

METHYL ISOBUTYL KETONE

CAS # 108-10-1

ORAL RISK ASSESSMENT DOCUMENT



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EXECUTIVE SUMMARY

Methyl Isobutyl Ketone– Oral Risk Assessment CAS # 108-10-1			
PARAMETER	LEVEL	UNITS	DERIVED
NOAEL (no-observed-adverse-effect level)	1,000	mg/kg-day	From a subchronic gavage study in rats.
Oral RfD (oral reference dose)	1	mg/kg-day	From a subchronic gavage study in rats with a 1,000x uncertainty factor.
TAC (total allowable concentration)	7	mg/L	From the oral RfD, for a 70 kg adult drinking 2 L/day with a default 20% Relative Source Contribution for drinking water.
SPAC (single product allowable concentration)	0.7	mg/L	From the TAC, assuming 10 drinking water sources of methyl isobutyl ketone.
STEL (short term exposure level)	100	mg/L	From a subchronic gavage study in rats adjusted for a 10 kg child drinking 1 L/day.
KEY STUDY	MAI (Microbiological Associates, Inc.). 1986. Subchronic toxicity of methyl isobutyl ketone in Sprague Dawley rats. Final Report. Study No. 5221.04. Performed by Microbiological Associates, Inc. for Research Triangle Institute. Unpublished report dated July 15, 1986.		
CRITICAL EFFECT	No effects considered adverse were observed after subchronic gavage exposure to methyl isobutyl ketone in rats.		
UNCERTAINTY FACTORS	• 10x for interspecies extrapolation • 10x for intraspecies extrapolation • 10x for extrapolation from a less-than-lifetime study to a lifetime exposure duration • 1x for extrapolation from a LOAEL to a NOAEL • 1x for database deficiencies. Therefore, the total uncertainty factor was 1,000x.		
TOXICITY SUMMARY	Limited data in humans were identified. Short-term, subchronic, reproduction, and developmental toxicity data via oral or inhalation exposure in laboratory animals were identified, along with a chronic inhalation bioassay in rats and mice. Subchronic gavage exposure in rats caused transient narcosis, liver and kidney weight increases, altered blood parameters, and renal nephropathy. Increased narcosis, liver and kidney weights and altered blood parameters were observed after subchronic inhalation of methyl isobutyl ketone. Maternal and fetotoxicity were observed in developmental inhalation studies. Two subchronic oral studies in rats were available, but a drinking water study evaluated an insufficient number of animals, used only one concentration, and included only females. Increased liver and kidney weights and nephropathy were observed without hepatic histopathology in a standardized subchronic gavage study in rats. The increased liver weight was likely an adaptive response to metabolizing high bolus doses of methyl isobutyl ketone. The renal effects after subchronic gavage were attributed to spontaneous nephropathy and/or alpha-2μ-globulin nephropathy, since they were limited to male rats. The transient narcosis observed after gavage but not after drinking water exposure at the same dose was attributed to bolus dosing. Further, narcosis was inconsistently observed after inhalation exposure and most observations of narcosis after subchronic oral, intraperitoneal or inhalation exposures were present only during the first few to several weeks of exposure, but not at study termination. Gestational inhalation exposure resulted in reduced fetal body weight in rats and mice, reduced ossification in rats, and increased fetal death in mice. The non-neoplastic and neoplastic lesions observed in male and female F344 rats and B6C3F1 mice chronically inhaling methyl isobutyl ketone were attributed to spontaneous nephropathy and/or alpha-2μ-globulin nephropathy, thus not considered relevant to humans and/or were within historical control ranges, thus not considered toxicologically significant. However, interpretation of the effects in male rats chronically inhaling methyl isobutyl ketone was confounded by reduced survival. The subchronic gavage study was selected as the key since it was a standardized study, and the oral route was preferred for derivation of an RfD, since there were no kinetic or metabolism data in humans which could be used to reduce route to route or interspecies extrapolation. The weight of genotoxicity evidence suggests that methyl isobutyl ketone is not genotoxic. Since no epidemiological studies in humans or chronic oral data in animals were identified, the <i>data are inadequate for an assessment of human carcinogenic potential</i> of methyl isobutyl ketone after oral exposure.		
CONCLUSIONS	Based on the concurrence of oral and inhalation data in laboratory animals, and on the uncertainty factors selected to account for interspecies extrapolation, intraspecies variability, and database deficiencies, the drinking water action levels developed in this risk assessment are protective of public health.		

3

1.0 INTRODUCTION

This document has been prepared to allow toxicological evaluation of the unregulated contaminant **methyl isobutyl ketone** in drinking water, as an extractant from one or more drinking water system components evaluated under NSF/ANSI 61 (2005), or as a contaminant in a drinking water treatment chemical evaluated under NSF/ANSI 60 (2005). Both non-cancer and cancer endpoints have been considered, and risk assessment methodology developed by the U.S. Environmental Protection Agency (U.S. EPA) has been used.

Non-cancer endpoints are evaluated using the reference dose (RfD) approach (Barnes and Dourson, 1988; Dourson, 1994; U.S. EPA, 1993; U.S. EPA, 2002), which assumes that there is a threshold for these endpoints that will not be exceeded if appropriate uncertainty factors (Dourson et al., 1996; U.S. EPA, 2002) are applied to the highest dose showing no significant effects. This highest dose is derived from human exposure data when available, but more often is derived from studies in laboratory animals. Either the no-observed-adverse-effect level (NOAEL) taken directly from the dose-response data, or the calculated lower 95% confidence limit on the dose resulting in an estimated 10% increase in response (the LED₁₀ or BMDL₁₀ from benchmark dose programs) can be used (U.S. EPA, 2003a). The lowest-observed-adverse-effect level (LOAEL) can also be used, with an additional uncertainty factor, although the benchmark dose approach is preferred in this case. The RfD is expressed in mg/kg-day. It is defined by the U.S. EPA as “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 effects during a lifetime” (Barnes and Dourson, 1988; U.S. EPA, 1993; U.S. EPA, 2005a).

NSF uses the RfD to derive three product evaluation criteria for non-cancer endpoints. The total allowable concentration (TAC), generally used to evaluate the results of extraction testing normalized to static at-the-tap conditions, is defined as the RfD multiplied by the 70 kg weight of an average adult assumed to drink two liters of water per day. A relative source contribution (RSC), to ensure that the RfD is not exceeded when food and other non-water sources of exposure to the chemical are considered, is also applied in calculating the TAC. The relative source contribution should be data derived, if possible. Alternately, a 20% default contribution for water can be used (U.S. EPA, 1991a). The TAC calculation is then as follows:

$$\text{TAC (mg/L)} = \frac{[\text{RfD (mg/kg-day)} \times 70 \text{ kg}] - [\text{total contribution of other sources (mg/day)}]}{2 \text{ L/day}}$$

or

$$\text{TAC (mg/L)} = \frac{\text{RfD (mg/kg-day)} \times 70 \text{ kg}}{2 \text{ L/day}} \times 0.2 \text{ (RSC)}$$

The single product allowable concentration (SPAC), used for water treatment chemicals and for water contact materials normalized to flowing at-the-tap conditions, is the TAC divided by the estimated total number of sources of the substance in the drinking water treatment and

1 distribution system. In the absence of source data, a default multiple source factor of 10 is used.
2 This accounts for the possibility that more than one product in the water and/or its distribution
3 system could contribute the contaminant in question to drinking water.
4

5 Finally, a short-term-exposure level (STEL), at a higher level than the TAC, may be calculated
6 for contaminants such as solvents expected to extract at higher levels from new product, but also
7 expected to decay rapidly over time. The STEL is calculated from the NOAEL or the LED₁₀ of
8 an animal study of 14- to 90-days duration, with uncertainty factors appropriate to the duration of
9 the study. The contaminant level must decay to a level at or below the TAC under static
10 conditions, or to a level at or below the SPAC under flowing conditions within 90 days, based on
11 the contaminant decay curve generated from over-time laboratory extraction data.
12

13 Endpoints related to cancer are evaluated using modeling to fit a curve to the appropriate dose-
14 response data (U.S. EPA, 1996a; U.S. EPA, 1999; U.S. EPA, 2003b; U.S. EPA, 2005b). If there
15 is sufficient evidence to use a non-linear model, the LED₁₀ or BMDL₁₀, divided by the
16 anticipated exposure, is calculated to give a margin of exposure. If there is insufficient evidence
17 to document non-linearity, a linear model drawing a straight line from the LED₁₀ or BMDL₁₀ to
18 zero, is used as a default. If a linear model (generally reflecting a genotoxic carcinogen) is used,
19 a target risk range of 10⁻⁶ to 10⁻⁴ is considered to be safe and protective of public health (U.S.
20 EPA, 1991a). For the purposes of NSF/ANSI 60 (2005) and 61 (2005), the TAC is set at the 10⁻⁵
21 risk level, and the SPAC is set at the 10⁻⁶ risk level. Use of a higher risk level is not ruled out,
22 but would generally require documentation of a benefit to balance the additional risk.
23

24 The RfD, TAC, SPAC, and STEL values derived in this document are based on available health
25 effects data and are intended for use in determining compliance of products with the
26 requirements of NSF/ANSI 60 (2005) and 61 (2005). Application of these values to other
27 exposure scenarios should be done with care, and with a full understanding of the derivation of
28 the values and of the comparative magnitude and duration of the exposures. These values do not
29 have the rigor of regulatory values, as data gaps are generally filled by industry or government
30 studies prior to regulation. Data gaps introduce uncertainty into an evaluation, and require the
31 use of additional uncertainty factors to protect public health.
32

33 The general guidelines for this risk assessment include those from the National Research Council
34 (NRC, 1983) and from The Presidential/Congressional Commission on Risk Assessment and
35 Risk Management (1997a, 1997b). Other guidelines used in the development of this assessment
36 may include the following: Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986),
37 Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996a), draft revised
38 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999), draft final Guidelines For
39 Carcinogen Risk Assessment (U.S. EPA, 2003b), Guidelines For Carcinogen Risk Assessment
40 (U.S. EPA, 2005a), Guidelines for Developmental Toxicity Risk Assessment (U.S. EPA, 1991b),
41 Guidelines for Reproductive Toxicity Risk Assessment (U.S. EPA, 1996b), Guidelines for
42 Neurotoxicity Risk Assessment (U.S. EPA, 1998), A Review of the Reference Dose and
43 Reference Concentration Process (U.S. EPA, 2002), Recommendations for and Documentation
44 of Biological Values for Use in Risk Assessment (U.S. EPA, 1988), and Health Effects Testing
45 Guidelines (U.S. EPA, 2006a).
46

The literature search strategy employed for this compound was based on the Chemical Abstract Service Registry Number (CASRN) and at least one common name. As a minimum, the following data banks were searched:

- ChemID Plus
- Registry of Toxic Effects of Chemical Substances (RTECS)
- Hazardous Substances Data Bank (HSDB)
- GENE-TOX
- Environmental Mutagen Information Center (EMIC)
- Developmental and Reproductive Toxicology (DART)
- TOXLINE – Core and Special
- TRI (Toxics Release Inventory)
- Chemical Carcinogenesis Research Information System (CCRIS)
- Medline (via PubMed)
- Integrated Risk Information System (IRIS)
- Syracuse Research Corporation Online Toxic Substance Control Act Database (TSCATS)
- Current Contents (as requested)

The literature search for this chemical was conducted on February 14, 2006. This document includes all relevant information retrieved as a result of that search.

2.0 PHYSICAL AND CHEMICAL PROPERTIES

Methyl isobutyl ketone is an aliphatic ketone with the following synonyms: 2-methyl-4-pentanone; 4-methyl-2-pentanone; 4-methylpentan-2-one; hexanone; hexone; isobutyl methyl ketone; isohexanone; isopropylacetone; methyl-2-pentanone; MIBK; MIK; Shell MIBK (Chemfinder, 2006; ChemIDPlus, 2006). It has the following structure, and physical and chemical properties listed in Table 1.



2.1 Organoleptic Properties

Methyl isobutyl ketone has a pleasant and sweet odor and taste (U.S. EPA, 2003c; WHO/IPCS, 1990).

Table 1. The physical and chemical properties of methyl isobutyl ketone

Property	Data	Reference
Empirical Formula	C ₆ H ₁₂ O	U.S. EPA, 2003c
CAS#	108-10-1	U.S. EPA, 2003c
Molecular Weight	100.16	U.S. EPA, 2003c
Physical State and Color	Colorless	U.S. EPA, 2003c
Melting Point	-84.7 °C	U.S. EPA, 2003c
Boiling Point	115.8 °C	U.S. EPA, 2003c
Density	0.8042 @ 20 °C	HSDB, 2005
Vapor Pressure	19.9 mmHg @ 25 °C	U.S. EPA, 2003c
Water Solubility	19 g/L @ 25 °C	U.S. EPA, 2003c
pK _a	Not located	-
n-Octanol/Water Partition Coefficient (log K _{ow})	1.31 (experimental)	U.S. EPA, 2003c
Henry's Law Constant (air/water partition)	1.38x10 ⁻⁴ atm-m ³ /mol	HSDB, 2006

3.0 PRODUCTION AND USE

3.1 Production

The commercial production of methyl isobutyl ketone involves acetone condensation, followed by catalytic hydrogenation (Johnson, 2004). In 1993, the United States alone produced 6.806 x 10⁷ kg (approximately 77 tons) of methyl isobutyl ketone (U.S. EPA, 2003c). Methyl isobutyl ketone is listed as a high production volume chemical in the European Union (OECD, 2001) and in the United States (U.S. EPA, 2006b).

3.2 Use

Methyl isobutyl ketone is used mainly as a solvent in cellulose-based and resin-based coating systems (U.S. EPA, 2003c). Methyl isobutyl ketone has been detected as an extractant from solvent-based coatings used in drinking water system components tested under NSF/ANSI 61 (2005). Methyl isobutyl ketone is also used as a separating agent for metals from solutions of their salts and in the mining industries to extract plutonium from uranium. Additionally, methyl isobutyl ketone is included in the formulation of pesticides and adhesives, and it is also used in wax/oil separation, leather finishing, textile coating, specialty surfactants for inks, as a denaturant for ethanol formulations, and in the production of rubber antioxidants (U.S. EPA, 2003c). Methyl isobutyl ketone also has limited use as a denaturant and solvent in cosmetic products (Johnson, 2004).

Methyl isobutyl ketone is currently permitted for use as a component of synthetic flavoring substances and adjuvants under Title 21 of the U.S. Code of Federal Regulations (21 CFR 172.515) and as a denaturant in alcohol and rum (27 CFR 21.161) at a maximum concentration of 4% (U.S. FDA, 2007).

4.0 ANALYTICAL METHODS

4.1 Analysis in Water

Methyl isobutyl ketone in water can be analyzed using gas chromatography with sample preparation by head-space sampling, liquid-liquid extraction, distillation, or stripping with an inert gas stream (WHO/IPCS, 1990). Methyl isobutyl ketone as an extractant from drinking water system components is determined by NSF International using a purge and trap gas chromatography method for volatile organic chemicals. The detection limit is 0.005 mg/L.

4.2 Analysis in Biological Matrices

Concentrations of methyl isobutyl ketone or its metabolites in the plasma and urine of human volunteers inhaling vapors of methyl isobutyl ketone have been measured using gas chromatography with or without mass spectrometric detection, an automated head-space air sampler and a capillary column, or flame ionization detection (DiVincenzo et al., 1976; Hjelm et al., 1990; Kawai et al., 2003). The limits of detection were not specified, with the exception of Hjelm et al. (1990), who reported a detection limit was 0.005 mmol/L (or 0.5 mg/L) using gas chromatography with an automated head-space air sampler and flame ionization detector.

Methyl isobutyl ketone in plasma, selected organs and tissues, and urine of laboratory rodents and/or guinea pigs has been measured following administration via gavage, inhalation, intraperitoneal injection, or epicutaneous injection. Gas chromatography with flame ionization or mass spectrometric detection was employed following extraction or other sample preparation (Johnson, 2004; DiVincenzo et al., 1976; Duguay and Plaa, 1995; Gingell et al., 2003; Granvil et al., 1994; Hjelm et al., 1991). The limits of detection were not specified.

5.0 SOURCES OF HUMAN AND ENVIRONMENTAL EXPOSURE

5.1 Sources of Human Exposure

Methyl isobutyl ketone exposure can occur through ingestion of fruits and meats that naturally contain this chemical (U.S. EPA, 2003c). Methyl isobutyl ketone is found in fruits, baked potatoes, cheese, milk, some meats, and some alcoholic beverages (WHO/IPCS, 1990), but the range of concentrations was not specified. The WHO/IPCS (1990) reported the following values: papaya, 8 µg/kg; beer, 10 to 120 µg/kg; and coffee, 6.5 mg/kg. Prolonged or repeated human exposure to methyl isobutyl ketone 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 used for wax/oil separation, leather finishing, textile coating, and surfactants for inks (U.S. EPA, 2003c).

5.2 Sources of Environmental Exposure

In the ambient atmosphere, methyl isobutyl ketone is expected to exist solely in the vapor phase with an estimated half-life of 27 hours (U.S. EPA, 2003c). Methyl isobutyl ketone is expected to have high mobility in soils, and volatilization from water surfaces is expected to be an important

fate process. Estimated volatilization half-lives for a model river and model lake are 9 hours and 6 days, respectively (U.S. EPA, 2003c).

6.0 COMPARATIVE KINETICS AND METABOLISM IN HUMANS AND LABORATORY ANIMALS

Kinetic and metabolism data for methyl isobutyl ketone have been critically reviewed by the U.S. EPA (2003e) and WHO/IPCS (1990). The following section relies on these reviews. Only recent publications that were not included in the review by the U.S. EPA (2003e) were fully reviewed by NSF International, and include Kawai et al. (2003) and Gingell et al. (2003).

Systemic absorption of methyl isobutyl ketone in rats after oral and inhalation exposure is rapid and dose-related (U.S. EPA, 2003c). The plasma elimination of methyl isobutyl ketone from blood of human volunteers after a two-hour inhalation exposure with light exercise was biphasic, with a half-life of 11 to 13 minutes during the first 30 minutes at exposures up to 200 mg/m³. The half-life in blood during the second elimination phase (60 and 180 minutes post-exposure) was 59 and 74 minutes. Methyl isobutyl ketone is likely to be widely distributed in the body, based on its rapid absorption into the bloodstream and on its blood and lipid solubility (U.S. EPA, 2003c). Concentrations of methyl isobutyl ketone and its principal metabolite, 4-hydroxy-4-methyl-2-pentanone, in rat plasma, liver, and lung tissue were positively dose-related after three daily oral or inhalation exposures. Methyl isobutyl ketone is rapidly metabolized in various tissues, including the brain. Total cumulative urinary excretion of methyl isobutyl ketone up to three hours post-exposure was dose-related in human volunteers (U.S. EPA, 2003c).

6.1 Absorption

6.1.1 Humans

The relative uptake of methyl isobutyl ketone from the gastrointestinal tract of humans has not been quantified (U.S. EPA, 2003c). According to the U.S. EPA (2003e), relative uptake of inhaled methyl isobutyl ketone ranged from 56.3-61.7% and was not related to exposure level in eight male volunteers inhaling 10 to 200 mg/m³ (unspecified purity) for two hours during light exercise. Total respiratory uptake during exposure was linearly related to exposure level (Hjelm et al., 1990).

6.1.2 Laboratory Animals

According to the U.S. EPA (2003e), plasma concentrations of methyl isobutyl ketone were 5.3, 8.4, or 16.1 µg/mL in rats one hour after the last of three daily gavage exposures to 1.5, 3.0, or 6.0 mmol/kg (purity 99.5% and reportedly equal to 150, 300, or 601 mg/kg-day). This finding indicated rapid and exposure related absorption into the bloodstream following oral dosing (Duguay and Plaa, 1995). The relative uptake of methyl isobutyl ketone from the gastrointestinal tract in animals has not been quantified (U.S. EPA, 2003c). Effects observed in laboratory animals following oral exposure to methyl isobutyl ketone provide qualitative evidence that it was absorbed from the gastrointestinal tract in toxicologically relevant quantities (U.S. EPA, 2003c).

In a study by Gingell et al. (2003), male CD rats were administered a single gavage dose of 500 mg/kg methyl isobutyl ketone (> 99.8% purity) in corn oil. This dose was in the range of doses used in previous oral metabolism studies of methyl isobutyl ketone. The number of animals was not specified, but may have been 13 since 26 rats were used in the study, which also included administration of methyl isobutyl carbinol. Blood samples were collected at various intervals up to 12 hours after dosing and concentrations of the parent compound and two metabolites, methyl isobutyl carbinol and 4-hydroxy-4-methyl-2-pentanone, in the blood were measured using gas chromatography/mass spectrometry. The study authors concluded that methyl isobutyl ketone was rapidly absorbed, since the peak plasma concentration ($C_{\max}$) of 0.644 mmol/L was reached 15 minutes after dosing.

According to the U.S. EPA (2003e), plasma methyl isobutyl ketone concentrations were 5.0, 8.1, and 14.3 $\mu\text{g/mL}$ in rats immediately following the last of three daily four-hour inhalation exposures to 200, 400, or 600 ppm methyl isobutyl ketone (819, 1,639, or 2,458 mg/m^3), indicating rapid and exposure related respiratory absorption into the bloodstream (Duguay and Plaa, 1995). Inhalation of 200, 400, or 600 ppm methyl isobutyl ketone for four hours in rats resulted in absorption of the same amount of methyl isobutyl ketone as from the oral administration of 1.5, 3.0, or 6.0 mmol/kg (equivalent to 150, 300, or 601 mg/kg-day), respectively (U.S. EPA, 2003c).

The U.S. EPA (2003e) reported that the dermal uptake rate in guinea pigs topically exposed to methyl isobutyl ketone peaked 10-45 minutes after the onset of a 150-minute exposure, and the maximum uptake rate averaged 1.1 $\mu\text{mol/min/cm}$ (Hjelm et al., 1991).

6.2 Distribution

6.2.1 Humans

According to the U.S. EPA (2003e), methyl isobutyl ketone 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 partition coefficients for methyl isobutyl ketone of 90 and 926, respectively, indicate significant blood and lipid solubility (Sato and Nakajima, 1979). Methyl isobutyl ketone partitions approximately equally between red blood cells and plasma in rat and human blood. In plasma, methyl isobutyl ketone is associated primarily with proteins rather than being dissolved in plasma water (Lam et al., 1990). High lipid solubility indicates that methyl isobutyl ketone may partition rapidly to lipid-rich tissues, such as nervous tissue.

According to the U.S. EPA (2003e), plasma elimination of methyl isobutyl ketone from blood, following a two-hour inhalation exposure in eight male volunteers undergoing light exercise, was biphasic, with a half-life of 11-13 minutes during the first 30 minutes post-exposure in subjects exposed to methyl isobutyl ketone (purity unspecified) at 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 male volunteers exposed to 100 and 200 mg/m^3 , respectively. Blood methyl isobutyl ketone levels were too low to permit the calculation of blood elimination half-lives in subjects exposed to 10 mg/m^3 .

6.2.2 Laboratory Animals

The U.S. EPA (2003e) reported that concentrations of methyl isobutyl ketone 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 three daily oral exposures up to 600 mg/kg-day or inhalation exposures up to 600 ppm (Duguay and Plaa, 1995). Methyl isobutyl ketone accumulated rapidly in brain tissue of mice that received a single intraperitoneal dose of 5 mmol/kg (approximately 500 mg/kg assuming 100% purity) methyl isobutyl ketone in corn oil, 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.

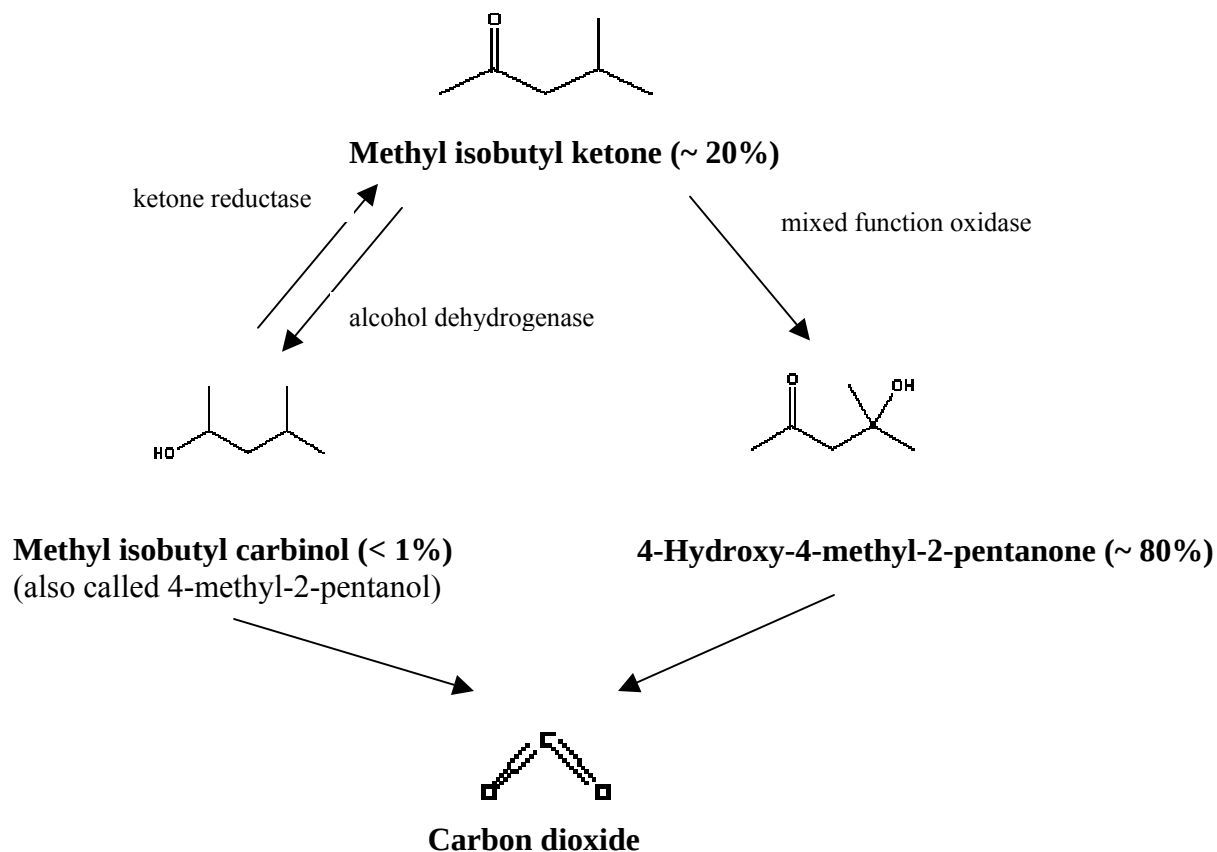
According to the U.S. EPA (2003e), the plasma elimination half-life of methyl isobutyl ketone in guinea pigs that were administered a single 450 mg/kg intraperitoneal dose was estimated to be 66 minutes, based on single blood samples collected from various guinea pigs up to 16 hours post-dosing (DiVincenzo et al., 1976). Within 16 hours, 4-Hydroxy-4-methyl-2-pentanone was cleared from the blood and only a trace amount of a second metabolite, 4-methyl-2-pentanol, was detected at any time.

Methyl isobutyl ketone was completely eliminated from the blood of mice within 90 minutes of injection of a single intraperitoneal dose of methyl isobutyl ketone (purity unspecified in corn oil) at 5 mmol/kg, which was approximately equivalent to 500 mg/kg, assuming 100% purity (Granvil et al., 1994). The blood concentration of 4-hydroxy-4-methyl-2-pentanone peaked at 60 minutes post-dosing and decreased at study termination of 90 minutes post-dosing.

6.3 Metabolism

6.3.1 Humans

According to the U.S. EPA (2003e), Hjelm et al. (1990) evaluated the metabolites, 4-methyl-2-pentanol and 4-hydroxy-4-methyl-2-pentanone, in the urine of human volunteers, since these metabolites had been reported in the blood of guinea pigs (DiVincenzo et al., 1976). Within a three-hour post-exposure period, neither metabolite was detected at a detection limit of 5 nmol/L. Blood levels of these potential metabolites were not quantified. Hjelm et al. (1990) suggested that the detection of methyl isobutyl ketone metabolites in the blood of guinea pigs, but not in the urine of humans, could be due to the lower dose of methyl isobutyl ketone used humans or to unspecified qualitative and/or quantitative differences in metabolism of methyl isobutyl ketone between man and guinea pig. Hjelm et al. (1990) further suggested that urinary excretion of the metabolites may be delayed and the three-hour post-exposure period may have been too short to permit detection of the metabolites in the urine. Hjelm et al. (1990) suggested that 4-methyl-2-pentanol (also called methyl isobutyl carbinol) and 4-hydroxy-4-methyl-2-pentanone may either undergo further metabolism to be eliminated as CO₂ via the lungs in humans, or intermediate metabolites may be stored in tissues. The authors of this risk assessment proposed part of the metabolic scheme depicted in Figure 1 based on Gingell et al. (2003) and Hjelm et al. (1990).



12 **Figure 1. Proposed metabolic scheme for methyl isobutyl ketone**
 13 **(based on Gingell et al., 2003; Hjelm et al., 1990)**

14 6.3.2 Laboratory Animals

17 The U.S. EPA (2003e) reported that DiVincenzo et al. (1976) identified 4-methyl-2-pentanol and
 18 4-hydroxy-4-methyl-2-pentanone as methyl isobutyl ketone metabolites in the blood of guinea
 19 pigs. Vézina et al. (1990) found that either single or repeated oral doses of methyl isobutyl
 20 ketone induced significant increases in hepatocellular cytochrome P-450 content and the hepatic
 21 activities of aniline hydroxylase and 7-ethoxycoumarin O-deethylase in rats, suggesting that the
 22 liver is involved in the metabolism of methyl isobutyl ketone. Similarly, Brondeau et al. (1989)
 23 reported increased hepatic cytochrome P-450 content and glutathione-S-transferase activity in
 24 rats, but not in mice inhaling a single dose of methyl isobutyl ketone. Hepatic total cytochrome
 25 P-450 concentrations were significantly increased in New Zealand male rabbits treated orally
 26 with 5 mmol methyl isobutyl ketone/kg (equivalent to 500 mg/kg) daily for three days (Kobusch
 27 et al., 1987). Furthermore, hepatic mixed-function oxidase activities of aminopyrine N-
 28 demethylation, 7-ethoxycoumarin dealkylation, and aniline hydroxylation were increased
 29 significantly.

Raymond and Plaa (1995) showed that oral administration of 6.8 mmol/kg methyl isobutyl ketone (678 mg/kg, based on reported 99.5% purity) in male Sprague-Dawley rats resulted in induction of cytochrome P-450. WHO/IPCS (1990) reported that there was a significant increase in aniline hydroxylase activity and cytochrome P-450 content in the liver of five hens that continuously inhaled vapors of methyl isobutyl ketone (purity unspecified) at 4,100 mg/m³ (1,000 ppm) for 50 days (Abou-Donia et al., 1985; Brondeau et al., 1989). In rats that inhaled vapors of methyl isobutyl ketone (purity unspecified) from 2,440 to 12,400 mg/m³ (595 to 3,020 ppm) for an unspecified timeframe, increased liver cytochrome P-450 content and glutathione- S-transferase activity were observed (WHO/IPCS, 1990).

In a study by Gingell et al. (2003), male CD rats were administered a single gavage dose of 500 mg/kg methyl isobutyl ketone (> 99.8% purity) in corn oil. This dose was in the range of doses used in previous oral metabolism studies for methyl isobutyl ketone. The number of animals was not specified, but may have been 13 since 26 rats were used in the study, which also included administration of methyl isobutyl carbinol. Blood samples were collected at various intervals up to 12 hours after dosing, and concentrations of the parent compound and two metabolites, methyl isobutyl carbinol and 4-hydroxy-4-methyl-2-pentanone, were measured using gas chromatography/mass spectrometry.

The study authors detected only three chemicals in the plasma, which included methyl isobutyl ketone and the two metabolites: methyl isobutyl carbinol and 4-hydroxy-4-methyl-2-pentanone. The major metabolite in the blood was 4-hydroxy-4-methyl-2-pentanone, based on a peak plasma concentration (C_{max}) of 2.03 mmol/L (approximately 80% of the administered dose), which was reached 9 hours after dosing. Methyl isobutyl carbinol was detected in the blood at less than 1% of the administered dose (C_{max} of 0.014 mmol/L). The study authors proposed part of the metabolic scheme depicted in Figure 1.

6.4 Elimination/Excretion

6.4.1 Humans

According to the U.S. EPA (2003e), total cumulative urinary excretion of methyl isobutyl ketone up to three hours following a two-hour inhalation exposure was concentration-related in eight male volunteers inhaling vapors of methyl isobutyl ketone (purity unspecified) from 10 to 200 mg/m³ while undergoing light exercise. Total cumulative urinary excretion was 0.04 µmol at 10 mg/m³ and 1.21 µmol at 200 mg/m³ (Hjelm et al., 1990).

Kawai et al. (2003) measured the concentration of methyl isobutyl ketone in the urine of 27 furniture plant volunteers (19 men and eight women) inhaling workplace air that contained various solvents including methyl isobutyl ketone. Eleven unexposed controls (six men and five women) were also included for baseline measurements. The other solvents in the air included: methyl ethyl ketone, acetone, toluene, three xylene isomers, and ethyl benzene. Urine samples were collected from the volunteers after a single eight-hour exposure. The eight-hour time weighed average concentration was 1.8 ppm (arithmetic mean) or 0.7 ppm (geometric mean). The study authors reported that an average of ~ 0.12% of the methyl isobutyl ketone dose absorbed through the lungs was excreted as methyl isobutyl ketone in the urine.

6.4.2 Laboratory Animals

No studies examining the potential for urinary, respiratory, and/or fecal elimination or excretion of methyl isobutyl ketone in laboratory animals were identified.

6.5 Physiologically-Based Pharmacokinetic Models

According to the U.S. EPA (2003e), there are no toxicokinetic models currently available for methyl isobutyl ketone.

6.6 Conclusions Regarding Comparative Kinetics and Metabolism

No kinetic data in humans after oral exposure to methyl isobutyl ketone were identified for a comparison to the oral data identified in laboratory animals. Further, although a human blood/air partition coefficient of 90 for methyl isobutyl ketone inhalation has been reported (Sato and Nakajima, 1979), no animal blood:air partition coefficients were located, and sufficient comparative data in humans and laboratory animals could not be identified to determine the fraction of the inhaled dose absorbed after inhalation exposure to methyl isobutyl ketone.

7.0 EFFECTS ON HUMANS

7.1 Case Reports

No case reports were located related to oral exposure of humans to methyl isobutyl ketone. According to the U.S. EPA (2003e), a 16-year-old male presented with polyneuropathy approximately eight weeks after he had used spray paint containing methyl ethyl ketone and methyl isobutyl ketone several times in a closed space (AuBuchon et al., 1979). The polyneuropathy was characterized by a burning paresthesias in the extremities and acute segmental demyelination of the sural nerve with Schwann cell hyperplasia

Grober and Schaumburg (2000) reported that occupational exposure to methyl isobutyl ketone during a six-year period of repeated high exposures in a 44-year-old male caused lasting impairment of memory. The patient worked in a poorly ventilated indoor solvent extraction facility, where he was exposed for eight hours daily to ambient vapors of methyl isobutyl ketone in excess of 100 ppm (410 mg/m³). During this phase, he also climbed into a large chamber and became drenched in solvent several times each day. Neuropsychological tests administered six times over 10 years demonstrated a stable pattern of cognitive impairment. Dynamic imaging studies suggested persistent central nervous system dysfunction. The study authors concluded that chronic, high-level, occupational methyl isobutyl ketone exposure could cause a persistent cognitive syndrome best explained by impaired working memory.

7.2 Epidemiological Studies

Several studies of occupational exposure to solvent vapor mixtures that included methyl isobutyl ketone have reported various neurological effects (U.S. EPA, 2003c). Acute neurological symptoms and effects on neurobehavioral tests were observed after occupational inhalation (Tsai

et al., 1997; Valciukas et al., 1985). These publications were not further reviewed, since the possible synergistic effect of the other solvents in the mixture could not be fully ascertained.

7.2.1 Controlled Exposure Studies

According to the U.S. EPA (2003e), four acute inhalation studies with methyl isobutyl ketone in human volunteers indicated transient sensory irritation, neurological effects, and/or detection of a strong odor during exposure (Dick et al., 1992; Esso Research and Engineering Company, 1965; Hazleton Laboratories, Inc., 1965; Hjelm et al., 1990; Iregren et al., 1993).

8.0 EFFECTS ON LABORATORY ANIMALS AND *IN VITRO* TEST SYSTEMS

Short-term, subchronic, reproduction, and developmental toxicity data via oral or inhalation exposure in laboratory animals were identified. Subchronic gavage exposure in animals caused transient narcosis after dosing, liver and kidney weight increases, altered blood parameters, and renal nephropathy. Subchronic inhalation data also identified transient narcosis, liver and kidney weight increases, and altered blood parameters. Two subchronic oral studies were available. However, a drinking water study evaluated an insufficient number of animals and used a single dose level administered exclusively to females. In a standardized subchronic gavage study, liver and kidney weight increases, altered blood parameters, and nephropathy were observed in the absence of hepatic histopathology. Reduced fetal body weight in rats and mice, reduced ossification in rats, and increased fetal death in mice were observed after gestational inhalation exposure in the presence of maternal toxicity, which included reduced body weight and transient lethargy during exposure.

The weight of evidence from available *in vivo* and *in vitro* genotoxicity data suggests that methyl isobutyl ketone is not genotoxic. A chronic inhalation bioassay in rats and mice was available. After F344 rats inhaled methyl isobutyl ketone vapors for two years, the NTP (2006) concluded that the non-neoplastic renal lesions observed were characteristic of α -2u-globulin accumulation in male rats and nephropathy in female rats. Further, the NTP (2006) considered there to be *some evidence of carcinogenic activity* of methyl isobutyl ketone in male rats, based on the incidence of renal tubule tumors. The NTP (2006) considered that the incidence of mononuclear cell leukemia in high-dose males may have been treatment-related, and considered there to be *equivocal evidence of carcinogenic activity* in female rats based on two renal mesenchymal tumors at the high dose, but none in controls. After B6C3F1 mice inhaled methyl isobutyl ketone vapors for two years, the NTP (2006) concluded that there was *some evidence of carcinogenic activity* of methyl isobutyl ketone in male and female mice based on the incidences of hepatocellular adenomas and carcinomas.

8.1 Limited-Exposure Effects

8.1.1 Irritation and Sensitization Studies

Immediate signs of nasal irritation were observed in guinea pigs inhaling methyl isobutyl ketone at 16,800 ppm, while little or no irritation was observed at 1,000 ppm (HSDB, 2006; CMA, 1981). A single 10-hour occluded application of neat methyl isobutyl ketone to the skin of

1 rabbits produced only transient erythema, which occurred immediately after the application and
2 persisted for up to 24 hours. Daily applications of 10 mL on 10 cm² skin for seven days caused
3 drying and flaking of the skin surface (WHO/IPCS, 1990; Krasavage et al., 1982). When 500
4 mg methyl isobutyl ketone was applied to the skin of rabbits, moderate irritation was observed
5 after 24 hours (HSDB, 2006; RTECS, 2005). No further details were reported.

6 7 **8.1.2 Ocular Exposure Studies** 8

9 Immediate signs of eye irritation were observed in guinea pigs inhaling methyl isobutyl ketone at
10 16,800 ppm, while little or no irritation was observed at 1,000 ppm (HSDB, 2006; CMA, 1981).
11 A 0.1 mL solution of neat methyl isobutyl ketone (purity unspecified) produced some irritation
12 within 10 minutes after instillation in the rabbit eye (WHO/IPCS, 1990; Krasavage et al., 1982).
13 Inflammation and swelling occurred within eight hours, and inflammation, swelling, and exudate
14 were present at 24 hours, but the symptoms disappeared by 60 hours. The RTECS (2005)
15 database included information that 40 mg methyl isobutyl ketone instilled into the eyes of rabbits
16 produced severe irritation. No further details were reported.

17 18 **8.2 Single-Exposure Studies** 19

20 **8.2.1 Oral Exposure** 21

22 Oral LD₅₀ values for methyl isobutyl ketone of 2,080 to 5,700 mg/kg in the rat, 1,500 to 1,900
23 mg/kg in the mouse, and 1,600 to 3,200 mg/kg in the guinea pig have been reported
24 (ChemIDPlus, 2006; Johnson, 2004). Some of the values were reported in mL/kg, and thus were
25 converted to mg/kg assuming 100% purity, since purity was not specified.

26 27 **8.2.2 Other Exposure Routes** 28

29 Several studies investigating the acute intraperitoneal, intravenous, inhalation, and/or dermal
30 toxicity of methyl isobutyl ketone in rats, mice, rabbits, guinea pigs, cats, and/or dogs have been
31 reviewed by Johnson (2004) and/or CMA (1981). Only the studies reporting LD₅₀ or LC₅₀
32 values are reported here (Eastman Kodak, 1982; Zakhari et al., 1977). The remaining acute
33 toxicity studies were not further reviewed, since they did not report LD₅₀ values and their
34 relevance to the determination of an oral RfD for methyl isobutyl ketone were limited (Batyrova,
35 1973; McOmie and Anderson, 1949; Smyth et al., 1951; Specht et al., 1940). According to
36 Johnson (2004), the intraperitoneal LD₅₀ for methyl isobutyl ketone is 590 mg/kg in mice, 1,140
37 mg/kg in rats, and 919 mg/kg in guinea pigs (Eastman Kodak, 1982; Zakhari et al., 1977). Some
38 of the values were reported in mL/kg, and thus were converted to mg/kg assuming 100% purity,
39 since purity was not specified. The LC₅₀ for methyl isobutyl ketone is 74 mg/L (approximately
40 300 mg/m³) in mice (Zakhari et al., 1977).

8.3 Short-Term Exposure Studies

8.3.1 Oral Exposure

In a range-finding study, methyl isobutyl ketone (purity unspecified) was administered to three 4-week-old female HLA Wistar rats per dose at 0.5 or 1% in their drinking water for seven days (Carnegie-Mellon Institute of Research, 1977a; 1983). Two groups of three rats each served as controls, but the rationale for two control groups was not specified. The study authors estimated these doses to be equivalent to 396 and 652 mg/kg-day, based on measured body weights and water consumption. Mortality and clinical signs were monitored. Food and water consumption were measured. Body weight was measured. Gross necropsies were conducted on unspecified organs.

At 396 mg/kg-day, pale and/or mottled kidneys were observed in all three of the treated rats and all three of the control rats in one control group and two control rats in the other control group.

At 652 mg/kg-day, a statistically significant reduction in mean body weight gain of 53% and 67%, compared to the first and second control group, respectively, was observed. The study authors considered this effect to be reflective of the 16% and 13% (mean of both control groups) reductions in mean water and food intake, respectively. Further, the study authors considered the reduced water intake to be related to palatability. Pale or mottled kidneys were observed in two of three treated rats but not in either control group.

According to Johnson (2004), a Russian-language study by Batyrova (1973) reported that the administration of increasing oral doses of methyl isobutyl ketone (unspecified purity) as an emulsion in a 2% starch solution resulted in the death of 9 of 10 mice by Day 24 of dosing. The first deaths were noted on Day 8 after a total dose of 3,820 mg/kg. In most of the animals, severe clonic-tonic spasms occurred prior to death, and the mean administered lethal dose was 9,350 mg/kg. No further details were reported.

8.3.2 Inhalation Exposure

Six F344 rats per sex and dose inhaled methyl isobutyl ketone vapors (> 99% purity) in whole-body chambers at mean measured concentrations of 0, 101, 501, or 1,996 ppm (0, 410, 2,053, or 8,178 mg/m³) six hours/day, five days/week, for approximately two weeks (Phillips et al., 1987; Bushy Run Research Center, 1982). The first five days and the remaining four days of exposure were separated by a two-day non-treatment period. Mortality and clinical signs were monitored daily and body weights were measured on the first, fifth, seventh and ninth exposure days. Kidney, heart, liver, lung, and testes weights were measured at study termination and relative organ-to-body weight ratios were calculated. Gross necropsies were conducted on all rats, and targeted histopathological examinations--which included the nasal cavity, trachea, liver, kidneys, and lungs--were conducted on all control and high-dose rats. The liver and kidneys were also examined in low- and mid-dose rats.

At 501 ppm, a statistically significant increase in mean relative liver weight (8%) compared to controls was observed in male rats and hyaline droplet formation was observed in the kidneys of all mid-dose male rats but none of the controls.

At 1,996 ppm, lethargy (first exposure day only) and lacrimation (exposure days one and six) were observed at a scattered (unspecified) incidence. Increased mean absolute (28% in males) and relative (36% in males, 8% in females) liver weights were observed and increased mean absolute (13%) and relative (11%) kidney weights were observed in male rats. Hyaline droplet formation and epithelial regeneration of proximal convoluted tubules were observed in the kidneys of all high-dose male rats but in none of the controls or 1/6 of the controls, respectively. An increased number (qualitative assessment only) of mitotic figures was observed in the liver of 2/6 males and 1/6 females. The study authors considered the low dose of 101 ppm as the NOEL, but did not identify a NOAEL.

Six B6C3F1 mice per sex and dose inhaled mean measured methyl isobutyl ketone vapors (> 99% purity) in whole-body chambers at 0, 101, 501, or 1,996 ppm (0, 410, 2,053, or 8,178 mg/m³) six hours per day and five days per week for approximately two weeks (Phillips et al., 1987; Bushy Run Research Center, 1982). The first five days and the remaining four days of exposure were separated by a two-day non-treatment period. Mortality and clinical signs were monitored daily and body weights were measured on the first, fifth, seventh and ninth exposure days. Kidney, heart, liver, lung, and testes weights were measured at study termination and relative organ-to-body weight ratios were calculated. Gross necropsies were conducted on all mice, and targeted histopathological examinations,--which included the nasal cavity, trachea, liver, kidneys, and lungs-- were conducted on all control and high-dose mice. The liver and kidneys were also examined in low- and mid-dose mice.

At 1,996 ppm, lethargy (second and fifth exposure days only) was observed at a scattered (unspecified) incidence. Increased mean absolute (12%) and relative (13%) liver weights and absolute (8%) kidney weight in female mice and decreased mean relative (10%) kidney weight in male mice were observed. Hepatic glycogen depletion was observed in 4/6 female mice and increased hepatic mitosis was observed in 1/6 female mice. The study authors did not identify a NOEL or NOAEL.

According to the U.S. EPA (2003e), in an unpublished study by Hazleton Laboratories, Inc. (1968; 1966), 10 young adult albino CD-derived rats per sex and dose inhaled vapors of methyl isobutyl ketone (purity unspecified) at 0 or 0.75 mg/L (180 ppm or 750 mg/m³) six hours per day and five days per week for two weeks. Mortality and clinical signs were monitored. Body weights were measured. Lung, liver, kidney, adrenal, and spleen weights were measured at study termination. Histopathology--including the lung, liver, kidney, adrenal, and spleen-- was conducted in three rats per sex. Increased mean relative liver weights of an unspecified magnitude were observed in male rats in the absence of hepatic histopathology.

According to Johnson (2004) and the U.S. EPA (2003e), reporting on an unpublished study by Hazleton Laboratories, Inc. (1966; 1968; 1992), 10 young adult albino CD-derived rats per sex and dose inhaled vapors of methyl isobutyl ketone (purity unspecified) at 0 or 4.53 mg/L (1,105 ppm or 4,530 mg/m³) six hours per day and five days per week for four weeks. Mortality and clinical signs were monitored. Body weights were measured. Lung, liver, kidney, adrenal, and

spleen weights were measured at study termination. Hematology and gross necropsies (brain, pituitary, trachea, thyroid, parathyroid, lungs, heart, liver, spleen, stomach, duodenum, large intestine, small intestine, adrenals, kidneys, urinary bladder, and gonads) were conducted. Histopathology--including the lung, liver, kidney, adrenal, and spleen--was conducted in three rats per sex.

Exposed females had a significant increase in mean relative adrenal weights (unspecified magnitude), a 1.4% increase in lymphocytes, and a 1.0% decrease in segmented neutrophils compared to controls. Cytoplasmic eosinophilic droplets in the proximal convoluted tubule epithelium were observed in two of the three exposed males but not in the controls. No further details were provided.

In an unpublished range-finding study by Wright-Patterson Air Force Base Aerospace Medical Research Laboratory (MacEwen et al., 1971), four monkeys, eight dogs, 40 mice, and 50 rats inhaled mean concentrations of methyl isobutyl ketone (unspecified purity) in whole-body chambers at 0 or 100 ppm continuously for two weeks. Control groups consisted of three monkeys, four dogs, 20 mice, or 25 albino rats. Although the sex of the animals evaluated was not specified, they were assumed to be all males, since this was a range-finding study for a subchronic study (MacEwen et al., 1971), in which all the animals evaluated were males. Mortality and clinical signs were monitored regularly. At study termination, body weight and heart, lung, liver, spleen, and kidney weights were recorded, and organ-to-body weight ratios were calculated. Hematology, clinical chemistry, and urinalysis were conducted. Electroencephalographic measurements were conducted on all animals, and one monkey per group had cortical electrodes implanted to evaluate central nervous system effects. Gross necropsies and histological examinations were conducted on unspecified organs. Only effects in rats were observed, and thus, the experiment was repeated at 200 ppm methyl isobutyl ketone in rats.

At 100 ppm, a slight decrease in mean body weight (unspecified magnitude and statistical significance) and significant increases in mean absolute (12%) and relative (8%) kidney weights were observed in treated rats compared to controls. At 200 ppm, statistically increased mean absolute (68%) and relative (16%) kidney and absolute (9%) and relative (7%) liver weights, and increased mean relative (4%) heart weights were observed. Nephrosis of the proximal tubules was observed in the kidneys of an unspecified number of rats at both 100 and 200 ppm.

According to Johnson (2004), when 10 mice inhaled air saturated with methyl isobutyl ketone vapor at 74-98 mg/L for 15 to 20 minutes each day, six mice died. Deaths occurred on post-inhalation Days 1, 6, and 9 (McOmie and Anderson, 1949). The original unpublished study was not available and no further details were provided.

CMA (1981) also reported that the inhalation of methyl isobutyl ketone vapors at 20,000 ppm for 20 minutes per day for 15 days resulted in the death of six out of 10 mice. No further details were provided.

8.3.3 Dermal Exposure

Johnson (2004) reviewed an unpublished study by McOmie and Anderson (1949) in which seven applications of neat methyl isobutyl ketone (purity unspecified) at 3 mL/kg each were made for durations between five to 12 hours to a 100-cm² area of shaved skin on each of two rabbits. The exposures occurred over a period of 15 to 21 days. Assuming 100% purity, each rabbit received approximately 3,000 mg/kg methyl isobutyl ketone. At microscopic examination, there were no pathologic changes in the internal organs. Local changes in the skin consisted of polymorphonuclear infiltration in the upper dermis. Hair follicles appeared normal, and there was no evidence of sloughing of keratin. No systemic effects were noted. No further details were reported.

8.4 Long-Term and Chronic Exposure Studies

8.4.1 Subchronic Studies

8.4.1.1 Oral

In an unpublished study by MAI (1986), 30 six-week old Sprague-Dawley rats per sex and dose were administered methyl isobutyl ketone (purity unspecified) in corn oil via gavage at 0, 50, 250, or 1,000 mg/kg-day for 13 weeks. The dose selection was based on a preliminary 14-day study in which the highest dose of 1,000 mg/kg-day produced mean body weight decreases of less than 10%. Food and water were available *ad libitum*. Mortality was monitored daily and clinical signs were monitored weekly. Body weight and food consumption were measured weekly. Ophthalmological examinations were conducted before the start of exposure and at study termination. Heart, liver, spleen, brain, kidney, testes, ovary, adrenals, thyroid, and parathyroid weights were recorded at study termination. Interim (Week 7) and terminal (Week 14) hematology (hematocrit, hemoglobin, erythrocytes, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, total and differential leukocyte counts, prothrombin time, activated partial thromboplastin time, and reticulocyte counts), clinical chemistry (alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, lactate dehydrogenase, blood urea nitrogen, globulin, albumin/globulin ratio, glucose, direct and total bilirubin, total cholesterol, total serum protein, carbon dioxide, calcium, chloride, potassium, and sodium), and urinalysis (appearance, protein, bilirubin, urobilinogen, glucose, ketones, pH, specific gravity, color, volume, and microscopic appearance) were conducted on 10 rats per sex and dose. Gross necropsies were conducted on all rats at study termination. Histopathological examinations were performed on all control and high-dose rats, and included: adrenals, aorta, bone and bone marrow, brain, cecum, colon, duodenum, esophagus, eye, heart, ileum, jejunum, kidneys, liver, lung, lymph nodes, mammary glands, skeletal muscle, sciatic nerve, ovaries, pancreas, pituitary, prostate, salivary gland, spinal cord, spleen, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus. Renal histology was also evaluated in mid-dose rats. This study was included in the reviews by Johnson (2004), the U.S. EPA (2003e), and WHO/IPCS (1990).

This study appears to meet current health effects testing guidelines (U.S. EPA, 2006a), with some minor exceptions. For instance, the lungs were not weighed; clinical chemistry did not

1 include blood creatinine, gamma glutamyl transferase, sorbitol dehydrogenase, magnesium, or
2 phosphorous; and urinalysis did not include occult blood, sediment, or albumin. Further,
3 histology did not include the seminal vesicles or skin.

4
5 One control female was found dead during Week 5 of fungal meningoencephalitis.

6
7 At 250 mg/kg-day, mean terminal (6%) protein and alanine aminotransferase (28%) were
8 increased in female rats compared to controls. Mean relative (11% in males and 6% in females)
9 kidney weights were increased compared to controls.

10
11 At 1,000 mg/kg-day, two females died, one at Week 6 (cause of death unknown) and the other at
12 Week 13 (gavage error). Transient lethargy was observed in both sexes for a few hours
13 following dosing, although the effect decreased in incidence and severity during the study. No
14 further details were provided. Food consumption of both sexes increased significantly (7 to 22%
15 in males and 7 to 18% in females) during the second half of the study (Weeks 5 through 13) and
16 mean terminal food consumption was statistically lower than controls by 18% in males and 9%
17 in females. High-dose males had a non-statistically significant decrease in mean terminal body
18 weight (10%) and a statistically significant decrease in mean terminal body weight gain (9%)
19 compared to controls. In high-dose females, although mean body weight at all study weeks and
20 mean terminal body weight gain (Week 13) were not statistically different from controls, mean
21 body weight gain was statistically increased from 10% to 16% in high-dose females from Weeks
22 8 through 12. Various statistically significant clinical measurements and organ weight changes
23 indicative of liver and kidney effects were also observed in high-dose animals (Table A1;
24 Appendix A) and included increased mean terminal hemoglobin (6%) and hematocrit (8%),
25 alkaline phosphatase (46%, interim), alanine aminotransferase (42%, interim; 25%, terminal),
26 albumin (6%, terminal), total protein (8%, interim; 9%, terminal), and lymphocytes (28%,
27 terminal) in females, increased blood-urea-nitrogen (27%, interim), mean cell volume (4%,
28 interim), and serum potassium (25%, terminal) in males, decreased serum glucose (27%,
29 terminal), albumin/globulin ratio (16%, interim) and lymphocyte count (16%, terminal) in males,
30 increased serum cholesterol (23%, interim) in males and (37% interim; 39%, final) in females ,
31 and increased terminal urinary protein and ketones in males and females (data not provided).
32 Further, increases in mean absolute (25%, males; 28%, females) and relative (29%, males; 50%,
33 females) liver weights, absolute (22% in males and 18% in females) and relative (25% in males
34 and 18% in females) kidney weights, absolute (17% in males and 11% in females) and relative
35 (23% in males and 10% in females) adrenal weights, and relative testes weights (8%) in males
36 were observed. Histology revealed a statistically increased incidence of nephropathy of minimal
37 severity, described as multifocally distributed cortical tubular alteration of swollen or
38 hyperchromatic and flattened epithelial cells in male rats. Further, eosinophilic casts in dilated
39 tubules were associated with the cortical foci and corticomedullary junction. This effect was
40 observed in 4/20, 3/20, and 16/20 male rats in the control, low- and high-dose groups,
41 respectively, with statistical significance reached at the high-dose. For comparison, the effect
42 occurred in 1/20, 3/20, and 4/20 female rats in the respective groups. The study authors
43 considered this effect to be age-related general nephropathy, possibly exaggerated by treatment
44 in high-dose males. Thus, the authors considered methyl isobutyl ketone a weak nephrotoxin at
45 1,000 mg/kg-day in male rats. They also concluded that treatment-related effects occurred at the

high-dose and, to a lesser extent, at the mid-dose. Thus, they identified the low dose of 50 mg/kg-day as the NOEL, but did not identify a NOAEL.

Five four-week-old female HLA Wistar rats per dose received drinking water *ad libitum* containing 0 or 1,040 mg/kg-day methyl isobutyl ketone (purity unspecified) for 120 days (Carnegie-Mellon Institute of Research, 1977a; 1977b). The dose was based on the saturation concentration of 1.3% (v/v). Food and water were available *ad libitum*. Two groups of three rats each served as controls and received tap water. Mortality and clinical signs were monitored regularly and food and water consumption were measured regularly (unspecified intervals). Body weight was measured regularly. Terminal liver and kidney weights were measured. Gross necropsies were conducted. Histopathology included the sciatic nerve, brachial plexi, lumbosacral spinal ganglia, anterior and posterior thigh muscles, larynx, nasal cavity, brain, spinal cord, heart, lymph nodes, lungs, spleen, liver, and kidney.

This study does not meet current health effects testing guidelines (U.S. EPA, 2006a), since only five female mice were evaluated at only one dose level. Further, hematology, clinical chemistry, and urinalysis were not conducted, and all of the recommended major organs were not weighed at study termination. Histological examination did not include all of the recommended organs and tissues.

Statistical increases in mean absolute (18% and 11%, depending on control group) and relative (22% and 17%, depending on control group) kidney weights were observed in treated rats (unspecified magnitude) compared to controls. Histopathological examination revealed Harderian Gland dacryoadenitis in 1/5, focal myocarditis in 1/5, and renal tubular cell hyperplasia in 1/5 treated rats, but not in controls. A NOAEL was not reported from this study.

8.4.1.2 Inhalation

Fourteen Fischer 344 rats per sex and concentration inhaled methyl isobutyl ketone (> 99% purity) in whole-body chambers at mean measured concentrations of 0, 50, 252, or 1,002 ppm (0, 205, 1,033, or 4,106 mg/m³) six hours per day and five days per week for 14 weeks (Phillips et al., 1987; Bushy Run Research Center, 1983a; 1983b). Mortality and clinical signs were monitored daily. Body weights were measured weekly. During non-exposure periods, food and water were available *ad libitum*. Water consumption was measured at unspecified intervals for all exposure concentrations. Terminal hematology (hematocrit, hemoglobin concentration, erythrocytes, total and differential leukocytes, platelets, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration) and clinical chemistry (alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, blood urea nitrogen, blood creatinine, glucose, lactate dehydrogenase, sorbitol dehydrogenase, total bilirubin, total cholesterol, total serum protein and albumin, globulin, and electrolytes) were conducted on all rats. Urinalysis (total protein, glucose, creatinine, occult blood, sediment, and volume) was conducted on 10 rats per sex and dose. Kidney, heart, liver, lung, and testes weights were measured. Gross necropsies and targeted histopathological examinations, which included the nasal cavity, trachea, liver, kidneys, and lungs, were conducted on all rats. Histopathology, which included the adrenals, aorta, bone and bone marrow, brain, cecum, cervix, colon, duodenum, esophagus, epididymis, eye, heart, ileum, jejunum, kidneys, liver, lung, lymph

nodes, mammary glands, skeletal muscle, peripheral nerves, ovaries, pancreas, pituitary, prostate, salivary gland, seminal vesicle, skin, spinal cord, spleen, stomach, testes, thymus, thyroid/parathyroid, trachea, uterus, vagina, urinary bladder, and gross lesions, was conducted on control and high-dose rats.

This study appears to meet current health effects testing guidelines (U.S. EPA, 2006a), with some exceptions. For instance, brain and ovary weights were not measured. Further, clinical measurements did not include gamma glutamyl transferase, chloride, magnesium, or phosphorus, and differential leukocyte counts were determined using only control and high-dose rats. Also, urinalysis did not include pH, specific gravity or ketones, or albumin and bilirubin specifically, although total protein was measured.

Renal tubular mineralization was observed in 3/14 control female rats and renal tubular epithelial regeneration was observed in 4/14 control male rats, but the severity of the effects was not indicated

At 50 ppm, mean absolute liver weight (7%) was significantly increased in low-dose males. Renal tubular mineralization was observed in 5/14 female rats and renal tubular epithelial regeneration was observed in 2/14 male rats. At 50 ppm and above, hyaline droplet formation, ranging from minimal to moderately severe, was observed in the kidneys of all male rats, and the severity of the lesion generally increased with exposure level.

At 252 ppm, mean terminal body weight (2%) and absolute kidney weights were significantly increased (4%) in female rats. Serum cholesterol (23%) and urine glucose (37%) were significantly increased in male rats when compared to controls. Renal tubular mineralization was observed in 2/14 female rats.

At 1,002 ppm, mean terminal body weight (4%) was statistically increased in females compared to controls. Mean platelet numbers were significantly increased by 13% compared to controls in male rats, and eosinophil counts in female rats were significantly decreased by 57% compared to controls. Serum cholesterol was significantly increased by 35% compared to controls in male rats. There was a significant increase in mean absolute (13%) and relative (9%) liver weight in male rats. Urine glucose was significantly increased in male (55%) and female (26%) rats, and urine protein was significantly increased (28%) in male rats when compared to controls. Renal tubular mineralization was observed in 7/14 female rats, renal tubular epithelial regeneration was observed in 5/14 male rats, and renal tubular nephritis was observed in 1/14 male rats.

According to the study authors, since no histological lesions were observed in the liver and no changes were seen in serum liver enzymes and bilirubin in any exposure group, the increased liver weights may have been an adaptive response to increased hepatic metabolic activity rather than a toxic effect. The U.S. EPA (2003e) attributed the alpha-2 μ -globulin hyaline droplet formation to be unique to male rats and not relevant to humans for the purposes of risk assessment (U.S. EPA, 1991c). It should be noted, though, that the U.S. EPA (2003e) indicated the presence of alpha-2 μ -globulin hyaline droplet formation in the 14-week inhalation study by Phillips et al. (1987), but review of the Phillips et al. (1987) published study and the corresponding Bushy Run Research Center (1983) unpublished study by the authors of this risk

assessment found only the notation of hyaline droplet formation (Phillips et al., 1987) and/or renal tubular epithelial regeneration (Bushy Run Research Center, 1983) in male rats from which the U.S. EPA (2003e) may have inferred the presence of alpha-2μ-globulin.

Fourteen B6C3F₁ mice per sex and concentration inhaled methyl isobutyl ketone (> 99% purity) in whole-body chambers at mean measured concentrations of 0, 50, 252, or 1,002 ppm (0, 205, 1,033, or 4,106 mg/m³) six hours per day and five days per week for 14 weeks (Phillips et al., 1987; Bushy Run Research Center, 1983a; 1983b). Mortality and clinical signs were monitored daily. Body weights were measured weekly. During non-exposure periods, food and water were available *ad libitum*. Terminal hematology evaluation (hematocrit, hemoglobin concentration, erythrocytes, total and differential leukocytes, platelets, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration) was conducted on all mice. Kidney, heart, liver, lung, and testes weights were measured. Gross necropsies and targeted histopathological examinations, which included the nasal cavity, trachea, liver, kidneys, and lungs, were conducted on all rats. Histopathology, which included the adrenals, aorta, bone and bone marrow, brain, cecum, cervix, colon, duodenum, esophagus, epididymis, eye, gall bladder, heart, ileum, jejunum, kidneys, liver, lung, lymph nodes, mammary glands, skeletal muscle, peripheral nerves, ovaries, pancreas, pituitary, prostate, salivary gland, seminal vesicle, skin, spinal cord, spleen, stomach, testes, thymus, thyroid/parathyroid, trachea, uterus, vagina, urinary bladder, and gross lesions, was conducted on control and high-dose mice.

This study appears to meet current health effects testing guidelines (U.S. EPA, 2006a), with some exceptions. For instance, brain and ovary weights were not measured. Further, clinical chemistry and urinalysis were not conducted.

At 252 ppm, a significant increase in mean absolute liver weight (8%) was observed in male mice compared to controls.

At 1,002 ppm, one male mouse died during the 14th week of the study, but no gross or histopathological lesions were observed and a cause of death could not be determined. A significant increase in mean absolute (7%) and relative (11%) liver weight was observed in male mice compared to controls. According to the study authors, since no histological lesions were observed in the liver and no changes were seen in serum liver enzymes or bilirubin in any exposure group, the increased liver weights may have been an adaptive response to increased hepatic metabolic activity rather than a toxic effect.

Twenty male Sprague-Dawley rats per concentration inhaled methyl isobutyl ketone (unspecified purity) at 0, 250, 750, or 1,500 ppm (0, 1,024, 3,073, or 6,146 mg/m³) six hours per day and five days per week for 13 weeks (David et al., 1999; Eastman Kodak Company, 1996). One half of the rats at each exposure level were maintained on a restricted diet and the other half were fed *ad libitum*. Mortality and clinical signs were monitored regularly. Food consumption (*ad libitum*-fed only) and body weight were measured. Liver and kidney weights were measured at study termination. Gross necropsies, including the brain, spinal cord with dorsal and ventral roots, dorsal and ventral ganglia, sciatic nerve, tibial nerve, kidney, and liver, were conducted. Although this was a neurotoxicity study, a summary of systemic effects is included in this section.

1 This study does not meet current health effects testing guidelines (U.S. EPA, 2006a), since it was
2 intended as a neurotoxicity study. Only liver and kidney weights were measured and blood
3 parameters were not measured. Histopathology was not conducted as well.

4
5 At 250 ppm, mean relative liver weight (9%) was significantly higher than controls in treated rats
6 on restricted diets. Mean relative liver (9%) and kidney (109%) weights were significantly
7 higher than controls in treated *ad libitum*-fed rats.

8
9 At 750 ppm, reduced activity during exposure was observed in an unspecified number of rats for
10 the first eight weeks, but not the final weeks of treatment. Mean absolute (9%) and relative
11 (13%) liver weights were significantly higher than controls in treated rats on restricted diets. In
12 *ad libitum*-fed rats, mean absolute (17%) and relative (11%) liver weights and mean absolute
13 (15%) and relative (9%) kidney weights were significantly higher when compared to controls.

14
15 At 1,500 ppm, reduced activity during exposure was observed in an unspecified number of rats
16 for the first 10 weeks, but not the final three weeks of treatment. Terminal mean body weight of
17 restricted-diet rats was significantly greater than controls (6%). In *ad libitum*-fed rats, mean
18 relative liver (16%) and kidney (14%) weights were significantly higher compared to controls.
19 In food-restricted rats, mean absolute liver weight (10%) was significantly higher compared to
20 controls.

21
22 In an unpublished study by Wright Patterson Air Force Medical Laboratories (MacEwen et al.,
23 1971), 100 male Wistar rats, two male *Macaca mulatta* (rhesus) monkeys, and eight male beagle
24 dogs per concentration inhaled vapors of methyl isobutyl ketone (purity unspecified)
25 continuously at 100 ppm (410 mg/m³) for up to 90 days. A control group included 56 male rats
26 and an unspecified number of male animals of the other species. The exposure level was
27 selected on the basis of a two-week, continuous exposure, range-finding study (MacEwen et al.,
28 1971). Toxicological endpoints evaluated in this study were not fully specified, but included at
29 least biweekly hematology (hematocrit, hemoglobin, erythrocyte, leukocyte, sodium, calcium,
30 chloride, phosphorus, and potassium) and clinical chemistry (at least cholesterol, bilirubin,
31 albumin, total protein, uric acid, blood urea nitrogen, glucose, alkaline phosphatase, creatinine,
32 and glucuronidase), and liver function tests (dogs only) at study termination, which involved the
33 intravenous injection of bromsulphalein, followed by determination of the dye concentration 15
34 minutes later. Gross necropsy and histopathological examination of control and treated animals
35 included: the heart, lung, brain, liver, spleen, kidney, adrenal glands, and pituitary gland. After
36 the 90-day exposure period, two of the eight exposed dogs were subjected to a 60-day recovery
37 period prior to sacrifice.

38
39 This study does not meet current health effects testing guidelines (U.S. EPA, 2006a), since only
40 male animals were evaluated and it does not appear that urinalysis was conducted. Further,
41 histopathology did not include all of the recommended organs and tissues.

42
43 In rats, significant increases in mean absolute (8%) and relative (7%) liver, and absolute (16%)
44 and relative (15%) kidney weights were observed in the absence of hepatic histopathology.
45 Hyaline droplet degeneration of the renal proximal tubules with occasional foci of tubular
46 necrosis was observed in each of the 100 treated rats, including those that were removed from