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TOXICOLOGICAL REVIEW

OF

METHYL ISOBUTYL KETONE

(CAS No. 108-10-1)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

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U.S. Environmental Protection Agency
Washington DC

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FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to methyl isobutyl ketone. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of methyl isobutyl ketone.

In Section 6, the U.S. Environmental Protection Agency (EPA) has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose response. Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at 202-566-1676.

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This document and summary information on IRIS have received peer review by both EPA scientists and independent scientists external to EPA. Subsequent to external review and incorporation of comments, this assessment has undergone an Agency-wide review process whereby the IRIS Program Director has achieved a consensus approval among the Office of Research and Development; Office of Air and Radiation; Office of Prevention, Pesticides, and Toxic Substances; Office of Solid Waste and Emergency Response; Office of Water; Office of Policy, Economics, and Innovation; Office of Children's Health Protection; Office of Environmental Information; and the Regional Offices.

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Summaries of the external peer reviewers' comments and the disposition of their recommendations are in Appendix A.

1. INTRODUCTION

This document presents background and justification for the hazard and dose-response assessment summaries in EPA's Integrated Risk Information System (IRIS). IRIS Summaries may include an oral reference dose (RfD), inhalation reference concentration (RfC) and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for noncancer dose-response assessments. The RfD is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis but may not exist for other toxic effects such as some carcinogenic responses. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer effects during a lifetime. The inhalation RfC is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). It is generally expressed in units of mg/m^3.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral exposure and inhalation exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg-day. The *unit risk* is the quantitative estimate in terms of either risk per µg/L drinking water or risk per $µg/m^3$ air breathed. Another form in which risk is presented is a drinking water or air concentration providing cancer risks of 1 in 10,000; 1 in 100,000; or 1 in 1,000,000.

Development of these hazard identification and dose-response assessments for methyl isobutyl ketone has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines that were used in the development of this assessment may include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991a), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), *Draft Revised Guidelines for Carcinogen Assessment* (U.S. EPA, 1999), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), (proposed) *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998b, 2000a), *Science Policy*

Council Handbook: *Risk Characterization* (U.S. EPA, 2000b), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c), and *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000d).

The literature search strategy employed for this compound was based on the CASRN and at least one common name. At a minimum, the following databases were searched: RTECS, HSDB, TSCATS, CCRIS, GENE-TOX, DART/ETIC, EMIC, TOXLINE, CANCERLIT, and MEDLINE. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through January 2003.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Common synonyms for methyl isobutyl ketone (MIBK) include 4-methyl-2-pentanone, 2-methyl-4-pentanone, 4-methyl pentan-2-one, 2-methyl propyl methyl ketone, isopropyl acetone, isobutyl methyl ketone, 4-methyl-2-oxopentane, hexone, and hexanone (HSDB, 2000). Some relevant physical and chemical properties of MIBK are shown in Table 2-1.

MIBK is used mainly as a coating solvent in cellulose-based and resin-based coating systems (Braithwaite, 1995). It is also used as a separating agent for metals from solutions of their salts and in the mining industries to extract plutonium from uranium. MIBK is also used in the production of paints, pesticide formulations, adhesives, wax/oil separation, leather finishing, textile coating, and specialty surfactants for inks and as a denaturant for ethanol formulations. Another increasingly important use of MIBK is in the production of rubber antioxidants (Braithwaite, 1995). MIBK is also naturally present in fruits (Furia and Bellanca, 1975) and meats (Ramarathnam et al., 1991). MIBK is currently approved for use as a component of synthetic flavoring substances and adjuvants (21 CFR 171.515) and as a denaturant in alcohol and rum (27 CFR 21.161) at a maximum concentration of 4%. In 1993, the United States alone produced 6.806×10^7 kg of MIBK (USITC, 1994).

In the ambient atmosphere, MIBK is expected to exist solely in the vapor phase (Bidleman, 1988), based on a measured vapor pressure of 19.9 mm Hg at 25°C (Daubert and Danner, 1989). Vapor-phase MIBK is degraded by reaction with photochemically produced hydroxyl radical (Atkinson, 1989), with an estimated half-life of 27 hours. MIBK is expected to have high mobility in soils based on an estimated K_{oc} value of 11, determined using a structure estimation method that uses molecular connectivity indices (Meylan et al., 1992).

Volatilization from water surfaces is expected to be an important fate process, based on MIBK's estimated Henry's Law constant of 1.4×10^{-4} atm m^3/mole, determined from its vapor pressure (Daubert and Danner, 1989) and water solubility (Yalkowsky and Dannenfelser, 1992). Estimated volatilization half-lives for a model river and model lake are 9 hours and 6 days, respectively (Meylan and Howard, 1991).

Due to MIBK's low octanol water partition coefficient of 1.31, it is not expected to bioconcentrate significantly in living organisms. Biodegradation in the environment is expected to occur relatively rapidly. MIBK, present at 100 mg/L, reached 84% of its theoretical biological oxygen demand in 2 weeks using an activated sludge inoculum at 30 mg/L in the Japanese MITI test (Chemicals Inspection and Testing Institute, 1992).

Table 2-1. Physical and Chemical Properties of MIBK

Property		Reference
CASRN	108-10-1	NIOSH, 1997
Empirical formula	$C_6H_{12}O$	Verschueren, 1996
Molecular weight	100.16	Budavari, 1996
Physical state	Liquid	Lewis, 1997
Color	Colorless	Lewis, 1997
Odor/Taste	Pleasant/Sweet	Verschueren, 1996
Boiling point (°C)	115.8	Lewis, 1997
Melting point (°C)	-84.7	Budavari, 1996
Log Kow	1.31	Hansch et al., 1995
Vapor pressure (at 25°C)	19.9 mm Hg	Daubert and Danner, 1989
Water solubility(at 25°C)	19 g/L	Yalkowsky and Dannenfelser, 1992
Explosive Limits	LEL = 1.4% UEL = 7.5%	Lewis, 1997
Conversion factors	1 ppm = 4.1 mg/m^3 1 mg/m^3 = 0.244 ppm	Verschueren, 1996

The general population may be exposed to MIBK through the use of commercial products such as paints, adhesives, and pesticides containing MIBK and through ingestion of fruits and meats that contain MIBK as a natural component. The 8-hour time-weighted average threshold limit values (TWA/TLVs) and permissible exposure limits (PELs) for MIBK are 50 and 100 ppm, respectively (NIOSH, 1997). The odor threshold concentration in air is reported to range from 0.10 and 0.68 ppm (Verschueren, 1996).

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

3.1. ABSORPTION

3.1.1. Gastrointestinal Absorption

Plasma MIBK concentrations were 5.3, 8.4, and 16.1 µg/mL in rats at 1 hour after the last of 3 daily gavage exposures to 1.5, 3.0, and 6.0 mmol/kg MIBK (150, 300, or 601 mg/kg-day), indicating rapid and exposure level-related oral absorption into the bloodstream (Duguay and Plaa, 1995). The relative uptake of MIBK from the gastrointestinal tract has not been quantified in humans or in animals in studies located for this assessment. Effects observed in laboratory animals following oral exposure to MIBK provide qualitative evidence that it is absorbed from the gastrointestinal tract in toxicologically relevant quantities.

3.1.2. Respiratory Tract Absorption

Relative uptake of inhaled MIBK ranged from 56.3 to 61.7% and was not related to exposure level in human volunteers exposed to concentrations of 10 to 200 mg/m^3 for 2 hours during light exercise; total respiratory uptake (mmol MIBK) during exposure was linearly related to exposure level (Hjelm et al., 1990). In rats, plasma MIBK concentrations were 5.0, 8.1, and 14.3 µg/mL immediately following the last of 3 daily 4-hour inhalation exposures to 200, 400, or 600 ppm MIBK (819, 1639, and 2458 mg/m^3)[1], indicating rapid and exposure level-related respiratory absorption into the bloodstream (Duguay and Plaa, 1995). In the rat, inhalation exposures to atmospheric concentrations of 200, 400, or 600 ppm MIBK for 4 hours resulted in absorption of the same amount of MIBK as from the oral administration of 1.5, 3.0, or 6.0 mmol/kg, respectively.

3.1.3. Dermal Absorption

The percutaneous uptake rate in guinea pigs exposed epicutaneously to MIBK peaked at 10 to 45 minutes after the onset of a 150-minute exposure; the maximum uptake rate ranged from 0.11 to 2.0 µmol/min/cm and averaged 1.1 µmol/min/cm (Hjelm et al., 1991).

3.2. DISTRIBUTION

MIBK is likely to be widely distributed in the body because it is absorbed readily into the bloodstream after inhalation exposure (Hjelm et al., 1990), and human blood/air and oil/air

[1] Throughout this report, inhalation exposure levels that were originally reported in ppm or mg/L units were converted to mg/m^3 for ready comparison of exposure-effect data between studies. Conversions from mg/L were based on the following equation: *mg/m^3 = 1000 x (mg/L)*. Conversions from ppm were based on the following general equation (Snyder and Andrews, 1996): *mg/m^3 = (ppm)(molecular weight)/24.45*; where, the molecular weight used for MIBK was 100.18 g/mol.

partition coefficients for MIBK of 90 and 926, respectively, indicate significant blood and lipid solubility (Sato and Nakajima, 1979). MIBK partitions approximately equally between red blood cells and plasma in rat and human blood; in plasma MIBK is associated primarily with proteins rather than being dissolved in plasma water (Lam et al., 1990). High lipid solubility indicates that MIBK may partition rapidly to lipid-rich tissues, such as nervous tissue.

Concentrations of MIBK and its principal metabolite, 4-hydroxy-4-methyl-2-pentanone, in rat plasma, liver, and lung tissue were positively related to exposure level shortly after the last of 3 daily oral or inhalation exposures (Duguay and Plaa, 1995).

MIBK accumulated rapidly in brain tissue of mice that received a single intraperitoneal dose of 5 mmol MIBK/kg, peaking at 30 minutes post-exposure, but it was completely eliminated from the brain by 90 minutes post-exposure (Granvil et al., 1994). The brain concentration of 4-hydroxy-4-methyl-2-pentanone continued to increase throughout the 90-minute post-exposure period.

3.3. METABOLISM

DiVincenzo et al. (1976) identified 4-methyl-2-pentanol and 4-hydroxy-4-methyl-2-pentanone as MIBK metabolites in blood of guinea pigs. Hjelm et al. (1990) evaluated for these metabolites in the urine of human volunteers and found them both to be at concentrations below the detection limit of 5 nmol/L within a 3-hour post-exposure period. Blood levels of potential MIBK metabolites were not quantified in the Hjelm et al. (1990) study. Hjelm et al. (1990) suggested the source of the apparent discrepancy for why MIBK metabolites were detected in the blood of guinea pig but not in the urine of humans could be the lower dose of MIBK used in the Hjelm et al. (1990) study, or perhaps the qualitative and/or quantitative differences in metabolism of MIBK between man and guinea pig. In addition, the urinary excretion of the metabolites may be delayed and therefore the 3-hour post-exposure period may have been too short to permit detection of the metabolites in the urine. Hjelm et al. (1990) suggested that, in humans, 4-methyl-2-pentanol and 4-hydroxy-4-methyl-2-pentanone may either undergo further metabolism to be eliminated as CO_2 via the lungs or intermediate metabolites may be stored in tissues.

Vézina et al. (1990) found that either single or repeated oral doses of MIBK induced significant increases in hepatocellular cytochrome P-450 content and the hepatic activities of aniline hydroxylase and 7-ethoxycoumarin O-deethylase in rats, suggesting that the liver is involved in the metabolism of MIBK. Similarly, Brondeau et al. (1989) reported increased hepatic cytochrome P-450 content and glutathione-S-transferase activity in rats (but not mice) exposed once by inhalation to MIBK. Hepatic total cytochrome P-450 concentrations were significantly increased in New Zealand male rabbits treated orally with 5 mmol MIBK/kg daily for 3 days (Kobusch et al., 1987). Furthermore, the hepatic mixed-function oxidase activities for aminopyrine N-demethylation, 7-ethoxycoumarin dealkylation, and aniline hydroxylation were increased significantly.

3.4. ELIMINATION AND EXCRETION

The half-life of MIBK in the serum of guinea pigs administered a single 450 mg/kg intraperitoneal dose was estimated to be 66 minutes, based on single blood samples collected from different guinea pigs at intervals up to 16 hours post-dosing (DiVincenzo et al., 1976). 4-Hydroxy-4-methyl-2-pentanone was cleared from the blood within 16 hours, and only a trace amount of a second metabolite, 4-methyl-2-pentanol, was detected at any time.

MIBK was completely eliminated from the blood of mice within 90 minutes of injection of a single intraperitoneal dose of 5 mmol/kg (Granvil et al., 1994); the blood concentration of 4-hydroxy-4-methyl-2-pentanone peaked at 60 minutes post-dosing and was decreasing at the termination of the study at 90 minutes post-dosing.

In humans, elimination of MIBK from blood following cessation of a 2-hour inhalation exposure with light exercise was biphasic, with a half-life of 11 to 13 minutes during the first 30 minutes post-exposure in subjects exposed to 100 or 200 mg/m^3 (Hjelm et al., 1990). The half-life in blood during the second elimination phase (60 and 180 minutes post-exposure) was 59 and 74 minutes in subjects exposed to 100 and 200 mg/m^3, respectively. Blood MIBK levels were too low to permit the calculation of blood elimination half-times in subjects exposed to 10 mg/m^3. Total cumulative urinary excretion of MIBK up to 3 hours post-exposure was exposure-related in human volunteers and ranged from 0.04 µmol at the 10 mg/m^3 exposure level to 1.21 µmol at 200 mg/m^3 (Hjelm et al., 1990).

3.5. PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELS

There are no toxicokinetic models currently available.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

On December 14, 2001, after internal peer review of this document, the Agency articulated its interim policy on the use of third-party studies submitted by regulated entities (U.S. EPA, 2001). For these purposes, EPA is considering "third party studies" as studies that have not been conducted or funded by a federal agency pursuant to regulations that protect human subjects. Under the interim policy, the Agency will not consider or rely on any such human studies (third-party studies involving deliberate exposure of human subjects when used to identify or quantify toxic endpoints such as those submitted to establish a NOAEL or NOEL for systemic toxicity of pesticides) in its regulatory decision making, whether previously or newly submitted. Some of the supporting studies discussed in this Toxicological Review are third-party studies; however, the scientific and technical strengths and weaknesses of these studies

were described before this Agency policy was articulated. In addition, the studies cited provide data that suggest and inform a public health concern for MIBK, but were not designed or used as principal studies in the derivation of any quantitative value for MIBK based on NOAELs or LOAELs. The Agency is requesting that the National Academy of Sciences conduct an expeditious review of the complex scientific and ethical issues posed by EPA's possible use of third-party studies that intentionally dose human subjects with toxicants to identify or quantify their effects.

4.1.1. Oral Exposure

No studies were located that reported oral exposures of MIBK in humans.

4.1.2. Inhalation Exposure

Several studies of occupational exposures to solvent vapor mixtures that included MIBK have reported various neurological effects. Decrements in neurobehavioral performance tests and increased prevalence of acute neurological symptoms were observed among shipyard painters exposed to complex solvent vapor mixtures when compared to an unexposed control group matched for age, sex, race, and education (Valciukas et al., 1985). Impairment in continuous performance, pattern comparison, and pattern memory were reported in paint factory workers exposed to solvent mixtures that included MIBK (Tsai et al., 1997). A 16-year-old male presented with polyneuropathy characterized by a burning paraesthaesias in the extremities and acute segmental demyelination of the sural nerve with Schwann cell hyperplasia approximately 8 weeks after he had used spray paint containing methyl ethyl ketone and MIBK several times in a closed space (AuBuchon et al., 1979). Collectively, these studies have limited usefulness for characterizing an exposure-response relationship for MIBK in humans, as exposure levels for individual solvents were not reported and the degree to which MIBK contributed to the observed effects from the solvent vapor mixtures is uncertain.

Four experimental studies of acute inhalation exposures to MIBK in human volunteers indicated transient sensory irritation, neurological effects, and/or strong odor sensation during exposure (Dick et al., 1992; Esso Research and Engineering Company, 1965; Hazleton Laboratories, Inc., 1965; Hjelm et al., 1990; Iregren et al., 1993). No decrements in task performance were observed in three studies (Dick et al., 1992; Hjelm et al., 1990; Iregren et al., 1993).

Groups of six adult volunteers were exposed via full face mask to 0.402, 0.915, 1.393, 1.68, 2.301, or 2.827 mg/L (402, 915, 1393, 1680, 2301, or 2827 mg/m^3) of MIBK during a 7-minute exposure period, followed 2 weeks later by a second 7-minute exposure to 0.845, 1.493, or 2.066 mg/L (845, 1493, or 2066 mg/m^3) (Esso Research and Engineering Company, 1965; Hazleton Laboratories, Inc., 1965). Volunteers indicated the presence and disappearance of eye, nose, and throat irritation throughout the exposures, which provided a continuous subjective assessment of irritation relative to known exposure levels. The incidence of volunteers reporting nose, eye, and throat irritation generally increased with exposure level; the thresholds for odor

and irritation were reported to be 402 and 1393 mg/m^3, respectively, estimated from graphs of the number of individual reports of irritation at various exposure levels.

Eight adult male volunteers were exposed in an exposure chamber on three separate occasions for 2 hours under conditions of light exercise to atmospheres of 10, 100, or 200 mg/m^3 MIBK, followed by 2-hour observation periods (Hjelm et al., 1990). No sham exposures were used as negative controls. The duration of the rest period between exposure occasions was not reported. Analysis of variance results indicated that both mean irritation index and mean index of neurological effects were marginally significantly different between exposure levels, with p-values of 0.16 and 0.1, respectively. Although no means comparison tests were reported, it was evident from a graphical presentation of results that both the mean index of subjectively reported irritation and the mean index of subjectively reported neurological symptoms (headache, nausea, and vertigo) generally increased with exposure level and decreased rapidly after cessation of exposure. Tabulated data indicated that neurological effects (vertigo) and sensory irritation effects each occurred in one of the eight volunteers at 10 mg/m^3. With exposure to 100 or 200 mg/m^3, three of the eight subjects reported nose and throat irritation and two reported headache and vertigo. No exposure-related effects were observed in mood ratings or in reaction time and simple addition performance tests.

In a study conducted by the National Institute for Occupational Safety and Health of the U.S. Department of Health and Human Services, a group of 13 adult male and 12 adult female volunteers was exposed in an environmental chamber to 100 ppm (410 mg/m^3) MIBK for two consecutive 2-hour exposure periods (Dick et al., 1992). Another group received placebo treatment during the exposure periods. Subjects underwent double-blind evaluations of performance on five psychomotor tests, one sensorimotor test, and a test of mood on the day before exposure, immediately prior to exposure, during each of the two consecutive 2-hour exposure sessions, immediately after exposure, and on the day following exposure. Subjective assessments of irritation and other symptoms were also solicited. No changes attributable to MIBK exposure were detected with respect to any of the performance tests or to the percentage of subjects experiencing various neurological or irritation symptoms, but a significant increase in percentage of subjects detecting a strong odor sensation was reported in the MIBK-treated group.

Simple reaction time performance, simple arithmetic test performance, mood rating, and heart rate were not related to exposure level in groups of six male and six female volunteers exposed to MIBK vapors in an exposure chamber at either 10 mg/m^3 (considered to be the control exposure level by the authors) or 200 mg/m^3 for 2-hour exposure periods at 1-week intervals for an unspecified total number of exposures (Iregren et al., 1993). Volunteers performed light exercise during the first 90 minutes and rested during the final 30 minutes of each exposure. Performance tests were conducted immediately prior to and following exposure, heart rate was monitored throughout exposure, and central nervous system (CNS) and irritation symptoms were assessed using a 17-point questionnaire. Sensory irritation ratings were not significantly different between the two exposure levels. Neurological symptoms were evaluated by questionnaire prior to, during, and following each exposure. An index of the prevalence and

intensity of neurological symptoms was significantly increased in the group exposed to 200 mg/m^3 as compared to the 10 mg/m^3 group.

4.2. PRECHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS

4.2.1. Oral Exposure

No chronic oral exposure studies were located.

In a study by Microbiological Associates, Inc. (MAI, 1986) groups of 30 male and 30 female Sprague-Dawley rats were administered MIBK by gavage in corn oil at daily dose levels of 0 (vehicle control), 50, 250, or 1000 mg/kg-day for 13 consecutive weeks and evaluated for exposure-related changes in body weight, food consumption, mortality, clinical signs, ophthalmological parameters, and terminal organ weights (heart, liver, spleen, brain, kidney, gonads, adrenals, thyroid, and parathyroid). The following evaluations were conducted in rats from each exposure level at interim (week 7) and final sacrifices: hematology, clinical chemistry, urinalysis, and comprehensive gross pathology. All tissue samples collected during gross necropsy in high-dose and control rats were evaluated for histopathologies, and kidney samples were also histologically evaluated in mid-dose rats.

Reversible lethargy was observed in rats of both sexes receiving 1000 mg/kg-day (but not at lower dose levels) for a few hours following dosing and reportedly decreased in incidence and severity during the study. Males in the high-dose group showed a slight (9%) but significantly decreased mean body weight gain as compared to controls during the last 2 weeks of exposure, whereas female body weight gain was significantly increased during 5 of the last 6 weeks of exposure. Both male and female food consumption was significantly increased during the second half of the exposure period. The only potentially exposure-related hematological effects observed were slight but statistically significant increases in hemoglobin (+6%) and hematocrit (+8%) at terminal sacrifice in females administered 1000 mg/kg-day and a 15% decrease in lymphocyte count in high-dose males at terminal sacrifice.

The lowest hepatic effect level that was observed in the oral exposure studies was 250 mg/kg-day for increased (+39%) serum glutamic-pyruvic transaminase (SGPT) in female rats at the terminal sacrifice. The following changes suggestive of adverse liver effects were observed at 1000 mg/kg-day at either interim and/or final sacrifice: increased SGPT (+73%, interim; +34%, terminal) in females as compared to controls, increased serum alkaline phosphatase (+84%, interim) in females, increased serum cholesterol in males (+30%, interim) and females (+59%, interim; +65%, final), increased terminal absolute (+34%, males; +39%, females) and relative (+42%, males; +38%, females) liver weights, decreased albumin/globulin ratio in males (-16%, interim), and minimally increased serum total protein in females (+9%, interim; +10%, terminal).

The only renal effect occurring at 250 mg/kg-day was increased terminal absolute or relative kidney weights in males and females, ranging from 6 to 12% over controls (MAI, 1986). The following changes suggestive of adverse kidney effects were observed at 1000 mg/kg-day: increased terminal absolute and relative kidney weights (from 25 to 34% in males and from 20 to 22% in females) as compared to controls, increased blood-urea-nitrogen (BUN) in males (+37%, interim), increased serum potassium in males (+34%, terminal), decreased serum glucose in males (-27%, terminal), and a reported increase in urinary protein and ketones in males and females at terminal sacrifice (summary data were not provided). Histological examination of kidney tissues revealed an increased incidence of male rats with mild nephropathy (multifocally distributed swollen or hyperchromatic and flattened renal cortical tubular epithelial cells) at 1000 mg/kg-day (16/20) as compared to controls (4/20) but no increase in such lesions in females.

Significantly increased relative adrenal weights in male (+29%) and female (+11%) rats and slightly increased relative testis weights (+9%) in males were also observed at 1000 mg/kg-day. No exposure-related histopathologic lesions were evident in the liver or adrenal glands nor in any other tissue that was examined, aside from the kidney. No treatment-related effects of any kind were observed at 50 mg/kg-day.

Groups of five 4-week-old female HLA Wistar rats were provided drinking water *ad libitum* containing either no MIBK or MIBK at saturation concentration (1.3% aqueous concentration; estimated by the study authors to be 1040 mg/kg-day dosage rate) for 120 days (Carnegie-Mellon Institute of Research, 1977a, b). The rats were evaluated for exposure-related changes in food and water consumption, body weight, general appearance and behavior, gross pathological examination, organ weights (liver, kidney), histopathology (sciatic nerve, brachial plexi, lumbo-sacral spinal ganglia, anterior and posterior thigh muscles, larynx, nasal cavity, brain, spinal cord, heart, lymph nodes, lungs, spleen, liver, and kidney), and performance in neurologic and neuromuscular function tests (balance, coordination, strength, and behavior). The only statistically significant finding was increased mean absolute and relative kidney weights in treated rats as compared to controls. No gross pathologies were observed in the kidneys. Histopathological examination revealed renal tubular cell hyperplasia in only one of five of the treated rats. No other histological lesions of the kidney were reported. No exposure-related histological changes were found in other organs.

In a preliminary range-finding study (Carnegie-Mellon Institute of Research, 1977a), groups of five 4-week-old female HLA Wistar rats were administered MIBK at 0.5 and 1% in drinking water for 7 days (estimated to be equivalent to 300 and 900 mg/kg-day, based on measured body weights and water consumption) and evaluated for changes in food and water consumption, general appearance and behavior, body weight, and gross pathology of unspecified extent. The only observed effects were significantly reduced body weight gain in rats receiving 1% MIBK (but not 0.5% MIBK) and pale or mottled kidneys in three of three rats receiving 0.5% MIBK and in two of three rats at 1% MIBK. However, it is unclear whether the kidney effects were treatment-related, because no control group was used.

4.2.2. Inhalation Exposure

No chronic inhalation exposure studies in animals were located. A two-year bioassay is currently being conducted by the National Toxicology Program but was not available for inclusion at the time of this toxicological review.

Phillips et al. (1987) and Bushy Run Research Center (1983a, b) exposed groups of 14 male and 14 female Fischer 344 rats and $B6C3F_1$ mice to measured mean concentrations of 0, 50, 252, and 1002 ppm (0, 205, 1033, and 4106 mg/m^3) MIBK for 6 hrs/day, 5 days/week, for 14 weeks and sacrificed the animals following their final exposure day. The following endpoints were evaluated: clinical signs, body weights, organ weights (kidneys, heart, liver, lungs, and testes), urinalysis, hematology, serum chemistry (including glucose and hepatic enzyme levels), complete gross pathology, targeted histopathology (nasal cavity, trachea, liver, kidneys, and lungs) in all animals and complete histopathology in control and high-exposure groups.

No effects of any kind were observed in rats or mice of either sex at 205 mg/m^3. Terminal body weights were significantly increased in female rats at $\geq$1033 mg/m^3. Mouse hematology was unaffected at all exposure levels, but platelet numbers in male rats were significantly increased at 4106 mg/m^3 by 13% over controls, and eosinophil number in female rats was significantly decreased at 4106 mg/m^3 by 57% as compared to controls. Serum cholesterol in male rats was significantly increased at the 1033 and 4106 mg/m^3 exposure levels by 23 and 35%, respectively, as compared to controls. Male rats and male mice showed a significant increase in absolute (+13%, rats; +7%, mice) and relative (+9%, rats; +11%, mice) liver weight at 4106 mg/m^3; absolute, but not relative, liver weight was also slightly increased in male mice (+8%) at 1033 mg/m^3. No histological lesions were observed in the liver and no changes were seen in serum liver enzymes and bilirubin in any exposure group; thus, the observed liver enlargement may have been an adaptive response to increased hepatic metabolic activity rather than a toxic effect.

Urine glucose was significantly increased in male rats at 1033 mg/m^3 (+37%) and 4106 mg/m^3 (+55%) and in female rats at 4106 mg/m^3 (+26%). Significantly increased urine protein (+28%) was also observed in male rats at 4106 mg/m^3. The only renal histological lesion observed was hyaline droplet formation in all male rats; the severity of the lesion generally increased with exposure level. The U.S. EPA has concluded that renal $alpha_{2u}$-globulin hyaline droplet formation is unique to male rats and is probably not relevant to humans for the purposes of risk assessment (U.S. EPA, 1991b).

David et al. (1999) and Eastman Kodak Company (1996) exposed groups of 20 male Sprague-Dawley rats to 0, 250, 750, or 1500 ppm (0, 1024, 3073, and 6146 mg/m^3) MIBK for 6 hrs/day, 5 days/week, for 13 weeks. One half of the rats at each exposure level was maintained on a restricted diet and the other half were fed *ad libitum*. All treatment groups were evaluated for changes in clinical signs, food consumption, body weight, organ weights (liver and kidney), and gross pathology (brain, spinal cord with dorsal and ventral roots, dorsal and ventral ganglia, sciatic nerve, tibial nerve, kidney, and liver); no histopathology was conducted. The treatment

groups on restricted diets also underwent daily schedule-controlled operant behavioral (SCOB) testing from 4 days prior to exposure (to establish baseline responses), throughout the 13-week exposure period, and for 2 weeks following cessation of exposure. The SCOB testing in rats on the restricted diet consisted of fixed-ratio and fixed-interval schedules of reinforcement (food pellets) after appropriate response (lever press) to a cue (light or sound stimulus).

Reduced activity during exposure was observed for the first 10 weeks (but not the final 3 weeks) of treatment in animals exposed to 6146 mg/m^3 and to a lesser degree in animals exposed to 3073 mg/m^3 during the first 8 weeks of treatment. No other treatment-related clinical signs were reported. Terminal mean body weights of restricted-diet rats were significantly greater than those of controls in groups exposed to 3073 or 6146 mg/m^3. Terminal body weights of *ad libitum*-fed rats were 5–7% greater in exposed groups as compared to control groups, although no significant differences were observed. Mean relative liver weight was significantly higher in the 1024 and 3073 mg/m^3 groups (but not in the 6146 mg/m^3 group) among the restricted diet rats as compared to controls; among *ad libitum*-fed rats, both mean relative liver and kidney weights were significantly higher in the 3073 and 6146 mg/m^3 groups as compared to the control group.

Food consumption was not consistently affected in rats fed *ad libitum*; food consumption was not measured in the rats fed restricted diets. Among restricted-diet rats, no significant differences between treatment groups and the control group were observed in any of the SCOB test measurements. No exposure-related gross pathologies were observed in either the restricted-diet or the *ad libitum*-fed treatment groups.

Groups of 100 Wistar rats, two rhesus monkeys, and eight beagle dogs (sex of test animals was not identified) were exposed continuously to 100 ppm (410 mg/m^3) MIBK over a 90-day period (MacEwen et al., 1971; MacKenzie, 1971; Vernot et al., 1971). A control group included 56 rats and an unspecified number of animals from the other species. The exposure level was selected on the basis of the results of a 2-week continuous exposure range-finding study in which no effects were observed in rats, mice, monkeys, or dogs other than significantly increased mean relative heart, liver, and kidney weights and mottled kidneys in rats at 200 ppm (820 mg/m^3), increased mean relative kidney weight in rats at 410 mg/m^3, and toxic nephrosis in the proximal tubules of rats at both exposure levels (MacEwen et al., 1971; Vernot et al., 1971). The range-finding study evaluated the following endpoints in at least one test species: lethality, body weight, clinical chemistry, hematology, EEG, gross pathology, blood pH and gases, and absolute and relative organ weights (heart, lung, liver, spleen, kidney), as well as clinical symptomatology. Toxicological endpoints that were evaluated in the 90-day study were not fully specified, but presumably were at least as extensive as in the range-finding study. The 90-day assay additionally included liver function tests and histopathological evaluations (liver, kidney, brain, heart, lung, spleen, and unspecified endocrine glands).

The only effects reported in the 90-day study included significantly increased mean relative liver and kidney weights in rats; focal chronic renal inflammation in one of the two exposed monkeys; and hyaline droplet degeneration of kidney proximal tubules (sex not

specified, but assumed to be male), with "occasional" tubular necrosis foci occurring after as few as 15 days of exposure in rats and decreasing in severity at weekly interim sacrifices. Complete reversal of the renal hyaline droplet lesion was seen in animals sacrificed at 3 to 4 weeks post-exposure.

Groups of 10 male and 10 female young Charles River albino rats were exposed to 0 or 4.53 mg/L (4530 mg/m^3) MIBK for 6 hrs/day, 5 days/week, for 4 weeks or to 0 or 0.75 mg/L (750 mg/m^3) under the same intermittent exposure schedule for 2 weeks (Hazleton Laboratories, Inc., 1966, 1968). No significant exposure-related changes in clinical signs, body weight, organ weights (lungs, liver, kidneys, adrenals, and spleen), hematological parameters, and gross appearance of organs (brain, pituitary, trachea, thyroid, parathyroid, lungs, heart, liver, spleen, stomach, duodenum, large intestine, small intestine, adrenals, kidneys, urinary bladder, and gonads) were observed in the exposed animals as compared to controls after the 4-week exposure to 4530 mg/m^3 MIBK. Histopathology (lungs, liver, kidneys, adrenals, and spleen in three male and three female rats in each exposure group) revealed cytoplasmic eosinophilic droplets in proximal convoluted tubule epithelium of two of the three exposed male rats after the 4-week exposure but not in females or controls. The only effect observed after the 2-week exposure to 750 mg/m^3 MIBK was increased relative liver weights in males; no renal histopathological lesions were reported.

Groups of six male and six female rats and mice were exposed for 6 hrs/day, 5 days/week, for 9 days to measured concentrations of 0, 101, 501, or 1996 ppm (0, 410, 2053, or 8178 mg/m^3) MIBK (Bushy Run Research Center, 1982). Groups were evaluated for changes in clinical signs, body weight, organ weights (liver, lungs, kidneys, and testes), ophthalmology, gross pathology, and histopathology. The only exposure-related effects observed were periocular wetness in rats exposed to 8178 mg/m^3, increased relative liver weights in male rats at 2053 and 8178 mg/m^3 and in female rats and female mice at 8178 mg/m^3, increased kidney weights in male rats and female mice at 8178 mg/m^3, and hyaline droplet degeneration in kidneys of male rats exposed to 2053 or 8178 mg/m^3, with epithelial regeneration of proximal convoluted tubules in the high-exposure group. No effects of any kind were observed in the 410 mg/m^3 exposure group.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES

No oral developmental or reproductive toxicity assays were available for this assessment.

Reproductive toxicity of MIBK was evaluated in a two-generation inhalation study in Crl:CD®(SD)BR rats (WIL Research Laboratories, 2000). Groups of 30 male and 30 female F_0 rats were exposed whole-body to MIBK vapors at mean measured concentrations of 0, 491, 999, and 1996 ppm (0, 2012, 4093, and 8178 mg/m^3) for 6 hrs/day for 70 consecutive days prior to mating and throughout mating. F_0 females were further exposed until gestation day 20 and again during lactation days 5 to 21; pups were not directly exposed during lactation. Litters were culled to four per sex on lactation day 4. At weaning on lactation day 21, groups of 30 male and 30 female F_1 rats at each exposure level were randomly selected, including at least one male and

one female from each viable litter. Beginning at 7 days after weaning, selected F_1 rats were exposed to mean measured concentrations of 0, 506, 1002, and 2006 ppm (0, 2073, 4105, and 8219 mg/m^3) using the same exposure schedule that was used for the F_0 rats.

F_0 and F_1 parental rats were evaluated for reproductive endpoints (estrous cycle regularity and duration, sperm count, production rate, motility, morphology, mating and fertility indices, number of days between pairing and coitus, gestation length, parturition, litter size, pup sex ratio, and ovarian follicle counts and corpora lutea in control and high-exposure females), survival, clinical signs, startle response, food consumption, body weight, organ weights, comprehensive gross pathology, and histopathology of major organ systems and all gross lesions (10/sex in control and high-exposure groups and all rats that died prior to terminal sacrifice).

F_1 and F_2 pups were evaluated for developmental endpoints, including post-natal survival (both before and after resumption of maternal exposures during lactation), clinical signs, body weight, and external anatomical integrity (skeletal examinations were conducted in pups that had abnormal external changes). F_1 pups were also evaluated for balanopreputial separation in males and vaginal perforation in females. Complete gross pathology evaluations were performed in F_1 pups that were not selected for mating and in all F_2 pups. The report did not mention any examination of hematology, blood chemistry, and urine chemistry in parental groups or offspring of either generation.

Parental survival in both generations was unaffected by exposure. Statistically significant ($p < 0.05$), transient deviations of body weight gain from control levels were observed in high-dosed F_0 female rats during weeks 1-2 of the study only. High-exposure F_1 parental female body weights were depressed through mating, but not throughout gestation and lactation. F_1 parental males showed transient depressed body weight at 2073 and 4105 mg/m^3 and consistently depressed body weight at 8219 mg/m^3, in spite of elevated food consumption (g food consumed/kg bw/day) in the 8219 mg/m^3 exposure group. No exposure-related effect on body weight gain was seen in parental rats of either generation throughout gestation and lactation. Among F_0 rats, increased relative liver weights (males and females at 8178 mg/m^3) and increased relative kidney weights (males at $\geq$2012 mg/m^3; females at $\geq$4093 mg/m^3) were observed. Significantly increased relative adrenal weights were also observed in F_0 females at 8178 mg/m^3; and significant increased relative and absolute ovarian weights were observed in F_0 females at 8178 mg/m^3 (relative weight, $p \leq 0.01$; 0.031 g in control versus 0.040g at 2045 mg/m^3, a greater than 20% difference; and absolute weight, $p \leq 0.01$; 0.096 g in control versus 0.1194 g at 2045 mg/m^3, also a greater than 20% difference). In the F_1 parental groups, significant increases in relative liver weight (males at $\geq$4105 mg/m^3; females at 8219 mg/m^3) and relative kidney weight (males at $\geq$2073 mg/m^3; females at 8219 mg/m^3) were observed, and significantly increased relative seminal vesicle, right testis, left cauda epididymis, and adrenal glands were seen in F_1 parental males at 8219 mg/m^3. The incidence of rats with centrilobular hepatocellular hypertrophy (considered by the authors to be an adaptive response) was significantly increased in F_0 males at $\geq$4093 mg/m^3 and in F_1 parental males at $\geq$4105 mg/m^3.

The prevalence of nephropathy characterized by inflamed and thickened basophilic tubule membranes was significantly increased in F_0 males exposed to 8178 mg/m^3 and in F_1 parental males at ≥4105 mg/m^3. F_1 parental males also showed increased prevalence of acidophilic spherical inclusions/droplets in the renal cortical tubular epithelium at ≥4105 mg/m^3, but the authors reported that there was no evidence of fully developed alpha$_{2u}$-globulin-related renal tubular lesions. No other exposure-related gross or microscopic tissue changes were observed in F_0 or F_1 parental groups.

Signs suggestive of CNS depression were observed in mid- and high-exposure parental groups in both generations. Reduced startle response during exposure was observed in F_0 males and females at ≥4093 mg/m^3, in F_1 parental males at ≥4105 mg/m^3, and in F_1 parental females at 8219 mg/m^3. Transient unsteady gait and prostration were observed among F_1 parental males and females approximately 1 hour following exposure to 8219 mg/m^3 on several days prior to mating, but the effect attenuated with repeated exposures; similar neurological symptoms were not reported in F_0 rats.

The only effect reported in offspring was significantly depressed body weights on day 14 post-partum in F_1 and F_2 male and female pups in the mid- and high-exposure groups; however, pup body weights were not different from those of controls on days 7 and 21 post-partum. No other exposure-related changes were observed in any reproductive or developmental endpoint in either generation, including an absence of anatomical changes in F_0 and parental F_1 reproductive organs.

Developmental and maternal toxicity were evaluated in groups of 35 pregnant Fischer 344 rats and 30 pregnant CD-1 mice exposed by inhalation to 0, 300, 1000, or 3000 ppm (0, 1229, 4106, 12,292 mg/m^3) MIBK for 6 hrs/day on gestation days 6 through 15 (preliminary study report was submitted to EPA under the Toxic Substances Control Act (TSCA) from Bushy Run Research Center, 1984; Tyl et al., 1987). Animals were sacrificed on gestation day 21 (rats) or 18 (mice). Dams were evaluated for exposure-related changes in clinical signs, body weight, food consumption, organ weights (kidney, liver, and gravid uterus), and reproductive parameters; fetuses were evaluated for exposure-related changes in body weight and viability, and for external, skeletal, and thoracic and peritoneal visceral alterations.

Maternal mean body weight, weight gain, and food consumption were significantly decreased in rats exposed to 12,292 mg/m^3 (but not to 4106 mg/m^3 or lower) during the exposure period, but they had recovered to control levels by the day of sacrifice; maternal body weight was not affected in mice. Maternal clinical signs observed in rats or mice included coordination loss, hindlimb weakness, paresis, irregular gait, hypoactivity, ataxia, unkempt fur, negative tail or toe pinch, piloerection, lacrimation, or red perioral encrustation. These clinical signs were observed only during the exposure period and only at 12,292 mg/m^3. Three maternal deaths (12% of the animals in the group) occurred in mice exposed to 12,292 mg/m^3 after the first exposure on gestation day 6; no further deaths occurred in that group, and no exposure-related deaths occurred in the other mouse or rat exposure groups. Neonates from those dams were not considered in the final evaluation. Statistical analyses by the authors were per dam or per litter.

No exposure-related effects were observed in rats or mice with respect to numbers of corpora lutea, total implants, percent implantation loss, live fetuses per litter, nonviable implants per litter, percent live fetuses, and sex ratio. In mice, there was an increased mean number of dead fetuses per litter at 12,292 mg/m^3 (0.6 per litter compared to 0.1 in controls).

Fetal body weights (litter weight, male weight per litter, and female weight per litter) were significantly reduced in rats exposed to 1229 (the mean by 3%) and 12,292 mg/m^3 (the mean by 6%) but not to 4106 mg/m^3; and in mice, fetal body weights were statistically significantly reduced at 12,292 mg/m^3 (the mean by 13%) but not at 4106 mg/m^3 or below. The authors indicated that the reduction in rat fetal body weight was confounded by a skewed distribution of litter size, whereby higher doses had very small litters and smaller litters had varied mean weights across dose, while lower-dosed dams appeared to have larger litters and larger litters showed a dose-dependence in mean weight. There was no statistically significant increase in the number of rat or mouse fetuses per litter. The authors decided the reductions in rat fetal body weight was not treatment-related.

No exposure-related change in the incidence of malformations of any type were observed in rat and mouse fetuses. The number of litters with observations indicating retarded skeletal ossification was significantly increased to various degrees in both rats and mice at 12,292 mg/m^3 relative to controls for a variety of skeletal endpoints, with scattered increases in litters with retarded ossification at lower exposure levels that were not considered by the authors to be exposure-related.

4.4. OTHER STUDIES

4.4.1. Neurotoxicity

Studies that evaluated neurological effects in humans from MIBK exposures are presented in Section 4.1.

A 13-week inhalation study in diet-restricted rats with regular schedule-control operant behavioral testing found no evidence of effects in behavioral test performance and no gross pathologies in various nervous system tissues, although overall activity level was reduced during exposure at higher levels (David et al., 1999). Study details are provided in Section 4.2.2.

A group of six young adult rats (strain and sex not specified) were exposed whole-body to vapors of commercial grade MIBK (containing approximately 3% methyl n-butyl ketone [MnBK] as a contaminant) 6 hrs/day, 5 days/week, for up to 5 months at a measured concentration of 1500 ppm (6146 mg/m^3) (Spencer et al., 1975). A group of three rats served as controls. Animals were observed during exposure for changes in body weight and for neurological clinical signs of toxicity. Histological evaluations were performed in the following CNS and peripheral nervous system (PNS) tissues: tibial nerve of the hindlimb, ulnar nerve of the forelimb, peroneal and sural nerves of the lower thigh, lumbosacral dorsal root ganglion with dorsal and ventral roots, lumbar and cervical spinal cord, medulla, and cerebellum. Slight

narcosis was observed in the rats during the exposures, but recovery time was not reported. No cumulative neurological effects were observed after 5 months of exposure, and no histopathologies were observed in CNS and proximal PNS tissues. However, while there was no evidence of frank distal nerve fiber degeneration, the most distal portions of tibial and ulnar nerve showed evidence of increased dilated mitochondrial remnants filled with glycogen in the axons, invaginations of adaxonal Schwann cells, and focal swellings in the MIBK-treated rats. Spencer et al. (1975) considered this neuropathology to be "minimal" and hypothesized that it actually may have been due to the contaminant MnBK rather than to MIBK. This is a plausible explanation because MnBK is a well-documented neurotoxicant (Spencer et al., 1980). No other effects related to MIBK exposure were reported.

Groups of 10 male Swiss OF1 mice were exposed whole-body in an inhalation chamber to 0, 662, 757, 807, or 892 ppm (0, 2712, 3102, 3307, or 3655 mg/m^3) MIBK for single 4-hour exposures and subjected immediately afterward to the behavioral despair swimming test, in which the decrease in total time of immobility during the first 3 minutes in a water bath was used as an indication of behavioral toxicity (DeCeaurriz et al., 1984). Immobility time was significantly lowered at all but the lowest exposure level. The degree of decrease was exposure-related, declining to 70% of the control immobility time at the highest MIBK exposure level. The exposure concentration at which 50% decrease in immobility was expected to occur was estimated to be 803 ppm (3290 mg/m^3), using an analysis based on the linear regression of percentage decrease in immobility on logarithm of exposure concentration. No other observations of effects of MIBK exposure was reported in this study.

No clear effect on mean variable interval response rate was observed in a schedule-controlled operant behavioral test in diet-restricted male Sprague-Dawley rats immediately after a single 3-hour, whole-body exposure to 25 ppm (102 mg/m^3) (N = 7), or 50 ppm (205 mg/m^3) MIBK (N = 1), or in tests performed at various times up to 12 days post-exposure (Garcia et al., 1978; Geller et al., 1978). No other observations were reported.

Two studies of task performance in baboons yielded conflicting results. Perceptual acuity and discrimination performance were evaluated in a match-to-sample task in groups of juvenile baboons (reportedly two per group) exposed whole-body in an inhalation chamber to 0, 25, 35, 50, or 75 ppm (0, 102, 143, 205, or 307 mg/m^3) MIBK. The exposure schedule during the 7-day exposure period was not clearly reported (Geller et al., 1978). No clear exposure-related effects were observed in task performance, although one baboon exposed to 205 mg/m^3 MIBK consistently showed an increase in extra responses as compared to controls in five separate behavioral testing occasions during the 7-day exposure period. A similar match-to-sample task performance study reported depressed extra response rate in three of four baboons (as compared to controls) and increased mean response time in all four baboons (as compared to pre-exposure levels) exposed whole-body in an inhalation chamber to 205 mg/m^3 MIBK continuously for 7 days (Geller et al., 1979). No other exposure levels were reported, but the same test animals had been exposed to 100 ppm (295 mg/m^3) methyl ethyl ketone (MEK) 1 month previously for 7 days, which potentially affected responses in the MIBK study. No other effects were reported in either study.

4.4.2 Genotoxicity

A battery of genotoxicity assays submitted individually to EPA under TSCA and published collectively in O'Donoghue et al. (1988) yielded mostly negative responses. MIBK did not induce revertant point mutations in five *Salmonella* tester strains (TA98, TA100, TA1535, TA1537, and TA1538), either in the presence or absence of Aroclor 1254-induced rat liver microsomal enzymatic activation (MAI, 1984a). Mutant frequencies were also not affected in the L5178Y TK+/- mouse lymphoma mutagenesis assay in the presence of Aroclor-induced rat liver S-9 (MAI, 1984b). No dose-response relationship was observed in cultures exposed to MIBK in the absence of exogenous metabolic activation, although mutation frequency was significantly elevated in three out of six of the MIBK-treated cultures in the absence of S-9 metabolic activation (MAI, 1984b). A second L5178Y TK+/- mouse lymphoma mutagenesis assay yielded similar results; negative in the presence of S-9 and equivocal in the absence of S-9 (MAI, 1984c).

MIBK was also negative in the unscheduled DNA synthesis assay in rat primary hepatocytes (MAI, 1984d) and in the micronucleus cytogenetic assay in mice administered MIBK intraperitoneally at 0.73 mL/kg (the dose level selected as the LD_{20} on the basis of a preliminary toxicity study) (MAI, 1984e). MIBK induced significantly increased frequency of morphological transformations in BALB/3T3 mouse embryo cells at the highest exposure level in the absence of exogenous metabolic activation, but not at lower exposure levels and not in the presence of exogenous metabolic activation (MAI, 1984f). A second cell transformation assay in BALB/3T3 mouse embryo cells using slightly higher exposure levels was negative in both the presence and absence of exogenous metabolic activation (MAI, 1984f).

The following additional set of genotoxicity assays also yielded negative results: reverse mutation assays in five strains of *Salmonella typhimurium* and three strains of *Escherichia coli* in both the presence and absence of exogenous metabolic activation, a mitotic gene conversion test in *Saccharomyces cerevisiae* in both the presence and absence of exogenous metabolic activation, and a structural chromosome damage assay in cultured rat liver cells (Brooks et al., 1988; Shell Oil Company, 1982).

Additional studies confirmed that the number of revertant *Salmonella typhimurium* colonies was not increased in five strains exposed to MIBK as compared to negative controls in both the presence or absence of exogenous metabolic activation (Goodyear Tire and Rubber Company, 1982; Litton Bionetics, Inc., 1978).

4.4.3. Potentiation and Other Interaction Studies

Studies in animals have shown that MIBK administered either orally or by inhalation potentiates cholestasis (arrested bile flow) induced by taurolithocholic acid (Dahlström-King et al., 1990; Duguay and Plaa, 1993; Duguay and Plaa, 1997b; Plaa and Ayotte, 1985), manganese (Vézina and Plaa, 1987), manganese-bilirubin co-treatment (Duguay and Plaa, 1993, 1997a, b; Vézina and Plaa, 1987), or lithocholic acid (Joseph et al., 1992).

Acute oral pretreatment with MIBK caused potentiated rat hepatotoxicity that was induced by carbon tetrachloride, as indicated by MIBK dose-related increases in plasma alanine aminotransferase (ALT) activity and bilirubin concentrations at a given carbon tetrachloride dose (Pilon et al., 1988; Raymond and Plaa, 1995a). Interestingly, an inverse dose-response relationship between the dosages of MIBK and carbon tetrachloride was demonstrated by Pilon et al. (1988); that is, as the dose of carbon tetrachloride increased, the potentiating dose of MIBK to produce the same severity of injury decreased.

Liver damage (as indicated by increased activity of liver enzymes in plasma and increased severity of hepatocellular inflammation and necrosis) was increased in rats administered a chloroform challenge after oral pretreatment with either MIBK or one of its metabolites, 4-methyl-2-pentanol or 4-hydroxymethyl isobutyl ketone. Treatment with only chloroform or only MIBK or one of its metabolites resulted in a relatively mild hepatotoxic response (Vézina et al., 1990).

Carbon tetrachloride-induced nephrotoxicity was potentiated by oral pretreatment with MIBK, as measured by in vitro accumulation of *p*-aminohippuric acid in renal cortical tissue collected after treatments were completed (Raymond and Plaa, 1995a).

Modulation of hexachlorobenzene (HCB)-induced hepatic porphyria by MIBK was investigated in the rat (Krishnan et al., 1992). In that study, two dosing schedules were used in female Sprague-Dawley rats to investigate the possible potentiating properties of MIBK on the porphyrinogenic effect of HCB. The first schedule involved simultaneous administration of HCB (50 mg/kg-day, po, 5 days/week for 6 weeks) and MIBK (7.5 mmol/kg/day, po, 3 days/week for 6 weeks). The second schedule involved an initial dosing of 25 or 50 mg HCB/kg/day for 12 consecutive days, followed by the administration of 7.5 mmol MIBK/kg every other day for 27 days. When administered simultaneously with HCB, MIBK reduced the severity of HCB-induced porphyria, but when given sequentially after HCB accumulation, it enhanced the porphyrinogenic response.

These results suggest that the effect of combined exposure to HCB and MIBK on hepatic porphyria depends on the sequence of the administration of both chemicals and that the mechanism involved in this interaction may invoke both the induction and inhibition of specific hepatic isoenzymes by MIBK. MIBK can modify the porphyrinogenic potential of HCB, but time and sequence of administration of the chemicals on the outcome of the interaction occur. In addition, there was a reduction in the intensity of porphyria and an increase in the time to onset of porphyria when MIBK and HCB were given simultaneously.

The influence of pretreatments with ketonic solvents on the methemoglobinemia (mHb) induced by N, N-dimethylaniline (DMA) was also investigated by Krishnan et al. (1989). DMA produces mHb after being metabolically transformed. Male Sprague-Dawley rats were pretreated with MIBK (7.5 mmol/kg, po) once or as three consecutive doses. DMA (0.8 or 2.4 mmol/kg, intraperitoneal) was administered 18 hours later. Both dose regimens enhanced significantly the mHb produced by DMA; the 3-day pretreatment exerted a greater influence

than the 18-hour pretreatment. These results indicate that MIBK can increase the toxicity of DMA and suggest that it might act like other microsomal enzyme inducers.

4.4.4. Mode of Action Studies

No studies were located that evaluated potential mode of action for developmental toxicity of MIBK.

MIBK increases serum cholesterol in rats after both inhalation and oral exposures. Joseph et al. (1992) found that although acute oral exposure to MIBK did not significantly affect bile flow in rats, bile salt and cholesterol secretion rates were significantly lower than in untreated controls. Decreased hepatocellular secretion of cholesterol to the bile may affect serum cholesterol levels. Duguay and Plaa (1997a) provided evidence that retention of newly synthesized endogenous cholesterol in the liver was significantly increased after inhalation exposure, which also could impact serum cholesterol.

MIBK has been shown in several studies to induce adverse neurological symptoms in animals without causing observable permanent changes in nervous system tissues. The mode of action of MIBK-induced neurological effects was explored in an in vitro experiment using isolated mouse synaptosomes (Huang et al., 1993). The ability of monoketones, including MIBK, to inhibit β-adrenergic receptor binding and Na^+-K^+-ATPase activity was positively related to the ability of the parent material to penetrate the synaptic membrane preparations because of their lipophilicity. The authors proposed that the monoketones increased lipid fluidity in the synaptic membrane, thereby disrupting the function of receptor proteins and membrane enzymes. The in vitro IC_{50} (the concentration necessary to achieve 50% inhibition) values for MIBK inhibition of receptor binding and enzyme activity in prepared mouse synaptosomes were 46 and 43 μM (approximately 4.61 and 4.31 mg/L), respectively (Huang et al., 1993).

MIBK has been found to potentiate cholestasis induced by various chemicals. Duguay and Plaa (1997a) implicated MIBK-induced increased cholesterol content in the bile canalicular membrane (and in various other liver fractions) as a factor in MIBK potentiation of cholestasis in rats that were pre-treated by MIBK inhalation for 4 hrs/day for 3 days prior to challenge with manganese-bilirubin.

MIBK-induced potentiation of hepato- and nephrotoxicity caused by carbon tetrachloride may be related to the findings that MIBK pre-treatment increased P-450 content in liver and kidney microsomes and increased carbon tetrachloride covalent binding. The P-450 enzyme species increased were those isoenzymes associated with increased aminopyrine N-demethylation activity in both the liver and kidney and increased benzphetamine N-demethylation activity in the liver (Raymond and Plaa, 1995b).

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION

4.5.1. Oral Exposure

No studies were located regarding health effects in humans associated with oral exposures to MIBK, and no chronic oral exposure studies in animals were located.

A number of effects suggestive of liver, kidney, and CNS involvement have been observed in animals following subchronic repeated oral exposures (Table 4-1), but the effects did not show a clear, toxicologically relevant continuum of severity or marked progression of response with increasing dose. Collectively, a variety of clinical blood chemistry, urine chemistry, and organ weight changes that occurred after subchronic gavage exposure (MAI, 1986) or drinking water exposure (Carnegie-Mellon Institute, 1977a, b) suggest adverse changes in the liver and kidney. When the effects are considered individually, however, the degree of their biological adversity is uncertain, particularly since treatment-related corroborative gross pathologies or histopathological lesions were not observed.

The prevalence of transient lethargy, a clearly adverse effect occurring at 1000 mg/kg-day in the subchronic gavage study (MAI, 1986), was not quantified, and no treatment-related lesions were observed in the brain, spinal cord, and sciatic nerve in this study. Subchronic drinking water exposure to 1040 mg/kg-day did not elicit similar neurological signs and no lesions were observed after comprehensive neuropathological examination (Carnegie-Mellon Institute, 1977a,b), suggesting that the neurological signs observed in the gavage study may have been an artifact of the bolus mode of oral administration causing temporarily high blood MIBK levels. Thus, no lowest-observed-adverse-effect level (LOAEL) was identified in either the subchronic gavage or subchronic drinking water study.

Table 4-1. Summary of effects at exposure levels reported in laboratory animals following repeated oral exposures to MIBK[a]

Species (Sex)	Exposure Schedule	Reported Exposure Levels (mg/kg-day)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effects	Reference
Rat (M/F)	Daily gavage in corn oil for 13 weeks	0, 50, 250, 1000	1000	ND	At 50 mg/kg-day: No effects At 250 mg/kg-day: Minimally increased SGPT (F) and relative kidney weights (M,F) At 1000 mg/kg-day: Increased interim (M,F) and terminal (F) serum cholesterol; minimally increased SGPT and serum alkaline phosphatase (F); increased absolute and relative liver, kidney, and adrenal weights (M,F); increased relative testis weight (M); increased incidence of nephropathy (M); transient lethargy (M,F)	Microbio-logical Associates, Inc, 1986
Rat (F)	Ad libitum drinking water, 120 days	0, 1040	1040	ND	At 1040 mg/kg-day: Increased absolute and relative kidney weights; renal tubular hyperplasia in one of five exposed vs. zero of five controls. Clinical chemistry was not evaluated in this study, but several neurological endpoints were examined. No adverse changes were found in tests of neuromuscular function or in histological examinations of PNS and CNS tissues.	Carnegie-Mellon Institute, 1977a, b

[a] No chronic oral exposure studies were located. No other studies were located that examined neurological, developmental, or reproductive endpoints in humans or animals after oral exposure to MIBK.

NOAEL = no-observed-adverse-effect level
LOAEL = lowest-observed-adverse-effect level
ND = not determined
SGPT = serum glutamic-pyruric transaminese
PNS = peripheral nervous system
CNS = central nervous system

The effects observed at 250 mg/kg-day in the subchronic gavage study (increased SGPT and increased relative kidney weights), although statistically significant, are not clearly biologically adverse. Although SGPT activity level has been shown to be positively correlated with degree of hepatocellular necrosis in rats (Hoffman et al., 1989), changes in SGPT that are less than several-fold, such as occurred in the MAI (1986) study, can be difficult to interpret in the absence of corroborative liver histopathologies. Also, mean SGPT in females did not increase in a dose-related manner. The terminal absolute and relative kidney weight increases reported in males and females at 250 mg/kg-day ranged from 6 to 12% over controls. In the absence of corroborative kidney lesions or changes in urinalysis parameters at 250 mg/kg-day, the kidney weight changes are not clearly attributable to adverse anatomical or functional changes in the kidney.

Of the effects observed at 1000 mg/kg-day after subchronic gavage exposure, the increased serum cholesterol (+65% above controls) in female Sprague-Dawley rats appears to be the most biologically significant endpoint among a number of effects potentially associated with liver or kidney changes (MAI, 1986). The observed mean terminal serum cholesterol level of 162 mg/dL at 1000 mg/kg-day was elevated, not only as compared to the study control group (MAI, 1986), but also as compared to a reference range of mean control values of 66 to 97 mg/dL compiled by the Charles River Laboratory (2000) from 10 subchronic oral exposure studies conducted from 1993 to 1998 in groups of 15 12-week old female Sprague-Dawley rats. In the MAI (1986) study, mean serum cholesterol in females increased in each exposure group between the interim sacrifice at 7 weeks and the terminal sacrifice after 13 weeks of exposure (+23 to 44%), indicating that the severity of the effect increased with duration of exposure and suggesting that this effect may be more pronounced in a lifetime exposure study.

Significantly increased serum cholesterol was also observed in male rats at the interim sacrifice in the subchronic gavage study (+30%) and in another strain of rat after subchronic inhalation exposure (Phillips et al., 1987). The liver synthesizes most of the endogenous cholesterol found in blood plasma (Guyton, 1976) and takes up cholesterol from the blood (Moslen, 1996), so it is plausible that hypercholesterolemia is indicative of adverse changes in the liver. Joseph et al. (1992) found that, although bile flow rates were unaffected, hepatocellular cholesterol secretion rates into bile were significantly lower in rats administered acute oral doses of MIBK than in untreated controls. Impeded cholesterol elimination via biliary excretion could potentially affect serum cholesterol levels. Interference with cholesterol metabolism in other parts of the body cannot be ruled out as a possible contributing mechanism of the observed hypercholesterolemia. In spite of relatively strong evidence that hypercholesterolemia occurs in animals after oral gavage exposure, increased cholesterol was not identified as the critical effect, because there was no clear continuum of severity in related effects, such as adverse histological changes in the liver.

Serum alkaline phosphatase increases of 7- to 10-fold have been reported in rats within 24 hours of cholestasis induced by surgical bile duct ligation (Hoffman et al., 1989). The increase in serum alkaline phosphatase observed in female rats (+84%) at 1000 mg/kg-day (MAI, 1986) is therefore consistent with reports that MIBK potentiates chemical-induced

cholestasis in laboratory animals (see section 4.4.3). However, serum alkaline phosphatase activity in females was demonstrably elevated above study controls only at the interim sacrifice; no significant changes were observed in males.

Absolute and relative organ weight changes were minimal after subchronic gavage exposure to 250 mg/kg-day, but increases in liver, kidney, and adrenal weights ranged from 20 to 42% in males or females at 1000 mg/kg-day (MAI, 1986). Because no histopathologies were reported in the liver or adrenals of either sex or in the kidneys of females, the observed weight changes may be tentatively attributed to adaptive rather than adverse changes until chronic exposure data become available.

The observed changes in BUN, serum potassium, and serum glucose in male rats at 1000 mg/kg-day (MAI, 1986) may be related to the observed increased prevalence of mild nephropathy. Descriptions of the kidney lesions in males mentioned multifocal swollen or hyperchromatic and flattened tubular epithelial cells, but they did not mention hyaline droplet formation. Renal hyaline droplet lesions in male rats have been reported in inhalation exposure studies (Bushy Run Research Center, 1982; MacEwen et al., 1971; Phillips et al., 1987). It is possible that the renal lesions observed after oral gavage exposure were related to hyaline droplet formation, but the reported observations in the MAI (1986) study do not confirm this hypothesis. Hyaline droplet formation in male rats is not considered to be relevant to human health for the purposes of risk assessment (U.S. EPA, 1991b). Because the kidney lesions in male rats are of unlikely relevance to human health, the observed clinical chemistry changes in males that may be associated with renal changes are also of unlikely relevance.

Reversible lethargy suggestive of neurological effects was observed in rats administered 1000 mg/kg-day by gavage for 13 weeks, but not in rats administered at lower gavage exposure levels (MAI, 1986) and not in rats exposed in drinking water at approximately 1040 mg MIBK/kg/day for 120 days (Carnegie-Mellon Institute of Research, 1977a, b). Prevalence data for lethargy were not provided.

A 120-day drinking water exposure to approximately 1040 mg MIBK/kg/day resulted in increased mean absolute (+18%) and relative (+22%) kidney weights in female rats as compared to controls, but no liver or other treatment-related effects occurred (Carnegie-Mellon Institute of Research, 1977a, b). No pathologies were observed in comprehensive gross and histopathological examinations in the subchronic drinking water study; however, pale or mottled kidneys were observed in a drinking water range-finding study in rats consuming approximately 300 or 900 mg MIBK/kg/day for 7 days (Carnegie-Mellon Institute of Research, 1977a).

Rats appeared to be more susceptible to effects associated with liver, kidney, and neurological changes after gavage exposure than after drinking water exposure. A greater number of renal effects as well as liver and neurological symptoms were observed after gavage exposure to 1000 mg/kg-day than after drinking water exposure to 1040 mg/kg-day. Comparison of administration routes was not possible at lower exposure levels, because the drinking water study evaluated only one exposure level. MIBK is thought to be metabolized via

cytochrome P-450 metabolic pathways (Vézina et al., 1990). Bolus doses of MIBK may have resulted in blood MIBK levels that saturated the enzymatic metabolic pathways, resulting in repeated occurrences of temporarily higher blood MIBK (and/or MIBK metabolite) levels than those achievable from continuous drinking water exposure.

The 120-day drinking water study (Carnegie-Mellon Institute of Research, 1977a, b) may more appropriately simulate potential human exposures than the gavage study (MAI, 1986), but it did not include blood or urine chemistry evaluations, and it evaluated effects only at 1040 mg/kg-day. No exposure levels comparable to the gavage exposure levels of 50 or 250 mg/kg-day were used in the drinking water study. The only effect observed in the drinking water study was increased kidney weights, which was also observed in the gavage study at comparable and lower exposure levels. The drinking water study evaluated a greater variety of neurological endpoints than did the gavage study, including histological evaluations in a variety of PNS and CNS tissues as well as several performance tests of neuromuscular function. Although no neurological effects were observed in the drinking water study at 1040 mg/kg-day, transient lethargy was observed immediately following gavage administrations of 1000 mg/kg-day. This effect may have been due to a temporary peak in blood MIBK (and/or metabolite) levels near the time of gavage dosing. It is not clear whether chronic exposure or more extensive examination of neurological endpoints would have identified nervous system lesions at gavage exposures up to 1000 mg/kg-day.

4.5.2. Inhalation Exposure

No studies were available that provided reliable MIBK exposure-response data in humans from chronic or subchronic inhalation exposures. Studies of workers exposed repeatedly to mixtures of solvents that include MIBK have associated various neuropathies and decrements in neurobehavioral performance tests with exposure. However, the results are not sufficient for establishing causality or characterizing an inhalation exposure-response relationship in humans, because exposure levels for individual solvents were not reported or the degree to which MIBK contributed to the observed effects is uncertain.

No chronic duration inhalation exposure studies in animals were available. Table 4-2 highlights information regarding effect levels for repeated inhalation exposure studies in animals. Following guidance provided in U.S. EPA (1994b) for identifying the critical effect, all effect levels in Table 4-2 were converted to continuous-exposure Human Equivalent Concentration

Table 4-2. Summary of effects at $LOAEL_{HEC}$ reported in laboratory animals following repeated inhalation exposure to MIBK to be used for identifying the critical effect in animals

Species (Sex)	Exposure Schedule	Reported Exposure Levels (HEC exposure levels) (mg/m^3)	NOAEL (HEC NOAEL)[a] (mg/m^3)	LOAEL (HEC LOAEL)[a] (mg/m^3)	Effects at HEC Exposure Levels	Reference
Rat (M/F)	6 hrs/day, 5 days/week, 14 weeks	0, 205, 1033, 4106 (0, 37, 185, 733)	4106 (733)	ND	At 37 mg/m^3: No significant effects At 185 mg/m^3: Females, 2% increase in body weight over controls; males, 23% increase in serum cholesterol, 37% increase in urinary glucose, mild hyaline droplet lesions in kidneys At 733 mg/m^3: Females, 5% increase in body weights, 26% increase in urinary glucose, 57% decrease in eosinophil number; males, 13% increase in platelet number, 35% increase in serum cholesterol, 28% increase in urinary protein, 55% increase in urinary glucose, increased absolute (13%) and relative (9%) liver weights, increased severity of renal hyaline droplet lesions	Phillips et al., 1987
Mouse (M/F)	6 hrs/day, 5 days/week, 14 weeks	0, 205, 1033, 4106 (0, 37, 185, 733)	4106 (733)	ND	At 37 mg/m^3: No significant effects At 185 mg/m^3: Increased absolute liver weight (8%) in males At 733 mg/m^3: Increased absolute (11%) and relative (10%) liver weights in males	Phillips et al., 1987
Rat (M)	6 hrs/day, 5 days/week, 13 weeks	0, 1024, 3073, 6146 (0, 183, 549, 1098)	6146 (1098)	ND	At 549 mg/m^3: Reduced activity during first 8 weeks of exposure; increased relative kidney and liver weights; increased terminal body weights At 1098 mg/m^3: Reduced activity during first 10 weeks of exposure; increased terminal body weights; increased relative kidney and liver weights	David et al., 1999

Species (Sex)	Exposure Schedule	Reported Exposure Levels (HEC exposure levels) (mg/m^3)	NOAEL (HEC NOAEL)[a] (mg/m^3)	LOAEL (HEC LOAEL)[a] (mg/m^3)	Effects at HEC Exposure Levels	Reference
Rat (M/F)	Multi-generation reproductive toxicity assay. In both generations: 6 hrs/day, 70 days prior to mating, then throughout mating and gestation and most of lactation until weaning	F_0: 0, 2012, 4093, 8178 (0, 503, 1023, 2045) F_1: 0, 2073, 4105, 8219 (0, 518, 1026, 2055)	F_0: 8178 (2045) F_1: 8219 (2055)	F_0: ND F_1: ND	F_0: At 503 mg/m^3: Males, increased relative kidney weight At 1023 mg/m^3: Males, increased relative kidney weight, centrilobular hepatocellular hypertrophy, reduced startle response. Females, increased relative kidney weight, reduced startle response. Offspring, transient depressed pup weight At 2045 mg/m^3: Males, increased relative kidney and liver weights, increased prevalence of centrilobular hepatocellular hypertrophy and nephropathy, reduced startle response. Females, increased relative adrenal, kidney, ovary, and liver weights, reduced startle response. Offspring, transient depressed pup weight F_1: At 518 mg/m^3: Males, increased relative kidney weight At 1026 mg/m^3: Males, increased relative liver and kidney weights, increased prevalence of centrilobular hepatocellular hypertrophy and nephropathy, reduced startle response. Offspring, transient depressed pup weight At 2055 mg/m^3: Males, increased relative liver, kidney, testis, cauda epididymis, seminal vesicle, and adrenal weights; increased prevalence of centrilobular hepatocellular hypertrophy and nephropathy; reduced startle response; transient unsteady gait and prostration. Females, increased relative liver and kidney weights, reduced startle response, transient unsteady gait and prostration. Offspring, transient depressed pup weight	WIL Research Labs, 2000

Species (Sex)	Exposure Schedule	Reported Exposure Levels (HEC exposure levels) (mg/m^3)	NOAEL (HEC NOAEL)[a] (mg/m^3)	LOAEL (HEC LOAEL)[a] (mg/m^3)	Effects at HEC Exposure Levels	Reference
Rat (NS)	continuous, 90 days	0, 410 (0, 410)	410 (410)	ND	At 410 mg/m^3: Increased mean relative liver and kidney weights, hyaline droplet renal proximal tubule degeneration	MacEwen et al., 1971
Dog (NS)	continuous, 90 days	0, 410 (0, 410)	410 (410)	ND	At 410 mg/m^3: No effects	MacEwen et al., 1971
Monkey (NS)	continuous, 90 days	0, 410 (0, 410)	410 (410)	ND	At 410 mg/m^3: Focal chronic renal inflammation in one of two monkeys	MacEwen et al., 1971
Rat (M/F)	6 hrs/day, 5 days/week, 2 weeks	0, 750 (0, 134)	750 (134)	ND	At 134 mg/m^3: Increased relative liver weights in males	Hazleton Labs, Inc., 1966, 1968
Rat (M/F)	6 hrs/day, 5 days/week, 4 weeks	0, 4530 (0, 409)	4530 (409)	ND	At 409 mg/m^3: No effects	Hazleton Labs, Inc., 1966, 1968
Rat (M/F)	6 hrs/day, 5 days/week, 9 days	0, 410, 2053, 8178 (0, 73, 367, 1460)	8178 (1460)	ND	At 73 mg/m^3: No effects At 367 mg/m^3: Increased relative liver weight in males, renal hyaline droplet degeneration At 1460 mg/m^3: Periocular wetness, increased relative liver weights in males and females; renal hyaline droplet formation in males with tubule epithelial regeneration	Bushy Run Research Center, 1982
Mouse (M/F)	6 hrs/day, 5 days/week, 9 days	0, 410, 2053, 8178 (0, 73, 367, 1460)	8178 (1460)	ND	At 73 mg/m^3: No effects At 367 mg/m^3: Increased relative liver and kidney weights in females At 1460 mg/m^3: Increased relative liver and kidney weights in females	Bushy Run Research Center, 1982

Species (Sex)	Exposure Schedule	Reported Exposure Levels (HEC exposure levels) (mg/m^3)	NOAEL (HEC NOAEL)[a] (mg/m^3)	LOAEL (HEC LOAEL)[a] (mg/m^3)	Effects at HEC Exposure Levels	Reference
Rat (F)	6 hrs/day, each gd 6-15	0, 1229, 4106, 12,292 (0, 307, 1026, 3073)	4106 (1026)[b]	12,292 (3073)[b]	At 307 and 1026 mg/m^3: No treatment-related effects At 3073 mg/m^3: Maternal effects, reduced body weight and body weight gain, hypoactivity, ataxia, lacrimation. Fetal effects, reduced fetal body weight, delayed skeletal ossification	Tyl et al., 1987
Mouse (F)	6 hrs/day, each gd 6-15	0, 1229, 4106, 12,292 (0, 307, 1026, 3073)	4106 (1026)[b]	12,292 (3073)[b]	At 307 and 1026 mg/m^3: No treatment-related effects At 3073 mg/m^3: Maternal effects, hypoactivity, ataxia, lacrimation. Fetal effects, increased fetal death, reduced fetal body weight, delayed skeletal ossification	Tyl et al., 1987
Rat (NS)	6 hrs/day, 5 days/week, 5 months	0, 6146 (0, 1098)	6146 (1098)	ND	At 1098 mg/m^3: Slight narcosis during exposure	Spencer et al., 1975

[a] HECs were calculated according to EPA guidance (U.S. EPA, 1994b) for category 3 gases by adjusting intermittent exposure levels to a continuous exposure basis (see text) and multiplying the result by a ratio of the animal blood gas partition coefficient for MIBK to the human blood gas partition coefficient as follows:

$$NOAEL_{HEC}\ (mg/m^3) = NOAEL_{ADJ}\ (mg/m^3) \times (H_{b/g})_A/(H_{b/g})_H,$$

where

$NOAEL_{HEC}$ = the NOAEL (or LOAEL) expressed in mg/m^3, dosimetrically adjusted for differences between humans and animals in absorptivity of MIBK into blood,

$NOAEL_{ADJ}$ = the NOAEL (or LOAEL) expressed in mg/m^3, adjusted for exposure schedule to estimate equivalent continuous exposure concentration, and

$(H_{b/g})_A/(H_{b/g})_H$ = the ratio of blood:gas partition coefficients of MIBK for the animal value to human value.

EPA guidance (U.S. EPA, 1994b) indicates that the default value of the $(H_{b/g})_A/(H_{b/g})_H$ ratio should be set equal to 1 if blood:air partition coefficient data are not available for either humans or animals. As no animal blood:air partition coefficients were located, the $LOAEL_{HEC}$ and $NOAEL_{HEC}$ values for MIBK were set equal to the continuous duration-adjusted exposure concentrations in all cases.

[b] Exposure concentrations in the Tyl et al. (1987) developmental toxicity assay were duration-adjusted to derive HEC exposure levels (U.S.EPA, 1994b). This methodology differs from previous EPA practice where the Guidelines for Developmental Toxicity Risk Assessment (U.S. EPA, 1991a) noted that most developmental assessments did not perform dosimetric timing adjustments. This previous science-policy practice had been based on the premise that developmental effects for a number of agents were more likely to depend on peak exposure concentrations. Further evaluation had indicated that developmental effects for a number of agents may be a function of area under the curve or AUC. Hence, in the absence of specific information on the dose-response timing sensitivity for MIBK, i.e, peak versus A.C., EPA has chosen to perform a dosimetric adjustment, consistent with public health protection and the science-policy set forth in US EPA (2002). In the Tyl et al. (1987) study, the daily exposure cycle was comprised of 6 hours of exposure followed by 18 hours of no exposure in rats and mice. Therefore, experimental values in Tyl et al. (1987) were duration-adjusted by a factor of 6/24 to provide estimated equivalent continuous exposure levels using the equation described in section 4.5.2.

LOAEL = lowest-observed-adverse effect level
HEC = human equivalent concentration
NOAEL = no-observed-adverse-effect level
ND = not determined
NS = gender not specified

(HEC)[2] values to permit comparisons between studies with different exposure schedules. The available inhalation data summarized in Table 4-2 show that the only $LOAEL_{HEC}$ for adverse effects in animals was 3073 mg/m^3 for reduced fetal body weight and delayed ossification in rats and mice and increased fetal death in mice after inhalation exposure to MIBK at 6 hrs/day on gestation days 6 to 15. The corresponding no-observed-adverse-effect level $(NOAEL)_{HEC}$ was 1026 mg/m^3 (Tyl et al., 1987). The developmental effects were the most clearly adverse effects in the substantial database of subchronic MIBK inhalation studies in animals. In a two-generation inhalation reproductive toxicity assay in rats that included pre-mating, mating, gestational, and lactational exposures to up to 8219 mg/m^3 (2055 mg/m^3 HEC), no MIBK-induced effects were observed in either generation in the number of pups with gross external malformations at birth, number of stillbirths, number of live pups, body weight on post-natal day 1, or survival to post-natal day 4 (WIL Research Laboratories, 2000).

The following discussion provides an evaluation of effects that may be associated with adverse changes to the liver, kidney, and CNS that were reported in animals at exposure levels comparable or lower than those associated with developmental effects. On the basis of data provided in the subchronic inhalation database, the liver, kidney, and CNS effects were not

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[2] Guidance for extra-respiratory effects of category 3 gases was used for the MIBK assessment (U.S. EPA, 1994b). Category 3 gases typically induce extra-respiratory effects, because they are considered nonreactive (i.e., having low propensity for dissociation or metabolism to reactive forms) in the respiratory tract and have relatively low water solubility, which would promote rapid partitioning into the bloodstream and transport away from respiratory tissues. MIBK is classified as a category 3 gas for this assessment on the basis of the following observations: (1) toxicological data from repeated exposure studies indicate that MIBK is not appreciably reactive in biological tissues, as no histological effects were observed in either upper or lower respiratory tissues in animals at exposure concentrations significantly higher than those that induce sensory irritation (Hazleton Laboratories, Inc., 1966, 1968; Phillips et al., 1987); (2) although MIBK is considered to be soluble in water (Topping et al., 1994; Yalkowsky and Dannenfelser, 1992), it is absorbed readily into the bloodstream from inhalation exposures (Hjelm et al., 1990), and human blood/air and oil/air partition coefficients for MIBK of 90 and 926, respectively, indicate significant blood and lipid solubility (Sato and Nakajima, 1979); (3) MIBK partitions equally between red blood cells and plasma in rat and human blood—only 20% of plasma MIBK and only 25% of RBC MIBK was dissolved in water (Lam et al., 1990); and (4) mechanism-of-action data suggest that neurological effects from MIBK exposures may be related to the ability of MIBK to penetrate the nerve membranes because of its lipophilicity, thereby disrupting the function of imbedded receptor proteins and enzymes (Huang et al., 1993).

Effect levels for repeated-exposure studies were duration-adjusted to provide estimated equivalent continuous exposure levels using the following equation (U.S. EPA, 1994b):

$NOAEL_{ADJ}$ (mg/m^3) = E (mg/m^3) x D(hrs/24 hrs) x W(days/7day),

where,

$NOAEL_{ADJ}$	=	the NOAEL (or LOAEL) expressed in mg/m^3, adjusted for exposure schedule to estimate equivalent continuous exposure concentration,
E	=	experimental exposure level, expressed in mg/m^3,
D	=	number of hours exposed per day/24 hours, and
W	=	number of days exposed per weeks/7 days.

considered to be clearly adverse and therefore were considered to be of uncertain relevance to effects in humans after chronic exposures.

Evidence indicating that an MIBK-induced increase in serum cholesterol occurred in animals after subchronic inhalation exposure is relatively strong, but increases were not dramatic and the effect was not sufficiently adverse to be considered the critical effect in the absence of a continuum of increasingly severe related effects, such as liver lesions, at higher exposure levels. Moreover, it is uncertain if the increases in serum cholesterol in rats following exposure to MIBK have any relevance to humans for more severe effects, like cardiovascular toxicity, since it is generally acknowledged that the rat model is not considered a good species for predicting cardiovascular effects in humans and because the rat, like the mouse, is relatively resistant to hyperlipidemia and atherosclerosis (Sipes et al., 1997; Loeb and Quimby, 1999). The HEC exposure level of 185 mg/m^3 was associated with increased serum cholesterol and hepatomegaly in 26-week-old male Fischer 344 rats after a 13-week intermittent inhalation exposure (Phillips et al., 1987). The observed mean serum cholesterol levels of 59 and 65 mg/dL at 185 and 737 mg/m^3 HEC exposures, respectively, were elevated not only as compared to the study control group (Phillips et al., 1987), but also as compared to other relevant reference values.

Both age and method of blood collection may affect measured clinical chemistry values. Charles River Laboratory (1984) provided baseline mean control values of 46, 34, and 26 mg/dL for male Fischer 344 rats of 6–8, 19–21, and 32–34 weeks of age, respectively, and Neptun et al. (1985) reported mean serum cholesterol values of 54.3 and 55.3 mg/dL for untreated adult male Fischer 344 rats using the same blood collection method (retroorbital bleeding) as that employed by Phillips et al. (1987).

Although adverse gross or histopathological liver lesions were not observed in any animal inhalation study, Duguay and Plaa (1997a) provided evidence that increased retention of newly synthesized hepatocellular cholesterol occurred in male rats after acute inhalation exposure to 200 and 600 ppm MIBK (819 and 2458 mg/m^3; 4 hrs/day for 3 days) but not after acute exposure to 100 ppm (410 mg/m^3). They found that MIBK-induced accumulation of newly synthesized cholesterol in the bile canalicular membrane apparently altered the membrane lipid dynamics, possibly potentiating cholestasis that was induced by other chemicals.

Interference with cholesterol metabolism in other parts of the body cannot be ruled out as a possible contributing mechanism of hypercholesterolemia. In spite of relatively strong evidence indicating that hypercholesterolemia occurs in rats after subchronic repeated inhalation exposures to MIBK, in the absence of histopathological changes in the liver the effect was not considered to be clearly adverse. The available database does not provide sufficient information to indicate whether chronic inhalation exposures would cause more severe liver effects in animals or if the hypercholesterolemia observed in rats would have severe cardiovasular consequences in humans.

Hepatomegaly in mice was also associated with the HEC exposure levels of $\geq$185 mg/m^3, but there was no evidence of any other hepatic effect in mice at any exposure level; thus, the

mouse hepatomegaly may be considered simply an adaptive response to prolonged hepatic metabolism of MIBK (Phillips et al., 1987).

Increased urinary glucose occurred in male rats at 185 mg/m^3 and in both sexes at a higher exposure level (Phillips et al., 1987), suggesting that the ability of the kidney proximal tubule to resorb sugars was impaired, particularly since blood sugar levels were unaffected at the same exposure levels. In this study, alpha$_{2u}$-globulin hyaline droplet formation, but no other renal lesions was observed in male rats at the same exposure levels that induced glucosuria. However, renal hyaline droplet formation is not considered by EPA to be relevant to humans for the purposes of risk assessment, because alpha$_{2u}$-globulin is produced in male rats to much greater degrees than in female rats or humans (U.S. EPA, 1991b). The impaired renal function indicated by glucosuria may be attributable to the hyaline droplet lesions in male rats (Phillips et al., 1987). Glucosuria observed in high-exposure females may be indicative of nascent adverse kidney changes but are not corroborated by observations of kidney histopathologies. Glucosuria occurred at exposure concentrations lower than those associated with the kidney weight increases observed in several studies.

Effects associated with adverse changes in the liver and kidney generally occurred at lower concentrations than neurological effects in repeated exposure animal studies (see Table 4-2). The principal effects associated with neurological impairment in animals were behavioral changes (e.g., hypoactivity, ataxia, and unsteady gait) that were only observed during exposure events in repeated exposure studies. The lowest HEC exposure level at which neurological effects were observed in animals was 549 mg/m^3 for reduced activity in rats that only occurred during the daily 6-hour exposure events (David et al., 1999). Because the neurological symptoms are acute effects that occur during exposure events, it may not be appropriate to adjust exposure concentrations in repeated exposure experiments to continuous exposure equivalents for these effects. Indeed, neurological symptoms were observed during exposure events in rats at the exposure duration-adjusted concentration of 549 mg/m^3 (actually repeated exposure to 3073 mg/m^3) (David et al., 1999), but no neurological effects were seen in rats, dogs, and monkeys that were exposed continuously to an exposure level (410 mg/m^3) comparable to the duration-adjusted level (MacEwen et al., 1971; MacKenzie, 1971; Vernot et al., 1971).

Neurological clinical signs attenuated with repeated exposure over 13 weeks (David et al., 1999), possibly reflecting an adaptive increase in the capacity for hepatic metabolism of MIBK. Repeated exposures at higher MIBK concentrations induced more severe symptoms, such as ataxia, coordination loss, hindlimb weakness, paresis, and irregular gait (Bushy Run Research Center, 1984; Tyl et al., 1987; WIL Research Laboratories, 2000). However, the database of subchronic inhalation animal studies—including one comprehensive histological evaluation of CNS and PNS tissues (Spencer et al., 1975)—includes no reports of MIBK-induced adverse effects in gross and histological examinations of nervous system tissues (Carnegie-Mellon Institute of Research, 1977a, b; David et al., 1999; Hazleton Laboratories, Inc., 1966, 1968; MacEwen et al., 1971; MacKenzie, 1971; Phillips et al., 1987; Vernot et al., 1971), or in batteries of neurobehavioral task performance tests (David et al., 1999; Garcia et al., 1978; Geller et al., 1978).

MIBK may induce the observed neurological symptoms by causing transient nerve membrane changes. On the basis of experiments in isolated mouse synaptosomes, Huang et al. (1993) proposed that monoketones, including MIBK, may disrupt the function of embedded enzymes and receptor proteins in nerve cell membranes by increasing the fluidity of the membrane. The transient nature of observed neurological symptoms corresponds with the observation that blood levels of MIBK increase during exposure but rapidly drop off after cessation of an exposure event (Hjelm et al., 1990) because MIBK is rapidly metabolized (Granvil et al., 1994). MIBK levels peaked quickly in the brain, and then MIBK was rapidly eliminated from brain tissue in mice administered a single intraperitoneal injection, whereas the brain levels of a principle MIBK metabolite, 4-hydroxy-4-methyl-2-pentanone, continued to increase throughout a 90-minute post-exposure period (Granvil et al., 1994). No studies were located that evaluated the ability of MIBK metabolites to induce neurological effects.

Transient neurological symptoms (e.g., headache, vertigo, and nausea) have also been subjectively reported by human volunteers during experimental acute inhalation exposures at exposure levels as low as 100–200 mg/m^3 (Hjelm et al., 1990; Iregren et al., 1993). Information concerning effects in humans after acute experimental exposures is summarized in Table 4-3. There is some uncertainty about whether an RfC based on a $NOAEL_{HEC}$ of 1026 mg/m^3 for developmental effects in mice would be protective of irritation and neurological effects that may occur with exposure to MIBK at much lower air concentrations. However, the RfC of 3 mg/m^3, which is based on a $NOAEL_{HEC}$ of 1026 mg/m^3 for developmental effects in mice, is more than thirty-fold lower than the LOAEL of 100 mg/m^3 reported for acute CNS and irritation effects in humans that may occur with exposure to MIBK at much lower air concentrations and therefore may be protective of these effects. Animal studies that examined neurological endpoints (e.g., motor activity or coordination) after repeated exposure to airborne MIBK have reported effect levels only at much higher concentrations than the acute exposure levels (100–200 mg/m^3) that elicited neurological and irritation symptoms in humans (Hjelm et al., 1990; Iregren et al., 1993).

Two factors may have influenced the fact that neurological effects in humans were reported at much lower exposure concentrations than in animals. First, the LOAEL values of 100 and 200 mg/m^3 for neurological effects in human volunteers were reported under exposure conditions that included light exercise (Hjelm et al., 1990; Iregren et al., 1993), whereas no animal study included exercise during exposure. Acute inhalation exposures in humans to MIBK at 410 mg/m^3 did not elicit neurological symptoms in the absence of exercise (Dick et al., 1992). The light exercise may have effectively increased MIBK absorption rate by increasing the respiration rate, which would increase the total mass of MIBK gas to contact respiratory absorptive tissues per unit time. Second, the method of measuring neurological symptoms in humans (reporting of vertigo, headache, or nausea) may be a more sensitive method for detecting neurological effects than the method employed for animals (objective observation of changes in motor activity or coordination).

Table 4-3. Summary of effects in humans at LOAEL concentrations following acute inhalation exposure to MIBK

Exposure Schedule	Exposure Levels (mg/m^3)	NOAEL (mg/m^3)	LOAEL (mg/m^3)	Notes	Reference
7 mins, 2 times at 2-week interval	402, 915, 1363, 1680, 2301, or 2827 (first exposure); 845, 1493, or 2066 (second exposure)	915	1393	At 1393 mg/m^3: LOAEL is a threshold for eye, nose, or throat irritation reported during exposure.	Esso Research and Eng. Co., 1965; Hazleton Labs, Inc., 1965
2 hrs (light exercise)	10, 100, 200	10 [a]	100	Prevalence of CNS effects increased with exposure level. At 100 mg/m^3: three of eight subjects reported nose and throat irritation; two of eight reported vertigo and headache. A similar prevalence of symptoms was reported at 200 mg/m^3.	Hjelm et al., 1990
4 hrs	0, 410	410	NA	At 410 mg/m^3: The NOAEL is for psychomotor test performance, irritation, and general CNS symptoms.	Dick et al., 1992
2 hrs; unspecified number at weekly intervals (light exercise)	10, 200	10	200	At 200 mg/m^3: An index of self-reported CNS symptoms (such as fatigue) was significantly increased at 200 mg/m^3 as compared with the index at 10 mg/m^3 exposure. Increased sensory irritation was also noted.	Iregren et al., 1993

[a] Only one of eight subjects reported eye, nose, and throat irritation and vertigo at this exposure level.

NOAEL = no-observed-adverse-effect level
LOAEL = lowest-observed-adverse-effect level
CNS = central nervous system
NA = not available

In summary, delayed ossification in rats and mice and reduced fetal body weight and increased fetal death in mice were identified as the critical effects in a substantial database of repeat-dose inhalation studies; the $LOAEL_{HEC}$ for developmental effects was 3073 mg/m^3 and the corresponding $NOAEL_{HEC}$ was 1026 mg/m^3 (Tyl et al., 1987). In a two-generation inhalation reproductive toxicity assay in rats, no treatment-related developmental effects were observed in neonates at exposure levels up to 8219 mg/m^3 (2055 mg/m^3 HEC). The only effect reported in offspring was significantly depressed body weights on day 14 post-partum in F_1 and F_2 male and female pups in the mid- and high-exposure groups, but pup body weights were comparable to controls on days 7 and 21 post-partum. There were no other exposure-related changes observed in any reproductive or developmental endpoint in either generation, including an absence of anatomical changes in F_0 and parental F_1 reproductive organs. Effects that may be associated with adverse changes to the liver, kidney, and CNS were also reported in numerous subchronic inhalation studies at comparable or lower exposure levels. Interestingly, most of these effects appeared to be occurring within the same range of exposure levels. In spite of the evidence that these effects occurred following subchronic exposures to MIBK, none of these effects showed a clear, toxicological continuum of severity and/or marked progression of response with increasing dose, nor were there any treatment-related corroborative gross pathologies or histopathological lesions. The available subchronic database does not provide sufficient information to indicate whether chronic inhalation exposures would cause more severe effects in animals. Therefore, while not at all being dismissed, until further chronic inhalation data becomes available, the liver, kidney, and CNS effects were not considered to be clearly adverse and therefore were considered to be of uncertain relevance to effects in humans from chronic exposures.

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION

Under the draft revised cancer guidelines (U.S. EPA, 1999), the data for MIBK are inadequate for an assessment of human carcinogenic potential. This characterization is based on the absence of both cancer epidemiology studies in humans and carcinogenicity assays in animals. The results of genotoxicity tests in a range of assay systems yielded mostly negative responses.

4.7. SUSCEPTIBLE POPULATIONS AND LIFESTAGES

4.7.1 Possible Childhood Susceptibility

There were no human studies located that indicate the relative sensitivity of children and adults to the toxic effects of MIBK. No specific data are available that assess the potential age-related differences in susceptibility to MIBK between younger and older animals. However, available results from an animal inhalation developmental toxicity study in rats and mice (Tyl et al., 1987) suggest that MIBK crosses the placenta and produces developmental effects. Additionally, transient CNS effects were reported in numerous animal subchronic inhalation studies (Bushy Run Research Center, 1984; Tyl et al., 1987; WIL Research Laboratories, 2000); however, no morphological changes in CNS tissues were observed and a definitive neurotoxicity study has not been conducted. Since there are no neurotoxicity studies of young animals

available and because the developing CNS can be more sensitive, there is a concern for potential childhood susceptibility to developmental neurotoxicity.

4.7.2 Possible Gender Susceptibility

The limited human studies available provide no data to suggest that gender differences in toxicity might occur as a result of exposure to MIBK. Likewise, no studies in animals designed specifically to examine the possible gender differences in susceptibility to MIBK-induced effects were located. No gender-specific susceptibility was observed in offspring in either the developmental (Tyl et al., 1987) or two-generation reproductive toxicity studies (WIL Research Labs, 2000). There were, however, some possible indications of gender-related differences observed in the animal subchronic toxicity studies. In a repeat-dose subchronic oral toxicity study in rats by Microbiological Associates, Inc (MAI, 1986), increases in serum glutamic-pyruvic transaminase, serum alkaline phosphatase, and terminal serum cholesterol were seen in females, but not in males. Possible gender-related differences in susceptibility were also observed in animals following repeated inhalation exposures for a variety of effects such as, body weights, liver and kidney weights, and urinary glucose and serum cholesterol levels (Phillips et al., 1987; Bushy Run Center, 1982; WIL Research Labs, 2000); however, no consistent pattern in these differences was apparent.

4.7.3 Other

There were no human studies located that indicate the relative sensitivity of elderly adults or adolescents to the toxic effects of MIBK and no specific studies in animals were located that assessed possible age differences in susceptibility to MIBK-induced effects. However, given the observation that effects that may be associated with changes to the liver, kidney, and CNS have been reported in numerous subchronic inhalation studies in animals, it is possible that elderly people or those with liver or kidney function impairment or with hypercholesterolemia might be more susceptible to MIBK. Since MIBK-induced potentiation of hepato- and nephrotoxicity has been demonstrated, the potential also exists for increased susceptibility for liver or kidney toxicity following exposure to MIBK in combination with certain other solvents.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

No oral RfD was developed for MIBK because no critical effect was identified after subchronic exposure. As no chronic oral studies were available, it is uncertain whether chronic exposure at the same exposure levels would have induced biologically significant adverse effects. The database was limited to two subchronic oral exposure studies: one 13-week gavage exposure study (MAI, 1986) and one 90-day *ad libitum* drinking water study (Carnegie-Mellon Institute, 1977a, b). Effects that may be associated with changes in the liver or kidney occurred

at approximately 1000 mg/kg-day in both studies and at 250 mg/kg-day in the gavage study, but the effects were not considered to be clearly adverse for reasons discussed in section 4.5.1.

Transient lethargy, a clear adverse effect, was also reported at 1000 mg/kg-day in the subchronic gavage study, but severity and prevalence data by dose level were not provided, so it was not clear whether the effect was significantly elevated from control levels. In addition, the lethargy was rated as only minimal to mild to moderate, and it was only observed in close association with the gavage exposures not with drinking water exposure. Thus, acute lethargy may have been an artifact of the bolus method of administration that probably resulted in repeated, temporarily high blood levels of MIBK (or metabolites). The relevance of bolus oral exposures to likely chronic oral exposure scenarios in humans is uncertain. Thus, no quantitative risk assessment was conducted for the transient lethargy endpoint.

An RfD for MIBK that was previously on IRIS was withdrawn in 1991. The health effects data for MIBK were reviewed by EPA at that time and were determined to be inadequate for derivation of an oral RfD.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1. Choice of Principal Study and Critical Effect with Rationale and Justification

The principal inhalation study was a developmental toxicity assay in rats and mice conducted by Tyl et al. (1987). The study reported a clear $LOAEL_{HEC}$ of 3073 mg/m^3 for delayed skeletal ossification in mice and rats and reduced fetal body weight and increased fetal death in mice. The corresponding $NOAEL_{HEC}$ was 1026 mg/m^3. Several additional effects observed at 3073 mg/m^3 (but not at 1026 mg/m^3) in the Tyl et al. (1987) study included hypoactivity, ataxia, and lacrimation in mouse and rat dams and reduced maternal body weight and body weight gain in rats. In a two-generation reproductive toxicity assay in rats, no neonatal developmental effects (number of offspring with gross external malformations at birth, number of stillbirths, number of live births, body weight on post-natal day 1, or survival to post-natal day 4) were seen in either generation of rats exposed to air concentrations of MIBK up to 8219 mg/m^3 (2055 mg/m^3 HEC) for 6 hrs/day for 70 days prior to mating, throughout mating, and during most of gestation and lactation (WIL Research Laboratories, 2000).

CNS-related effects and irritation were reported in other studies at exposure levels lower than 3073 mg/m^3, but they occurred only during exposure and, in the absence of histopathologies in nervous system tissues, were considered to be primarily acute responses with uncertain relevance to chronic effects in humans from long-term exposure to MIBK (see discussion in section 4.5.2). As discussed in section 4.5, the human neurotoxicity data were not selected as the basis for the chronic RfC, primarily because the exposure durations were very short and the relevance to effects after lifetime exposures in humans is unknown.

Effects that may be associated with changes in the liver and kidney were also observed in animals at exposure concentrations less than the $NOAEL_{HEC}$ of 1026 mg/m^3 in the developmental

toxicity assay, but for reasons discussed in section 4.5.2 they were not considered clearly adverse.

5.2.2. Methods of Analysis—Including Models

A substantial database of subchronic studies exists for evaluating inhalation exposure effects in animals (see Table 4-2). No chronic inhalation exposure studies in animals were located. Several studies, in particular the only continuous exposure study, evaluated only one exposure level and therefore did not provide sufficient data to fit a benchmark dose (BMD) model. The NOAEL-LOAEL approach was used to identify the critical effect from inhalation exposure in animals. Empirically based effect levels were identified in several studies that used multiple exposure levels, including the principal study.

The method used to review the entire data array to decide on the critical effect was to convert all inhalation effect levels to HEC exposure levels following EPA guidance (U.S. EPA, 1994b) and identify the lowest HEC exposure level associated with clearly adverse effects. By this method of comparison, the lowest HEC adverse-effect level was identified as 3073 mg/m^3 in the Tyl et al. (1987) study. Among the effects observed at that exposure level, the critical effects were reduced fetal body weight, skeletal variations, and increased fetal death in mice and skeletal variations in rats (see further discussion in section 4.5.2). Because litter-specific fetal weights were not reported even in the raw data tables, affected litters could not be identified and methods (including BMD) for analyzing these weight-related results—either with appropriate adjustments for covariates (e.g., litter size, dam weight) or in combination with skeletal variations data (rats)—could not be applied. Additionally, skeletal variations could not be grouped in the EPA analysis.

The incidence of litters with at least one fetus having a specific skeletal variation was statistically evaluated in Tyl et al. (1987), and a significantly increased litter incidence was seen in both rats and mice at 3073 mg/m^3 for a number of fetal skeletal endpoints. Fetal skeletal endpoints for which there was no statistically significant increased litter incidence were excluded from further evaluations. In the high-exposure group for each species, percent litters with at least one responder varied widely between skeletal endpoints (e.g., in rats, the lowest percent responding litters was 26.1% in the high-exposure group for unossified sternebra 5, and the highest it was 100% for generally fragile fetal skeleton), suggesting differences in sensitivity between skeletal endpoints. Statistical comparisons between the control group and the other exposure levels concerning the incidence of fetuses showing specific skeletal effects also were not provided in Bushy Run Research Center (1984) or in Tyl et al. (1987).

BMD methodology was considered for estimating an RfC for MIBK. The only potential data sets were the exposure-response data for eight fetal skeletal endpoints (Bushy Run Research Center, 1984; Tyl et al., 1987). The eight endpoints were selected for BMD modeling on the basis of apparent differences in sensitivity that were reflected in calculated differences between the control and high-exposure groups in percent litters with at least one responder. For example, in rats, the difference in percent litters having at least one fetus with unossified sternebra 5 was

21.9% between controls (4.2%) and the high-exposure group (26.1%), whereas the percent litters having fragile skeletons was 0% in controls and 100% in the high-exposure group, for a difference of 100%. Thus, in rats, the fragile skeleton endpoint appears to be more sensitive to MIBK exposure than the unossified sternebra 5 endpoint. The difference in percent responding litters ranged from 21.9 to 100% in rats and from 22.7 to 63.4% in mice; endpoints at both extremes of apparent sensitivity were selected for BMD modeling in order to capture the range of endpoint sensitivity. This difference measure is a rough gauge of sensitivity, and analyses based on it may diverge from ones that might have been based on a statistically derived measure, had such been available.

The specific skeletal variation endpoints in rats that were selected for modeling were unossified sternebra 5 (low sensitivity), unossified anterior arch of the atlas (moderate sensitivity), and a generally fragile skeleton (less cleared tissue) (high sensitivity). The specific skeletal variation endpoints in mice that were selected for modeling were unossified hindlimb distal phalanges (low sensitivity), poorly ossified metatarsals (low sensitivity), bilobed sternebra 6 (high sensitivity), unossified hindlimb proximal phalanges (high sensitivity), and bilobed supraoccipital (high sensitivity). A single scattered variation or site is not, however, ordinarily considered a good basis for an RfC. Because individual fetus data for skeletal variations in rats or mice were not available, groupings of skeletal outcomes, which might have allowed the derivation of a BMC for the collection of outcomes, could not be performed.

Increased fetal death in mice was not selected for BMD modeling, because the magnitude of the mean increased fetal mortality, although statistically significant, was very small in the high-exposure group (0.6 dead fetuses per litter) as compared to controls (0.1 dead fetuses per litter), and there was no trend apparent with exposure level. No BMD software (BMDS) modeling was performed on the rat fetal weight data, because the magnitude of change from the control mean value was small (< 10% decrease) in the 3073 mg/m^3 group, and no individual data were available. In mice, the percent reduction in body weight between the control group and the 3073 mg/m^3 exposure group ranged from 10 to 14%, but, again, individual litter data were not available, and the reported dam-grouped data gave insufficient basis for BMD modeling.

Although individual outcome benchmark concentrations (BMCs) could be derived, as just described, these are not regarded as a satisfactory basis for an RfC. Although it might be suitable to use some particular skeletal variation together with fetal weight and fetal death in a multivariate model, this is still an exploratory area of methods development, and we do not have sufficient information to carry it out. Consequently, none of the BMCs that were developed were used and no details of the BMC development are provided because they were not considered suitable for deriving the RfC; however, the study data are available in Bushy Run Research Center (1984). A more traditional approach based on NOAEL/LOAEL perspective was implemented. The RfC is derived in section 5.2.3, based on the values shown in section 5.2.1.

5.2.3. RfC Derivation—Including Application of Uncertainty Factors

An RfC of 3 mg/m^3 was derived on the basis of effects observed in fetuses after repeated exposure on gestation days 6 to 15 (Tyl et al., 1987). The RfC was based on developmental effects in fetuses reported in a toxicity assay in which maternal exposure occurred only during gestation. The $NOAEL_{HEC}$ of 1026 mg/m^3 was divided by an uncertainty factor (UF) of 300 to derive the RfC (1026 $mg/m^3 \div 300 = 3.4$ mg/m^3). For interspecies extrapolation a value of 3 was used, because following EPA guidance (U.S. EPA, 1994b), animal-to-human dosimetric adjustment of exposure levels was done. A value of 10 for intraspecies variability was used. An additional UF of 10 for database deficiency was also applied, based on the lack of developmental neurotoxicity data and definitive neurotoxicity data in general, and on the lack of any chronic toxicity data. A two-year bioassay is currently being conducted by the National Toxicology Program, but was not available for inclusion at the time of this toxicological assessment; this study might have permitted further consideration of the liver, kidney, and CNS effects reported in numerous subchronic inhalation studies. Following EPA guidance (U.S. EPA, 1991a), a UF of 10 for extrapolation from subchronic to chronic exposure was not used in deriving the RfC, because exposure concentration during certain time windows of development may be more important than the total duration of the exposure period for determining developmental outcome, as critical developmental stages may occur within brief time windows during gestation.

5.2.4. Previous Inhalation RfC Assessment

An assessment in support of the derivation of an RfC for MIBK was not previously conducted.

5.3. CANCER ASSESSMENT

As discussed in Section 4.6, the data on the carcinogenicity of MIBK are inadequate for an assessment of human carcinogenic potential. No cancer epidemiology studies in humans and no carcinogenicity assays in animals were located. Therefore, a quantitative assessment of carcinogenic potential for MIBK is not appropriate. The results of genotoxicity tests in a range of assay systems yielded mostly negative responses.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

Prolonged or repeated human exposures to MIBK may occur in the workplace or in the home through the use of numerous commercial products such as paints, adhesives, and pesticides as well as agents for wax/oil separation, leather finishing, textile coating, and surfactants for inks and through ingestion of fruits and meats that contain MIBK as a natural component.

The database concerning effects in humans after MIBK exposure is limited. Severity of sensory irritation and transient neurological symptoms were exposure-related in humans, although MIBK did not affect performance in neurobehavioral tests in volunteers at similar or higher exposure concentrations (Hjelm et al., 1990; Dick et al., 1992; Iregren et al., 1993). In these studies, human susceptibility to MIBK-induced neurological symptoms appeared to increase when subjects performed light exercise during inhalation exposure. No other reliable information is available concerning MIBK-induced effects in humans.

The animal database is more extensive, but it has some deficiencies related to assessing health effects from chronic (i.e., lifetime) exposure. No oral or inhalation chronic exposure, oral or inhalation carcinogenicity, oral multi-generation reproductive, or oral developmental studies in animals were available. Subchronic oral exposure in animals induced effects associated with the liver, kidney, blood, and nervous system (see Table 4-1). Similarly, a substantial subchronic inhalation database (see Table 4-2) identifies MIBK-induced effects associated with the following tissues: liver, kidney, blood, nervous system, and the developing fetus.

The similarity and breadth of effects from subchronic oral and inhalation exposure in animals indicates that MIBK potentially affects numerous organ systems. A constellation of effects from both the subchronic inhalation and oral assays were suggestive of adverse changes in the liver, kidney, and CNS, but because these effects did not show a clear, toxicological continuum of severity and/or marked progression of response with increasing dose or any treatment-related corroborative gross pathologies or histopathological lesions, they were not considered to be clearly adverse and were considered to be of uncertain relevance to effects in humans after chronic exposures. The developmental effects in rats and mice after gestational inhalation exposure, are considered to be the most clearly adverse effects in the animal database.

Only one subchronic drinking water assay was available in animals (Carnegie-Mellon Institute of Research, 1977a, b), and it was limited in several ways, including the use of only one MIBK exposure level, a small number of rats per group, and evaluation of only females. An adequately designed repeated oral gavage study (MAI, 1986) identified a greater number of effects in animals than occurred after continuous drinking water exposure at higher or comparable exposure levels. Bolus doses of MIBK may have resulted in blood MIBK levels that saturated the enzymatic metabolic pathways, resulting in repeated occurrences of temporarily higher blood MIBK (and/or MIBK metabolite) levels than those achievable from drinking water exposure. The relevance of bolus oral exposures to potential human oral exposure scenarios is uncertain.

Available toxicokinetic data indicate that MIBK is readily absorbed into the blood after exposure by any route (Hjelm et al., 1990, 1991; Duguay and Plaa, 1995), blood MIBK level is related to oral or inhalation exposure level (Duguay and Plaa, 1995), and MIBK is rapidly metabolized in various tissues, including the brain, after cessation of exposure (Duguay and Plaa, 1995; Granvil et al., 1994). MIBK toxicokinetics, together with mechanism of action data indicating MIBK-induced disruption of nerve membrane integrity (Huang et al., 1993), potentially explain the observation that neurological symptoms in humans and animals are

transient, occurring only during or immediately following exposure. There is no evidence in the current database to suggest that irreversible changes occur in nervous system tissues from oral or inhalation exposures, although it is possible that such changes might be discernable after chronic exposures.

No data were located regarding the existence of an association between cancer and MIBK exposure in humans, but studies of the in vivo and in vitro genotoxicity of MIBK overwhelmingly provided negative responses.

6.2. DOSE RESPONSE

No oral RfD was derived; no critical effect was identified, for reasons discussed in section 5.1.

An RfC of 3 mg/m^3 was developed by dividing the $NOAEL_{HEC}$ of 1026 mg/m^3 by a UF of 300. The critical effects after repeated subchronic inhalation exposure in an adequately designed developmental inhalation bioassay in which the dams were exposed for 6 hours/day on gestation days 6 to 15 (Tyl et al., 1987) were reduced fetal body weight and skeletal variations in rats and mice and increased fetal death in mice at the $LOAEL_{HEC}$ of 3073 mg/m^3. A number of liver and kidney effects, as well as hematological effects, and signs of transient neurological involvement were observed at comparable exposure levels either in the principal study or in several other subchronic inhalation exposure studies. A NOAEL-LOAEL assessment of the relative adversity of numerous observed effects identified the critical adverse effects (reduced fetal body weight and fetal skeletal variations) in a substantial subchronic inhalation exposure-response data array.

Confidence in the principal inhalation study is medium because the study used an adequate number of study animals and exposure levels to evaluate a comprehensive set of developmental endpoints. Confidence in the critical effects of reduced fetal body weight and skeletal variations in mice and rats and increased fetal death in mice is medium because the developmental effects were clearly adverse and indicated a clear threshold for developmental effects. A constellation of effects from subchronic inhalation assays, however, are suggestive of adverse changes in the kidney, liver, and CNS and occurred at comparable exposure levels. The kidney, liver, and CNS effects are collectively suggestive of adverse changes but individually are not sufficiently adverse to be considered critical effects in the absence of clearly adverse changes such as gross or histopathological lesions.

Confidence in the inhalation toxicity database is low to medium because it comprises a number of well-designed subchronic toxicity, neurotoxicity, and reproductive/developmental toxicity animal bioassays, but no data were available for lifetime exposures that would be useful for definitive evaluation of the biological significance of observed liver, kidney, and CNS effects. No studies have evaluated immunotoxicity in laboratory animals, although existing bioassays provide no suggestion that immune effects are expected to occur in association with MIBK exposure. The database included evidence of transient neurological effects in humans at acute exposure levels that were lower than the $NOAEL_{HEC}$ of 1026 mg/m^3 in mice that was used

to derive the RfC and also lower than the lowest exposure level that showed neurological effects in animals from repeated inhalation exposures. However, the derived RfC of 3 mg/m^3 is more than 30-fold lower than the acute exposure LOAEL of 100 mg/m^3 for subjectively reported irritation and neurological symptoms in humans and is lower than the NOAEL of 10 mg/m^3 for subjective irritation/neurological effects in humans. Overall confidence in the RfC is low to medium.

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APPENDIX A. EXTERNAL PEER REVIEW—SUMMARY OF COMMENTS AND DISPOSITION

The support document and IRIS summary for methyl isobutyl ketone (MIBK) have undergone both internal peer review by scientists within EPA and a more formal external peer review by scientists in accordance with EPA guidance on peer review (U.S. EPA, 1998b, 2000a). Comments made by the internal reviewers were addressed prior to submitting the documents for external peer review and are not part of this appendix. The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. A summary of significant comments made by the external reviewers and EPA's response to these comments follows.

(1) General Comments

The three external reviewers offered editorial comments and many minor but valuable suggestions, all of which have been incorporated into the text when feasible. Substantive scientific comments are addressed below.

A. Comment: Additional data/studies recommended for inclusion.

One reviewer recommended no additional data/studies that were directly pertinent or useful for the purposes of hazard identification or dose-response assessment. A second reviewer made suggestions for the inclusion of several animal studies relating to MIBK-potentiation of toxic effects. A third reviewer suggested investigating the status of a 2-year inhalation bioassay in rats and mice currently being conducted by the National Toxicology Program (NTP), as well as a mortality study in steelworkers exposed to MIBK conducted by Dr. K. Mallin at the University of Illinois' Public Health and Epidemiology-Biometry Department. Additionally, this third reviewer also recommended the inclusion of several secondary sources: (1) International Programme on Chemical Safety (IPCS)/World Health Organization (WHO) 1990 reference on the irritant effects reported in long-term occupational exposure studies in workers; (2) Dutch Expert Committee for Occupational Standards 1991 report summarizing MIBK toxicity; and (3) the ECETOC working group's 1987 joint assessment of commodity chemicals citing MIBK toxicity.

Response to comments: Additional studies regarding MIBK potentiation were incorporated into the assessment, as recommended by one reviewer. The NTP website was searched for the status of studies in progress, and it appears that the 2-year bioassay is not final. Dr. K. Mallin was contacted, and she was not aware of having conducted any mortality studies in steelworkers with specific exposures to MIBK. The remaining references that were also recommended by the third reviewer were looked into for possible inclusion in the toxicological assessment. However, as they were not found to add any new substantive information, these secondary references were not incorporated into the document.

B. Comment: For the RfD and RfC, has the most appropriate critical effect been chosen (i.e., that adverse effect appearing in a dose-response continuum)?

With respect to the RfD, two reviewers agreed that no oral RfD could be calculated due to the reasons cited by EPA. One reviewer agreed that the oral toxicity data were inadequate to determine a critical effect but suggested using a weight-of-evidence approach, whereby a combination of data from several studies is used to identify a critical effect and a minimal LOAEL.

With respect to the RfC, one reviewer agreed with EPA's selection of the critical effect for the RfC and concluded that increased hepatic cholesterol and hepatomegaly following subchronic inhalation exposures to MIBK are not clear indications of potential adverse effects and are more than likely adaptive responses. This reviewer went on to say that Raymond and Plaa (1995a, b) showed that oral administration of 6.8 mmol MIBK/kg in male rats resulted in induction of cytochrome P-450 and suggested that it is likely that the hepatomegaly observed after subchronic inhalation of MIBK was a reflection of an increase in cytochrome P-450, which is considered an adaptive response and not a critical event leading to potential adverse effects. The second reviewer, while also in agreement with EPA's conclusions that observed effects in the liver, kidney, and CNS are probably adaptive and should not be used as the critical effects following subchronic inhalation exposure, asserted that selection of an appropriate critical effect for the noncancer assessment is difficult. This reviewer concluded that the database on MIBK is somewhat diffuse and had ambiguities that preclude the selection of a reliable critical effect at all. The third reviewer recommended that EPA reconsider the critical effect chosen for the RfC. This third reviewer commented that the database for MIBK does not lend itself to the identification of a single critical effect from a single critical study and suggested pooling the results from several studies (and co-critical studies) in order to identify a NOAEL.

Response to comments: The identification of a critical effect from the existing database for MIBK is problematic. EPA is aware of this and provided extensive discussion relating to this issue**.** A constellation of effects from both subchronic inhalation and oral assays were suggestive of adverse changes in the kidney, liver, and CNS. These effects did occur at lower exposures levels than the critical effect selected (delayed ossification, reduced fetal body weight, and increased fetal death in mice and delayed ossification in rats). Because these effects did not show a clear toxicological continuum of severity and/or marked progression of response with increasing dose or any treatment-related corroborative gross pathologies or histopathological lesions, however, they were not considered to be clearly adverse and were therefore considered to be of uncertain relevance to effects in humans from chronic exposures. Therefore, as suggested by the third reviewer, the pooling of results from several studies in order to develop the RfC would not be appropriate in this instance. The developmental effects, while occurring at higher exposure levels than the effects from the subchronic inhalation studies, were considered to be clearly adverse and indicated a clear threshold for developmental effects. As a result, no changes were made to the assessment as a consequence of these comments.

C. Comment: Has the noncancer assessment been based on the most appropriate study? This study should present the critical effect in the clearest dose-response relationship. If not, what other study (or studies) should be chosen and why?

One reviewer agreed with the conclusions reached by EPA on the selection of the most appropriate study for the critical effect from the existing database for MIBK. The other two reviewers agreed with EPA that few of the several measured responses on the liver and kidney from both acute and subchronic oral and inhalation studies followed a clear dose-response relationship with clear, persistent toxicological and/or pathological effects and were therefore unsuitable for use as the critical effect; however, both reviewers did not agree with EPA's final selection of the critical effect. Both reviewers argued that the developmental effects did not show a clear, dose-response relationship because the effects occurred at the highest dose only. One reviewer argued that the developmental endpoints identified as critical effects, especially delayed ossification, may also be considered as adaptive, minimal, or of uncertain relevance to effects in humans, because no anatomical, pathological, or histological lesions were reported in any exposed fetuses. The other reviewer argued that the developmental effects (delayed ossification, reduced fetal body weight, and increased fetal death in mice and delayed ossification in rats) occurred at the highest dose only and in the presence of maternal toxicity (12% maternal death in the case of the mice) and were therefore secondary to maternal toxicity. This reviewer suggested that several studies (Phillips et al., 1987; David et al., 1999; and WIL Research Labs, 2000) be listed as co-critical and that a weight-of-evidence approach be used to identify a NOAEL and a LOAEL. Following this logic, this reviewer pooled the results of these studies and identified a $NOAEL_{HEC}$ of 185 mg/m^3 from the Phillips et al. (1987) study on the basis of minimal effects indicative of an effect on the liver and kidney, including increases in serum cholesterol and urinary glucose (males only).

Response to comments: EPA does consider delays in ossification as an adverse developmental effect. When evaluating the critical effect for MIBK, EPA used a weight-of-evidence approach and considered the totality of effects at the highest concentration as co-critical (delays in ossification, decreases in fetal body weight, and increased fetal death). Although there were signs of maternal toxicity (12% maternal mortality) in mice at that same concentration, the deaths occurred in three dams after the first exposure on gestation day 6 only; no further deaths occurred in that group, and no exposure-related deaths occurred in the other mouse or rat exposure groups. Furthermore, the neonates from those dams were not considered in the final evaluation. A constellation of developmental effects at the highest dose from the Tyl et al. (1987) study were considered by EPA to represent the most appropriate endpoints for use in the noncancer toxicological assessment of MIBK. As a result, no changes were made to the assessment as a consequence of these comments.

D. Comment: Are there other data that should be considered in developing the uncertainty factors (UFs) or the modifying factor? Do you consider that the data support use of different (default) values than those proposed?

One reviewer agreed with the UFs applied by EPA. A second reviewer had no pertinent comments. A third reviewer felt that the selection of UFs was appropriate, but that the issue of exposures to mixtures or interactions needed to be further addressed in the text of the toxicological review.

Response to comments: Further expansion of the discussions on potentiation and other interaction studies, as suggested by one reviewer (also see comment A), were made.

E. Comment: Do the confidence statements and the weight-of-evidence statements present a clear rationale and accurately reflect the utility of the studies chosen, the relevancy of the effects to humans, and the comprehensiveness of the data? Do these statements make sufficiently apparent all the underlying assumptions and limitations of these assessments? If not, what needs to be added?

One reviewer agreed with the confidence statements. A second reviewer had no pertinent comments. The third reviewer did not agree with the confidence statements because this reviewer did not agree with the selection of the critical effect and suggested that results from several studies (and co-critical studies) be pooled in order to identify a NOAEL. This same reviewer also suggested that the human data be given more weight in the selection of the RfC. This reviewer did not think it appropriate to calculate an RfC and then apply UFs to compensate for effects in humans seen at lower levels.

Response to comments: Although it is preferable to use human studies as the basis for the dose-response derivation, no studies were available that provided reliable MIBK exposure-response data in humans from chronic or subchronic inhalation exposures. Studies of workers exposed repeatedly to mixtures of solvents that included MIBK have associated various neuropathies and decrements in neurobehavioral performance tests with exposure. However, the results are not sufficient for establishing causality or characterizing an inhalation exposure-response relationship in humans because exposure levels for individual solvents were not reported and/or the degree to which MIBK contributed to the observed effects is uncertain. In the case of MIBK, the database included evidence of transient neurological effects in humans at acute exposure levels that were considerably lower than the $NOAEL_{HEC}$ of 1026 mg/m^3 in mice that was used to derive the RfC and were also lower than the lowest exposure level that showed neurological effects in animals from repeated inhalation exposures. However, the derived RfC of 3 mg/m^3 is more than thirty-fold lower than the acute exposure LOAEL of 100 mg/m^3 for subjectively reported irritation and neurological symptoms in humans and is lower than the NOAEL of 10 mg/m^3 for subjective irritation/neurological effects in humans. The human neurotoxicity data were not selected as the basis for the chronic RfC primarily because the exposure durations were very short. The relevance to effects after lifetime exposures in humans is unknown. As a result, UFs were applied to account for recognized uncertainties in the extrapolation from experimental data conditions to human scenarios.

F. Comment: Do you agree with the methods of analysis and the benchmark dose (BMD) methodology/calculations that were used to evaluate dose-response data for the chosen critical effects?

One reviewer agreed with the BMD methodology applied in order to derive an RfC. The second reviewer offered no pertinent comments. The third reviewer did not agree with the methodology used and was of the opinion that the critical effects selected were not suitable for use in BMD modeling. This third reviewer suggested using a NOAEL/LOAEL approach, with the critical effect being all of the developmental endpoints, to identify the highest exposure concentration from the critical study; in other words, that the totality of the developmental data from the critical study be used to identify a LOAEL rather than selecting a single endpoint for use in BMD modeling.

Response to comments: Upon re-evaluation of the methodology used in deriving the RfC, EPA agrees with the suggestion of using a NOAEL/LOAEL approach made by the third reviewer. The document was adjusted accordingly.

(2) Chemical-Specific Comments

A number of effects suggestive of adverse changes to the liver, kidney, and CNS were observed in animals following subchronic repeated oral and inhalation exposures at comparable or lower exposure levels than those that were associated with developmental effects (identified as the critical effects in a substantial database of subchronic inhalation studies), but these effects did not show a clear, toxicological continuun of severity and/or marked progression of response with increasing dose nor were there any treatment-related corroborative gross pathologies or histopathological lesions. In the absence of data from chronic-exposure studies, these effects were not considered to be clearly adverse and therefore were considered to be of uncertain relevance to effects in human from chronic exposures.

A. Comment: Do you agree with that conclusion? Is sufficient rational given to support that conclusion?

One reviewer agreed with the conclusions made by EPA that effects such as increases in serum cholesterol, increases in hepatic cholesterol, and hepatomegaly were adaptive responses and not considered as a critical event leading to potential adverse effects. This reviewer went on to say that, "there are no data that clearly show the changes in cholesterol status produced by MIBK exposure are detrimental to rats, in and of themselves...one can speculate about the consequences, but there are no definitive, detrimental results observed." The second reviewer was also in agreement with EPA that the observed effects on the liver, kidney, and CNS are probably adaptive and should not be used as the critical effects in the database of subchronic inhalation studies. The third reviewer contended that the database for MIBK does not lend itself to the identification of a single critical effect from a single critical study but thinks that a weight-of-evidence approach for liver, kidney, and CNS effects represents the critical effect(s). This reviewer went on by saying that, "...while some effects may be adaptive (e.g., hepatocellular

hypertrophy), this certainly cannot be said about all of the effects (e.g., increased serum cholesterol and urinary glucose, CNS effects lasting 8-10 weeks)...several effects are clearly minimal, especially in the absence of histopathology...however, a constellation of effects in the liver, kidney, and CNS represents the most appropriate endpoint for use in the noncancer risk assessment of MIBK."

Response to comments: All three reviewers essentially agreed with EPA's decision not to use data on the liver, kidney, or CNS as the basis for the critical effect, and essentially for the same reasons. The third reviewer did suggest pooling the results from these studies by using a weight-of-evidence approach. EPA does not believe in this particular instance that a weight-of-evidence approach using the existing database of effects from subchronic studies is appropriate for MIBK, because these effects (liver, kidney, and CNS) did not show a clear, toxicological continuum of severity and/or marked progression of response with increasing dose or show any treatment-related corroborative gross pathologies or histopathological lesions; as a result, they were not considered to be clearly adverse and therefore were considered to be of uncertain relevance to effects in humans from chronic exposures. For example, CNS-related effects and irritation were reported in animal studies, but they occurred only during exposure and, in the absence of histopathologies in nervous system tissues, were considered to be primarily acute responses with uncertain relevance to chronic effects in humans from long-term exposure to MIBK. Similarly, effects that may be associated with changes in the liver and kidney were also observed in animals but were lacking in any evidence of histopathology (for further discussion, see section 4.5.2). As a result, no changes were made to the assessment as a consequence of these comments.

OVERALL RECOMMENDATION

One reviewer stated that the document is acceptable with revisions. Two reviewers had some major comments pertaining to the benchmark modeling and the use of developmental effects in general. EPA agreed with the comments on the benchmark modeling and addressed the concerns about the use of developmental toxicity in deriving the RfC.

New References:

Kobusch, A.B., B. Bailey, and P. du Souich. (1987) Enzyme induction by environmental agents: effect on drug kinetics. In: Plaa, G.L., P. du Souich, and S. Erill, eds. Interactions Between Drugs and Chemicals in Industrial Societies. Amsterdam: Elsevier Science Publishers, pp. 29–42.

Krishnan, K., J. Brodeur, G.L. Plaa, and M. Charbonneau. (1992) Modulation of hexachlorobenzene-induced hepatic porphyria by methyl isobutyl ketone in the rat. Toxicol Letters 61:167-174.

Krishnan, K., J. Brodeur, P. du Souich, and G.L. Plaa. (1989) Influence of pretreatments with ketonic solvents on the methemoglobinemia (mHb) induced by N,N-dimethylaniline (DMA). Toxicologist 9:249 (Abstract 987).