBK is used at a concentration of 21%, specifically in a nail correction pen (volume = 3 ml) (CTFA 2000).

Cosmetic products containing MIBK are applied to the nail and may come in contact with skin adjacent to the nail or the ocular and nasal mucosae. These products could be used on a weekly basis, and could be applied frequently over a period of several years.

MIBK is included in the CTFA *List of Japanese Cosmetic Ingredients* (Santucci 1999). It has precedent for use without restrictions in nail makeup preparations. In Japan, MIBK is not used in cleansing preparations, hair care preparations, treatment preparations, make-up preparations, fragrant preparations, suntan/sunscreen preparations, eyeliner preparations, lip preparations, oral preparations, and bath preparations.

MIBK is not included among the substances listed as prohibited from use in cosmetic products marketed in the European Union (European Economic Community 1999).

Noncosmetic Use

MIBK is used primarily in industrial coating solvents, lube oil dewaxing, and in rare metal refining (EPA 1979). It is also used in public health environmental studies for determining the presence of heavy metals in air and in biological materials. For example, lead in air and biological materials can be extracted with MIBK and then analyzed by atomic absorption spectrophotometry (Zakhari et al. 1977).

MIBK has been approved as denaturant in denatured alcohol and rum, with specifications for its acidity, color, distillation range, odor, and specific gravity (27 CFR 21.161). Specifications for the composition of completely denatured alcohol formulas (27 CFR 21.21; 21.22; 21.23; 21.24) and specially denatured spirit formulas (27 CFR 21.31; 21.32; 21.49) containing MIBK are available. According to these specifications, established by the Bureau of Alcohol, Tobacco, and Firearms, the maximum concentration of MIBK that is listed for use as a denaturant of alcohol is 4.0%.

MIBK is also listed in the *National Formulary* as an alcohol denaturant that is used as an excipient for drugs (Committee of Revision of the United States Pharmacopeial Convention 2000).

MIBK has been approved for use as a component of synthetic flavoring substances and adjuvants (21 CFR 172.515) and as a component of adhesives that are present in articles intended for use in packaging, transporting, or holding food (21 CFR 175.105), and as an optional component (solvent-use only) of polysulfide polymer-polyepoxy resins that form the food-contact surface of articles intended for packaging, transporting, or holding dry food (21 CFR 177.1650).

BIOLOGICAL PROPERTIES

Fate of Inhaled MIBK

Hjelm et al. (1990) exposed eight male volunteers (18 to 35 years old; weights = 68–90 kg) to MIBK (concentrations of 2.4 ppm [10 mg/m^3], 24.4 ppm [100 mg/m^3], and 48.8 ppm [200 mg/m^3]) for 2 h during light physical exercise on three different occasions. Exposures took place in a 12-m^3 exposure chamber. Pulmonary retention of MIBK was described as fairly constant throughout the exposure period. The relative pulmonary uptake of MIBK was ≈60%, and total pulmonary uptake increased linearly with increasing exposure concentrations. Average values for uptake were 0.2 mmol at 10 mg/m^3, 1.7 mmol at 100 mg/m^3, and 3.2 mmol at 200 mg/m^3. At the end of exposure, blood concentrations of MIBK increased linearly with increasing uptake. No tendency toward saturation kinetics was observed over the range of doses tested. The apparent blood clearance was 1.6 L/h/kg at all exposure concentrations. The concentration of MIBK in the urine was higher than that noted in arterial blood both at 0.5 h and 3 h after exposure. Only 0.04% of the total dose was eliminated unchanged in the urine within 3 h post exposure. Results concerning the irritation potential (nose and throat) of MIBK and effects on the central nervous system that were recorded during the study are included in the section on Short-Term Inhalation Toxicity later in this report.

Fate of MIBK Applied to the Skin

Hjelm et al. (1991) evaluated the percutaneous absorption of MIBK using eight outbred female guinea pigs. Initially, to determine blood clearance values, MIBK was infused into each animal at a rate of 0.478 μmol MIBK per minute, corresponding to 0.680 to 0.928 μmol/minute/kg body weight, for 30 min. The average blood clearance of MIBK was 201 ml/min/kg body weight.

After a 2.5-h nontreatment period, the percutaneous absorption part of the study was begun. Hair on the back of each animal was clipped and epicutaneous exposure (150 min) was carried out by filling a glass cylinder, secured to the application site, with 1 ml of MIBK. Arterial blood was analyzed for MIBK using gas chromatography. A maximum percutaneous uptake rate of 1.1 μmol/min/cm^2 was reached at 10 to 45 min after the initiation of exposure. A decrease in the uptake rate to 0.56 μmol/min/cm^2 was noted during the latter part of exposure (75 to 135 min after the initiation of exposure).

Distribution

Using a mass-spectrometric method, Dowty, Laseter, and Storer (1976) demonstrated MIBK in human maternal blood samples collected immediately after delivery. The authors interpreted this finding as indicating the potential for MIBK to enter the umbilical cord and cross the placenta.

In vitro partition coefficients of 70 to 90 between blood and air have been reported (Sato and Nakajima 1979; Hjelm et al. 1990). Sato and Nakajima (1987) reported the following partition coefficients for MIBK: 90 (MIBK into blood), 79 (MIBK into water), and 926 (MIBK into oil).

Bellanca et al. (1982) reported that MIBK was detected in the brain, liver, lung, vitreous fluid, kidney, and blood (at concentrations ranging from 0.14 to 0.52 and 0.04 to 0.22 mg/100 g, respectively) in workers who died after exposure to several volatile organic solvents during spray painting.

Regardless of the route of administration, Duguay and Plaa (1995) reported that the amount of MIBK detected in the plasma and liver of Sprague-Dawley rats was proportional to the initial MIBK administered dose. Based on a linear-regression analysis for plasma and liver concentrations versus dose, the correlations were statistically significant. A dose-related increase in MIBK concentration in the lungs was also noted.

Metabolism

DiVincenzo, Kaplan, and Dedinas (1976) evaluated the metabolism of MIBK using male guinea pigs (weights = 250–450 g). A single dose of the test substance (450 mg/kg in corn oil) was administered intraperitoneally to each animal and blood samples were collected at 1, 2, 4, 6, 8, 12, and 16 h post dosing. After centrifugation of the samples, sera were assayed within 48 h. 4-Hydroxy-4-methyl-2-pentanone (HMP) and 4-methyl-2-pentanol (4-MPOL) were MIBK metabolites identified in the serum by gas chromatography and confirmed using gas chromatography–mass spectrometry. The authors stated that HMP and 4-MPOL result from the oxidation and reduction of MIBK, respectively. The serum half-life and total clearance time for parent MIBK were 66 min and 6 h, respectively. The total clearance time for HMP was 16 h. It was also stated that the hydroxylation products of MIBK, such as 4-MPOL, are expected either to be conjugated with sulfate or glucuronic acid and excreted in the urine or to enter intermediary metabolism to be converted to carbon dioxide.

Lande et al. (1976) reported that enzymatic ketonic reduction of MIBK to the alcohol 4-MPOL occurs in the liver, and that conjugation with glucuronic acid can occur prior to elimination in the urine.

DiVincenzo et al. (1980) demonstrated that 16 h was required for the elimination of both HMP and 4-MPOL metabolites.

According to Hjelm et al. (1990), inhaled MIBK accumulates in adipose tissue, because it is easily soluble in blood and has a high affinity for fat.

Proteins, chiefly hemoglobin, are the major carriers of MIBK in the blood (Lam et al. 1990).

According to the WHO, the structure of MIBK precludes the metabolic production of 2,5-hexanedione, a neurotoxic agent formed from methyl *n*-butyl ketone and hexane (WHO 1990).

Granvil, Sharkawi, and Plaa (1994) studied the metabolic fate of MIBK using groups of eight male Charles River CD-1 mice. The animals received a single intraperitoneal (IP) injection of 5 mmol/kg MIBK. MIBK was dissolved in corn oil, and the injection volume was 10 ml/kg. The animals were killed by decapitation and blood and brain samples were collected at 15, 30, 60, and 90 min post injection. The principal metabolites were 4-MPOL (reduction product) and 4-hydroxy-4 methyl-2-pentanone (oxidation product). The concentration of the reduction product in the brain was twice that seen in the blood at 15- and 30-min time intervals.

Duguay and Plaa (1995) reported that the MIBK metabolite 4-MPOL increased in a dose-related manner in the plasma, following oral or inhalation exposure using Sprague-Dawley rats. When MIBK was administered by gavage, 4-MPOL was not detected in the plasma, liver, or lung. However, following inhalation exposure, 4-MPOL was detected in all of the tissues. The authors concluded that metabolite concentrations were influenced by the route of MIBK administration.

Excretion

Zlatkis and Liebich (1971) reported that MIBK can also be eliminated unchanged in the urine. As indicated above, the metabolism of MIBK is an oxidative-reductive metabolic conversion.

Human volunteers were exposed to 100 ppm (410 mg/m^3) MIBK for 4 h in an environmental chamber. This group represented one of four groups exposed to MIBK, methyl ethyl ketone, or mixtures of the two. Ninety-eight male and female subjects were randomly assigned to the four exposure groups. Steady-state blood concentrations of MIBK were attained after

2 h of exposure. Blood and breath samples collected at 90 min post exposure indicated that most of the absorbed MIBK had been eliminated from the body (Dick et al. 1990).

Effect on Enzyme Activity

Lapin et al. (1982) stated that MIBK (50 and 200 mM) inhibited the enzyme activity of creatine kinase from rat muscle and adenylate cyclase from rat brain in vitro. Cunningham, Sharkawi, and Plaa (1989) reported that MIBK reduced the activity of mouse (CD-1 mice) liver alcohol dehydrogenase in vitro.

According to Raymond and Plaa (1995a), the oral administration to male rats of 1362 mg/kg MIBK in 5% polyoxyethylated castor oil produced increased renal cytochrome P-450 and aniline hydroxylase activity and increased liver and renal aminopyrine *N*-demethylase activity. No histopathology was noted. The authors were uncertain about the toxicological significance of these findings.

Effect on Cholestatic Activity

Plaa and Ayotte (1985) evaluated the effect of MIBK on the acute cholestatic response (change in bile flow) induced by sodium taurolithocholate using Sprague-Dawley rats (weights = 250–300 g). Animals were pretreated with MIBK daily with oral doses of 3.75 or 7.5 mmol/kg at a dose volume of 10 ml/kg for 3 or 7 days. Sodium taurolithocholate in a vehicle consisting of albumin, dextrose, and NaCl was then injected intravenously (5 to 25 mg/kg). Control animals were pretreated with corn oil. MIBK potentiated the decrease in bile flow that was induced by sodium taurolithocholate (TLC). Pretreatment with MIBK (7.5 mmol/kg oral doses) for three days, followed by intravenous (IV) dosing with sodium taurolithocholate (15 mg/kg) resulted in a 79% decrease in bile flow, compared to the 55% decrease in bile flow that was induced by similar pretreatment with corn oil followed by dosing with sodium taurolithocholate. Decreased bile flow was not noted in rats dosed only with MIBK.

The effect of MIBK on the cholestatic activity of manganese, with or without bilirubin, was evaluated by this same laboratory (Vézina, Ayotte, and Plaa 1985; Vézina and Plaa 1987, 1988) using male Sprague-Dawley rats. MIBK was administered by gavage at doses ranging from 188 to 1502 mg/kg once daily for 1, 3, or 7 days. MIBK was not cholestatic over the range of doses tested. However, it potentiated the cholestasis induced by a manganese-bilirubin combination, administered 18 h after dosing with MIBK. MIBK dosing for 3 or 7 days caused dose-related enhancement of cholestasis that had been induced by the manganese-bilirubin combination. Potentiation of the cholestasis induced by manganese alone was noted after dosing with 750 mg MIBK/kg for 3 days. In other experiments, two metabolites of MIBK (HMP and 4-MPOL) also potentiated the cholestatic effect of manganese or the manganese-bilirubin combination in male Sprague-Dawley rats.

Dahlstrom-King et al. (1990) demonstrated the potentiation of taurolithocholic acid–induced reduction in bile flow after oral dosing with MIBK (same procedure) in rats. Study results also indicated that this effect is not caused by alteration of the kinetics of taurolithocholic acid.

Joseph et al. (1992) evaluated the effect of MIBK on bile flow in male Sprague-Dawley rats. During oral exposure, the rats received MIBK in corn oil at the following doses for 3 days: 1.5 mmol/kg (0.5 MED [minimal effective dose]), 3 mmol/kg (1 MED), and 6 mmol/kg (2 MED). The MED was defined as the smallest dose of MIBK that potentiated the cholestatic response. Inhalation exposure consisted of exposure to 200, 400, or 600 ppm MIBK for 4 h.

In this study, cholestasis induced by taurolithocholic acid or manganese-bilirubin combinations was enhanced following oral or inhalation exposure to MIBK. When compared to control rats, the decrease in bile flow was more pronounced and lasted longer in rats preexposed to MIBK. Additionally, the oral administration of 1.5, 3.0, or 6 mmol/kg or 4 h of inhalation exposure to 200, 400, or 600 ppm resulted in equivalent MIBK plasma concentrations. No observable diminution in bile flow was noted in rats that received MIBK (alone) either orally or by inhalation for 3 days (Duguay and Plaa 1993). It has been suggested that MIBK potentiates lithocholate-induced cholestasis by reducing the bile salt pool and interfering with the rate of hepatic secretion of bile salts (Joseph et al. 1992).

In a study by Duguay and Plaa (1997) using male Sprague-Dawley rats, MIBK inhalation potentiated taurolithocholic acid (30 μmol/kg) and manganese-bilirubin (4.5 mg/kg Mn and 25 mg/kg bilirubin) induced cholestasis in a dose-related manner. The rats were exposed to MIBK for 3 days (4 h per day) at concentrations equivalent to 0.5, 1.0, 1.5, or 2.0 times the MEC (minimal effective concentration). The MEC was estimated to be 400 ppm for 3 days of exposure (4 h/day) to MIBK.

Antimicrobial Activity

The threshold concentration of MIBK for the inhibition of bacterial (*Pseudomonas putida*) growth was 275 mg/L in a 16 h study (WHO 1990).

ANIMAL TOXICOLOGY

Acute Oral Toxicity

Mice

McOmie and Anderson (1949) evaluated the acute oral toxicity of MIBK in five fasted mice (weights not stated). MIBK was administered intragastrically as a 10% to 40% emulsion in 1% aqueous Tergitol (dose volume = 0.2 ml/10 g). The Tergitol used in the emulsion is defined as the sodium sulfate derivative of 3,9-diethyl tridecanol-6. MIBK caused excitement and generalized involuntary movements; light anesthesia was also induced. At necropsy of mice that died, hyperemia of the stomach wall and duodenum was a common finding. The congested area usually extended throughout the length of the gut and along the mesenteric blood vessels. At microscopic examination, albuminous degeneration of the liver was the most significant finding. An LD_{50} of 1.5 ml/kg was reported.

Batyrova (1973) stated that the average lethal dose for MIBK in mice dosed orally (stomach tube) was 2.85 (2.638–3.078) g/kg. The number, strain, and weights of the animals tested were not stated.

Zakhari et al. (1977) evaluated the acute oral toxicity of MIBK using six groups of eight CF-1 male mice (weights = 23–34 g). Doses ranging from 0.9 to 3.5 g/kg per group were administered. An LD_{50} of 1.90 ± 0.68 g/kg was reported. All animals in the highest dose group died, whereas the mortality rate was 13% in the 0.9-g/kg dose group.

Rats

Acute oral LD_{50} values of 2.08 (1.91–2.27) g/kg (Smyth, Carpenter, and Weil 1951); 4.6 (3.932–5.382) g/kg (Batyrova 1973); and 5.7 ml/kg (CMA 1981) have been reported in studies involving rats.

Panson and Winek (1980) evaluated the acute oral toxicity of MIBK using six rats (three males, three females; body weights = 200–226 g). Each animal was given a single dose of 1 ml/kg and then observed over a 24-h period. Necropsy was then performed. All of the rats died instantly. Lung weights ranged from 1.42 to 2.51 g (mean = 1.84) and the lung weight/body weight ratio ranged from 0.70 to 1.23 (mean = 0.88). In most of the animals, 25% of the lung tissue (all right lobes and caudal lobe included) was hemorrhagic. In one animal, 50% of the lung tissue was hemorrhagic. A blood clot at the base of the heart was also noted in one animal. Thus, MIBK may be aspirated into the lungs when swallowed.

The Exxon Chemical Company (1982) evaluated the aspiration hazard and toxicity of MIBK using five male albino rats (weights = 179–267 g). The animals were anesthetized with diethyl ether vapor to the point of apnea, and 0.2 ml of the test substance was placed in the oral cavity of each. Next, the animals were held in a vertical position with mouths held open and nostrils closed at end of expiration phase of breathing cycle. The nostrils were closed to promote entry of the test material into the trachea. Negative controls were dosed with tap water. At 24 h post dosing, the lungs were removed from animals that died and surviving animals that were killed under ether anesthesia by exsanguination from the abdominal aorta. Some of the animals (number not stated) died; all deaths were due to respiratory arrest, cardiac failure, or both, rather than pulmonary edema. None of the negative-control animals died. It was concluded that MIBK presents a potential aspiration hazard.

Guinea Pigs

An acute oral LD_{50} in the range between 1600 and 3200 mg/kg has been reported for guinea pigs (CMA 1981).

Acute Intraperitoneal Toxicity

Mice

Zakhari et al. (1977) evaluated the acute intraperitoneal toxicity of MIBK using six groups of 8 to 10 CF-1 male mice (weights = 20–23 g). The doses injected per group ranged from 0.25 to 1.25 g/kg. An acute IP LD_{50} of 0.59 ± 0.23 g/kg was reported at 24 h post injection. All animals in the 1.25-g/kg dose group died, whereas no deaths occurred in the 0.25-g/kg dose group. These authors also reported that the IP administration of MIBK to cats caused pulmonary vascular effects. The threshold dose for these effects was 8 mg/kg. However, bronchoconstriction was not noted after IP administration of MIBK at doses ranging from 4 to 32 mg/kg in the cats.

Guinea Pigs

Divincenzo and Krasavage (1974) evaluated the hepatotoxicity of MIBK was evaluated using mature guinea pigs. The test substance (in corn oil) was injected intraperitoneally at doses of 500 and 1000 mg/kg (four animals per dose). Blood was drawn at 24 h post injection and the animals were then killed. Serum ornithine carbamyl transferase (OCT) activity in the blood was measured using a spectrophotometric procedure. OCT is an enzyme that is found predominantly in the liver, and is released into the blood stream whenever liver cells are ruptured. MIBK induced a slight, but insignificant increase in serum OCT activity. One of the animals dosed with 1000 mg/kg MIBK died. Serum OCT activity in this animal was comparable to that observed at the 500-mg/kg dose. Neither histologic evidence of liver damage nor lipid deposition was observed in any of the guinea pigs tested.

Multiple Species

The Eastman Kodak Company (1982a) evaluated the acute intraperitoneal toxicity of MIBK using four groups of six male rats (Carworth Farms; weights = between 128 and 210 g) and four groups of six male guinea pigs (strain not stated; weights = 210–770 g). Both groups of six animals received MIBK in doses of 0.5, 1.0, 2.0, and 4.0 ml/kg body weight, respectively. The following signs were observed in rats after dosing: weakness, ataxia, prostration, dyspnea, and vasodilation. Signs indicative of demyelination or other nervous system damage were not observed. Deaths (rats) occurred anywhere from less than one day to six days after dosing, and the time to death was inversely related to the dose administered. The mortality data were as follows: 6/6 (4.0 ml/kg), 6/6 (2.0 ml/kg), 1/6 (1.0 ml/kg), and 0/6 (0.5 ml/kg). An acute IP LD_{50} (rats) of 1.14 ml/kg was reported. For guinea pigs, the authors reported that the signs of intoxication and time to death were similar to the data reported for rats above. The mortality data were as follows: 6/6 (4.0 ml/kg), 4/6 (2.0 ml/kg), 3/6 (1.0 ml/kg), and 2/6 (0.5 ml/kg). An acute IP LD_{50} of 0.919 ml/kg was reported.

Acute Intravenous Toxicity

Zakhari et al. (1977) injected MIBK intravenously into nine male cats to determine whether the pulmonary effects noted following inhalation (study described earlier) were limited to this route of exposure. Geometrically increasing doses of MIBK ranging from 4 to 128 mg/L were injected (single injection per dose). Dosing with 4 mg/kg MIBK resulted in no response.

MIBK (8 mg/kg) induced a 17% increase in mean pulmonary arterial pressure and a 34% decrease in mean pulmonary arterial flow. The authors stated that these results were indicative of an intense increase in pulmonary vascular resistance (84%), and that, most likely, this increase in resistance was caused by pulmonary vasoconstriction.

In another experiment, MIBK was injected intravenously into eight male cats to determine whether bronchoconstriction could be produced by this route of exposure. The IV injection of MIBK at doses ranging from 4 to 32 mg/kg did not result in an increase in pulmonary resistance or transpulmonary pressure. The authors concluded that bronchoconstriction, previously reported to be induced by MIBK inhalation, was not observed after intravenous dosing. Both a precipitous hypotension and apneic response (unexpected results) were observed simultaneously after the injection of 64 mg/kg MIBK (Zakhari et al. 1977).

Acute Dermal Toxicity

The acute dermal toxicity of MIBK was evaluated using two rabbits. Undiluted MIBK was applied (10 h of exposure) either by flooding the test site or placement of a cotton pad impregnated with the test substance. Signs of systemic effects were not noted during the 10-day observation period. At microscopic examination, there were no pathologic changes in the internal organs that resulted from exposure to MIBK. Irritation reactions are reported in the section on Skin Irritation later in this report (McOmie and Anderson 1949).

Acute Inhalation Toxicity

Mice

A concentration-dependent decrease in respiratory rate during 5 min of exposure to MIBK was noted in male Swiss OF_1 mice. A 50% decrease in the respiratory rate (RD_{50}) was noted after exposure to MIBK at a concentration of 3195 ppm. In this study, the reflex decrease in the respiratory rate of mice was measured as an index of sensory irritation (De Ceaurriz et al. 1981). In an earlier study, the decreased respiratory rate induced by MIBK was said to have been due to a narcotic effect (Specht et al. 1940).

McOmie and Anderson (1949) exposed six groups of mice (6 to 33 mice/group) to MIBK (saturated air-vapor mixture) at concentrations ranging from 43 to 100 mg/L of air (20°C). Each group received a single exposure and the duration ranged from 0.25 to 22.6 h.

Mortality data were provided for three of six groups. In the group exposed to 82 mg/L for 0.5 h, 18 of 33 animals died. In the group exposed to 86 mg/L for 1 h, 21 of 22 animals died. And in the group exposed to 82 mg/L for 1.25 h, 5 of 10 died. Deaths occurred by 10 h post exposure. Signs of irritation (e.g., closed eye, pawing at nose) were reported for all animals tested. Intense excitement and rapid, shallow respiration was noted after 3 min of exposure. The behavior of some mice ranged from convulsive movements to depression, with some animals lying prone. Furthermore, in the group of 33 mice, 30 animals had a loss of righting reflex in 30 min. At microscopic examination of animals that died, damage to the lung was the most common finding. Congestion, and, in some instances, hemorrhage and pneumonia were noted. Congestion noted in the liver and kidney was not as severe (McOmie and Anderson 1949).

Batyrova (1973) reported that narcosis was induced in all mice (number, weights, and strain not stated) exposed to MIBK (15 mg/L of air) for 2 h. An exposure concentration of 23.3 (18.49–29.36) mg/L was classified as moderately fatal.

Zakhari et al. (1977) evaluated the acute inhalation toxicity of MIBK using five groups of 10 CF-1 male mice (weights = 20–23 g). The animals were exposed to various concentrations of the test substance (1.0% *v/v* [41 mg/L] to 3.0% *v/v* [123 mg/L]) in a 10-L glass chamber. The mortality rate was determined after 45 min of exposure. Exposure to 1.0% *v/v* MIBK and to a saturated concentration of 3% MIBK resulted in no deaths and a mortality rate of 80%, respectively. The LC_{50} (95% fiducial limit) was 74.2 ± 25.8 mg/L.

The CMA (1981) reported that the acute inhalation toxicity of MIBK was evaluated using 10 mice. Exposure to 19,500 ppm MIBK induced anesthesia within 30 min. Recovery from this effect was noted within 5 min after exposure was discontinued. MIBK (concentrations above 20,000 ppm) also induced anesthesia within 30 min, which was followed by death of most of the animals. Congestion of the lungs was observed at gross necropsy.

Cometto-Muñiz and Cain (1991) stated that the RD_{50} (mice) for MIBK is 3195 ppm. The RD_{50} is defined as the concentration of an irritant that is expected to cause a 50% decrease in respiratory rate. Alarie (1966) had noted that the measurement of a decrease in respiratory rate of experimental animals (specifically mice) exposed to airborne irritants serves as an index of sensory irritation.

Rats

Smyth, Carpenter, and Weil (1951) evaluated the acute inhalation toxicity of MIBK (4-Methyl-2-Pentanone) using two groups of six rats (weights and strain not stated). The two groups were exposed to test concentrations of 2000 and 4000 ppm, respectively, for 4 h. None of the animals exposed to 2000 ppm died, whereas, 4000 ppm resulted in death of all six animals.

Batyrova (1973) exposed rats (number, weights, and strain not stated) to MIBK at a concentration of 0.2 mg/L for 4 h. The threshold concentration for inhalation intoxication (change in conditioned reflex activity, using the maze procedure) was 0.2 mg/L.

The CMA (1981) stated that all rats (number and strain not stated) exposed to 21,000 ppm MIBK for 55 minutes died. Rats exposed to 4,000 ppm MIBK for 6 h experienced loss of coordination and prostration.

Guinea Pigs

Specht (1938) evaluated the acute inhalation toxicity of MIBK was evaluated using 10 female guinea pigs (weights ≈300 g).

The animals were exposed to the following concentrations of MIBK in a 1-cm^3 inhalation chamber: 2.8 volume % (saturation), 1.68 volume %, 1.0 volume %, 0.3 volume %, and 0.1 volume %. Death occurred within 4 h at a concentration of 1.0 volume % and at progressively shorter periods at higher concentrations. At an exposure concentration of 1.68%, 9 of the 10 animals died by 142 min post exposure. Marked irritation, indicated by lacrimation and salivation, was observed in guinea pigs at higher concentrations. The animals that died during exposure were subjected to gross and microscopic evaluations. Gross changes were described as slight and consisted mainly of congestion, especially in the brain and lungs. At microscopic examination, a fine droplet fatty metamorphosis was present in many liver cells. However, most liver cells were normal and many sections of the liver had no pathology. No abnormalities in the kidneys or heart were observed. However, congestion and hypertrophy of the spleen was evident. Both gross and microscopic pathology was described as slight, resembling that of most acute reactions to solvent exposures. Gross findings in survivors of the study were not different from those noted in controls.

This same laboratory (Specht et al. 1940) exposed female guinea pigs to MIBK at concentrations of 1000 ppm (4100 mg/m^3), 16,800 ppm (69,000 mg/m^3), and 28,000 ppm (115,000 mg/m^3) for 24 h. A decrease in the respiratory rate (narcotic effect during first 6 h) and minimal ocular or nasal irritation were noted during exposure to 1000 ppm MIBK. The following signs were noted at higher concentrations: ocular and nasal irritation, salivation, lacrimation, ataxia, progressive narcosis, and death. Half of the animals exposed to the highest test concentration (28,000 ppm) died within 45 min of exposure. The following observations were made in some of the animals that were subjected to necropsy/microscopic examination: fatty livers and congestion of the brain, lungs, and spleen. No damage to the heart and kidneys was noted.

Cats

Batyrova (1973) determined the threshold for MIBK-induced irritation of the lungs using cats (number, weights, and strain not stated) with fistulae of the parotid gland. Salivation served as the index for respiratory irritation. After 15 min of exposure, the irritation threshold was between 0.25 and 0.50 mg/L.

Zakhari et al. (1977) evaluated the pulmonary and systemic vascular response following inhalation exposure to MIBK using nine male cats (weights = 2.9–3.4 kg). MIBK was volatilized by injection of a measured volume into a stream of air entering a breathing bag. The bag was connected to the inlet of a respirator and a cat was exposed to MIBK vapor (5 min/concentration) according to a sequence of increasing concentrations (*v*/*v*): 0.01% (0.41 mg/L), 0.05% (2.05 mg/L), 0.10% (4.1 mg/L), 0.25% (10.25 mg/L), 0.5% (20.5 mg/L), and 1.0% (41.0 mg/L).

Compared to controls, an MIBK concentration–dependent increase in mean pulmonary arterial pressure and pulmonary vascular resistance was observed.

Pulmonary vasoconstriction was noted at all concentrations of MIBK. The lowest test concentration (0.01% MIBK) caused a small, but significant, increase (2%, $p < .05$) in mean pulmonary arterial pressure. The greatest increase in mean pulmonary arterial pressure (9% increase) and pulmonary vascular resistance (18% increase) were noted during exposure to 1% MIBK. The preceding changes were accompanied by a steady recovery of mean pulmonary arterial flow to control levels. Concerning systemic effects, changes in mean arterial pressure were variable (i.e., no overall pattern observed). Mean left atrial pressure remained unchanged. Nonsignificant increases (3% to 4%) in mean arterial pressure and systemic vascular resistance were noted during the inhalation of MIBK concentrations ranging from 0.01% to 0.25%. MIBK (1%) induced a nonsignificant 4% to 5% decrease in mean arterial pressure and systemic vascular resistance.

In this same report, the effect of MIBK inhalation on respiratory responses was evaluated using eight male cats (weights = 2.7–3.3 kg). The animals (free-breathing, close-chest) were exposed to the following vapor concentrations of MIBK (*v*/*v*): 0.01%, 0.05%, 0.10%, 0.25%, 0.5%, and 1.0% (5 min exposure/test concentration). Like the preceding experiment, these vapor concentrations were prepared in breathing bags. A constant stream of vapor was provided by a pump that was placed between the vapor bag and the inlet port (connected to the tracheal cannula).

Compared to controls, MIBK induced significant changes in airway resistance and transpulmonary pressure. The first statistically significant increases in transpulmonary pressure and airway resistance were observed during ventilation with 0.10% and 0.05% MIBK, respectively. These two parameters reached a maximum during the inhalation of 0.5% MIBK. Decreased dynamic compliance, beginning with the inhalation of 0.05% MIBK and reaching a maximum during 0.25% MIBK inhalation, was also noted. The magnitude of these bronchopulmonary responses was classified as somewhat attenuated during the inhalation of 1.0% MIBK. Bronchoconstriction was the primary finding in these inhalation experiments. This effect was characterized by a small but statistically significant increase in pulmonary resistance and no increase in tracheal air flow (Zakhari et al. 1977).

Dogs

Zakhari et al. (1977) administered the following concentrations of MIBK to dogs of either sex (weights = between 18 and 24 kg) via the inlet of a respirator: 0.01%, 0.05%, 0.10%, 0.25%, 0.50%, and 1.0%. The duration of exposure to each concentration was 5 min. The chest of each animal was opened and various hemodynamic parameters were studied.

At a concentration as low as 0.05%, MIBK induced an increase in mean pulmonary arterial pressure (5.1% increase), effective mean pulmonary arterial pressure (5.8% increase), and pulmonary vascular resistance (6.9% increase). The increase in each parameter was intensified at higher concentrations.

Exposure concentrations up to 0.5% induced either no effect or a nonsignificant decrease in myocardial contractility. However, exposure to 1% MIBK induced a significant decrease (15.9%) in myocardial contractility. Significant decreases in left ventricular pressure and systemic vascular resistance were also noted. The observed increases in heart rate were described as consistent and concentration dependent, but statistically nonsignificant. Decreases in mean pulmonary arterial flow, stroke volume, and stroke work were observed at high concentrations of exposure (0.5% and 1.0% MIBK) (Zakhari et al. 1977).

Short-Term Oral Toxicity

Batyrova (1973) reported that the administration of increasing oral doses of MIBK (emulsion in 2% starch solution) resulted in the death of 9 of 10 mice by day 24 of dosing. The first animal deaths were noted on day 8 (total dose of MIBK = 3.82 g/kg). In most of the animals, severe clonic-tonic spasms occurred prior to death. The total average lethal dose was 9.35 g/kg.

The Carnegie Mellon Institute of Research (1983) administered MIBK at concentrations of 0.5% and 1.0% in drinking water to two groups of three Wistar female rats (4 weeks old), respectively, for 7 days. Two groups of three rats served as untreated controls. Pale kidneys were noted in two of three rats dosed with 1% MIBK, and all three rats dosed with 0.5% MIBK had pale, mottled kidneys. Similar findings were reported for both untreated control groups. The authors concluded that no evidence of gross pathologic effects was observed in animals dosed with MIBK. The results of the subchronic study are included in the section on Subchronic Oral Toxicity later in this report.

Short-Term Dermal Toxicity

McOmie and Anderson (1949) made seven applications (3 ml/kg each, 5 to 12 h) of undiluted MIBK to a 100-cm^2 area of shaved skin on each of two rabbits over a period of 15 to 21 days. At microscopic examination, there were no pathologic changes in the internal organs that resulted from exposure to MIBK. Local changes in the skin consisted of polymorphonuclear infiltration in the upper dermis. Hair follicles appeared normal, and there was no evidence of sloughing of keratin. No systemic effects were noted. Irritation reactions are included in the section on Skin Irritation later in this report.

Short-Term Inhalation Toxicity

Mice

McOmie and Anderson (1949) subjected 10 mice to 15 20-min exposures to a saturated air–MIBK vapor mixture (74 to 98 mg/L of air). Deaths (six animals) occurred on days 1, 6, and 9 post inhalation.

CMA (1981) stated that, following daily exposures to 20,000 ppm MIBK for 15 days (20 min/day), 6/10 exposed mice died.

The Bushy Run Research Center (1982) exposed three groups of $B6C3F_1$ mice (six males, six females) to MIBK at concentrations of 101 ppm (44 mg/m^3), 501 ppm (2050 mg/m^3), and 1996 ppm (8180 mg/m^3), respectively, for 9 days (6 h/day). A fourth group served as the untreated control. The first 5 days and the remaining 4 days of exposure were separated by a 2-week nontreatment period. A fourth group served as the control.

At the highest exposure concentration (1996 ppm MIBK), an increase in liver weight (as a % of body weight) was observed in female mice, but not in male mice. A significant increase in both absolute and relative kidney weights (females) and a decrease in relative kidney weight (males) were also noted in the 1996 ppm exposure group. No ophthalmological lesions or alterations in body weight resulted from exposure to 1996 ppm MIBK. No statistically significant effects on liver weight, kidney weight, or other organ weights were observed in mice exposed to 501 ppm MIBK.

At a concentration of 101 ppm, a statistically significant decrease in liver weight (as a % of body weight) was observed in male mice but not in female mice. No significant changes in kidney weight or other organ weights were noted in male or female mice exposed to 101 ppm MIBK. Compared to controls, no statistically significant histologic lesions were observed at any of the concentrations tested (Bushy Run Research Center 1982).

Rats

The Bushy Run Research Center (1982) exposed three groups of F-344 rats (six males, six females) to MIBK at concentrations of 101 ppm (44 mg/m^3), 501 ppm (2050 mg/m^3), and 1996 ppm (8180 mg/m^3), respectively, for 9 days (6 h/day). A fourth group served as the untreated control. The first 5 days and the remaining 4 days of exposure were separated by a 2-day nontreatment period.

In the highest dose group (1996 ppm), an increase in liver weight (as % of body weight) and a significant increase in both absolute and relative kidney weights were noted in male and female rats. Epithelial regeneration of the proximal convoluted tubules was also noted at 1996 ppm. No ophthalmological lesions or alterations in body weight resulted from exposure to 1996 ppm MIBK. In the 501-ppm exposure group, a nonsignificant increase in kidney weight and a statistically significant increase in liver weight were observed in male rats, but not in female rats. In both 501- and 1996-ppm exposure groups, hyaline droplet formation was observed in the kidneys of male rats. No microscopic abnormalities were noted in rats exposed to 101 ppm MIBK (Bushy Run Research Center 1982).

Phillips et al. (1987) conducted a 2-week probe study on MIBK using male and female Fischer-344 rats and B6C3F1 mice; six males, six females per group per species. Groups within each species were exposed to MIBK at concentrations of 100, 500, and 2,000 ppm, respectively, 6 h per day, 5 days/week for 2 weeks. A fourth group per species served as the untreated control. None of the animals died. A slight increase in both

absolute and relative liver weight was noted in male rats exposed to 2000 ppm MIBK. The only microscopic changes reported were increases in regenerative tubular epithelium and hyaline droplets in the kidneys of male rats exposed to 500 or 2000 ppm MIBK.

Hazleton Labs, Inc. (1992) evaluated the short-term inhalation toxicity of MIBK using young adult albino rats (Charles River Caesarian-derived strain; 10 males, 10 females). The animals were exposed to MIBK at a concentration of 4.53 mg/L of air 5 days per week (6 h/day) for 4 weeks. The control group was exposed to filtered room air. No signs of irritation (i.e., nasal bleeding) were observed in any of the test animals throughout the entire exposure series, although slight nasal bleeding was noted in the control group. Compared to the control group, exposed females had a significantly higher adrenal/body weight ratio. Additionally, exposed females had a 1.4% increase in lymphocytes and a 1.0% decrease in segmented neutrophils.

Multiple Species

In a range-finding study, a laboratory at the Wright-Patterson Air Force Base Aerospace Medical Research Laboratory (1971) continuously exposed 4 monkeys, 8 dogs, 40 mice, and 50 rats to a mean concentration of 100 ppm MIBK for 2 weeks. Control groups consisted of 3 monkeys, 4 dogs, 20 mice, and 25 albino rats.

A comparison of the results for test and control groups revealed no signs of toxicity during exposure, no differences in cortical activity (based on electroencephalogram [EEG]), no differences in hematologic or clinical chemistry measurements between dogs or monkeys, and, no differences at gross examination of tissues. However, compared to controls, a significant increase in kidney weight and in the kidney-to-body weight ratio ($p < .01$) was noted in rats exposed to MIBK. Growth was also slightly depressed in rats.

When this experiment was repeated at a higher level of exposure (200 ppm MIBK), the following statistically significant effects were reported: increased kidney weight and kidney-to-body weight ratio ($p < .01$), increased liver weight and liver-to-body weight ratio ($p < .01$), and increased heart-to-body weight ratio ($p < .05$). In both experiments (rats), the kidneys were primarily affected. At microscopic examination, toxic nephrosis of the proximal tubules was observed in tissues from rats exposed to 100 and 200 ppm MIBK (Wright-Patterson Air Force Base Aerospace Medical Research Laboratory 1971).

Subchronic Inhalation Toxicity

Mice

In a Union Carbide Corporation (1983) study, subsequently reported by Phillips et al. (1987), three groups of $B6C3F_1$ mice (14 males, 14 females) were also exposed to the same concentrations of MIBK in the rat study described above. A fourth group served as the control.

Growth retardation was not observed in any of the animals tested. A slight increase in liver weight (~11%) and in the liver weight per body weight ratio was noted in male mice exposed to 1000 ppm MIBK. Liver weight was also slightly increased in male mice exposed to 250 ppm MIBK. Exposure to 1000 ppm MIBK resulted in no hepatic lesions at gross necropsy or microscopic examination and urinalysis and serum chemistry values were normal.

Rats

Batyrova (1973) exposed a group of 70 rats to MIBK at concentrations ranging from 86 to 127 mg/m^3 (average concentration = 115 ± 14 mg/m^3) 5 days per week (4 h/day) for 4.5 months.

An increase in the time required for traversing a maze was observed in rats with a previously developed and reinforced food reflex. The conditioned reflex was less clearly developed and easily slowed during the action of random external stimuli. Disruption of the speed of extinction of the elementary defensive reflex (in Lyubimov chamber) and the ability to practice the reflex at the end of exposure was also noted. Other findings included narcosis, disruption of the detoxifying function of the liver, and decreased eosinophil count. Compared to controls, the number of eosinophils in the blood of test animals was noticeably less. It was also noted that the adrenaline load and painful irritation yielded no differences in the eosinopenic reaction in test and control rats.

The author also reported decreased weight of the liver and adrenal glands in animals exposed to MIBK compared to controls. Weight coefficients of internal organs were compared at 2 months after initiation of exposure, at the end of exposure, and at 1 to 2 months after the end of exposure. Disruption of blood circulation and dystrophic changes in the parenchymatous elements (up to necrobiosis) were detected in the central nervous system and in the most important internal organs.

In another experiment by this author, subchronic exposure to MIBK at a concentration of 30 mg/m^3 induced insignificant changes in the function of the central nervous system, which is said to be most sensitive to the effects of MIBK (Batyrova 1973).

In a Union Carbide Corporation (1983) study, subsequently reported by Phillips et al. (1987), three groups of F-344 rats (14 males and 14 females in each group) were exposed to 50 ppm (205 mg/m^3), 250 ppm (1025 mg/m^3), and 1000 ppm (4100 mg/m^3) MIBK 5 days per week (6 h/day) for 90 days. A fourth group served as the untreated control. Growth retardation was not observed in any of the animals tested. A slight increase in liver weight (~11%) and in the liver weight per body weight ratio was noted in male rats exposed to 1000 ppm MIBK. It is important to note that exposure to 1000 ppm MIBK resulted in no hepatic lesions at gross necropsy or microscopic examination and that urinalysis and serum chemistry values were normal. However, an increase in the number of hyaline droplets in the proximal tubular cells of the kidney was noted in male rats of the 250- and 1000-ppm exposure groups. No other gross or microscopic changes in the kidney were observed. The authors stated

that the significance of an increase, compared to controls, in the occurrence of hyaline droplets in male rats was not known. Additionally, it was noted that the presence of hyaline droplets did not appear to be associated with major alterations in kidney function.

According to Alden et al. (1984), increased hyaline droplet formation is thought to be related to a rat-specific protein, α-2u-globulin, which is found predominantly in male rats. Alden (1986) indicated that the hyaline droplet, renal effects observed in male rats exposed to MIBK may be specific to the male rat, and, therefore, these effects (in male rats) do not constitute an appropriate model for man.

In a commentary on the Union Carbide study above, EPA (1991) noted that evidence of increased renal α-2u-globulin levels (indicative of alpha 2u-globulin nephropathy) was not reported.

Multiple Species

The Wright-Patterson Air Force Base Aerospace Medical Research Laboratory (1971) conducted a subchronic inhalation toxicity study of MIBK in three species: rats, dogs, and monkeys. Male Wistar albino rats (100), male Beagle dogs (8), and male *Macaca mulatta* monkeys (2) were exposed to 410 mg/m^3 MIBK vapor (100 millimoles/25 m^3) for 90 days in an altitude chamber. The control group (no MIBK exposure) was maintained in a separate altitude chamber. Liver function tests (dogs only) involved the intravenous injection of bromsulphalein, followed by determination of the dye concentration 15 min later. Tissue sections from the following organs (test and control animals) were subjected to gross and microscopic examination: heart, lung, brain, liver, spleen, kidney, adrenal glands, and pituitary gland.

The results of clinical chemistry and hematology tests on dogs and monkeys revealed no biologically significant differences between test and control animals. There were also no significant differences in liver function test results between test and control dogs. Gross examination also revealed no differences in the tissues examined (heart, lung, brain, liver, spleen, kidney, adrenal glands, and pituitary gland) between test and control animals.

Microscopic examination of kidney sections revealed hyaline droplets in one test and one control dog, fat in a few tubules at the corticomedullary junction in dogs (classified as common finding in untreated dogs), and focal chronic inflammation of the kidney in one monkey. Statistically significant increases in liver and kidney weights and organ-to-body weight ratios for these tissues were noted in rats exposed to MIBK. This increase in liver weight was not associated with any pathological changes. However, microscopic examination of kidneys revealed hyaline droplet degeneration of the proximal tubules (with occasional foci of tubular necrosis) in each of the 100 rats exposed to MIBK, including those that were removed from the inhalation chamber after 15, 22, 28, 71, and 85 days. It is important to note that a trend toward a linear progression of hyaline droplet degeneration during exposure was observed, but that this pattern was not associated with all animals. Additionally, the hyaline droplets appeared larger with time. This observation was thought to have resulted from the coalescence of smaller droplets.

Microscopic examination of rat kidneys, removed after 15 days of exposure, indicated a gradual reversion of tubular damage with time. Kidney damage was completely reversed in rats observed up to 60 days post exposure. Recovery from MIBK-induced kidney lesions was also noted in rats that were serially killed for reversibility studies after 90 days of exposure. However, recovery was not as rapid as that noted in animals exposed for shorter periods. Growth rate (rats) was unaffected by continuous exposure to MIBK (Wright-Patterson Air Force Base Aerospace Medical Research Laboratory 1971).

Subchronic Oral Toxicity

In a study reported by the Carnegie Mellon Institute of Research (1983), the subchronic oral toxicity of MIBK was evaluated using five Wistar female rats (4 weeks old). MIBK was administered at a concentration of 1.3% in drinking water daily for 120 days (MIBK dose = 1.04 g/kg/day). Two groups of five rats that received tap water served as untreated controls. Neurological evaluations for any treatment-related effects during the study included observations of any changes in balance, strength, coordination, or behavior. The animals were killed by CO_2 narcosis and subjected to gross and microscopic examination. Body weight gain in animals dosed with MIBK was not significantly different from that noted in controls. MIBK induced a statistically significant increase in relative and absolute kidney weight. No significant gross lesions were noted in any of the tissues examined. Tubular cell hyperplasia was noted in the kidney of one of the five rats. Results concerning the neurotoxicity of MIBK are included in the section on Neurotoxicity later in the report text.

WHO (1990) described a subchronic oral toxicity study of MIBK using three groups of Sprague-Dawley rats (30 males, 30 females). The test substance was administered to the three groups in doses of 50, 250, and 1000 mg/kg, respectively, daily for 13 weeks. All animals that survived were killed at the end of the dosing period. Ten animals (five males, five females) from each treatment group were subjected to gross and microscopic examination. In the highest dose group (1000 mg/kg), nephrotoxicity and increased liver and kidney weights were observed in males and females. Hepatic lesions were not observed at microscopic examination. These effects were significantly less pronounced in females and males of the 250-mg/kg dose group, and were not observed in the 50-mg/kg dose group. Thus, the 50-mg/kg dose of MIBK was considered the no-observed-effect level.

Subchronic Dermal Toxicity

Malyscheva (1988) evaluated the dermal toxicity of MIBK (in sunflower oil) using white rats (males, number not stated). The test substance was applied to the tail (lower 2/3) of each animal daily in doses of 300 mg/kg. Intermittent application involved 600-mg/kg doses. The duration of the study was 4 months.

Control animals were dosed with sunflower oil according to the same test procedures.

The intermittent application of MIBK resulted in an undulatory increase in the activity of copper-containing oxidase. At 1 month of daily application, there was a sharp increase (82.4%) in enzyme activity. This was not a consistent finding following this type of exposure because a 7% to 20% increase in enzyme activity was noted in other groups dosed according to the same procedure. At 2 months of daily application, a 160% increase in enzyme activity was noted.

In the control group and the group subjected to intermittent administration for 2 months, the increase in enzyme activity was 60% to 70%. Following 3 months of administration, the increases in enzyme activity were as follows: intermittent administration (39.67%), daily administration (101.93%), and control group (32.6%).

An increase in the number of binuclear hepatocytes, reduced mitotic activity of these cells, and an increase in the number of hepatocytes with pathology were observed in the liver. Morphological changes in the internal organs (liver, adrenal glands, spleen, and testes) and skin of white rats were noted after monotonic administration of MIBK. The following changes were observed in the liver: increase in number of binuclear hepatocytes, decrease in mitotic activity of the cells, and an increase in the number of hepatocytes with pathology. A reduction in the lipid content of the cortical layer was observed in the adrenal glands and changes in the spleen included a reduction in the number of reactive centers in follicles and an increase in the number of iron-containing pigments in the area of the red pulp. Changes in the testes included a reduction in the number of spermatocytes, spermatids, and spermatozoa. The daily application of MIBK caused changes in the skin. Mitotic activity in the basal layer and the sprout layer of the hair follicles was reduced, and the thickness of the horny layer of the epidermis and the granular cell layer was increased. Following intermittent exposure, similar, but smaller, changes in the skin and liver were observed (Malyscheva 1988).

Chronic Intraperitoneal Toxicity

MIBK was injected intraperitoneally into rats (strain and number not specified) five times per week for 35 weeks. During the first 2 weeks the animals were injected with doses of 10, 30, or 100 mg/kg body weight. For the remainder of the study, the doses were doubled. After 3 to 4 weeks of dosing, body weight gain suppression was noted. Transient narcosis was observed during the first 4 weeks of treatment with the highest dose (100 mg/kg) (CMA 1981).

Effect of MIBK on the Hepatotoxicity of Other Agents

1,2-Dichlorobenzene

Brondeau et al. (1989) investigated the effect of MIBK on the hepatotoxicity of inhaled 1,2-dichlorobenzene (DCB) using male Sprague-Dawley rats and OF1 mice. The aim of this study was to compare the interactive liver responses of MIBK with DCB and to explain them in terms of metabolic changes. Thus, in rats, the mitochondrial enzyme glutamate dehydrogenase (GLDH) was assessed in serum as a quantitative sign of hepatic necrosis, and liver cytochrome P-450 content and glutathione-*S*-transferase (GST) activity as indicators of phase I and phase II metabolic effects, respectively. Three groups of rats (five per group) were exposed to MIBK at concentrations of 595, 1280, and 3020 ppm, respectively, for 4 h in 200-L inhalation chambers. After an 18-h nontreatment period, the rats were exposed to 377 ppm DCB for 4 h. Three groups of eight mice were exposed to MIBK at concentrations of 664, 1477, and 3260 ppm, respectively, followed by exposure to 263 ppm DCB, according to the same procedure. Control mice and rats inhaled air only. At 24 h post exposure to DCB, the rats were exsanguinated from the abdominal aorta, and serum GLDH activity was measured. The livers were removed, homogenized, centrifuged, and the microsomal pellet that resulted from ultracentrifugation was assayed for cytochrome P-450 activity. The mice were killed at 48 h post exposure to DCB. Liver glucose-6-phosphatase (G-6-Pase) staining intensity was measured in the periportal, mediolobular and centrilobular areas.

Exposure of rats to MIBK alone did not cause any modifications in serum GLDH activity. However, exposure to DCB alone increased GLDH activity. Preexposure to 595, 1280, or 3020 ppm MIBK caused a dose-related increase in DCB-induced GLDH activity. Compared to controls, MIBK caused a dose-dependent elevation of rat liver cytochrome P-450 content. Rat liver GST activity was also increased following exposure to MIBK.

In the mice, neither MIBK nor DCB alone induced any constant effect on G-6-Pase staining intensity. However, successive exposure to MIBK and DCB induced a significant decrease in G-6-Pase staining intensity, varying from 29% to 49% when compared to the DCB-only exposed group.

The authors summarized their results as follows: MIBK increased liver cytochrome P-450 content and GST activity, but did not affect serum GLDH activity in rats. Preexposure to MIBK enhanced the DCB-induced increase in serum GLDH activity, whereas the increases in cytochrome P-450 content and GST activity were identical to those resulting from exposure to MIBK alone. In mice, MIBK interacted with DCB on centrolobular liver G-6-Pase (Brondeau et al. 1989).

Chloroform

According to Vézina, Ayotte, and Plaa (1985), a single oral dose of MIBK administered to male Sprague-Dawley rats enhanced the hepatotoxicity of a single IP dose of chloroform that was administered 24 h later. The no-observed-effect level of MIBK was 375 mg/kg, and 560 mg/kg was the minimal-effect level.

A more recent oral study by Vézina et al. (1990) exposed male Sprague-Dawley rats to MIBK and its two major metabolites, 4-MPOL and 4-hydroxymethyl isobutyl ketone. The authors

reported significant increases in liver damage induced by chloroform (0.5 ml/kg, dissolved in corn oil to yield 10 mL/kg). Three doses of each chemical were administered orally to three groups of six animals, respectively (total of 9 groups) 24 h before dosing with chloroform. The minimally effective dose of MIBK and each of the two metabolites that was needed for potentiation of chloroform-induced hepatotoxicity was approximately 5 mmol/kg. Liver damage was demonstrated by elevation of the plasma activity of two transferases, alanine aminotransferase and ornithine carbamoyl transferase, and by the severity of the morphological changes (necrosis and inflammation) observed.

In a second series of experiments, these same authors studied the enzyme inducing properties of MIBK. They assayed cytochrome P-450 liver content and the activity of aniline hydroxylase, 7-ethoxycoumarin *O*-deethylase, and aminopyrine *N*-demethylase. The liver content of cytochrome P-450 and the oxidation of aniline and 7-ethoxycoumarin were significantly increased following a single oral dose (7.5 mmol/kg or greater) or multiple doses (5.0 and 7.5 mmol/kg/day for 5 days) of MIBK. Repetitive administration of MIBK also caused an increase in the activity of aminopyrine demethylase. MIBK also caused a significant increase in the 52.1- and 54.1-kDa microsomal proteins, which probably corresponded to cytochrome P-450 isozymes (Vézina et al. 1990).

Other Agents

Krishnan et al. (1992) evaluated the effect of MIBK on hexachlorobenzene (HCB)-induced hepatic porphyria using groups of female Sprague Dawley rats (weights = 125–150 g). The first dosing schedule consisted of the simultaneous oral administration of HCB (50 mg/kg in 10 ml/kg corn oil daily, 5 days per week) and MIBK (7.5 mmol/kg in 10 ml/kg corn oil daily, 3 days per week) for 6 weeks. The second dosing schedule consisted of initial oral dosing with 25 or 50 mg HCB/kg daily for 12 consecutive days. Dosing with HCB was followed by oral dosing with 7.5 mmol MIBK every other day for 27 days. The simultaneous administration of HCB and MIBK resulted in a reduction in the severity of HCB-induced porphyria. The sequential administration procedure for both chemicals (MIBK dosing after initial dosing with HCB) resulted in enhancement of the porphyrinogenic response. The authors concluded that the effect of combined exposure to HCB and MIBK on hepatic porphyria depends on the sequence of administration of both chemicals. Furthermore, it was suggested that the mechanism involved in this interaction may invoke both the induction and inhibition of specific hepatic isoenzymes by MIBK.

Raymond and Plaa (1995b) dosed groups of 12 male Sprague-Dawley rats (weights = 175–200 g) orally with the hepatotoxicant, carbon tetrachloride (in corn oil) 18 h after oral dosing with MIBK in corn oil. MIBK was administered at potentiator dosages of 0.3, 1.5, 3.0, 12.0, or 20 mmol/kg, and carbon tetrachloride was administered at dosages of 0.005, 0.01, 0.05, 0.1, and 0.5 ml/kg. The extent of potentiation of carbon tetrachloride–induced hepatotoxicity in male rats was found to be dependent on MIBK and carbon tetrachloride concentrations. MIBK administration induced a dose-dependent potentiation of carbon tetrachloride toxicity. Hepatotoxicity was indicated by an increase in plasma alanine transaminase activity and the concentration of bilirubin.

The MED of MIBK decreased 10-fold when the dose of carbon tetrachloride was increased from 0.01 ml/kg to 0.1 ml/kg. The MED was defined as the smallest dose of a potentiator that was able to produce a statistically significant enhanced response to carbon tetrachloride–induced injury. The results of this study suggested that a given level of liver injury induced by a ketone-haloalkane combination could be evaluated on the basis of the potentiator × hepatotoxicant product (Pilon, Brodeur, and Plaa 1988).

Ocular Irritation

McOmie and Anderson (1949) evaluated the ocular irritation potential of undiluted MIBK using one rabbit. Reactions were scored according to the Draize scale (0 to 110). Draize irritation scores were 8, 3, and 1 at 1, 24, and 72 h post instillation, respectively. The test substance induced conjunctivitis, with some edema and corneal injury. Light accommodation was unaffected, and pupillary damage was not observed. The eye was described as grossly normal at day 7 post instillation

CMA (1981) stated that ocular irritation was observed within 10 min after instillation of undiluted MIBK (0.1 ml) into the rabbit eye. Inflammation and conjunctival swelling were noted within 8 h post instillation. Inflammation, swelling, and exudate were evident at 24 h; however, reactions had cleared by 60 h.

The Exxon Chemical Company (1982) evaluated the ocular irritation potential of MIBK using six albino rabbits. Undiluted MIBK (0.1 ml) was instilled into the left conjunctival sac of each animal. Untreated eyes served as controls. Reactions were scored at 1, 4, and 24 h and at 2, 3, 4, and 7 days post instillation according to the Draize scale (0 to 110). Additional readings at 10 and 14 days were taken, depending on the types of reactions that were observed. Blinking was observed in all six animals immediately after instillation. One animal had slight iritis at 1 and 4 h, which had cleared by 24 h. Slight to moderate conjunctivitis was noted in all rabbits from 1 h to day 2 post instillation. Reactions had cleared within 4 days post instillation. Corneal reactions were not observed throughout the experiment in any of the animals tested. MIBK induced slight, transient ocular irritation.

Kennah et al. (1989) studied the ocular irritation potential of MIBK using New Zealand albino rabbits (four to six animals). The test substance (0.1 ml) was instilled into the conjunctival sac of one eye of each animal. Untreated eyes served as controls. The cornea, iris, and conjunctiva were scored at days 1, 2, 3, 7, 10, 14, and 21 post instillation. A Draize score was computed at each observation period by averaging the total scores of all rabbits tested. Draize scores of 5 and 2 (110 max) were reported for 100% and 2% MIBK, respectively. It was concluded that MIBK induced mild ocular irritation in rabbits.

Gautheron et al. (1994) evaluated the ocular irritation potential of undiluted MIBK in the Draize test using four to six rabbits. The test substance (0.1 ml) was instilled into the conjunctival sac of one eye of each animal. A Draize score of 5 (maximum = 110) was reported.

Skin Irritation

McOmie and Anderson (1949) made seven applications (3 ml/kg each, 5 to 12 h) of undiluted MIBK to a 100-cm^2 area of shaved skin on each of two rabbits over a period of 15 to 21 days. Drying of the skin and some exfoliation were the only reactions observed.

These same authors applied undiluted MIBK (10 h of exposure) to the skin of two rabbits either by flooding the test site or placement of a cotton pad impregnated with the test substance. The reactions observed were classified as immediate (moderate erythema) and delayed (erythema persisting for 24 h). Additional study results are included in the earlier section on Acute Dermal Toxicity (McOmie and Anderson 1949).

Batyrova (1973) reported that the immersion of the ear of a rabbit and the tails of mice in pure MIBK for 2 h resulted in pronounced inflammation and necrosis of the tissues. In another experiment in the same study, no noticeable skin changes were observed in guinea pigs subjected to brief exposures to MIBK over a period of 3 months.

In a series of studies reported by CMA (1981), rabbits (shaved skin) were patch tested with MIBK in a single, 10-h occlusive patch test. Erythema was observed for up to 24 h post application. Drying and flaking of the skin surface were observed after MIBK was applied to the skin of rabbits daily (10 ml/day) for 7 days. Slight skin irritation was observed after undiluted MIBK (5 and 10 ml) was applied (under occlusive wrap) to depilated skin of guinea pigs for 24 h. Reportedly, there was no clinical evidence of absorption. MIBK (500 mg) induced moderate irritation of rabbit skin after a contact period of 24 h. The application of MIBK (2 ml) to the backs of guinea pigs daily for 31 days caused desquamation, but no clinical or histologic evidence of toxic neuropathy.

The Exxon Chemical Company (1982) evaluated the skin irritation potential of MIBK using 12 albino rabbits. The application sites of six animals were abraded. Four gauze patches (adhesive backing), each containing 0.5 ml MIBK, were applied to clipped abdominal skin of each animal. Patches were secured with dental damming and gauze binders for 24 h. Reactions were scored at 24 and 72 h post application according to the following scales: 0 (no erythema) to 4 (severe erythema [beet redness] to slight eschar formation [injuries in depth]) and 0 (no edema) to 4 (severe edema [raised more than 1.0 ml, extending beyond the area of exposure]). At 24 h post application, very slight erythema was observed at three intact skin sites (three animals respectively) and no signs of irritation were noted in the remaining three animals (intact skin sites). Slight or well-defined erythema at all abraded sites (six animals) was noted at 24 h; very slight edema was observed in two of the animals. At 72 h post application, very slight erythema was observed in two animals (abraded application sites); no signs of irritation were observed in the remaining animals (abraded or intact skin). MIBK induced slight, transient erythema (primary irritation score = 0.75).

NEUROTOXICITY

Oral Dosing

The Carnegie Mellon Institute of Research (1983) administered MIBK to each of five Wistar female rats (4 weeks old) at a concentration of 1.3% in drinking water daily for 120 days (MIBK dose = 1.04 g/kg/day). Two groups of five rats that received tap water served as untreated controls. Neurological evaluations for any treatment-related effects during the study included observations of any changes in balance, strength, coordination, or behavior. The animals were killed by CO_2 narcosis and subjected to gross and microscopic examination. MIBK did not induce any significant neurologic alterations. Additionally, no discernible neurotoxic gross effects were noted. Additional results for this subchronic study are included in the section on Subchronic Oral Toxicity earlier in this report.

Nagano et al. (1988) reported that the maximum motor-fiber conduction velocity in the tail nerve of male rats (number and strain not stated) was unaffected by treatment with MIBK (601 mg/kg, 5 times/week for 55 weeks). However, treatment with MIBK (201 mg/kg) facilitated the neurotoxic effect of methyl *n*-butyl ketone (401 mg/kg).

Intraperitoneal Injection

In a study by the Eastman Kodak Company (1977), the neurotoxicity of MIBK was evaluated using three groups of 12 Sprague-Dawley albino rats. The three groups were injected intraperitoneally with MIBK (10% in corn oil) at doses of 10, 30, and 100 mg/kg, respectively for 2 weeks. At the end of the 2-week period, the doses were increased to 20, 60, and 200 mg/kg, respectively. The new doses were injected intraperitoneally 5 days per week for 33 weeks. The test groups were referred to as low-, mid-, and high-dose groups. Control rats (group of 12) were injected with corn oil for 2 weeks and then distilled water for the remainder of the study. At the end of the study, some of the surviving animals in the highest dose group were killed and tissues (sciatic, tibial, peroneal, and sural nerves and interosseous muscles from the right hindlimb) subjected to microscopic examination. Tissues (spinal cord, medulla, and sciatic nerve) from some of the survivors of all dose groups were also examined microscopically.

The mortality rate per dose level was comparable to that noted in the control group. A significant decrease (>10%, compared to control group) in mean body weight gain was noted only in the high-dose group. This finding was first noted after 17.5 weeks and persisted to the end of the study. The following non-neural lesions were observed in test animals: chronic respiratory disease (2 rats—high dose; 1 rat—low dose), peritonitis

(4 rats—high dose), bone marrow hyperplasia (1 rat—high dose), and increased splenic hematopoiesis (1 rat—high dose). These pathologic changes were either spontaneous occurrences or were due to an irritative property of the test substance. Tissue lesions (neural and non-neural) were not observed in the control group. Except for a transient anesthetic effect in the high-dose group, observed initially after 1 month of dosing, no neurologic signs were observed in either of the test groups. At microscopic examination, senile changes in the nucleus gracilis of the medulla oblongata were observed in one mid-dose and one low-dose animal, but not in high-dose or control animals. It was concluded that MIBK did not induce peripheral neuropathy when injected intraperitoneally at doses up to 200 mg/kg (Eastman Kodak Company 1977).

Sharkawi et al. (1994) studied the effect of MIBK on the duration of ethanol-induced loss of righting reflex and on ethanol elimination using two groups of seven Charles River CD-1 mice (weights not stated). MIBK was dissolved in corn oil and injected intraperitoneally (2.5 or 5.0 mmol/kg) 30 min before ethanol (4 g/kg, intraperitoneally). MIBK significantly prolonged the duration of ethanol-induced loss of righting reflex when administered at a dose of 5 mmol/kg. The concentrations of ethanol in blood and in the brain upon return of the righting reflex were similar in MIBK-treated and control animals. MIBK did not induce ataxia or loss of righting reflex in any of the mice at doses of 2.5 or 5 mmol/kg (Cunningham et al. 1989). MIBK (5 mmol/kg) also prolonged the duration of ethanol-induced loss of righting reflex in a more recent study involving CD-1 mice.

Intravenous Injection

Tham et al. (1984) evaluated the influence of MIBK on the vestibulo-oculomotor reflex (VOR) of female Sprague-Dawley rats (number not stated; weights = 250–300 g). The effect of MIBK on VOR was studied by recording nystagmus that was induced by accelerated rotation. The VOR connects the labyrinth with the eye muscles via the brainstem, thereby eliciting ocular movements in response to acceleration or deceleration of the head. The test substance (in an emulsion of lipids) was administered by continuous intravenous infusion for 60 min. Test concentrations varied between 0.1% and 10%. MIBK had a depressive effect on the VOR. The threshold limit for this effect was 0.2 mM/L (20 ppm) at an infusion rate of 30 μM/kg/min. It was suggested that solvents cause depression or excitation of the VOR by interaction with central pathways in the reticular formation and the cerebellum.

Subcutaneous Injection

Spencer and Schaumburg (1976) reported a study in which 4 cats (weights = 2–3 kg) were injected subcutaneously with 150 mg undiluted MIBK/kg body weight twice daily, 5 times/week, for up to 8.5 months. Injection sites on the back were rotated. The composition of the test substance was as follows: MIBK (98.79%), methyl *n*-butyl ketone (0.94%), acetone (0.02%), other light impurities (0.14%), and heavy impurities (0.11%). A group of four control cats received subcutaneous doses of saline (0.2 ml/kg) 5 days per week for up to 5 months. None of the animals died. Biopsies were taken from the right and left hind feet after 45 and 135 days of dosing with MIBK. Biopsy results indicated no detectable damage to nerve tissues.

The Eastman Kodak Company (1982b) evaluated the neurotoxicity of MIBK using four purebred, male Beagle dogs (9 to 30 months old). Each dog was injected subcutaneously with a dose of 300 mg/kg daily for approximately 11 months and then subjected to electromyographic examination. No evidence of neurotoxicity was noted in either of the four dogs tested.

In another study using dogs, the neurotoxicity of MIBK was evaluated (four dogs; mean age = 13 months). MIBK was >98% pure and also consisted of 0.9% methyl *n*-butyl ketone and trace amounts of 4-methyl-2-hydroxypentane. The test substance was administered subcutaneously at a dose of 150 mg/kg twice daily for a year. The animals were necropsied at the end of the study. No evidence of systemic toxicity or neurotoxicity was observed in any of the animals tested (Eastman Kodak Company 1992).

Inhalation Exposure

Rats

Spencer et al. (1975) studied the neurotoxicity of MIBK in six young adult rats (ages and strain not stated). The animals were exposed to 1500 ppm MIBK in an 18.5-L glass exposure chamber for up to 5 months. The animals were then killed and tissues (muscle, brain, and peripheral nerves) subjected to gross and microscopic evaluation. Weight gain was described as normal. Slight narcosis was observed during exposure. However, no signs of neurological dysfunction were noted at the end of the exposure period. Consistently, many axons containing large numbers of dilated, glycogen-filled mitochondrial remnants, adaxonal Schwann cell invaginations, and rare focal swellings were noted in the most distal portions of the tibial and ulnar nerves. Distal nerve fiber degeneration was not observed. Results of examination of sampled areas of the central nervous system and proximal parts of the peripheral nervous system were unremarkable. The authors stated that the neuropathological changes noted may have been related to the presence of 3% methyl *n*-butyl ketone in the commercial grade of MIBK that was used in this study.

In a study by Geller, Rowlands, and Kaplan (1978), the effect of inhaled MIBK on the lever-pressing behavior of Holtzmann, Sprague-Dawley male rats (~90 to 120 days old) on a match-to-sample discrimination task were evaluated. Rats were exposed to the test substance in chambers made of glass and steel. Animal weights were gradually reduced to 80% of the normal body weight and the animals were then trained to press a lever for a liquid food reward. A 2-min variable-interval schedule of reinforcement was used. The effect of 25 ppm MIBK on the variable response rate of one rat after the third hour of the experimental session was evaluated. The average response rate was 45 per

minute, which represented a 58% increase over the preexposure control rate of 26.5%. The response rate had not returned to control levels by day 7 post exposure.

De Ceaurriz et al. (1984) studied neurobehavioral effects of MIBK using 80 male Swiss, OF1 mice (40 controls, 40 test; weights = 20–25 g). Four test groups (10 mice/group) were exposed to test concentrations of 662, 757, 807, and 892 ppm, respectively, for 4 h in a 'behavioral despair' swimming test. At the end of each exposure period, mice were placed in a glass cylinder containing water. Neurobehavioral effects were determined by measuring the duration of immobility in this test. Transient periods of immobility were accompanied by periods of intensive swimming activity. The decrease in immobility time served as an indicator of MIBK-induced behavioral toxicity. Control groups were exposed concurrently to clean filtered air. A decrease in the duration of immobility (ID_{50} = 803 ppm) in the swimming test was reported after exposure to MIBK. The ID_{50} value was defined as the median active level that caused a 50% decrease in immobility.

In a study by Eastman Kodak Company (1996), published later by David et al. (1999), the neurotoxicity of MIBK in rats was evaluated in a 13-week (64 days of exposure) study using male Sprague-Dawley rats. Sixty five rats (CRL:CD (SD)BR/VAF Plus strain; 134 days old; weights = 338 ± 12 g) were restricted to 13 to 18 g of feed per day and used for schedule-controlled operant behavior (SCOB) testing. Systemic toxicity was evaluated using 64 rats of the same strain (68 days old; weights = 352 ± 12 g) that were fed ad libitum. Both sets of animals (20 per group) were exposed to MIBK at concentrations of 250, 750, or 1500 ppm 5 days per week (6 h/day) for 13 weeks. Untreated animals served as controls. Exposure was carried out in 420-L stainless steel and glass inhalation chambers. Each SCOB test session consisted of four fixed ratio (FR) sessions of 20 lever presses for each food pellet, followed by two fixed-interval (FI) sessions of 120 s for each food pellet. FR running rates, postreinforcement pause duration, FI response rates, and index of curvature values were presented as a mean for each animal, based on values determined on Tuesday through Friday of each week. The testing of SCOB animals continued post exposure for 2 weeks (i.e., through day 102). On day 107, 20 SCOB animals (5/group, selected at random) were perfused systemically for the collection of neurological tissues. The remaining SCOB animals were killed and necropsied on day 108. The animals in the systemic toxicity test were used for comparative purposes to determine whether feed restriction masked overt signs of systemic toxicity. These animals were killed and necropsied on day 88.

One death in a control animal was reported. Clinical signs observed during the study included minor piloerection and sialorrhea, and minimal to minor reduced activity (less movement, decreased alertness, and slower response to tapping on chamber wall). No statistically significant differences in FR running rate, FR pause duration, FI response rate, or index of curvature (each analyzed as % of baseline) were observed between test (all doses) and control groups. Thus, no differences in the performance of schedule-controlled operant behavior were noted.

Differences in body weight between animals fed ad libitum and controls were not statistically significant at either administered dose. However, for SCOB animals, only mean terminal body weights in groups exposed to 1500 ppm were significantly higher ($p \leq 0.05$) than those of controls. An increase in mean terminal body weights over those noted in controls was also reported for the 750-ppm exposure groups. Regarding organ weights of animals fed ad libitum, the mean absolute liver and kidney weights for all exposure groups and the relative (to body weight) liver and kidney weights for 750 and 1500 ppm exposure groups were statistically higher ($p \leq 0.05$) than control values. No other differences in organ weight were observed in animals fed ad libitum. In SCOB animals, the mean absolute liver weights for 750 and 1500 ppm exposure groups and the mean relative (to body weight) liver weights for 250 and 750 ppm exposure groups were statistically higher ($p \leq 0.05$) than control values. Mean absolute and relative (to body weight) kidney weights of all exposure groups were comparable to the control group. No other differences in organ weight were observed in SCOB animals.

At gross examination, no test substance–related changes were noted in SCOB animals or animals fed ad libitum. None of the tissues examined were examined microscopically. The results of this study indicate that repeated MIBK exposure did not induce changes in schedule controlled operant behavior. An exposure concentration of 1500 ppm MIBK was considered the no-observed-effect level (NOEL) for subchronic neurotoxicity (Eastman Kodak Company 1996; David et al. 1999).

Baboons

Geller et al. (1978) studied the effect of inhaled MIBK (25 to 75 ppm) on the behavior of young baboons (number and ages not stated) in a match-to-sample discrimination task. A match-to-sample task is an operant behavior procedure that measures perceptual acuity and discrimination performance. Two large stainless steel exposure chambers, for test and control animals respectively, were used. The test animals were exposed to MIBK over a 7-day period, whereas the controls were exposed to clean air. Each group was provided with an intelligence panel instrumented with a row of three round, translucent discs. Under the appropriate experimental conditions, pressing either of the two end discs would result in the release of a food pellet (reward). Each trial began with the illumination of one of the stimuli on the center key (probe stimulus). The following records were kept during the test procedure: number of probe stimuli presented during each 15-min segment, number of correct matching responses on the left and right keys and the number of incorrect responses on these keys, any extra responses, and the time required for a baboon to respond with a key press after a stimulus was activated (reaction time). Performance of the match-to-sample discrimination task was not impaired over the range of MIBK concentrations tested. However, it is important to note that one of the

baboons exposed to 50 ppm MIBK made extra responses on each day of testing. These changes were thought to reflect alterations in the animal's level of anxiety. No further increase in responses was noted when the test concentration was increased to 75 ppm for an additional 48 h. It was concluded that MIBK did not impair a baboon's ability to discriminate or remember stimuli.

In a subsequent study (Geller et al. 1979), the effect of inhaled MIBK on a delayed match-to-sample discrimination task was evaluated using four juvenile baboons (~2 years old). The animals were exposed to 50 ppm MIBK for 7 days. Two animals were exposed to the test substance and two animals served as controls (clean air exposure). Accuracy of performance was affected minimally. However, increased and decreased extra responses during the delay intervals were noted. Termination of the stimulus activated a timer for 2 min (defined as the delay interval). MIBK also caused a slowing of the response times for all four baboons during most or all of the exposure sessions. The authors stated that this effect could be an early manifestation of the incoordination and narcosis that is observed at much higher concentrations of MIBK.

In Vitro Study

Selkoe, Luckenbill-Edds, and Shelanski (1978) evaluated the neurotoxicity of MIBK using a clonal line of neuroblastoma (Neuro 2aE) derived from a spontaneously occurring murine tumor (C1300). Using light microscopy, it was determined that MIBK produced no discernible cytopathological changes in cells exposed to 0.1% MIBK for 10 days. At a concentration of 0.2%, MIBK induced a depression of growth rates; however, the cells appeared normal. MIBK (0.5%) caused widespread cell death. The cells that survived either appeared normal or a fine granular cytoplasm was observed.

GENOTOXICITY

The genotoxicity of MIBK has been evaluated in many assay systems. The results of those tests are presented in Table 2. In most assay systems, MIBK is not genotoxic. Equivocal results in a mouse lymphoma assay and a positive result in a cell transformation assay, however, were reported by O'Donoghue et al. (1988). The study and results are further described below.

O'Donoghue et al. (1988) present the results of several MIBK genotoxicity assays, including: Salmonella/microsome (Ames) assay, L5178Y/TK^{+}/$^{-}$ mouse lymphoma (ML) assay, BALB/3T3 cell transformation (cT) assay, unscheduled DNA synthesis (UDS) assay, and micronucleus (MN) assay.

The Ames test used the following *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 with and without metabolic activation. MIBK was tested at concentrations of 0.04, 0.1, 0.4, 1.0, and 4.0 μl/plate. The positive controls were as follows: 2-aminoanthracene (1.0 μg/plate), 2-nitro-*O*-phenylenediamine (10 μg/plate), sodium azide (5.0 μg/plate), 2-aminoanthracene (4.0 μg/plate), and 9-aminoacridine (75 μg/plate). DMSO served as the negative control. MIBK was not mutagenic in any of the strains. The positive controls were mutagenic.

MIBK was tested in the L5178Y/TK^{+}/$^{-}$ mouse lymphoma assay. The forward mutation frequency at the thymidine kinase locus in mouse lymphoma cells was evaluated. MIBK was tested at concentrations ranging from 0.32 to 4.2 μl/ml both with and without metabolic activation. Ethyl methanesulfonate (0.5 and 1.0 μl/ml) served as the positive control for assays without metabolic activation. 7,12-Dimethylbenz[a]anthracene (5.0 and 7.5 μl/ml) served as the positive control for cultures with metabolic activation. DMSO served as the negative control.

Results were negative when MIBK was tested with metabolic activation, but were equivocal when MIBK was tested at high concentrations without metabolic activation. A significant increase in the mutation frequency (at least 2× that noted in controls) was noted at concentrations of 1.8, 3.2, and 4.2 μl/ml. Both positive controls were mutagenic. The results of a second mouse lymphoma assay were also equivocal (without metabolic activation) at the highest doses tested. In this assay, a significant increase in the mutation frequency was noted at doses of 2.1, 2.9, and 3.7 μg/ml.

The authors noted that the mutation frequency in test cultures was not dose related and that repeat testing with replicate cultures did not result in a consistent positive effect. Furthermore, the greatest response to MIBK was noted at doses that resulted in 96% to 99% lethality. The authors noted that doses that result in 90% to 100% lethality may not be relevant in determining mutagenicity. They noted that if the doses that resulted in >90% lethality are not considered, then the few remaining increases were not concentration-dependent and the results would be considered negative. Regarding results for DMSO control cultures, it was noted that more than a twofold difference in mutation frequencies was observed when control cultures for the eight mouse lymphoma assays were compared. However, the results for DMSO control cultures were within historical control ranges for the testing laboratory. The authors also stated that the absence of increases in the mutagenic frequency in test cultures over that observed in the DMSO control range provides additional evidence for a negative conclusion on the mutagenic potential of MIBK.

MIBK was evaluated in the unscheduled DNA synthesis assay (rat hepatocytes). MIBK was tested at concentrations ranging from 0.010 to 100 μl/ml. The positive control was 2-acetylaminofluorene (2-AAF) at 2 and 20 μg/ml and DMSO served as the negative control. The test substance was classified as positive if it induced a dose-related response and at least one dose produced a significant increase in the average net nuclear grains (compared to control), or if the test substance induced a significant increase in the mean net nuclear grain count in at least two successive doses. MIBK did not induce a positive response, meaning that there was no significant increase in the net nuclear grain counts at any of the doses tested. It is important to note that because of the high level of toxicity at doses of 10 and 100 μl/ml,

TABLE 2
MIBK genotoxicity

Test system	Protocol and dose	Results	Reference
Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538	Preincubation assay (modification of procedure by Haworth et al. 1983) with and without metabolic activation; 0.1 ml	Negative	Zeiger et al. 1992
Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538; *Escherichia coli* strains WP_2 and WP_2 *uvr* A	Preincubation assay (Brooks and Dean 1981) with and without metabolic activation; up to 8000 μg/ml	Negative	Brooks, Meyer, and Hutson 1988
Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538; *Escherichia coli* strains WP_2 and WP_2 *uvr* A	Ames test with and without metabolic activation; up to 8000 μg/ml	Negative	Brooks, Meyer, and Hutson 1988
Salmonella typhimurium strains TA98, TA100, and TA1535	Ames test with and without metabolic activation; up to 0.1–2000 μg/plate	Negative	Goodyear Tire & Rubber Company 1982
Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538	Ames test with and without metabolic activation; 0.01–10 μl/plate	Negative	Litton Bionetics 1991
Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538; *Saccharomyces cerevisiae strain* D4	Ames test with and without metabolic activation; up to 5 μl/plate	Negative	Litton Bionetics 1977
Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538	Ames test with and without metabolic activation; 0.04–4 μl/plate	Negative	O'Donoghue et al. 1988
L5178Y/$TK^{+}/^{-}$ mouse lymphoma cells	Mouse lymphoma assay (Clive and Spector 1975; Clive et al. 1979) with and without metabolic activation; 0.32–4.2 μl/ml	Negative, with metabolic activation; equivocal, without metabolic activation	O'Donoghue et al. 1988

Rat hepatocytes	Unscheduled DNA synthesis assay (Williams 1977, 1979); 0.010-100 μl/ml	Negative	O'Donoghue et al. 1988
CD-1 mice	Micronucleus cytogenetic assay (in vivo)—mice dosed IP with 10 ml/kg; bone marrow samples obtained after animals killed	Negative	O'Donoghue et al. 1988
BALB/3T3 clone A31-1 mouse embryo cells	Cell transformation assay; with metabolic activation (1–4 μl/ml) and without metabolic activation (2–4.8 μl/ml)	Without metabolic activation, three type III foci in 15 dishes (statistically significant positive result) at highest dose. No transforming activity with metabolic activation	O'Donoghue et al. 1988
BALB/3T3 clone A31-1 mouse embryo cells	Cell transformation assay repeated with metabolic activation (2–5 μl/ml) and without metabolic activation (4–7 μl/ml)	Without metabolic activation, two type II foci in 15 dishes. No confirmation of preceding test results, because transformation frequency not significantly increased over that noted in negative control (phosphate-buffered saline) cultures. No transforming activity with metabolic activation	O'Donoghue et al. 1988
Saccharomyces cerevisiae strain JD1	Mitotic gene conversion assay with and without metabolic activation; up to 5 mg/ml	Negative	Brooks, Meyer, and Hutson 1988
Saccharomyces cerevisiae strain D61.M	Mitotic chromosome loss assay; 4.8–7.3 mg/ml	Negative	Zimmermann, Scheel, and Resnick 1989
Rat liver RL_4 cells	Chromosome damage assay; up to 8000 μl/ml	Negative	Brooks, Meyer, and Hutson 1988

it was not possible to determine the average net nuclear grains. Results for the positive control were classified as positive.

The mutagenicity of MIBK was evaluated in the micronucleus cytogenetic assay. A single dose of the test substance (in corn oil) was administered intraperitoneally (dose = 10 ml MIBK in corn oil/kg body weight) to groups of 10 CD-1 mice (5 males, 5 females per group). The animals were killed at 12, 24, or 48 h post dosing. The positive-control group was dosed with triethylene melamine (0.25 mg/kg) and examined at 24 h post dosing. Corn Oil served as the negative control. After the animals were killed, bone marrow samples were obtained and smears prepared. One thousand polychromatic erythrocytes were scored on coded slides for the presence of micronuclei. Micronucleated normocytes were also counted. MIBK was not mutagenic.

MIBK was also tested in the BALB/3T3 mouse embryo cell transformation assay. BALB/3T3 clone A31-1 cells were harvested during exponential growth. Based on the results of a preliminary cytotoxicity assay, the following concentrations were tested: 2, 4, 3.6, and 4.8 μl/ml (without metabolic activation) and 1.0, 2.0, and 4.0 μl/ml (with metabolic activation). The assay was repeated at concentrations of 4.0, 5.0, 6.0, and 7.0 μl/ml (without activation) and 2.0, 3.0, 4.0, and 5.0 μl/ml (with activation). At the end of the incubation period, transformation plates were fixed, stained, and scored for type II and type III foci. The transformation frequency for each treatment condition was expressed as the number of transformed foci per surviving cell. *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) served as the positive control. Phosphate buffered saline served as the negative control. Test results were classified as ambiguous. In the first assay, the 4.8-μl/ml dose of MIBK induced three type III foci in 15 dishes. This number of type III foci, together with a reduced cloning efficiency, yielded a positive statistical analysis in the nonactivated system. In cultures with metabolic activation, no transforming activity was present. When the assay was repeated, MIBK (dose = 5 μl/ml) induced two type III foci in 15 plates with 100% cell survival. Because the resulting transformation frequency was not significantly increased over that reported for the negative control, it was not possible to confirm the results of the first BALB/3T3 assay (without metabolic activation). Like the first assay, results for MIBK were negative with metabolic activation.

Taking into consideration the marginal response to MIBK at the highest cytotoxic concentration in the mouse lymphoma assay and the lack of reproducibility in the BALB 3T3 transformation assay, and based on negative results for MIBK in the Ames, unscheduled DNA synthesis, and micronucleus assays, the authors concluded that it is unlikely that MIBK would be genotoxic in mammalian systems (O'Donoghue et al. 1988).

CARCINOGENICITY

No studies of MIBK carcinogenic potential were found. MIBK, however, is among the chemicals that have been approved by the National Toxicology Program (NTP) for testing in a toxicology and carcinogenesis study (NTP 1999).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Dermal Exposure

Malyscheva (1988) applied MIBK to the tails (lower 2/3) of an unspecified number of male white rats daily (4 h/day) in doses of 300 or 600 mg/kg for 4 months. Changes in the testes included a reduction in the number of spermatocytes, spermatids, and spermatozoa. The magnitude of each reduction was not stated, and no statistical analysis of the results was included.

Inhalation Exposure

Tyl et al. (1987) evaluated the reproductive and developmental toxicity of MIBK in 100-day-old virgin male and virgin female Fischer 344 rats (NIH:(F-344)/H1aBR (F141 + 3)) and 6-week-old virgin male and virgin female CD-1 mice (outbred albino Crl:CD-1-(ICR)BR). The animals were mated and then divided into four groups per species. Three groups, 25 females in each group, per species were exposed to MIBK vapor at concentrations of 300, 1000, and 3000 ppm (mean analytical values of 305, 1012, and 2997 ppm), respectively, on gestation days 6 through 15. Group 4 animals served as untreated controls. On gestation day 21, the animals were killed and live fetuses examined for external, visceral, and skeletal alterations.

Overall the authors concluded that the results indicated that MIBK did not induce any treatment-related increases in embryotoxicity, or fetal malformations in pregnant Fischer 344 rats or CD-1 mice that inhaled MIBK at concentrations of 300, 1000, or 3000 ppm . There was also no evidence of treatment-related maternal toxicity in mice or rats exposed to 300 or 1000 ppm MIBK (Tyl et al. 1987).

Study results are presented separately for the two species below.

Mice

Of the 25 pregnant mice exposed to 3000 ppm MIBK, three died after the first exposure. No treatment-related changes in body weight were noted. Maternal body weight gain was significantly elevated only after exposure to 3000 ppm MIBK. Clinical observations, associated only with dams in this exposure group, included irregular gait, paresis (partial hindlimb paralysis), hypoactivity, ataxia, negative toe pinch, unkempt fur, and lacrimation. Significant increases in absolute (117.8% of controls) and relative (104.5% of controls) liver weight in the 3000-ppm exposure group were the only treatment-related changes in maternal organ weights that were noted. Neither maternal body weights (absolute or corrected for gravid uterine weight), gravid uterine weight, nor absolute or relative maternal kidney weight differed between either of the three test groups. No treatment-related findings were observed at gross necropsy.

The pregnancy rate was equivalent for control and test groups of mice. Twenty-two litters were evaluated in each exposure group. No treatment-related effects in the following gestational parameters were noted: number of corpora lutea, total implantations, viable or total nonviable implants per litter, % preimplantation loss, % live fetuses, and sex ratio (% males). Following exposure to 3000 ppm MIBK, a significant increase in the number of dead fetuses (but not early or late resorptions) per litter, compared to controls, was noted. A significant reduction in total body weight per litter was also noted following exposure to 3000 ppm MIBK. Compared to controls, no statistically significant, treatment-related increases in the number of fetuses or litters (with one or more affected fetuses) with individual malformations, pooled external, visceral, skeletal malformations, or total malformations in any treatment group were noted. This finding was true for all exposure groups. Visceral variations included an increase in the incidence of dilated lateral ventricles of the cerebrum and dilated renal blood vessels. An increased incidence (compared to controls) of reduced ossification (indicative of toxicity) in the vertebrae, sternebrae, limbs, and skull plates was observed after exposure to 3000 ppm.

Rats

None of the female rats died during the study, delivered early, or had aborted fetuses. Evidence of maternal toxicity included significant reductions in body weight and significantly reduced weight gain. Food consumption (g/dam/day) was significantly reduced in the 3000-ppm exposure group, and only during the exposure period. Exposure-related clinical signs, observed only at 3000 ppm, were as follows: loss of coordination, negative tail and/or toe pinch, paresis (partial hindlimb paralysis), muscular weakness in hindlimbs, piloerection, lacrimation, and red perioral encrustation. A slight but statistically significant elevation in maternal relative kidney weight (104% of controls) was observed in the 3000-ppm exposure group. The following parameters were unaffected by treatment: absolute kidney weight, relative and absolute liver weight, gravid uterine weight, and absolute or corrected body weight. No exposure-related findings were noted at gross necropsy.

The pregnancy rate in rats was slightly reduced in the highest dose group (65.7% at 3000 ppm), but was not significantly different from the control group. For the other two dose groups and the control group, the pregnancy rates were considered equivalent (86.2% for control, 86.7% at 300 ppm, and 80.6% at 1000 ppm). The litters evaluated were as follows: 25 controls, 26 at 300 ppm, 25 at 1000 ppm, and 23 at 3000 ppm. No treatment-related effects on the following parameters were noted: number of corpora lutea, total implantations, viable or nonviable implantations (resorptions or dead fetuses) per litter, % preimplantation loss, % live fetuses, and sex ratio (% males). At an exposure concentration of 3000 ppm, fetal body weight per litter (males, females, or total) was significantly reduced (≈93% to 94% of control values; $p < .001$). Fetal body weight was slightly reduced at 300 ppm (≈97% of control values; $p < .05$), but not at 1000 ppm. No statistically significant, treatment-related increases in the incidence of external, visceral, skeletal, or total malformations in rat fetuses were noted. An increased incidence of five skeletal variations involving the vertebrae, sternebrae, and distal limbs was noted following exposure to 3000 ppm MIBK. This finding was considered indicative of toxicity.

CLINICAL ASSESSMENT OF SAFETY

Acute Inhalation Toxicity

Twelve volunteers of both sexes were exposed to various concentrations of MIBK for 15 min. This duration of exposure was chosen because, presumably, it permitted an accurate observation of olfactory fatigue and increasing or decreasing irritation of mucous membranes. The sensory response limit was 100 ppm (410 mg/m^3), and the odor was found to be objectionable by most of the subjects at a concentration of 200 ppm (820 mg/m^3). MIBK (200 ppm) was also found to be irritating to the eyes during inhalation exposure (Silverman, Schulte, and First 1946).

The threshold for MIBK-induced irritation of the lungs was 0.03 to 0.1 mg/l after 1 min of respiration. The number and weights of the subjects involved in this study were not stated (Batyrova 1973).

Short-Term Inhalation Toxicity

Elkins (1959) reported symptoms of either nausea or respiratory irritation in workers exposed to 100 ppm MIBK (410 mg/m^3). Tolerance to this level of exposure was acquired during the work week, but was lost over the weekend. Complaints were largely eliminated when the level of exposure was reduced to 20 ppm (82 mg/m^3).

National Institute of Occupational Safety and Health (NIOSH) (1978) reported that workers exposed to 500 ppm MIBK for 30 min daily experienced weakness, loss of appetite, headache, burning eyes, stomach ache, nausea, vomiting, and sore throat. An enlarged liver and colitis were also observed in some of the workers. In another case, workers exposed to 100 ppm MIBK experienced nausea, headache, and respiratory irritation.

Hazleton Labs, Inc. (1982) reported that six subjects (19 to 49 years old) inhaled MIBK (six, 20-min exposures = exposure session) through face masks connected to ports on a 125-L aerosol chamber. Test concentrations for the series of six exposures ranged from 0.402 to 2.827 mg/L. The incidence of nasal, ocular, or throat irritation experienced by the subjects during one of the exposure sessions (results for exposure series 1 to 6 combined) is indicated as follows: nasal irritation (one to four subjects), ocular irritation (one to three subjects), and throat irritation (one to four subjects). The results for throat irritation are based on the testing of only four subjects (test concentration range = 1.363 to 2.827 mg/L).

The Shell Chemical Corporation (1983) stated that MIBK vapor causes irritation of both the conjunctival and nasal mucosa at concentrations near 200 ppm. Exposure to higher concentrations causes lacrimation (indicative of marked irritation).

WHO (1990) reported on an occupational exposure in which 19 workers inhaled MIBK at concentrations up to 500 ppm (2050 mg/m^3) for 20 to 30 min/day, and 80 ppm (328 mg/m^3) for the remainder of the work day. Half of the workers had symptoms of weakness, loss of appetite, headache, ocular irritation, stomach ache, nausea, vomiting, and sore throat. Insomnia, somnolence, heartburn, and intestinal pain were also reported by a few workers. Slightly enlarged livers were observed in four workers, and six workers had nonspecific colitis. No abnormalities were noted at clinical chemistry examination. Reportedly, work practices at this facility had improved greatly 5 years after this study was conducted. The highest levels of exposure to MIBK ranged from 100 to 105 ppm (410 to 430 mg/m^3), and the general concentration of exposure was 50 ppm (205 mg/m^3). However, gastrointestinal and central nervous system effects were reported by a few workers. Slight liver enlargement persisted in two workers, but the workers did not complain of the initial symptoms.

Hjelm et al. (1990) presented the results of exposing eight male volunteers (18 to 35 years old; weights = 68 to 90 kg) to MIBK at concentrations of 2.4 ppm [10 mg/m^3], 24.4 ppm [100 mg/m^3], and 48.8 ppm [200 mg/m^3] for 2 h during light physical exercise on three different occasions. Based on a questionnaire, nose and throat irritation were the most common symptoms. Neither symptom was experienced by more than three subjects at either of the three exposure concentrations. There were no significant, exposure-related effects on the performance of a simple reaction time task or a test of mental arithmetic. Results concerning the basic human toxicokinetics of MIBK are included in the section on Distribution and Excretion.

Neurotoxicity

Dick et al. (1992) evaluated neurobehavioral effects resulting from short-term inhalation exposure to MIBK using 10 male and 13 female subjects (18 to 32 years old). The 3-day test session began with a 2-h practice session on day 1, followed by 8 h of exposure to 100 ppm MIBK on day 2, and concluded with a 2-h postexposure session on day 3. Inhalation exposure to 100 ppm MIBK on day 2 (in an environmental chamber) was according to the following procedure: (1) 2-h preexposure period; (2) two 2-h exposure periods, experiments 1 and 2, respectively; and (3) 2-h postexposure period. Neurobehavioral tests administered during each of the two 2-h test periods consisted of the following: five psychomotor tests (choice reaction time [CRT], simple reaction time [SRT], visual vigilance, dual task, and short-term memory scanning), one neurophysiological test (eye blink reflex), and one sensorimotor test (postural sway).

The results of statistical analyses did not indicate any significant differences between male and female blood and breath concentrations of MIBK. Study results indicated that 4-h exposures to 100 ppm MIBK did not cause any significant neurobehavioral effects. The principal exposure-related effects were limited headache, nausea, throat irritation, and tearing. These authors also stated that the primary health hazards from acute MIBK inhalation are mucous membrane irritation of the eyes, nose, and respiratory tract at concentrations <500 ppm and central nervous system depression at higher concentrations (Dick et al. 1992).

Iregren, Tesarz, and Wigaeus-Hjelm (1993) studied the potential narcotic impact of MIBK on central nervous system (CNS) function. Heart rate, performance tests, and rating scales for local irritation, CNS symptoms, and mood were determined in six female and six male employees (ages = 19 to 47 years; all healthy) at the National Institute of Occupational Health. The 12 employees were exposed to 10 and 200 mg/m^3 concentrations of MIBK in a 12-m^3 exposure chamber. The subjects were exposed individually for 2 h, and exposure sessions were separated by a 1-week interval. Exposure started with a 90-min period of light physical exercise on a bicycle ergometer. During the last 30 min of exposure, the subjects were relaxing on a bed. Average MIBK concentrations in the exposure chamber were 201 $\pm$ 3 mg/m^3 and 11.9 $\pm$ 1.44 mg/m^3 for the two exposure levels.

The SRT performance test measured reaction time to an easily discriminable but temporally uncertain stimulus during 6 min, using a signal density of 16 signals per minute. Performance was evaluated with respect to level and variability of latencies to 80 stimuli. The results of the SRT test indicated no differences in performance that were attributed to exposure. Compared to the 10 mg/m^3 level of exposure, a decrease in heart rate (seven subjects), an increase in heart rate (four subjects), and no change in heart rate (one subject) were noted after exposure to 200 mg/m^3 MIBK. Thus, no consistent exposure-related effect on heart rate was identified. Mood ratings of activity and stress varied during exposure sessions. However, differences in these parameters were not noted between low and high concentrations of exposure.

The occurrence of symptoms of irritation and CNS symptoms was evaluated using a questionnaire. For irritation and CNS symptoms, the symptoms index was expressed as differences from the preexposure measurement. Symptoms of local irritation to the eyes and airways were not significantly different when the two exposure concentrations were compared; however, a clear trend toward a significant increase was noted. The occurrence and/or intensity of CNS symptoms increased with exposure. The authors concluded that 2 h of exposure to MIBK caused increased discomfort in the subjects tested, as measured by symptom ratings (Iregren, Tesarz, and Wigaeus-Hjelm 1993).

Gagnon, Mergler, and Lapare (1994) reported on the effect of MIBK on olfactory function in four volunteers (two men, two women; ages = 27 to 57 years old). The subjects were exposed to 20 and 40 ppm MIBK, respectively, in an 18.1-m^3 chamber for 7 h on each of 3 consecutive days. After a 25-day nonexposure period, a second identical exposure was performed. Olfactory

adaptation and an MIBK-induced transient, olfactory perception threshold shift were reported at both exposure concentrations. Symptoms of eye, nose, or throat irritation and headache were present in some of the subjects. The authors concluded that individuals exposed professionally or environmentally to certain organic solvents may suffer temporary loss of the sense of smell, which hinders odor detection.

Case Reports

van Joost et al. (1984) diagnosed contact dermatitis in a 40-year-old man who had worked in a chemical factory for approximately 2 years. In the workplace, he was exposed to a variety of chemicals that were used in the manufacture of pesticides. Patch tests of various chemicals were performed using International Contact Dermatitis Research Group routine batteries. Reactions were scored at 48 and 72 h. Patch test results for undiluted MIBK were negative.

Grober and Schaumburg (2000) reported persistent cognitive deficits for a 44-year-old male employee of a poorly ventilated, indoor solvent extraction facility who had been exposed to ambient concentrations of MIBK in excess of 100 ppm (8 h/day) for 6 years. The level of exposure to MIBK was twice the threshold limit value, short-term exposure limit of 50 ppm. The deficits noted included slowed information processing and impaired attention. The pattern of cognitive deficits was said to have been best accounted for by an impairment in the limited-capacity working memory system that supports the performance of activities of everyday life that are not routine. The presence of impaired working memory in the worker correlated with the functional magnetic resonance imaging (MRI) finding of diminished cerebral blood volume and diminished mean transit time in both frontal lobes, relative to the remainder of the cerebrum. Cognitive dysfunction was also noted in a coworker with the same history of exposure to MIBK. Most likely, the persistent cognitive deficits resulted from chronic exposure to MIBK. It is important to note that no symptoms were reported for other employees in the work area (same exposure) who wore protective breathing devices.

Occupational Safety

NIOSH (1978) proposed a time-weighted average (TWA) limit of 50 ppm MIBK (205 mg/m^3) in 1978. The Code of Federal Regulations (29CFR 1910.1000) includes the OSHA standard of 100 ppm MIBK (410 mg/m^3) established in 1983.

The American Conference of Governmental Industrial Hygienists (ACGIH 2000) recommended a threshold limit value–time-weighted average (TLV-TWA) of 50 ppm and a threshold limit value–short-term exposure limit (TLV-STEL) of 75 ppm for occupational atmospheric exposure to MIBK. The TLV-TWA is defined as the time-weighted average concentration for a normal 8-h workday and a 40-h workweek, to which nearly all workers may be repeatedly exposed, day after day, without adverse effect. The TLV-STEL is defined as the concentration to which workers can be exposed continuously for a short period of time without suffering from (1) irritation, (2) chronic or irreversible tissue damage, or (3) narcosis of sufficient degree to increase the likelihood of accidental injury, impair self-rescue or materially reduce worker efficiency, provided that the daily TLV-TWA is not exceeded.

Gray (2000) presented information suggesting that MIBK, as an industrial degreasing agent, removes lipid from the skin, causing reddening, scaling, blistering, and peeling, and is irritating to the eyes and respiratory tract. The author noted that, in the chemical industry, the use of skin and eye protection is advised when handling MIBK.

SUMMARY

MIBK is an aliphatic ketone that functions as both a denaturant and solvent in cosmetic products. One method of production is acetone condensation, followed by catalytic hydrogenation. MIBK may contain the following impurities: dimethyl heptane, methyl isobutyl carbinol, mesityloxide, acetic acid, 4-methyl-2-hydroxypentane, and methyl n-butyl ketone. According to the Chemical Manufacturers Association, MIBK producers indicated in 1999 that MnBK (known neurotoxin) is either not found in MIBK or is found in trace amounts (typically 0.01 to 0.06% and always less than 0.1%).

Frequency of use data provided by FDA in 1998 indicate that MIBK is used in two cosmetic products. However, use concentration data provided by CTFA in 2000 indicate that MIBK is used in one nail correction pen (volume = 3 ml) at a concentration of 21%.

According to regulations established by the Bureau of Alcohol, Tobacco, and Firearms, the maximum concentration of MIBK that is listed for use as a denaturant of alcohol is 4.0%. MIBK is also listed in the *National Formulary* as an alcohol denaturant that is used as an excipient for drugs.

The metabolites, 4-hydroxy-4-methyl-2-pentanone (MIBK oxidation product) and 4-methyl-2-pentanol (4-MPOL) (MIBK reduction product) were detected in blood samples from guinea pigs injected intraperitoneally with MIBK. Values for the serum half-life and total clearance time for MIBK that have been determined are 66 min and 6 h, respectively. Hydroxylation products of MIBK, such as 4-MPOL, are expected either to be conjugated with sulfate or glucuronic acid and excreted in the urine or to enter intermediary metabolism to be converted to carbon dioxide.

In a study in which MIBK was administered orally or by inhalation exposure to groups of guinea pigs, the amount of MIBK detected in the plasma and liver was proportional to the administered dose. The metabolite, 4-hydroxy MIBK was detected in the plasma regardless of the route of exposure. 4-MPOL was detected in the plasma after inhalation exposure, but not after oral doses of MIBK were administered. 4-MPOL and 4-hydroxy-4-methyl-2-pentanone were the principal MIBK metabolites in mice dosed intraperitoneally with MIBK.

The percutaneous absorption of MIBK was demonstrated in a study involving guinea pigs. A maximum percutaneous uptake rate of 1.1 μmol/min/cm^2 was observed at 10 to 45 min after the initiation of exposure.

Both gross and microscopic evidence of lung damage have been reported in acute inhalation toxicity studies in which mice and guinea pigs were exposed to MIBK. Increased pulmonary arterial pressure was noted in acute inhalation toxicity studies in which cats or dogs were exposed to MIBK. Bronchoconstriction was also observed in cats that inhaled MIBK.

Acute oral LD_{50} values of 4.6 (3.932–5.382) g/kg and 2.08 (1.91–2.27) g/kg have been reported for MIBK in studies involving rats. An acute oral LD_{50} of 1.5 ml/kg (mice) for MIBK has also been reported.

In an acute dermal toxicity study, undiluted MIBK was applied to the skin of two rabbits for 10 h either by flooding the test site or placement of a cotton pad impregnated with the test substance. Signs of systemic effects were not noted and no treatment-related pathologic changes were observed at microscopic examination of internal organs.

The following acute intraperitoneal LD_{50} values for MIBK have been reported: 1.14 ml/kg (rats), 0.59 $\pm$ 0.23 g/kg (mice), and 0.919 ml/kg (guinea pigs). Intraperitoneal injection of MIBK was associated with pulmonary vascular effects in cats.

Increased pulmonary arterial pressure, but not bronchoconstriction, was induced in cats dosed intravenously with MIBK.

In short-term exposure experiments, male and female Fischer-344 rats inhaled MIBK (concentrations up to ~2000 ppm) 6 h per day over a period of 9 days to 2 weeks. Concentrations of approximately 500 or 2000 ppm induced hyaline droplet formation in the kidneys of male rats, and epithelial regeneration of the proximal convoluted tubules was also noted at the highest concentration. Increases in liver and kidney weight were also observed at these concentrations. B6C3F1 mice exposed to MIBK according to the same procedure had increased liver weight (~2000 ppm, females only). An increase (females) and decrease (males) in kidney weight was also noted at this concentration. No changes in kidney weight occurred after exposure to ~500 ppm.

In another short-term test, monkeys, dogs, mice, and rats were exposed continuously (inhalation) to 100 or 200 ppm MIBK over a period of 2 weeks. Increased kidney weight and microscopic evidence of toxic nephrosis of the proximal tubules were reported only for rats, and this finding was noted at both concentrations of exposure. Increased liver weight (rats) was also noted after exposure to 200 ppm. Other study results indicated increased adrenal weight only in female albino rats exposed to MIBK at a concentration of 4.53 mg/L of air 5 days per week (6 h/day) for 4 weeks. No "clear-cut" test substance-related abnormalities were noted at gross necropsy.

No evidence of gross pathologic effects was observed in Wistar female rats that ingested MIBK at concentrations of 0.5% and 1.0% MIBK in drinking water for 7 days.

In a short-term dermal toxicity study, seven applications of undiluted MIBK (3 ml/kg each, 5 to 12 h) were made to the shaved skin of two rabbits over a period of 15 to 21 days. Local skin changes consisted of polymorphonuclear infiltration in the upper dermis. No systemic effects were noted.

The subchronic inhalation toxicity of MIBK was evaluated using groups of male and female F-344 rats. The animals were exposed to concentrations ranging from 50 to 1000 ppm 5 days per week (6 h/day) for 90 days. Increased liver weight was noted following exposure to 1000 ppm; however, hepatic lesions were not observed at gross or microscopic examination. Groups exposed to 250 or 1000 ppm MIBK had increased numbers of hyaline droplets in proximal tubule cells, which may be specific to the male rat. Male $B6C3F_1$ mice exposed to MIBK had increased liver weight following exposure to 250 or 1000 ppm, but hepatic lesions were not observed at gross or microscopic examination.

Increased liver and kidney weight was observed in male Wistar albino rats exposed to 410 mg/m^3 MIBK for 90 days. At microscopic examination, hyaline droplet degeneration of the proximal tubules was observed in kidneys from each of the 100 rats. Kidney damage was completely reversed in rats observed up to 60 days post exposure. In the same study (same dose and exposure duration), gross examination revealed no differences in tissues examined between test dogs and monkeys and controls. Liver function test results (dogs only) also indicated no differences between test and control dogs.

Nephrotoxicity and increased liver and kidney weight, but no evidence of hepatic lesions, was observed in male and female Sprague-Dawley rats dosed orally with 1000 mg/kg MIBK daily for 13 weeks. The 50-mg/kg dose (lowest dose) was considered the NOEL. No significant gross lesions and renal tubule cell hyperplasia were reported in a study involving rats that received daily oral doses of 1.04 g/kg MIBK (in drinking water) for 120 days.

In a subchronic dermal toxicity study, MIBK (in sunflower oil) was applied to white rats (lower 2/3 of tail) daily at doses of 300 or 600 mg/kg for 4 months. Skin changes included reduced mitotic activity in hair follicles and increased thickness of horny and granular cell layers of the epidermis. Changes in the spleen included a decrease in the number of reactive centers in follicles and an increase in the number of iron-containing pigments in the area of the red pulp. A reduction in the lipid content of the cortical layer was noted in the adrenal glands.

Inhalation exposure of Sprague-Dawley rats to MIBK (up to 3020 ppm) did not cause any modifications in serum GLDH activity. However, preexposure of rats to MIBK caused a dose-related increase in DCB-induced GLDH activity. In this study, the mitochondrial enzyme, GLDH was assessed in serum as a quantitative sign of hepatic necrosis. In other studies (oral dosing), the hepatotoxicity of chloroform in Sprague-Dawley rats was enhanced after the administration of MIBK or its major metabolites, 4-MPOL and 4-hydroxymethyl isobutyl ketone (minimally effective dose of each = 5 mmol/kg), and the

hepatotoxicity of carbon tetrachloride was also enhanced after MIBK administration (dose response; doses up to 20 mmol/kg).

The dermal administration of MIBK in sunflower oil to white rats (lower 2/3 of tail) at daily doses of 300 or 600 mg/kg for four months caused an increase in the number of binuclear hepatocytes, reduced mitotic activity of these cells, and an increase in the number of hepatocytes with pathology. Neither histologic evidence of liver damage nor lipid deposition was observed in mature guinea pigs injected intraperitoneally with a single dose of 500 or 1000 mg/kg MIBK.

MIBK had an additive effect on the hepatotoxicity of 1,2-dichlorobenzene, chloroform, hexachlorobenzene, and carbon tetrachloride.

Neuropathological changes in the most distal portions of the tibial and ulnar nerves were observed in young adult rats exposed (inhalation) to 1500 ppm MIBK for up to 5 months. The neuropathological changes observed may have been related to the presence of 3% methyl *n*-butyl ketone in the commercial grade of MIBK that was tested. No differences in the performance of schedule-controlled operant behavior were noted between Sprague-Dawley rats exposed to MIBK (inhalation) at concentrations up to 1500 ppm for 13 weeks and control rats. At gross examination, no test substance-related changes were noted.

In a 7-day study in which the effect of inhaled MIBK (25 to 75 ppm) on the behavior of young baboons was evaluated, it was concluded that MIBK did not impair each animal's ability to discriminate or remember stimuli presented in a match-to-sample discrimination task.

The oral dosing of rats with 601 mg/kg MIBK five times per week for 55 weeks had no effect on the maximum motor-fiber conduction velocity in the tail nerve.

MIBK did not induce peripheral neuropathy in groups of Sprague-Dawley albino rats injected intraperitoneally with MIBK (10% in corn oil) at doses up to 100 mg/kg for 2 weeks.

The intravenous infusion of female Sprague-Dawley rats with MIBK (in an emulsion) resulted in depression of the vestibulo-oculomotor reflex. The threshold limit for this effect was 0.2 mM/L (20 ppm) at an infusion rate of 30 μM/kg/min.

Based on the results of an electromyographic examination, neurotoxicity was not observed in male Beagle dogs injected with MIBK (300 mg/kg) daily for 11 months. No evidence of systemic toxicity or neurotoxicity was observed in dogs injected subcutaneously with MIBK (150 mg/kg) twice daily for a year. Tissue samples from the brainstem, nerves, and muscles were examined at necropsy. The test substance (98% pure) contained 0.9% methyl *n*-butyl ketone and trace amounts of 4-methyl-2-hydroxypentane. In another study, MIBK containing 0.9% methyl *n*-butyl ketone was injected subcutaneously (150 mg) into cats five times per week for up to 8.5 months. The analysis of biopsy specimens from the hind feet indicated no detectable damage to nerve tissues.

MIBK (0.5%) induced widespread cell death in a clonal line of neuroblastoma cells (Neuro 2aE) derived from a spontaneously occurring murine tumor (C1300). No discernible cytopathological changes and depressed growth rate were reported after exposure to concentrations of 0.1% and 0.2% MIBK, respectively.

The results of ocular irritation studies involving albino rabbits indicate that undiluted MIBK is an ocular irritant.

Single 24-h patch applications of undiluted MIBK induced reactions ranging from slight to moderate skin irritation in rabbits and slight skin irritation in guinea pigs. Repeated applications (seven) of undiluted MIBK over a 15- to 21-day period caused drying of the skin and exfoliation in rabbits. In another study, repeated applications of MIBK to guinea pigs (daily for 31 days) resulted in desquamation. The immersion of a rabbit's ear and tails from mice in pure MIBK for 2 h caused pronounced inflammation and necrosis.

The threshold concentration of MIBK for the inhibition of bacterial growth (*Pseudomonas putida*) was 275 mg/L in a 16-h study.

MIBK was not mutagenic in the Ames test (*Salmonella typhimurium* strains) or in the mitotic gene conversion assay (*Saccharomyces cerevisiae* strain) with or without metabolic activation. Mammalian mutagenicity test results (with or without metabolic activation) for MIBK in the following assays were also negative: mouse lymphoma, unscheduled DNA synthesis, micronucleus, cell transformation, and chromosome damage.

MIBK is among the chemicals that have been approved by the NTP for testing in a toxicology/carcinogenesis study.

MIBK did not induce any treatment-related increases in embryotoxicity or fetal malformations in pregnant Fischer 344 rats or CD-1 mice that inhaled MIBK at concentrations of 300, 1000, or 3000 ppm. There was evidence of treatment-related maternal toxicity only at the highest concentration tested. In another study, MIBK was applied to the skin (lower 2/3 of tail) of an unspecified number of male white rats daily (4 h/day) at doses of 300 or 600 mg/kg for four months. Changes in the testes included a reduction in the number of spermatocytes, spermatids, and spermatozoa.

Blood and breath samples were obtained from subjects who inhaled MIBK during six 20-min exposure sessions. Results at 90 min post exposure indicated that most of the absorbed MIBK had been eliminated from the body. In another group of subjects exposed to MIBK (inhalation) for 2 h during light physical exercise, the apparent blood clearance was 1.6 L/h/kg at all exposure concentrations. Only 0.04% of the total dose was eliminated unchanged in the urine within 3 h post exposure. MIBK was detected in the following tissues of subjects who died following exposure to several volatile organic solvents during spray painting: brain, liver, lung, vitreous fluid, kidney, and blood.

The threshold for MIBK-induced irritation of the lungs of human subjects was 0.03 to 0.1 mg/L after 1 min of respiration. Ocular irritation was noted in 12 volunteers exposed to 200 ppm MIBK (inhalation) for 15 min. Nasal, ocular, and throat irritation were experienced by no more than four of six volunteers subjected to six 20-min exposures (inhalation) to MIBK

at concentrations ranging from 0.402 to 2.827 mg/L. In another inhalation study, irritation of the nose and throat were the most common symptoms reported by three of the eight volunteers exposed to MIBK at concentrations up to 48.8 ppm during light physical exercise for 2 h.

Ocular irritation, nausea, and sore throat were experienced by approximately half of the 19 workers exposed to MIBK daily at concentrations up to 500 ppm for 30 min and 80 ppm for the remainder of the day. Slightly enlarged livers and nonspecific colitis were reported for 4 and 6 workers, respectively. In another study, symptoms of either nausea or respiratory irritation were reported by workers exposed to 100 ppm MIBK. Complaints were reduced substantially when the level of exposure was reduced to 20 ppm.

Exposure to 100 ppm MIBK for 4 h did not induce neurobehavioral effects in either of the 23 human subjects tested. In another study, the potential narcotic impact of MIBK on CNS function was evaluated using two groups of six subjects exposed to 10 mg/m^3 (control) and 200 mg/m^3 MIBK, respectively, for 2 h. No consistent exposure-related effect on heart rate was identified, and the results of the simple reaction time performance test indicated no exposure-related differences in performance.

In a case report, a 40-year-old worker (with contact dermatitis) at a chemical factory had a negative patch test reaction to undiluted MIBK. Findings in another case report indicated persistent cognitive deficits in a 44-year-old employee at an indoor solvent extraction facility who did not wear a protective breathing device.

The most recent occupational limits from the ACGIH recommended a TLV-TWA of 50 ppm and a TLV-STEL of 75 ppm for atmospheric exposure to MIBK.

DISCUSSION

MIBK is used as a solvent and denaturant in cosmetic products. The Panel expressed concern over the neurotoxicity potential of this ingredient, based on published data indicating that MnBK (methyl *n*-butyl ketone, a known neurotoxin) is present as an impurity in MIBK at concentrations as high as 3.0%. According to the Chemical Manufacturers Association, MIBK producers indicated in 1999 that MnBK is either not found in MIBK or is found in trace amounts (typically 0.01% to 0.06% and always less than 0.1%). After considering the new impurities data, data indicating that the only reported use of MIBK in cosmetics is in a nail correction pen (total volume of pen = 3 ml; 21% MIBK), and the observation that significant dermal absorption of the nail correction fluid would not be likely under normal use conditions, the Panel agreed that MIBK could be used safely as a solvent in nail polish removers in a controlled application system. However, given the known neurotoxic effects of MnBK, the Panel stressed the importance of continued efforts to limit the concentration of this impurity in MIBK. Furthermore, the Panel stressed the importance of avoiding inhalation exposure to MIBK, based on evidence of lung, kidney, or liver damage in animal studies and respiratory irritation or liver effects reported in human occupational exposure studies on MIBK.

Though one of the reported uses of MIBK in cosmetics is that of a denaturant, product data indicative of this function have not been provided. However, after noting that MIBK has been approved for use as a denaturant for alcohol, in keeping with the regulations established by the Bureau of Alcohol, Tobacco, and Firearms (27CFR21.21), the Panel agreed that MIBK could be considered safe for use as a denaturant in cosmetics at concentrations up to the maximum concentration of MIBK (4%) that is listed for use as a denaturant of alcohol. It is important to note that because of the established regulations, the Panel assumes that cosmetic product formulators use MIBK as a denaturant at concentrations that do not exceed 4.0%.

The Expert Panel is aware of an ongoing carcinogenicity study on MIBK that is being conducted by the National Toxicology Program, and agreed that the results will be reviewed by the Panel after the report has been made available to the public.

CONCLUSION

Based on the available animal and clinical data in this report, the CIR Expert Panel concludes that MIBK is safe as used in nail polish removers and as an alcohol denaturant in cosmetic products.

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