Amended Safety Assessment of MIBK as Used in Cosmetics

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*All interested persons are provided 60 days from the above release date **(i.e., until June 15, 2024)** to comment on this safety assessment, and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available for review by any interested party, and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Executive Director, Dr. Bart Heldreth.*

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ABBREVIATIONS

α2u	α2u-globulin
α2u-N	α2u-globulin-nephropathy
ACGIH	American Conference of Governmental Industrial Hygienists
AhR	aryl hydrocarbon receptor
BrdU	bromodeoxyuridine
BROD	benzyloxyresorufin-*O*-dealkylase
CAR	constitutive androstane receptor
CIR	Cosmetic Ingredient Review
CNS	central nervous system
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
CYP	cytochrome P450
Dictionary	web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI)
EGF	epidermal growth factor
ELISA	enzyme-linked immunosorbent assay
EROD	ethoxyresorufin-*O*-deethylase
EU	European Union
FDA	Food and Drug Administration
GC/MS	gas chromatography/ mass spectroscopy
ID_{50}	duration of immobility
LD_{50}	median lethal dose
MOA	mode of action
4-MPOL	4-methyl-2- pentanol
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no-observed-adverse-effect-level
NOEL	no-observed-effect-level
NR	not reported
OECD	Organisation for Economic Co-operation and Development
NTP	National Toxicology Program
OSHA	Occupational Safety and Health Administration
Panel	Expert Panel for Cosmetic Ingredient Safety
PND	postnatal day
PPAR-α	peroxisome proliferator-activated receptor-α
PROD	pentoxyresorufin-*O*-dealkylase
PXR	pregnane X receptor
RD_{50}	50% decrease in the respiratory rate
RDS	replicative DNA synthesis
STEL	short-term exposure limit
TG	test guideline
TLV	threshold limit value
TWA	time-weighted average
US	United States
VCRP	Voluntary Cosmetic Registration Program
VOR	vestibulo-oculomotor reflex

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of MIBK (aka methyl isobutyl ketone) as used in cosmetic formulations. MIBK is reported to function in cosmetics as a denaturant, fragrance ingredient, and solvent. The Panel considered the available data and concluded that MIBK is safe as used in nail polish removers and as an alcohol denaturant in cosmetics in the present practices of use and concentration described in this safety assessment.

INTRODUCTION

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), MIBK (aka methyl isobutyl ketone) is reported to function in cosmetics as a denaturant, fragrance ingredient, and solvent.[1] This ingredient was first reviewed by the Expert Panel for Cosmetic Ingredient Safety (Panel) in a safety assessment that was published in 2004.[2] At that time, the Panel issued a final report with the conclusion that MIBK is safe as used in nail polish removers and as an alcohol denaturant in cosmetic products, based on the available animal and clinical data in the report.

In accordance with its Procedures, the Panel evaluates the conclusions of previously issued reports approximately every 15 yr, and it has been at least 15 yr since this assessment was issued. In March 2023, the Panel determined that this safety assessment should be re-opened to include new carcinogenicity and toxicological data that were included in a National Toxicology Program (NTP) report; these studies were in progress at the time of the original report.

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an extensive search of the world's literature; a search was last conducted in October 2023. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Excerpts from the summary of the previous report on MIBK are disseminated throughout the text of this re-review document, as appropriate, and are identified by *italicized text*. (This information is not included in the tables or the summary section.)

CHEMISTRY

Definition and Structure

According to the *Dictionary*, MIBK (CAS No. 108-10-1) is the aliphatic ketone that conforms to the structure in Figure 1.[1] *MIBK has been described as a branched chain hydrocarbon that is photochemically reactive.*[2]

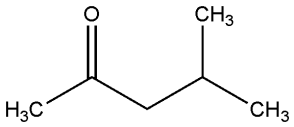

Figure 1. MIBK

Chemical Properties

MIBK occurs as a colorless liquid with a faint, ketonic, camphor odor.[2] *It has a molecular weight of 100.16 Da. Its solubility in water is 17 g/l (20°C); solubility has also been described as 2.04% by weight (28°C).*

Method of Manufacture

MIBK is manufactured by acetone condensation, followed by catalytic hydrogenation.[2] *Acetone is dimerized to diacetone alcohol at 0 to 20°C. Diacetone alcohol is then dehydrated at 100 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 to 165°C.*

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% mesityl oxide, < 0.002% acetic acid, and < 0.0002% non-volatiles.[2] *Another source indicates that MIBK is > 98% pure and contains 0.9% methyl n-butyl ketone and trace amounts of 4-methyl-2-hydroxypentane. A 3% concentration of the contaminant, methyl n-butyl ketone, in commercial MIBK has been noted. However, in 1999, 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%). Other impurities in MIBK include methyl amyl alcohol, acetone, and 3-methyl-2-butanone.*

USE

Cosmetic

The safety of the cosmetic ingredient addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of this ingredient in cosmetics and does not cover its use in airbrush delivery systems. Data included herein were obtained from the FDA's Voluntary Cosmetic Registration Program

(VCRP) database (frequency of use) and in response to a survey conducted by the Personal Care Products Council (Council) (maximum use concentrations). The data were provided by cosmetic product categories, based at that time on 21CFR Part 720. For most cosmetic product categories, 21CFR Part 720 does not indicate type of application and, therefore, airbrush application is not considered. Airbrush delivery systems are within the purview of the US Consumer Product Safety Commission (CPSC), while ingredients, as used in airbrush delivery systems, are within the jurisdiction of the FDA. Airbrush delivery system use for cosmetic application has not been evaluated by the CPSC, nor has the use of cosmetic ingredients in airbrush technology been evaluated by the FDA. Moreover, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety.

According to 2023 VCRP data, MIBK is reported to be used in 2 formulations, specifically an "other" manicuring preparation and an aftershave lotion;[3] however, no concentrations of use were reported in response to the survey conducted by the Council in 2022[4] (Table 1). The results of the concentration of use survey conducted in 2003 indicated MIBK was used at up to 21% in other manicuring preparations, specifically, in a nail correction pen; in the use of nail correction pens there may be dermal contact with the skin adjacent to the nail.[2]

Although products containing MIBK may be marketed for use with airbrush delivery systems, this information is not available from the VCRP or the Council survey. Without information regarding the frequency and concentrations of use of these ingredients (and without consumer habits and practices data or particle size data related to this use technology), the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems.

In the European Union (EU), MIBK is categorized in Annex II, the list of substances prohibited in cosmetic products, due to carcinogenic potential.[5,6]

Non-Cosmetic

MIBK is approved for direct addition to food for human consumption as a component of synthetic flavoring substances and adjuvants (21CFR172.515). It is also an approved indirect food additive when used as a component of adhesives that are present in articles intended for use in packaging, transporting, or holding food (21CFR175.105) and as a solvent of polysulfide polymer-polyepoxy resins that form the food-contact surface of articles intended for packaging, transporting, or holding dry food (21CFR177.1650). MIBK has been approved as a denaturant in denatured alcohol and rum, with specifications for its acidity, color, distillation range, odor, and specific gravity (27CFR21.117). (It should be noted that the original safety assessment, the CFR citation code for this specifications was 27 CFR 21.161; the update was effective January 19, 2001.[7]) According to specifications established by the Alcohol and Tobacco Tax and Trade Bureau, 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.

MIBK is used primarily in industrial coating solvents, lubricant oil dewaxing, and in rare metal refining.[2] It is also used in public health environmental studies for determining the presence of heavy metals in air and in biological materials.

TOXICOKINETIC STUDIES

Dermal Absorption

In Vitro

In vitro partition coefficients of 70 to 90 between blood and air have been reported for MIBK.[2] The following partition coefficients were also reported: 90 (MIBK into blood), 79 (MIBK into water), and 926 (MIBK into oil).

Animal

Dermal

The percutaneous absorption of MIBK (1 ml) was determined using 8 outbred female guinea pigs.[2] A maximum percutaneous uptake rate of 1.1 µmol/min/cm^2 was observed at 10 to 45 min after the initiation of exposure.

Absorption, Distribution, Metabolism, and Excretion

Using a mass-spectrometric method, the presence of MIBK in human maternal blood samples collected immediately after delivery was confirmed.[2] Findings indicated that MIBK has the potential to enter the umbilical cord and cross the placenta.

Animal

Oral

MIBK was administered by gavage to Sprague Dawley rats.[2] The metabolite 4-methyl-2-pentanol (4-MPOL) was not detected in the plasma, liver, or lung. The authors concluded that metabolite concentrations were influenced by the route of MIBK administration.

Plasma levels of MIBK were determined up to 12 h after a single oral dose of MIBK to male rats.[8] Twenty-six male Sprague-Dawley rats were orally administered a single dose of 5 mmol/kg in corn oil, by gavage, according to Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 417. Two or three blood samples (1 ml) were taken by orbital bleeding from each rat at each of the following times after dosing: 0.125, 0.25, 0.5, 0.75, 1, 1.5, 3, 4.5, 6, 9 and 12 h.

MIBK in plasma was determined using gas chromatography/mass spectroscopy (GC/MS). MIBK was rapidly absorbed into the systemic circulation following oral exposure, with a mean maximum plasma concentration of 0.644 mmol/l occurring at 0.25 h.

Inhalation

MIBK metabolite 4-MPOL increased in a dose-related manner in plasma following inhalation by Sprague-Dawley rats.[2] Following inhalation exposure, 4-MPOL was detected in the plasma, liver, and lung.

Parenteral

Male guinea pigs were administered a single intraperitoneal dose of 450 mg/kg MIBK in corn oil.[2] Blood samples were collected at 1, 2, 4, 6, 8, 12, and 16 h post-dosing. The serum half-life and total clearance times for parent MIBK were 66 min and 6 h, respectively.

The metabolic fate of MIBK using groups of 8 male Charles River CD-1 mice was assessed. The animals received a single intraperitoneal injection of 5 mmol/kg MIBK dissolved in corn oil, and the injection volume was 10 ml/kg. The principal metabolites were 4-MPOL (reduction product) and 4-hydroxy-4 methyl-2- pentanone. The concentration of the reduction product in the brain was twice that seen in the blood at 15- and 30-min time intervals.

Human

Inhalation

Eight male volunteers were exposed to MIBK in a 12 m^3 exposure chamber (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.[2] 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. The 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 and 3 h after exposure. Only 0.04% of the total dose was eliminated unchanged in the urine within 3 h post-exposure. When human volunteers were exposed to 100 ppm (410 mg/m^3) of MIBK for 4 h in an environmental chamber, blood and breath samples collected at 90 min post-exposure indicated that most of the absorbed MIBK had been eliminated from the body.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Dermal

In an acute dermal toxicity study, undiluted MIBK was applied to the skin of 2 rabbits for 10 h either by flooding the test site or via a cotton pad saturated with the test substance.[2] Signs of systemic effects were not noted, and no treatment-related pathologic changes were observed at microscopic examination of internal organs.

The acute dermal toxicity of MIBK was assessed in Crl: CD BR rats in accordance with OECD TG 402.[9] A semi-occlusive patch with 2000 mg/kg bw undiluted MIBK was applied to 5 male and 5 female rats for 24 h. Dermal reactions were recorded twice daily on days 2, 3, and 4, and once daily from the 5th to 14th day. Rats were weighed before dosing and on days 1, 8, and 15. At study termination, necropsy was performed, organ weights were recorded, and tissues were examined microscopically. No animals died during the test or observation period and no clinical signs of toxicity were noted. Additionally, there were no irritation reactions or other dermal changes at the sites of application of the test article. Body weight gains were not affected, and there were no macroscopic changes observed at necropsy. The acute median lethal dose (LD_{50}) was determined to be greater than the test dose of 2000 mg/kg bw.

In another study performed in accordance with OECD TG 402, rabbits were administered 20 ml/kg of MIBK dermally for 4 h.[9] An LD_{50} of > 20 ml/kg bw was reported. (No other details, including number of animals not stated or whether the test site was occluded, were available.)

Oral

In mice, oral LD_{50}s of 1.5 ml/kg (10 - 40% emulsion in a 1% aqueous emulsion of a sodium sulfate derivative of 3,9-diethyl tridecanol-6) and 1900 mg/kg were reported for MIBK.[2] In another study, the average lethal dose for MIBK in mice dosed orally (stomach tube) was 2805 mg/kg. In rats, acute oral LD_{50} values of 2080 mg/kg, 4600 mg/kg, and 5.7 ml/kg have been reported. In a study in which 6 rats were given a single dose of 1 ml/kg MIBK, all rats died instantly; in most of the animals, 25% of the lung tissue was hemorrhagic. The researchers stated that MIBK may have been aspirated into the lungs when swallowed. In a similar study, 0.2 ml MIBK was placed in the oral cavity of 5 male rats, and the animals were held with the nostrils closed to promote entry of the test material into the trachea. Some of the animals (number not stated) died within 24 h; all deaths were due to respiratory arrest, cardiac failure, or both, rather than pulmonary edema. It was concluded that MIBK presents a potential aspiration hazard. In guinea pigs, the acute oral LD_{50} was between 1.6 - 3.2 g/ kg.

Inhalation

During 5 min of exposure to MIBK, a concentration-dependent decrease in respiratory rate 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.[2] Mice

were exposed to a single exposure of MIBK (saturated air-vapor mixture) at concentrations ranging from 43 - 100 mg/l of air (20°C) for 0.25 - 22.6 h. Within 10 h post-exposure, 18 of 33 animals exposed to 82 mg/l for 0.5 h, 21 of 22 animals exposed to 86 mg/l for 1 h, and 5 of 10 animals exposed to 82 mg/l for 1.25 h died. In a study in which mice were exposed to MIBK (15 mg/l of air) for 2 h, narcosis was induced in all animals. An LC_{50} of 74.2 mg/l was reported in CF-1 male mice exposed to various concentrations of MIBK (1.0% v/v [41 mg/l] to 3.0% v/v [123 mg/l]) in a 10-l glass chamber. In another mouse study, exposure to 19,500 ppm MIBK induced anesthesia within 30 min, with recovery noted 5 min after exposure was discontinued; however, at concentrations >20,000 ppm, anesthesia followed by death occurred in most of the mice. In a 4-h inhalation study in rats (n = 6/ group), no animals exposed to 2000 ppm, but all animals exposed to 4000 ppm, died. In another 4-h inhalation study in rats, the threshold concentration for inhalation intoxication was 0.2 mg/l. Rats (number not stated) exposed to 21,000 ppm MIBK for 55 min died, and rats exposed to 4000 ppm MIBK for 6 h experienced loss of coordination and prostration. In guinea pigs, the acute inhalation toxicity of MIBK was evaluated by exposing groups of 10 animals to 0.1, 0.3, 1.0, 1.68, or 2.8 volume % (saturation) MIBK in an inhalation chamber. Death occurred within 4 h at a concentration of 1.0 volume %, and at progressively shorter periods at higher concentrations. In a study in which female guinea pigs were exposed to MIBK at concentrations of 1000 ppm (4100 mg/ m^3), 16,800 ppm (69,000 mg/m^3), or 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. Ocular and nasal irritation, salivation, lacrimation, ataxia, progressive narcosis, and death were observed at higher concentrations.

Short-Term Toxicity Studies

Dermal

Seven applications of undiluted MIBK (3 ml/kg each) were applied for 5 – 12 h to the shaved skin of 2 rabbits (100 cm^2 area) over a period of 15 - 21 d.[2] (Whether or not the applications were occluded was not specified.) Local skin changes consisted of polymorphonuclear infiltration in the upper dermis. No systemic effects were noted.

Oral

Administration of increasing oral doses of an 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), and the median lethal dose was 9.35 g/kg.[2] No evidence of gross pathologic effects was observed in female Wistar rats (3 rats/group) given 0.5 or 1.0% MIBK in drinking water for 7 d.

Inhalation

B6C3F_1 mice and F344 rats (6 males and 6 females/group) were exposed to MIBK at concentrations of 101 ppm (44 mg/m^3), 501 ppm (2050 mg/m^3), or 1996 ppm (8180 mg/m^3) for 6 h/d for 5 d, followed by a 2-wk non-treatment period, and then an additional 4 d of dosing.[2] In high-dose female mice and male and female rats, relative liver weights and absolute and relative kidney weights were increased; a decrease in relative kidney weight was reported for high-dose male mice. Compared to untreated controls, no statistically significant histologic lesions were observed in mice at any of the concentrations tested. Hyaline droplet formation was observed in the kidneys of mid- and high-dose male rats. In a study in which male and female B6C3F_1mice and F344 rats were exposed to 100, 500, or 2000 ppm MIBK for 6 h/d, 5 d/wk, for 2 wk, 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. In another study in which 10 mice were exposed daily to 20,000 ppm MIBK for 15 d (20 min/d); 6 animals died. No signs of nasal irritation were observed during an inhalation study in which albino rats exposed to MIBK at 4.53 mg/l air for 6 h/d, 5 d/wk, for 4 wk. In another short-term test, 4 monkeys, 8 dogs, 40 mice, and 50 rats were exposed continuously (inhalation) to 100 or 200 ppm MIBK over a period of 2 wk. Increased kidney weights 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.

Subchronic Toxicity Studies

Dermal

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 mo.[2] 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.

Oral

Nephrotoxicity and increased liver and kidney weights, but no evidence of hepatic lesions, were observed in male and female Sprague-Dawley rats dosed orally with up to 1000 mg/kg MIBK daily for 13 wk.[2] The 50-mg/kg dose (lowest dose) was considered the no-observed-effect level (NOEL). No significant gross lesions or renal tubule cell hyperplasia were reported in a study involving rats that received 1.3% MIBK in drinking water daily (1.04 g/kg/d) for 120 d. MIBK was administered to 3 groups of Sprague-Dawley rats (30 males, 30 females) at doses of 50, 250, and 1000 mg/kg, respectively, daily for 13 wk. All animals that survived were killed at the end of the dosing period. Ten animals (5 males, 5 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.

Inhalation

In an inhalation study, B6C3F$_1$ mice and F344 rats were exposed to 50 ppm (205 mg/m^3), 250 ppm (1025 mg/m^3), or 1000 ppm (4100 mg/m^3) MIBK for 6 h/d, 5 d/wk, for 90 d.[2] No hepatic lesions at gross necropsy or microscopic examination were observed in mice or rats, and urinalysis and serum chemistry values were normal. 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 groups. In a study in which rats were exposed via inhalation to 86 to 127 mg/m^{3}MIBK for 4 h/d, 5 d/wk, for 4.5 mo, some functional changes were noted. Groups of rats, dogs, and monkeys were exposed to 410 mg/m^3 MIBK vapor (100 mmol/25m^3) for 90 d in an altitude chamber. No biologically significant changes were reported for clinical chemistry and hematology parameters in dogs or monkeys. Microscopic examination of kidneys of rats revealed hyaline droplet degeneration of the proximal tubules (with occasional foci of tubular necrosis) in all animals exposed to MIBK, including those removed after 15, 22, 28, 71, or 85 d.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

MIBK was applied to the skin (lower 2/3 of tail) of an unspecified number of male white rats daily (4 h/d) at doses of 300 or 600 mg/kg for 4 mo.[2] Changes in the testes included a reduction in the number of spermatocytes, spermatids, and spermatozoa. In an inhalation study, MIBK did not induce any treatment-related increases in embryotoxicity or fetal malformations in pregnant Fischer 344 rats or CD-1 mice (3 groups, 25 females in each group per species) that inhaled MIBK for 6 h/d at concentrations of 0, 300, 1000, or 3000 ppm on gestation days 6 - 15. There was evidence of treatment-related maternal toxicity only at the highest concentration tested.

Inhalation

To investigate the potential impact of MIBK on reproductive performance, a two-generation reproduction study was conducted in Sprague-Dawley rats.[10] Four groups of 30 F_0 males and 30 F_0 females were randomly bred to produce an F_1 generation, and a replicate breeding procedure (avoiding sibling mating) was conducted to produce an F_2 generation. The F_0 and F_1 generations were approximately 7 wk and 4 wk old at their initiation of exposures, respectively. The rats were subjected to whole-body inhalation exposure of MIBK for 6 h/d, 7 d/wk, at concentrations of 0, 500, 1000, or 2000 ppm. F_0 and F_1 males were exposed for 70 d prior to mating and throughout mating until 1 d prior to euthanasia. F_0 and F_1 females were exposed for 70 d prior to mating and throughout mating, gestation, and lactation until 1 d prior to euthanasia. Exposure of the F_0 and F_1 dams was suspended for 5 d following parturition (lactation/postnatal days (PND) 0 - 4), and resumed on PND 5. The offspring of the F_0 and F_1 generations (F_1 and F_2 pups, respectively) were potentially exposed to MIBK both in utero and through nursing via maternal milk during PND 0 - 21. Exposures for all groups of F_1 weanlings were suspended between PND 22 and PND 27 because of the death of one male pup in the 2000 ppm group; this pup had clinical signs of central nervous system (CNS) depression indicative of a sedative effect (e.g., rocking, lurching, or swaying while ambulating). Exposures were reinitiated for all surviving animals on PND 28.

Detailed physical examinations were conducted weekly for parental animals (F_0 and F_1). All animals were observed twice daily for appearance and behavior and were examined for pharmacotoxic signs within 1 h after completion of exposure. Each male pup was examined for balanopreputial separation beginning on PND 35, and each female for vaginal perforation beginning on PND 25. The left testis and epididymis from all F_0 and F_1 males in all dose groups were evaluated for homogenization-resistant spermatid counts and sperm. Microscopic evaluations were performed on diverse tissues such as adrenal glands, prostate, brain, seminal vesicles, cervix, coagulating gland, uterus, ovaries, etc. Quantitative histopathologic evaluation of 10 sections of the inner third of the ovary (including enumeration of primordial follicles) was conducted on 10 F_1 females from the control and high-dose groups. Furthermore, a qualitative assessment was performed to identify the presence or absence of growing follicles, astral follicles, and corpora lutea.

No MIBK-related mortalities of adult rats occurred during the study, and no adverse effects on male and female reproductive function or indicators of sexual maturation were observed. The authors concluded that MIBK, at all exposure levels, did not affect any reproductive parameters or offspring growth and development. During the initial 2-wk of exposure at 2000 ppm, a reduction in body weight gains and a slight decrease in food consumption were observed in both generations. Additionally, in the 2000 ppm group, there was an increase in liver weights associated with centrilobular hypertrophy for both the F_0 and F_1 generations. Male rats exhibited increased kidney weights with hyaline droplets across all exposure concentrations, indicating male rat-specific nephropathy. For reproductive endpoints, the highest concentration tested, 2000 ppm, was considered the no-observed-adverse-effect level (NOAEL). Apart from acute sedative effects, the NOAEL for systemic effects in parental animals (excluding male rat kidney effects) was determined to be 1000 ppm, based on the temporary decrease in body weights and food consumption. Regarding neonatal toxicity, the NOAEL was determined to be 1000 ppm based on acute CNS depressive effects and the one death on PND 22.

GENOTOXICITY STUDIES

MIBK was not genotoxic in numerous assays, including several Ames tests (up to 8000 µg/ml, with and without metabolic activation), an unscheduled DNA synthesis assay in rat hepatocytes (up to 100 µl/ml), a chromosomal damage assay using rat

liver RL_4 cells (up to 8000 µl/ml), a mitotic gene conversion assay in Saccharomyces cerevisiae strain JD1 (up to 5 mg/ml, with and without metabolic activation), a mitotic chromosome loss assay in Saccharomyces cerevisiae strain D61.M (up to 7.3 mg/ml), and an in vivo mouse micronucleus test (10 ml/kg; intraperitoneal administration).[2] However, in a mouse lymphoma assay performed using L5178Y/$TK^{+/-}$ mouse lymphoma cells (0.32 - 4.2 µl/ml MIBK, with and without metabolic activation), results were negative with metabolic activation but equivocal without metabolic activation. In cell transformation assays with BALB/3T3 mouse embryo cells (up to 7 µl/ml without and 5 µl/ml with metabolic activation), no transforming activity was observed with metabolic activation, but positive results were reported without metabolic activation for 4.8 µl/ml MIBK.

CARCINOGENICITY STUDIES

Details on the inhalation carcinogenicity studies summarized below can be found in Table 2.

$B6C3F_1$ mice and F344/N rats (50/sex/group) were exposed to MIBK (greater than 99% pure) by inhalation (0, 450, 900, or 1800 ppm; whole-body, 6 h/d, 5 d/wk) for 2 yr.[11] Male and female mice exposed to MIBK had increased liver tumors, and the incidences of eosinophilic foci were significantly increased in female mice exposed to 450 and 1800 ppm MIBK. The incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) were significantly increased in male and female mice exposed to 1800 ppm. Male rats exposed to MIBK had tumors of the kidney, increased rates of hyperplasia of the kidney and adrenal gland, and mononuclear cell leukemia. The incidences of renal tubule hyperplasia were significantly increased in male rats exposed to 450 and 1800 ppm. Chronic nephropathy occurred in all male rats exposed to 1800 ppm and in 70 to 88% of exposed female rats. The incidences and severities of chronic nephropathy and mineralization in the renal papilla increased with increasing exposure concentration. Under the conditions of the 2-yr studies, there was *some evidence of carcinogenic activity* in male and female mice and male rats, and there was *equivocal evidence of carcinogenicity* in female rats. The liver was the primary site of MIBK-related toxicity in mice, and the kidney was the primary site of MIBK-related toxicity in rats (Table 3).

Mode of Action

Details of studies investigating the mode of action (MOA) underlying MIBK-induced tumors that are summarized below can be found in Table 4.

Inhalation

The MOA for the initiation of MIBK-induced liver tumors was investigated using male and female $B6C3F_1$, C57BL/6, and constitutive androstane receptor (CAR)/pregnane X receptor (PXR) knockout mice (16 sex/group).[12] These mice were exposed to either 0 or 1800 ppm MIBK via whole-body inhalation for 6 h/d, 5 d/wk, for a total of 10 d. The study concluded MIBK-induced hepatic effects are consistent with a phenobarbital-like MOA where the initiating events are activation of the CAR and PXR nuclear receptors and resultant hepatocellular proliferation, leading to rodent liver tumors. Overall, the MOA for rat and mouse liver tumor formation by phenobarbital and sodium phenobarbital and other CAR activators is considered qualitatively not plausible for humans.[12,13]Human hepatocytes are refractory to the mitogenic effects of CAR activator l (phenobarbital and sodium phenobarbital) and other CAR activators. These and other compounds do not stimulate replicative DNA synthesis (RDS) in cultured human hepatocytes and in in vivo studies performed in chimeric mice with humanized livers.[13]

Oral

To investigate whether MIBK operates through a non-genotoxic MOA to induce the male rat-specific renal tumor response following chronic exposure, 4 male and 4 female F344 rats were dosed by gavage with 0 or 1000 mg/kg MIBK in corn oil for 10 d.[14] In the positive control group, 4 male rats were dosed with 300 mg/kg D-limonene, a known inducer of α2u-globulin (α2u) nephropathy (α2u-N). The kidneys were removed and analyzed approximately 24 h after the final dose. MIBK caused an increase in protein droplets, accumulation of α2u, and renal cell proliferation in males, but not in females. The histological alterations caused by MIBK in male rat kidneys were similar to those induced by D-limonene, but they were of a milder degree. The investigators concluded that MIBK exerts renal effects through an α2u-N-mediated MOA, which is species and gender specific, occurring in male rates and not a concerning human risk.

In Vitro Cell Transformation

Two separate in vitro cell transformation studies were performed, one using cultured primary male C57BL/6 mouse hepatocytes and the other using cultured primary human hepatocytes.[9] Both types of hepatocytes were exposed to MIBK at 10 - 300 µM for 96 h. Certain nuclear receptors that link with carcinogenesis have been investigated, including CAR, PXR, aryl hydrocarbon receptor (AhR), and peroxisome proliferator-activated receptor-α (PPAR-α). Specifically, CAR, PXR, AhR, and PPAR-α were assessed by measurement of target genes, associated enzyme activities and cell proliferation. Phenobarbital and epidermal growth factor (EGF) were used as positive controls in the measurements of CAR activation and cell proliferation, respectively.

In the mouse hepatocyte study, cell viability was reduced at 10 µM (78% of controls) and at 300 µM (61% of controls). However, the researchers stated the finding at 10 µM was considered to be spurious in the absence of a concentration-response relationship. mRNA analysis revealed that cytochrome P450 (CYP) 2b10 mRNA expression was induced at all concentrations of MIBK, with a maximum of ~1.5 times at 300 µM. CYP1a2 mRNA expression showed marginal induction (~1.3 times) at 100 and 300 µM, without a clear concentration-response relationship. In contrast, MIBK treatment did not affect the mRNA expression of CYP3a11, CYP1a1, and CYP4a10. Furthermore, cell enzyme activities, including ethoxyresorufin-*O*-deethylase (EROD),

pentoxyresorufin-*O*-dealkylase (PROD), benzyloxyresorufin-*O*-dealkylase (BROD), and benzoquinone reductase, were assessed. PROD activity was increased (148% of controls) by exposure to 10 μM MIBK; however, this finding was considered questionable in the absence of any effects at higher concentrations. On the other hand, exposure to MIBK did not increase BROD, EROD, and benzoquinone reductase activities. Cells were also assessed for RDS immunohistochemically by bromodeoxyuridine (BrdU) incorporation. At any dose tested, MIBK did not induce RDS.

In the human hepatocyte study, cell viability was not reduced at any concentration. mRNA analysis revealed that CYP1A1 mRNA expression showed marginal induction (~1.3 times controls) at 300 μM MIBK in hepatocytes from one donor. CYP2B6 mRNA expression showed marginal induction (~1.6 times controls) at 300 μM MIBK in hepatocytes from one donor. In contrast, MIBK treatment did not affect the mRNA expression of CYP3A4 mRNA and CYP4A11 in hepatocytes from all three donors. Cell enzyme activities were also assessed. PROD, BROD, EROD, and benzoquinone reductase activity was not increased by exposure to MIBK in hepatocytes from any of the three donors. Exposure to MIBK at 300 μM increased RDS slightly (~1.7 times) in hepatocytes from one donor.

OTHER RELEVANT STUDIES

Neurotoxicity

MIBK (1.04 g/kg/d), administered to 5 female Wistar rats at a concentration of 1.3% in drinking water, did not induce any significant neurologic alterations.[2] 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/wk for 55 wk).

The neurotoxicity of MIBK was evaluated using 3 groups of 12 Sprague-Dawley albino rats. The 3 groups were injected intraperitoneally with MIBK (10% in corn oil) at doses of 10, 30, and 100 mg/kg for 2 wk. At the end of the 2-wk period the doses were doubled, and the new doses of 20, 60, and 200 mg/kg were injected intraperitoneally 5 d/wk for 33 wk. The following non-neural lesions were observed in test animals: chronic respiratory disease, peritonitis, bone marrow hyperplasia, and increased splenic hematopoiesis. It was concluded that MIBK did not induce peripheral neuropathy when injected intraperitoneally at doses up to 200 mg/kg.

The influence of MIBK on the vestibulo-oculomotor reflex (VOR) of female Sprague-Dawley rats (number not stated) was studied. The test substance 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.

Four cats were injected subcutaneously with 150 mg/kg bw undiluted MIBK twice daily, 5 times/wk, for up to 8.5 mo. A group of 4 control cats received subcutaneous doses of saline (0.2 ml/kg) 5 d/wk for up to 5 mo. No detectable damage to nerve tissues was observed. Four male Beagle dogs were injected subcutaneously with 300 mg/kg MIBK daily for 11 mo. No evidence of neurotoxicity was noted. In a similar study with 4 dogs, MIBK (>98% pure, with 0.9% methyl n-butyl ketone and trace amounts of 4-methyl-2-hydroxypentane) was administered subcutaneously at a dose of 150 mg/kg twice daily for a year. No evidence of systemic toxicity or neurotoxicity was observed in any of the animals tested.

The neurotoxicity of MIBK in 6 young adult rats was studied. The animals were exposed to 1500 ppm MIBK for up to 5 mo. No signs of neurological dysfunction were noted at the end of the exposure period.

The effect of inhaled MIBK on the lever-pressing behavior of Holtzmann, Sprague-Dawley male rats on a match-to sample discrimination task were evaluated. 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 min, 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.

The neurobehavioral effects of MIBK were studied using 80 male Swiss OF1 mice (40 controls, 40 test animals). Four test groups (10 mice/group) were exposed to test concentrations of 662, 757, 807, and 892 ppm for 4 h in a 'behavioral despair' swimming test. A decrease in the duration of immobility in the swimming test was reported after exposure to MIBK; the duration of immobility (ID_{50}) was 803 ppm. The ID_{50} value was defined as the median active concentration that resulted in a 50% decrease in immobility.

The neurotoxicity of MIBK in rats was evaluated in a 13-wk (64 d of exposure) study using male Sprague-Dawley rats. Rats (CRL:CD (SD)BR/VAF Plus strain animals; 20/group) were exposed to MIBK at concentrations of 250, 750, or 1500 ppm for 6 h/d, 5 d/wk, for 13 wk. Untreated animals served as controls. 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 NOEL for subchronic neurotoxicity.

The effect of inhaled MIBK (25 - 75 ppm) on the behavior of young baboons (number and ages not stated) was determined in a match-to-sample discrimination task. Test animals were exposed to MIBK over a 7-d period, whereas the controls were exposed to clean air. MIBK did not impair a baboon's ability to discriminate or remember stimuli. Similarly, in a delayed match-to-sample discrimination task using 4 baboons (~2 yr old), the animals were exposed to 50 ppm MIBK for 7 d and accuracy of performance was affected minimally.

The neurotoxicity of MIBK using a clonal line of neuroblastoma cells (Neuro 2aE) produced no discernible cytopathological changes in cells exposed to 0.1% MIBK for 10 d. At a concentration of 0.2%, MIBK induced a depression of growth rates; MIBK (0.5%) caused widespread cell death.

Nephropathy

As noted in the 'Carcinogenicity; Mode of Action' section of the report, MIBK was evaluated to assess its ability to induce specific measures of α2u-N in the kidneys of male and female rats compared to D-limonene, a known inducer of α2u-N.[14] In the study in which 4 male and 4 female F344 rats were administered corn oil (control) or MIBK (1000 mg/kg; 5 ml/kg) and another group of 4 male rats were administered D-limonene (300 mg/kg; 5 ml/kg) for 10 consecutive days by gavage, rats were euthanized approximately 24 h following the final dose, and the kidneys and a small section of duodenum were analyzed. Kidneys from the male rats exhibited similar rate of histological changes as seen in the kidneys from the D-limonene-treated male rats, including basophilic proximal convoluted tubule, increased hyaline droplet accumulation, and a minimal number of cell debris-containing pars recta tubules at the junction of the outer stripe of outer medulla and inner stripe of outer medulla. Also noted was a minimal increase in mitotic activity and nuclear variability in the cortex. There were no changes noted in the female rats.

The ability of MIBK to induce measures of α2u-N, including renal cell proliferation, was evaluated in 84 male and 84 female F344 rats following exposure to 0, 450, 900, or 1800 ppm.[15] Rats were exposed 6 h/d for 1 or 4 wk, and the kidneys were excised approximately 18-h post-exposure to evaluate hyaline droplet accumulation, α2u staining of hyaline droplets, renal cell proliferation, and quantitative renal α2u concentration. Hyaline droplet accumulation associated with MIBK was observed in the proximal convoluted tubules of all MIBK-exposed male, but not female, rats. Increasing MIBK concentration showed increasing hyaline droplets in terms of size and pattern disruption. Hyaline droplet accumulation was also prominent in the D-limonene positive control group. Males exposed to 1800 ppm MIBK for 4 wk had solitary tubules at the junction of the outer and inner stripes of the outer medulla containing eosinophilic granular debris, which were consistent with precursors of granular casts. There was an exposure-related increase in concentration of α2u in the male rats at both 1 and 4 wk of exposure. Total protein was not changed in the male rats exposed to MIBK, but an increase was observed following D-limonene administration. Counts of mitotic figures in the cortical proximal tubule cells were 10 times higher in male rats exposed to 1800 ppm MIBK compared to controls. Further in vitro analysis estimated the dissociation constant (to describe MIBK binding to α2u) to be 1.27×10^{-5} M, within range of other chemicals known to bind to α2u and cause nephropathy.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

Animal

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 tissue.[2] Undiluted MIBK was applied to the skin of 2 rabbits for 10 h; immediate (moderate erythema) and delayed (erythema persisting for 24 h) reactions were observed. In another study in which a single 10-h occlusive patch of MIBK was applied to the shaved skin of rabbits (number of animals not specified), erythema was observed for up to 24 h post-application. MIBK (500 mg) induced moderate irritation of rabbit skin after a contact period of 24 h. In a 24-h occlusive patch test using 12 albino rabbits (6 with intact and 6 with abraded sites), 24 h after dosing with 0.5 ml MIBK, very slight erythema was observed at 3 intact skin sites and all 6 animals with abraded sites had slight or well-defined erythema, with 2 animals having very slight edema. At 72 h post-application, very slight erythema was observed in 2 animals with abraded sites, and no signs of irritation were observed in the remaining animals (intact or abraded sites). The primary irritation score = 0.75. Drying and flaking of the skin surface were observed after 10 ml/d MIBK was applied to the skin of rabbits for 7 d. Seven applications (3 ml/kg each, 5 - 12 h) of undiluted MIBK were applied to a 100 cm^2 area of shaved skin on 2 rabbits over a period of 15 - 21 d; drying of the skin and exfoliation were observed.

In guinea pigs, slight skin irritation was observed after undiluted MIBK (5 and 10 ml) was applied (under occlusive wrap) to depilated skin for 24 h. Application of 2 ml MIBK to the backs of guinea pigs daily for 31 d caused desquamation, but no clinical or histologic evidence of toxic neuropathy. Guinea pigs subjected to brief exposures of MIBK over a period of 3 mo had no noticeable skin changes.

Sensitization

Animal

The skin sensitization potential of MIBK was assessed in female albino guinea pigs according to OECD TG 406; the test group comprised 20 animals and the control group comprised 10 animals.[9] Intradermal induction was carried out with 0.1 ml of 5% MIBK in vehicle (corn oil), and epicutaneous induction was performed with undiluted MIBK. Challenge exposure was conducted with 30% MIBK under occlusive conditions. Skin reactions were evaluated at 24 and 48 h. Test and control animals displayed normal body weight gain throughout the investigation. Local reactions (reddening and swelling) were observed in all treated animals. Some irritation reactions were also observed in the control animals. During the study there were insufficient details available to determine if the whole test area was abraded or only partially abraded. Therefore, results of skin irritation were deemed unreliable. Under the experimental conditions, MIBK produced no sensitizing reaction.

In another study a guinea pig maximization test was performed on 20 test animals (10 control) in accord with OECD TG 406.[9] Intradermal induction was carried out with 0.1 ml of 5% MIBK in vehicle (corn oil), and epicutaneous induction was

performed with semi-occlusive patches with 0.1 ml of 5% MIBK in vehicle (corn oil) applied neat to the skin on filter paper. Challenge was performed under occlusion (up to 48 h) with 30% MIBK in corn oil. No indication of skin sensitization was observed.

OCULAR IRRITATION STUDIES

Animal

The ocular irritation potential of undiluted MIBK was evaluated using 1 rabbit.[2] Reactions, scored according to the Draize scale (0 - 110), were 8, 3, and 1 at 1, 24, and 72 h post-installation, respectively; the test substance induced conjunctivitis, with some edema and corneal injury. Ocular irritation was observed within 10 min after instillation of undiluted MIBK (0.1 ml) into the eye of a rabbit, with inflammation and conjunctival swelling noted within 8 h, and inflammation, swelling, and exudate evident at 24 h. All reactions had cleared by 60 h. In another study, 6 albino rabbits were administered undiluted MIBK (0.1 ml) into the left conjunctival sac; untreated eyes served as controls; MIBK induced slight, transient ocular irritation. One-tenth ml of MIBK was instilled into the conjunctival sac of New Zealand albino rabbits (4 to 6 animals); untreated eyes served as controls. Effects on the cornea, iris, and conjunctiva were scored at 1 – 21 d post-instillation, and it was concluded that MIBK induced mild ocular irritation in rabbits. In another Draize test using 4 to 6 rabbits, undiluted MIBK (0.1 ml) was instilled into the conjunctival sac of one eye of each animal; a Draize score of 5/110 was reported.

A single-exposure ocular irritation study on MIBK was performed using 3 New Zealand White rabbits in accordance with OECD TG 405.[9] Undiluted MIBK (0.1 ml) was instilled into the conjunctival sac. Ocular changes were assessed at 30 min, and at 1, 4, 24, 48, and 72 h. MIBK caused changes of the conjunctivae (slight chemosis, ocular discharge) that resolved within 24 h. One rabbit had a minor disturbance of the corneal epithelium that resolved within 48 h. Under the conditions of the study, MIBK was considered to be slightly irritating to rabbit eyes.

A study was performed in accordance with OECD TG 405 in which 0.1ml of MIBK was instilled into one eye of each of 4 rabbits, and observations were made on days 1, 2, 3, and 7.[9] The overall mean scores were 0.08/4 for cornea opacity, 0/2 for iris lesion, 0.8/3 for redness of conjunctivae, and 0.17/4 for chemosis. MIBK was slightly irritating.

CLINICAL STUDIES

Twelve volunteers of both sexes were exposed to various concentrations of MIBK for 15 min.[2] 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). In another study, the threshold for MIBK-induced irritation of the lungs was 0.03 to 0.1 mg/l after 1 min of respiration (number of subjects not stated.)

Symptoms of either nausea or respiratory irritation were reported in workers (number not stated) 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).

Six subjects inhaled MIBK (six, 20-min exposures) through face masks connected to ports on a 125-l aerosol chamber. Test concentrations for the series of 6 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) was: nasal irritation (1 - 4 subjects), ocular irritation (1 - 3 subjects), and throat irritation (1 - 4 subjects). The results for throat irritation are based on the testing of only 4 subjects (test concentration range = 1.363 to 2.827 mg/l).

MIBK vapors have been reported to cause irritation of both the conjunctival and nasal mucosa at concentrations near 200 ppm. Exposure to higher concentrations caused lacrimation (indicative of marked irritation).

Eight male volunteers were exposed 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 3 subjects at any of the 3 exposure concentrations. There were no significant, exposure-related effects on the performance of a simple reaction time task or a test of mental arithmetic.

The neurobehavioral effects of MIBK resulting from short-term inhalation exposure was evaluated in 10 male and 13 female subjects (18- to 32-yr-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 post exposure session on day 3. 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.

The potential narcotic impact of MIBK on CNS function was studied. Heart rate, performance tests, and effects on local irritation, CNS symptoms, and mood were determined in 6 female and 6 male employees. 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-wk interval. The researchers concluded that 2 h of exposure to MIBK caused increased discomfort in the subjects tested, as measured by symptom ratings.

The occurrence of symptoms of irritation and CNS symptoms was evaluated using a questionnaire. 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 effects of MIBK on olfactory function in 4 volunteers were reported. Subjects were exposed to 20 and 40 ppm of MIBK in an 18.1-m^3 chamber for 7 h on each of 3 consecutive days. After a 25-d non exposure 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.

The potential narcotic impact of MIBK on CNS function was evaluated using two groups of 6 subjects exposed to 10 mg/m^3 (control) and 200 mg/m^3 MIBK for 2 h.[2] 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.

Case Report

In a case report, a 40-yr-old chemical factory worker with contact dermatitis had a negative patch test reaction to undiluted MIBK.[2] Findings in another case report indicated persistent cognitive deficits in a 44-yr-old 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/d) for 6 yr. The level of exposure to MIBK was twice the threshold limit value (TLV), short-term exposure limit of 50 ppm. The deficits noted included slowed information processing and impaired attention. Cognitive dysfunction was also noted in a coworker with the same history of exposure to MIBK.

Occupational Exposure

At the time of the original report, occupational limits from the American Conference of Governmental Industrial Hygienists (ACGIH) recommended a TLV– time-weighted average (TWA) of 50 ppm and a TLV–short-term exposure limit (STEL) of 75 ppm for atmospheric exposure to MIBK.[2] The National Institute for Occupational Safety and Health (NIOSH) proposed a TWA limit of 50 ppm MIBK (205 mg/m^3) in 1978. The Code of Federal Regulations (29CFR 1910.1000) included the Occupational Safety and Health Administration (OSHA) standard of 100 ppm MIBK (410 mg/m^3) established in 1983.

The short-term inhalation toxicity of MIBK in an occupational exposure was reported. Nineteen workers inhaled MIBK at concentrations up to 500 ppm (2050 mg/m^3) for 20 to 30 min/d, and 80 ppm (328 mg/m^3) for the remainder of the workday. Half of the workers had symptoms of weakness, loss of appetite, headache, ocular irritation, stomachache, nausea, vomiting, and sore throat. Insomnia, somnolence, heartburn, and intestinal pain were also reported by some of the workers (number not specified). 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.

MIBK was detected in the brain, liver, lung, vitreous fluid, kidney, and blood in workers who died after exposure to several volatile organic solvents during spray painting. Workers (number of subjects not stated) exposed to 500 ppm MIBK for 30 min daily experienced weakness, loss of appetite, headache, burning eyes, stomachache, 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.

The most recent occupational limits from the ACGIH recommend a TLV-TWA of 20 ppm (82 mg/m^3) and a TLV–STEL of 75 ppm (307 mg/m^3) for exposure to MIBK.[16] NIOSH lists a TWA limit of 50 ppm MIBK (205 mg/m^3), and also includes the OSHA standard of 100 ppm MIBK (410 mg/m^3).[17]

_ENREF_18 **SUMMARY**

MIBK is reported to function in cosmetics as a denaturant, fragrance ingredient and solvent. MIBK was previously reviewed by the Panel in a safety assessment that was published in 2004. At that time, the Panel issued a final report with the conclusion that MIBK is safe as used in nail polish removers and as an alcohol denaturant in cosmetic products. In accordance with its Procedures, the Panel evaluates the conclusions of previously issued reports approximately every 15 yr, and it has been at least 15 yr since this assessment was issued. In March 2023, the Panel determined that this safety assessment should be re-opened due to new carcinogenicity data available from the NTP; these studies were in progress at the time of the original report.

According to 2023 VCRP survey data, MIBK is reported to be used in 2 formulations, (other manicuring preparations and aftershave lotions). In response to a concentration of use survey conducted by the Council in 2022, no uses were reported. MIBK is categorized in Annex II of the EU, the list of substances prohibited in cosmetic products, due to carcinogenic potential.

In an absorption and metabolism study in which male Sprague-Dawley rats were orally administered a single dose of 5 mmol/kg bw of MIBK in corn oil, by gavage, MIBK was rapidly absorbed following oral exposure. The mean maximum plasma concentration was 0.644 mmol/l occurring at 0.25 h.

An acute dermal toxicity study of MIBK was performed in Crl:CD BR rats. Five male and 5 female rats were treated with 2000 mg/kg bw of undiluted MIBK under a semi-occlusive patch for 24 h; the LD_{50} was determined to be greater than the test dose of 2000 mg/kg. Rabbits (number of animals not stated) were administered 20 ml/kg of MIBK dermally for 4 h. An LD_{50} of > 20 ml/kg bw was reported.

A two-generation reproduction study was conducted to evaluate the effects of MIBK on reproductive performance. MIBK was administered to 30 Sprague-Dawley rats via whole-body inhalation at concentrations of 0, 500, 1000, or 2000 ppm, 6 h daily, for 70 d prior to mating. The authors concluded that MIBK, at all exposure levels, did not affect any reproductive parameters nor offspring growth or development. For reproductive endpoints, the highest concentration tested, 2000 ppm, was considered the NOAEL. Apart from acute sedative effects, the NOAEL for systemic effects in parental animals (excluding male rat kidney effects) was determined to be 1000 ppm. Regarding neonatal toxicity, the NOAEL was determined to be 1000 ppm.

Male and female $B6C3F_1$ mice and F344/N rats (50/sex/group) were exposed via inhalation (whole-body) to 0, 450, 900, or 1800 ppm MIBK for 6 h/d, 5 d/wk, for 2 yr. The incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) were significantly increased in male and female mice exposed to 1800 ppm. Male rats exposed to MIBK had tumors of the kidney, increased rates of hyperplasia of the kidney and adrenal gland, and mononuclear cell leukemia. Under the conditions of the 2-yr studies, there was *some evidence of carcinogenic activity* in male and female mice and male rats, and there was *equivocal evidence of carcinogenicity* in female rats. The liver was the primary site of MIBK-related toxicity in mice.

The MOA for the initiation of MIBK-induced liver tumors in mice was investigated in male and female $B6C3F_1$, C57BL/6, and CAR/PXR knockout mice. Mice were exposed to either 0 or 1800 ppm MIBK via whole-body inhalation for 6 h/d, 5 d/wk, for a total of 10 d. The study concluded MIBK-induced hepatic effects are consistent with a phenobarbital-like MOA; this MOA for rat and mouse liver tumor formation is considered not plausible for humans. The kidney was the primary site of MIBK-related toxicity in rats. MIBK was evaluated to assess its ability to induce specific measures of α2u-N in the kidneys of male and female rats compared to D-limonene, a known inducer of α2u-N. Kidneys from the male rats exhibited a similar rate of histological changes.

Cultured primary male C57BL/6 mouse hepatocytes and primary male human hepatocytes were exposed to MIBK (concentrations 10 - 300 µM) for 96 h. In the mouse study, cell viability was reduced at 10 µM but the results were considered spurious. CYP2b10 mRNA expression was induced at all concentrations of MIBK. CYP3a11, CYP1a, and CYP4a10 mRNA expression were unaffected. CYP1a2 expression was marginally induced. BROD, EROD, and benzoquinone reductase enzyme activity was not increased by exposure and exposure to MIBK did not induce RDS. In the human hepatocyte study, CYP1A1 and CYP2B6 mRNA expression was marginally induced at 300 µM in hepatocytes from one donor. CYP3A4 and CYP4A11 mRNA expression was unaffected by treatment with MIBK from all three donors. PROD, BROD, EROD and benzoquinone reductase activity was not increased by exposure to MIBK in hepatocytes from any of the three donors. However, exposure to MIBK at 300 µM increased RDS slightly (~1.7 times) in hepatocytes from one donor.

In the study in which MIBK was evaluated to assess its ability to induce specific measures of α2u-N in the kidneys of male and female rats as compared to D-limonene, 4 male and 4 female F344 rats were administered corn oil (control) or MIBK (1000 mg/kg; 5 ml/kg) and another group of 4 male rats were administered D-limonene, (300 mg/kg; 5 ml/kg) for 10 consecutive days by gavage. Kidneys from the male rats exhibited a similar rate of histological changes as seen in the kidneys from the D-limonene treated male rats. There were no changes noted in the female rats. The ability of MIBK to induce measures of α2u-N, including renal cell proliferation, was evaluated in 84 male and 84 female F344 rats following exposure to 0, 450, 900, or 1800 ppm MIBK for 6 h/d for 1 or 4 wk. Increased measures of α2u-N, renal cell proliferation and reversible binding of MIBK to α2u were observed in male rats; MOA studies indicated that MIBK-induced renal effects were consistent with a male rat-specific α2u-N mechanism.

The skin sensitization potential of MIBK was assessed in female albino guinea pigs; 20 animals comprised the test group and 10 animals comprised the control group. Intradermal induction was carried out with 0.1 ml of 5% MIBK in corn oil, epicutaneous induction was performed with undiluted MIBK, and challenge was conducted with 30% MIBK under occlusive conditions. MIBK produced no sensitizing reaction. In another study, a guinea pig maximization test was performed on 20 test animals (10 control). Intradermal induction was carried out with 0.1 ml of 5% MIBK in corn oil, and semi-occlusive patches with 0.1 ml of 5% MIBK in corn oil were used for epidermal induction. No indication of skin sensitization was observed.

A single-exposure ocular irritation study on MIBK was performed using 3 New Zealand White rabbits. Undiluted MIBK is considered to be slightly irritating to rabbit eyes. MIBK was also slightly irritating to the eyes in another study using 4 rabbits.

Occupational limits from the ACGIH recommend a TLV-TWA of 20 ppm (82 mg/m^3) and a TLV–STEL of 75 ppm (307 mg/m^3) for exposure to MIBK. NIOSH lists a TWA limit of 50 ppm MIBK (205 mg/m^3), and also includes the OSHA standard of 100 ppm MIBK (410 mg/m^3).

DISCUSSION

In accordance with its Procedures, the Panel evaluates the conclusions of previously issued reports approximately every 15 years. In 2004, the Panel published a final report with the conclusion that MIBK is safe as used in nail polish removers and as an alcohol denaturant in cosmetic products, based on the available animal and clinical data in that report. In March 2023, the Panel

determined that this safety assessment should be re-opened to include new carcinogenicity and toxicological data that were included in an NTP report; these studies were in progress at the time of the original report.

This amended assessment reviews the safety of MIBK as used in cosmetic formulations. The Panel concluded that MIBK is safe as used in nail polish removers and as an alcohol denaturant in cosmetics in the present practices of use and concentration described in this safety assessment. The Panel noted that one reported use of MIBK is in an aftershave lotion. In accordance with the conclusion reached by the Panel, use in this product type is safe if the function is as an alcohol denaturant. Additionally, no concentrations of use of MIBK have were reported in response to the survey conducted by the Council in 2022

Regarding the possible use of MIBK as a denaturant in cosmetics, the Panel noted that MIBK has been approved for use as a denaturant for alcohol. In keeping with the specification determined by the Alcohol and Tobacco Tax and Trade Bureau, the Panel agreed that MIBK could be considered safe for use as a denaturant of alcohol used in cosmetics at concentrations up to the maximum concentration of MIBK allowed for such purpose (i.e., 4%). For clarity, the designated concentration of 4% MIBK pertains exclusively to its utilization as a denaturant and not as the final concentration of the resultant cosmetic product.

The new studies that have been included from the NTP report did not raise concerns for the Panel. The MOA studies concluded that MIBK-induced hepatic effects are consistent with a phenobarbital-like MOA, where the initiating events are activation of the CAR and PXR nuclear receptors which results hepatocellular proliferation leading to rodent liver tumors. The Panel noted that concern for this effect was mitigated because the MOA for rat and mouse liver tumor formation initiated by phenobarbital and sodium phenobarbital and other CAR activators is considered not plausible for humans. The Panel also highlighted that the mechanism for renal cell proliferation observed due to the exposure to MIBK is only male rat-specific and not considered a human health hazard.

The Panel's respiratory exposure resource document (https://www.cir-safety.org/cir-findings) notes that airbrush technology presents a potential safety concern, and that no data are available for consumer habits and practices thereof. As a result of deficiencies in these critical data needs, the safety of cosmetic ingredients applied by airbrush delivery systems cannot be assessed by the Panel. Therefore, the Panel has found the data insufficient to support the safe use of cosmetic ingredients applied via an airbrush delivery system.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that MIBK is safe as used in nail polish removers and as an alcohol denaturant in cosmetics in the present practices of use and concentration described in this safety assessment.*

**Current concentrations of use are not reported; the expectation is that this ingredient would be used at concentrations comparable to that reported in the 2004 safety assessment, as listed in Table 1.*

TABLES

Table 1. Frequency (2023; 1998) and concentration (2022; 2000) of use by product category

	# of Uses		*Max Conc of Use (%)*	
	2023[3]	**1998[2]**	**2022[4]**	**2000[2]**
Totals	**2**	**2**	**NR**	**21**
Manicuring Preparations (Nail)				
Nail Polish and Enamel Remover	NR	2	NR	NR
Other Manicuring Preparations	1	NR	NR	21*
Shaving Preparations				
Aftershave Lotion	1	NR	NR	NR

NR – not reported

* MIBK was reported to be used at a concentration of 21%, specifically in a nail correction pen (volume = 3 ml); accordingly, some dermal contact would be expected.

Table 2. Inhalation carcinogenicity studies of MIBK

Animals/Group	Concentration/Dose	Procedure	Results	Reference
B6C3F$_1$ mice; 50/sex/group	0, 450, 900, or 1800 ppm	Mice were exposed whole body for 6 h/d, 5 d/wk, for 104 wk. Mice were housed in stainless steel chambers. Exposure valves in the chambers automatically opened and allowed vapors to flow through individual delivery lines to each exposure chamber. The vapor was then mixed and diluted with conditioned chamber air to achieve the desired exposure concentration. The total active mixing volume of each chamber was 1.7 m^3. MIBK concentrations were monitored by an on-line gas chromatograph. Samples were drawn every 28 min. Buildup and decay rates for chamber vapor concentrations were determined with animals present in the chambers. A T_{90} value of 12 min was selected for the studies. Chamber uniformity was monitored throughout the study. Animals were observed twice daily. Complete necropsies and microscopic examinations were performed on all mice. Complete histopathology was also performed.	Males - s*ome evidence of carcinogenic activity* Increased incidence of hepatocellular adenoma and hepatocellular adenoma or carcinoma at 1800 ppm Females - s*ome evidence of carcinogenic activity* Increased incidence of hepatocellular adenoma and hepatocellular adenoma or carcinoma at 1800 ppm. Increased incidence of eosinophilic foci in the liver at 450 and 1800 ppm. Female mice exposed to highest test concentration had decreased body weight.	[11]
F344/N rats 50/sex/group	0, 450, 900, or 1800 ppm	As above but performed with rats.	Males - s*ome evidence of carcinogenic activity* Increased incidences of renal tubule adenoma, adenoma/carcinoma in males exposed to 900 or 1800 ppm. Increased incidence of renal tubule carcinoma in males exposed to 1800 ppm. Increased incidence of renal tubule hyperplasia in males at 450 and 1800 ppm. Chronic nephropathy in all males at 1800 ppm. Transitional epithelial hyperplasia of renal pelvis in males exposed to 900 or 1800 ppm. Increased incidence of mineralization of renal papilla at all concentrations. Positive trend in incidences of mononuclear cell leukemia in males. Increased incidence in adrenal medulla hyperplasia in 1800 ppm Females - e*quivocal evidence of carcinogenic activity* Chronic nephropathy in 70 – 88% females at all concentrations. Two female rats exposed to 1800 ppm had renal mesenchymal tumors.	[11]

Table 3. Incidence of neoplastic and non-neoplastic lesions of the liver in mice and kidneys in rats [11]

Animals/Group	Neoplastic/ Non-Neoplastic	Effect	Chamber Control	450 ppm	900 ppm	1800 ppm
male $B6C3F_1$ mice; 50/sex/group	non neoplastic	eosinophilic focus	3/50	4/50	5/50	8/50
	neoplastic	hepatocellular adenoma	17/50	25/50	23/50	34/50
		hepatocellular carcinoma	12/50	12/50	10/50	9/50
		hepatocellular adenoma or carcinoma	27/50	34/50	28/50	37/50
female $B6C3F_1$ mice; 50/sex/group	non neoplastic	eosinophilic focus	4/50	11/50	10/50	14/50
	neoplastic	hepatocellular adenoma	13/50	15/50	20/50	23/50
		hepatocellular carcinoma	6/50	5/50	6/50	11/50
		hepatocellular adenoma or carcinoma	17/50	17/50	22/50	27/50
male F344/N rats; 50/sex/group	non neoplastic	renal tubule hyperplasia (standard eval)	1/50	11/50	3/50	18/50
		renal tubule hyperplasia (standard + extended eval combined)	1/50	14/50	7/50	21/50
		nephropathy	42/50	45/50	47/50	50/50
		pelvic transitional epithelium hyperplasia	1/50	5/50	6/50	19/50
		papilla mineralization	1/50	6/50	22/50	29/50
		adrenal medulla hyperplasia	13/50	18/48	18/50	24/50
	neoplastic	renal tubule adenoma (standard eval)	0/50	0/50	2/50	3/50
		renal tubule adenoma (standard + extended eval)	2/50	3/50	3/50	10/50
		renal tubular carcinoma (standard)	0/50	1/50	0/50	2/50
		renal tubular adenoma or carcinoma (standard and extended eval)	2/50	4/50	3/50	11/50
		mononuclear cell leukemia	25/50	26/50	32/50	35/50
female F344/N rats; 50/sex/group	non neoplastic	nephropathy	19/50	35/50	38/50	44/50
	neoplastic	malignant mesenchymal tumor	0/50	0/50	0/50	2/50

Table 4. Mode of action studies on MIBK-induced tumors

Animals/Group	Concentration/Dose	Procedure	Results	Reference
		Inhalation		
$B6C3F_1$ mice; 16/sex/group	0 or 1800 ppm	Mice were exposed whole body for 6 h/d, 5 d/wk, for a total of 10 d. Mice were implanted with an osmotic pump with 20 mg/ml of BrdU after day 1 of initial exposure.	Male and female $B6C3F_1$ mice showed an increase in liver weights that corresponded with hepatocellular hypertrophy and increased mitotic figures. Data shows induction of S-phase DNA synthesis. Gene expression showed maximally induced CAR-associated CYP2b10 and slightly increased PXR-associated CYP3a11 Compounds initiating liver tumors in rodents through the CAR MOA are not expected to be relevant in humans.	[12]
C57BL/6 mice; 16/sex/group	0 or 1800 ppm	As above.	Female C57BL/6 mice showed an increase in liver weights that showed hepatocellular hypertrophy and increased mitotic figures. Data shows induction of S-phase DNA synthesis Gene expression showed maximally induced CAR-associated CYP2b10 and slightly increased PXR-associated CYP3a11.	[12]
CAR/PXR KO mice; 16/sex/group	0 or 1800 ppm	As above.	No increase in induction of S-phase DNA synthesis. Mice exposed to 1800 ppm MIBK showed no evidence of activation of AhR, CAR, PXR or PPAR-α nuclear receptors via their associated transcripts.	[12]
		Oral		
F344 rats; 4/sex/group	0 or 1000 mg/kg in corn oil; 5 ml/kg positive controls: 300 mg/kg D-limonene males only)	To investigate whether MIBK operates through a non-genotoxic MOA to induce the male rat-specific renal tumor response following chronic exposure, rats were dosed by gavage for 10 consecutive days. The kidneys were removed approximately 24 h after the final dose. The left kidney was analyzed for histological alterations, which included the accumulation of protein (hyaline) droplets, staining for α2u, and the presence of proliferating cell nuclear antigen to determine renal cell growth rates. The right kidney was processed to measure total protein and α2u using ELISA. D-Limonene was used as the positive control in that it is an acknowledged inducer of α2u-N.	MIBK caused an increase in protein droplets, accumulation of α2u, and renal cell proliferation in males, but not in females. The histological alterations caused by MIBK in male rat kidneys were similar to those induced by D-limonene, but they were of a milder degree. The investigators concluded that MIBK exerts renal effects through an α2u-N-mediated MOA.	[14]

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