

**April 2010 ADDENDUM to Draft Methyl *teritary*-Butyl Ether  
Oral Risk Assessment Document, NSF International, February 2008**

The February 2008 draft risk assessment for methyl *teritary*-butyl ether (MTBE) by NSF International did not include the calculation of a risk value based on non-cancer endpoints. Non cancer endpoints were considered in the evaluation but were determined to be a less sensitive endpoint, compared to the cancer endpoint, and was therefore not included in the original assessment. This addendum represents the quantitative determination of non-cancer effects associated with oral exposure to MTBE.

The NSF International (2008) assessment for MTBE determined that there is “suggestive evidence of carcinogenic potential” after gavage exposure to MTBE in rats. This determination was based on an increase in Leydig cell tumors in male SD rats and leukemias/lymphomas (combined) in female SD rats that received MTBE via gavage for two years (Belpoggi et al., 1995). The non cancer assessment was based on a NOAEL from a ninety-day oral toxicity studies of methyl tertiary-butyl ether in Sprague-Dawley rats.

The Total Allowable Concentration (TAC) for MTBE based on non-cancer effects of 0.7 mg/L was calculated and exceeds the Total Allowable Concentration (TAC) for MTBE of 0.1 mg/L based on the cancer endpoint. For the purpose of evaluating cross linked polyethylene tubing/pipe for residential applications to NSF/ANSI Standard 61, the Total Allowable Concentration (TAC) for MTBE of 0.1 mg/L will be used because it is protective of both cancer and non cancer endpoints.

## TABLE OF CONTENTS

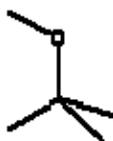
<b>1.0</b>	<b>PHYSICAL AND CHEMICAL PROPERTIES .....</b>	<b>1</b>
<b>1.1</b>	<b>Organoleptic Properties .....</b>	<b>1</b>
<b>2.0</b>	<b>PRODUCTION AND USE .....</b>	<b>2</b>
<b>2.1</b>	<b>Production .....</b>	<b>2</b>
<b>2.2</b>	<b>Use .....</b>	<b>2</b>
<b>3.0</b>	<b>ANALYTICAL METHODS.....</b>	<b>2</b>
<b>3.1</b>	<b>Analysis in Water .....</b>	<b>2</b>
<b>3.2</b>	<b>Analysis in Biological Matrices.....</b>	<b>3</b>
<b>4.0</b>	<b>SOURCES OF HUMAN AND ENVIRONMENTAL EXPOSURE .....</b>	<b>3</b>
<b>4.1</b>	<b>Sources of Human Exposure.....</b>	<b>3</b>
<b>4.2</b>	<b>Sources of Environmental Exposure.....</b>	<b>3</b>
<b>5.0</b>	<b>COMPARATIVE KINETICS AND METABOLISM IN HUMANS AND LABORATORY ANIMALS.....</b>	<b>4</b>
<b>5.1</b>	<b>Absorption .....</b>	<b>6</b>
<b>5.2</b>	<b>Distribution.....</b>	<b>6</b>
<b>5.3</b>	<b>Metabolism .....</b>	<b>7</b>
<b>5.4</b>	<b>Elimination/Excretion.....</b>	<b>9</b>
<b>5.5</b>	<b>Physiologically-based pharmacokinetic models.....</b>	<b>10</b>
<b>6.0</b>	<b>EFFECTS ON HUMANS .....</b>	<b>10</b>
<b>6.1</b>	<b>Case Reports.....</b>	<b>10</b>
<b>6.2</b>	<b>Epidemiological Studies.....</b>	<b>10</b>
<b>7.0</b>	<b>EFFECTS ON LABORATORY ANIMALS AND <i>IN VITRO</i> TEST SYSTEMS.....</b>	<b>11</b>
<b>7.1</b>	<b>Limited-Exposure Effects.....</b>	<b>12</b>
<b>7.2</b>	<b>Single-Exposure Studies .....</b>	<b>13</b>
<b>7.3</b>	<b>Short-Term Exposure Studies .....</b>	<b>13</b>
<b>7.4</b>	<b>Long-Term and Chronic Exposure Studies.....</b>	<b>18</b>
<b>7.5</b>	<b>Reproductive and Developmental Toxicity Studies .....</b>	<b>25</b>
<b>7.6</b>	<b>Studies of Immunological and Neurological Effects.....</b>	<b>33</b>
<b>8.0</b>	<b>RISK CHARACTERIZATION .....</b>	<b>35</b>
<b>8.1</b>	<b>Hazard Identification.....</b>	<b>35</b>
<b>8.2</b>	<b>TAC Derivation .....</b>	<b>42</b>
<b>8.3</b>	<b>SPAC Derivation.....</b>	<b>43</b>
<b>9.0</b>	<b>RISK COMPARISONS AND CONCLUSIONS .....</b>	<b>43</b>
<b>10.0</b>	<b>ADDENDUM REFERENCES: .....</b>	<b>44</b>

### ADDENDUM EXECUTIVE SUMMARY

<b>Methyl tertiary-Butyl Ether (MTBE) – Oral Risk Assessment CAS # 1634-04-4</b>			
<b>PARAMETER</b>	<b>LEVEL</b>	<b>UNITS</b>	<b>DERIVED</b>
<b>NOAEL</b> (no observed adverse effect level)	300	mg/kg-day	From a 13-week gavage study in SD rats
<b>Oral RfD</b> (oral reference dose)	0.1	mg/kg-day	From the NOAEL with a 3000x total uncertainty factor
<b>TAC</b> (total allowable concentration)	0.7	mg/L	For a 70 kg adult drinking 2 L/day with a 20% Relative Source Contribution
<b>SPAC</b> (single product allowable concentration)	0.07	mg/L	From the TAC, assuming 10 potential sources of MTBE in drinking water
<b>STEL</b> (short term exposure level)	Not determined	mg/L	Not applicable
<b>KEY STUDY</b>	Robinson, M., R.H. Bruner, and G.R. Olson. 1990. Fourteen- and ninety-day oral toxicity studies of methyl tertiary-butyl ether in Sprague-Dawley rats. <i>J Am Coll Toxicol.</i> 9(5):525-540.		
<b>CRITICAL EFFECT(S)</b>	The NOAEL was based on the approximate threshold associated with the induction of adaptive liver responses after subchronic gavage exposure to MTBE.		
<b>UNCERTAINTY FACTORS</b>	<p>Factors applied in calculating the oral RfD include:</p> <ul style="list-style-type: none"> <li>• 10x for interspecies extrapolation</li> <li>• 10x for intraspecies extrapolation</li> <li>• 1x for LOAEL to NOAEL extrapolation</li> <li>• 10x for subchronic to chronic extrapolation</li> <li>• 3x for database deficiencies</li> </ul> <p>The total uncertainty factor is therefore 3000x.</p>		
<b>TOXICITY SUMMARY</b>	<p>Oral toxicity data for MTBE in humans were limited to sensory irritation effects after occupational exposure or kinetic parameters after single-dose exposures. Gavage but not drinking water exposure to MTBE in laboratory rodents was associated with increased liver weights and altered blood parameters (blood urea nitrogen and cholesterol) accompanied by centrilobular hepatocyte hypertrophy. Increased mean kidney weights, hyaline droplet formation, and <math>\alpha</math>-2<math>\mu</math>-globulin immunoreactivity were observed in the proximal tubules of male rats after drinking water or gavage exposure. All investigations on nephrotoxicity associated with MTBE exposure are consistent with <math>\alpha</math>-2<math>\mu</math>-globulin nephropathy, which was not considered relevant to humans. The liver effects observed after subchronic gavage exposure were attributed to an adaptive mechanism by the liver to metabolizing bolus doses of MTBE since they were not observed after drinking water exposure. The effect of long-term exposure to MTBE at levels below the threshold that would elicit such adaptive responses is unknown. While adaptive mechanisms to metabolizing high-dose chemical exposures are usually reversible upon cessation of treatment, these mechanisms, if provoked for a sufficiently prolonged duration, may result in irreversible changes that are considered adverse and potentially relevant to humans. The NOAEL was considered 300 mg/kg-day based on the threshold associated with the induction of adaptive liver responses that occurred at 900 mg/kg-day after subchronic gavage exposure to MTBE. Although standardized chronic inhalation bioassays are available for MTBE, insufficient kinetics data are available to reliably extrapolate an inhalation concentration in rats to human equivalent oral doses.</p>		
<b>CONCLUSIONS</b>	<p>A physiologically-based pharmacokinetic model extrapolating oral rat doses to humans and additional studies examining potential modes of action would increase the confidence and reduce the uncertainty associated with the non-cancer risk levels derived herein. The relevance of the drinking water levels derived herein should be re-evaluated when the results of an ongoing two-year drinking water study becomes available.</p>		

## 1.0 PHYSICAL AND CHEMICAL PROPERTIES

MTBE is an aliphatic dialkyl ether with synonyms of 2-methoxy-2-methylpropane; 2-methyl-2-methoxypropane; ether, tert-butyl methyl; MTBE; methyl 1,1-dimethylethyl ether; methyl tert-butyl ether; methyl tertiary-butyl ether; propane, 2-methoxy-2-methyl-; t-butyl methyl ether; tert-butyl methyl ether (ChemIDPlus, 2003). It has trade names of 3 D Concord, Driveron, HSDB 5487, and UN 2398 (IPCS, 1998). It has the following structure, and physical and chemical properties listed in Table 1:



**Table 1. The physical and chemical properties of MTBE**

Property	Data	Reference
Empirical Formula	C <sub>5</sub> H <sub>12</sub> O	OEHHA, 1999
CAS#	1634-04-4	OEHHA, 1999
Molecular Weight	88.15	OEHHA, 1999
Physical State and Color	colorless liquid at room temperature	IPCS, 1998
Melting Point	-109°C	OEHHA, 1999
Boiling Point	55.2°C	IPCS, 1998
Density	0.7404 at 20°C	IPCS, 1998
Vapor Pressure	33,500 Pa at 25°C	IPCS, 1998
Water Solubility	51 g/L at 25°C	OEHHA, 1999
Dissociation Constant (pK <sub>a</sub> )	Not reported	
n-Octanol/Water Partition Coefficient (log K <sub>ow</sub> )	0.94-1.3 <sup>a</sup> 1.43 (estimated) <sup>b</sup>	<sup>a</sup> IPCS, 1998 <sup>b</sup> <a href="http://esc.syrres.com">http://esc.syrres.com</a>
Henry's Law Constant (air/water partition)	5.87 x 10 <sup>-4</sup> atm-m <sup>3</sup> /mole at 25°C	OEHHA, 1999

### 1.1 Organoleptic Properties

MTBE has a terpene-like odor (IPCS, 1998). Individual variability in sensitivity to taste and odor make it difficult to identify odor and taste thresholds for MTBE in water (ECB, 2002). IPCS (1998) has reported that the taste threshold for MTBE in water is 134 ppb. OEHHA (1999) has cited various sources that report odor thresholds for MTBE in water of between 2.5 to 680 ppb. The U.S. EPA (1997) recommended a drinking water level of 20-40 ppb for MTBE, based on averting taste and odor. More recent data by Suffet et al. (2007) suggests that the odor threshold for MTBE in water is ≥ 15 ppb.

## **2.0 PRODUCTION AND USE**

### **2.1 Production**

Industrially, MTBE is derived from the catalytic reaction of methanol and isobutylene over an acidic ion-exchange resin catalyst such as sulfonated styrene cross-linked with divinyl benzene in the liquid phase at 38-93°C and 100-200 psi (IPCS, 1998). It can also be prepared from methanol, t-butanol, and diazomethane.

MTBE is among the 50 highest production volume chemicals (IPCS, 1998). In 1999, total worldwide annual production of MTBE was about 21 million tons or 46.3 billion pounds (ECB, 2002). MTBE is a high production volume chemical in the United States (U.S. EPA, 2007) and European Union (2004).

### **2.2 Use**

It is anticipated that the use of MTBE will continue to increase (IPCS, 1998). North America is the largest consumer of MTBE, accounting for about two-thirds of the world's annual use (IPCS, 1998). In 1996, the US was the world's largest consumer of MTBE with a usage of 10.6 million tons (12.2 billion pounds) per year.

The major use of MTBE is as an oxygenated additive in gasoline, in which it is blended at 2 to 11.5% by volume (ECB, 2002). IPCS (1998) reports that MTBE has been added to gasoline in concentrations up to 17% by volume. Only a minor amount is used for other purposes, such as solvent instead of diethyl ether or diisopropyl ether in both the chemical and pharmaceutical industry and laboratories (ECB, 2002). Approximately 25% of gasoline in the USA is blended with MTBE (IPCS, 1998). MTBE is almost exclusively used to provide both octane enhancement and an increase in the oxygen content of gasoline. No approved uses for MTBE as a direct or indirect food additive were identified under Title 21 of the U.S. Code of Federal Regulations (U.S. FDA, 2010).

## **3.0 ANALYTICAL METHODS**

### **3.1 Analysis in Water**

Sorption/desorption, including purge and trap systems, and headspace procedures have been used to prepare water for analysis of MTBE (IPCS, 1998). The analytical methods for MTBE in water have been reviewed by IPCS (1998). These methods include the static headspace procedure using gas chromatography with photoionization detection (GC-PID) with a detection limit of 10.8  $\mu\text{g}/\text{m}^3$  and the purge and trap procedure using gas chromatography-mass spectrometry with detection limits ranging from 0.06 to 5  $\mu\text{g}/\text{L}$ . NSF International uses U.S. EPA (1995) method 502.2 employing gas chromatography for volatile compounds to detect MTBE as an extractant from drinking water system components tested to NSF/ANSI Standard 61 (2009). The reporting limit is 0.5  $\mu\text{g}/\text{L}$ .

### **3.2 Analysis in Biological Matrices**

MTBE is analyzed in biological matrices generally by gas chromatography, using a range of capillary columns and detector systems suited to the specific matrix (IPCS, 1998).

## **4.0 SOURCES OF HUMAN AND ENVIRONMENTAL EXPOSURE**

### **4.1 Sources of Human Exposure**

MTBE does not occur naturally in the environment (IPCS, 1998). Groundwater may become contaminated with MTBE through leaking underground storage tanks or spillage from overfilling of the storage tanks (ECB, 2002). In the USA, MTBE has been detected in storm water, surface water, including streams, rivers, and reservoirs, groundwater, and drinking water (IPCS, 1998). MTBE is infrequently detected in public drinking-water systems from groundwater. In all but three out of 51 systems in which it was reported, the concentration was  $\leq 20$   $\mu\text{g/L}$ . There are inadequate data to characterize the concentration of MTBE in public drinking-water systems from surface water. MTBE has been found at high levels (i.e.  $\geq 1,000$   $\mu\text{g/L}$ ) in a few private wells used for drinking water (IPCS, 1998). MTBE has been detected as an extractant from drinking water system components tested to NSF/ANSI 61 (2009) at normalized concentrations up to 0.2 mg/L.

Workers with potential exposure to MTBE include those involved in the production, distribution, and use of MTBE and MTBE-containing gasoline, including service station attendants and mechanics (IPCS, 1998). The sources of industrial occupational exposure to MTBE have been reviewed by ECB (2002) and include individuals involved in the production, formulation, transportation, or distribution of MTBE. These exposures include personnel employed at service stations, those involved in maintenance operations and automotive repairs, and individuals in the chemical or pharmaceutical industries in which MTBE is used as a solvent. Exposure of the public to MTBE can be principally by inhalation of fumes while refueling motor vehicles and drinking contaminated water (McGregor, 2006). Maximum internal doses resulting from such exposures are unlikely to exceed 0.05 mg/kg-day and will normally be very much lower.

### **4.2 Sources of Environmental Exposure**

MTBE may enter the environment during all phases of the petroleum fuel cycle (IPCS, 1998). Sources include auto emissions, evaporative losses from gasoline stations and vehicles, storage tank releases, pipeline leaks, other accidental spills, and refinery stack releases. Annual estimates of MTBE mass releases to the environment from all potential sources have not been reported in the scientific literature. However, releases from storage tanks, vehicular emissions, and evaporative losses from gasoline stations and vehicles are perceived to be important sources.

Concentrations of MTBE detected in storm water ranged from 0.2 to 8.7  $\mu\text{g/L}$  with a median of less than 1.0  $\mu\text{g/L}$ . For streams, rivers, and reservoirs, the range of detection was from 0.2 to 30  $\mu\text{g/L}$ , and the range of medians for several studies was 0.24 to 7.75

µg/L. MTBE has generally not been detected in deeper groundwater or in shallow groundwater in agricultural areas. When detected, the concentration is less than 2.0 µg/L. MTBE is more frequently found in shallow groundwater (top 5-10 feet of these aquifers) in urban areas. In this setting, the concentrations range from less than 0.2 µg/L to 23 mg/L, with a median value below 0.2 µg/L (IPCS, 1998).

## 5.0 COMPARATIVE KINETICS AND METABOLISM IN HUMANS AND LABORATORY ANIMALS

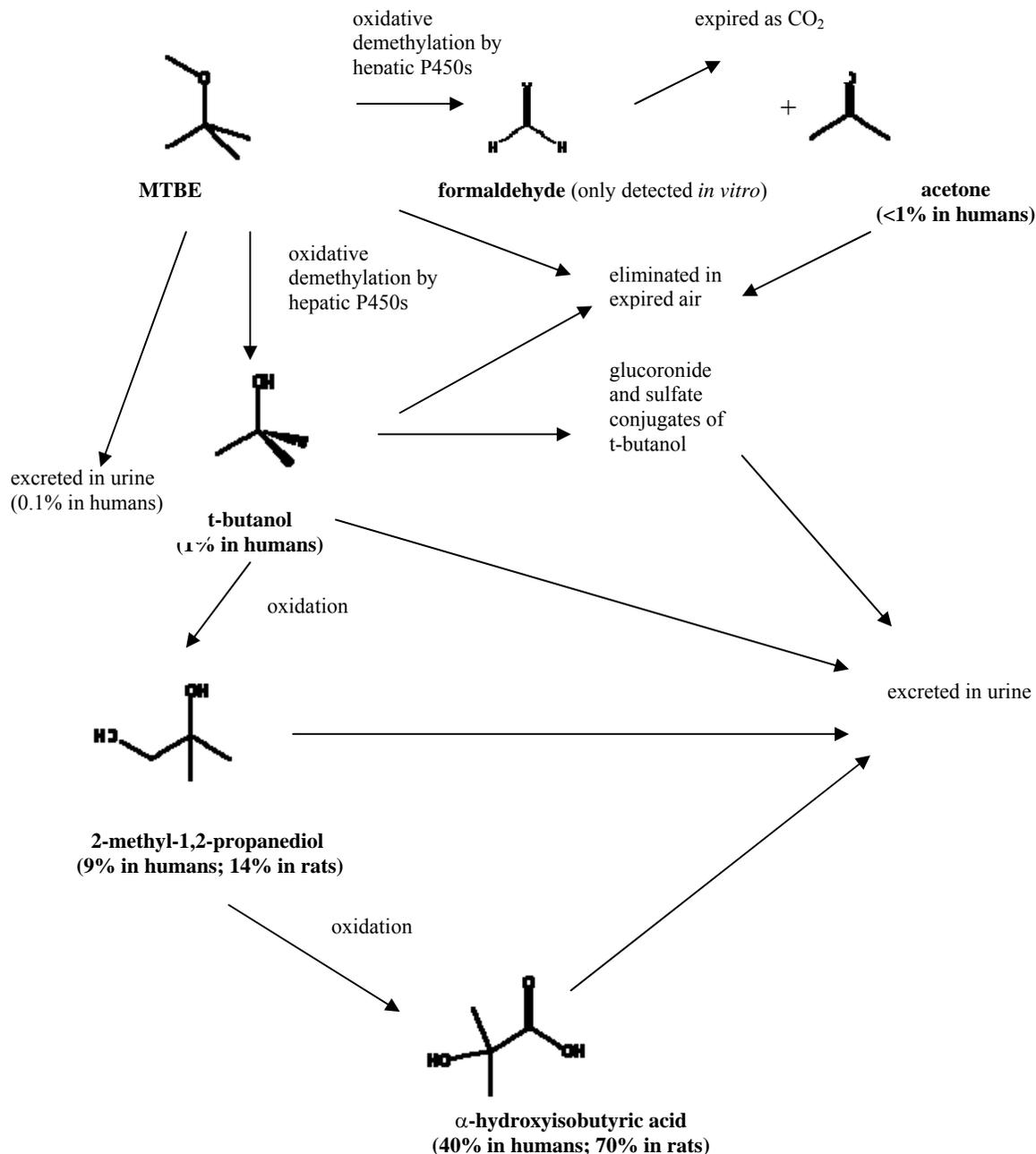
Numerous studies investigating the kinetics and metabolism of MTBE in humans and laboratory animals are available. These data have been reviewed by several regulatory organizations, including European Chemicals Bureau (ECB, 2002), Office of Environmental Health Hazard Assessment of the California EPA (OEHHA, 1999), the International Programme on Chemical Safety of the World Health Organization (IPCS, 1998), the European Center for Ecotoxicology and Toxicology of Chemicals (ECETOC, 1997), the Agency for Toxic Substances and Disease Registry (ATSDR, 1996), and Health Canada (1992). Several review articles on these data are also available in the scientific literature.

MTBE was absorbed into the blood of human volunteers who rapidly drank 2.8 mg MTBE in 250 mL Gatorade (Prah et al., 2004). Mean blood levels of MTBE peaked at 0.17 µmol/L between 15 and 30 minutes following administration and declined to at or below the detection limit (0.05 µmol/L) at the 24-hour sampling period. In human volunteers who rapidly drank 6.7 µl MTBE in “about 5 mg” of lemon-lime solution, peak blood levels of MTBE ranged from 5 to 15 ng/ml (0.06-0.17 µmol/l) (ECB, 2002).

In rodents, MTBE is well absorbed and distributed following oral administration (IPCS, 1998). Rapid and complete absorption across the gastrointestinal tract was observed in rats administered MTBE via gavage at 40 mg/kg (ECB, 2002). At 400 mg/kg oral exposure in rats, the percentage of total absorbed dose eliminated in expired air increased with a corresponding decrease in the percentage eliminated in urine, indicating a saturation of metabolism (IPCS, 1998).

*In vivo* studies on the metabolism of MTBE in humans and rats indicate qualitatively similar overall metabolism (ECB, 2002). MTBE is oxidatively demethylated by microsomal enzymes to t-butanol and formaldehyde, but the latter has only been shown *in vitro*. In rodents, the biotransformation of t-butanol has been shown to yield 2-methyl-1,2-propanediol and α-hydroxyisobutyric acid (Figure 1).

Figure 1. Proposed metabolic scheme of MTBE



The cytochrome P450-mediated biotransformation of MTBE has been explored in several *in vitro* studies with liver microsomes from humans, rats, and mice (ECB, 2002). Metabolism of MTBE by rat liver microsomes produced equivalent amounts of formaldehyde and t-butanol, and data strongly suggest that when expressed, CYP2B1 is the major enzyme involved in MTBE demethylation and that CYP2E1 may have a minor role.

Since these kinetic and metabolism data for MTBE in humans and laboratory animals have been reviewed previously, the current review focuses on only the new oral data since these reviews. Recent data confirm that MTBE is rapidly absorbed following oral administration. Approximately 30% of administered dose in humans was cleared by exhalation as unchanged MTBE and as t-butanol within 10-20 min. Less than 0.1% of the administered dose was recovered in expired air as acetone. Approximately 50% of the administered dose in humans was eliminated in the urine as unchanged MTBE (~0.1%), t-butanol (~1%), 2-methyl-1,2-propanediol (~9%), and 2-hydroxyisobutyrate (~40%).

## 5.1 Absorption

Previous data in humans or laboratory animals demonstrate that MTBE is rapidly absorbed following oral administration. Data by Prah et al. (2004), Amberg et al. (2001), and Dekant et al. (2001) confirm this observation. MTBE was rapidly absorbed from the gastrointestinal tract and a significant part of the administered dose was transferred into blood of human volunteers ingesting MTBE in water or Gatorade. No other recent data regarding the absorption of MTBE following oral exposure in humans or laboratory animals were identified.

## 5.2 Distribution

Recent data regarding the distribution of MTBE after oral exposure were limited to the measurement of MTBE and one of its metabolites, t-butanol, in blood after oral ingestion in human volunteers.

Fourteen healthy male volunteers ingested 2.8 mg MTBE (unspecified purity) in 250 mL Gatorade (Prah et al., 2004). Gatorade was used as the vehicle to mask the unpleasant taste of MTBE. Blood samples were collected after 0, 5, 15, 30, 45, 60, 75, 90, 105, 120, 180, 240, 360, and 1,440 minutes. Mean levels of MTBE and t-butanol in the blood were determined using gas chromatography/mass spectrometry. The plasma half-life of MTBE was determined. The area under the plasma concentration versus time curve was estimated for MTBE alone and for MTBE plus t-butanol.

Mean blood levels of MTBE peaked at 0.17  $\mu\text{mol/L}$  between 15 and 30 minutes following administration and declined to at or below the detection limit (0.05  $\mu\text{mol/L}$ ) at the 24-hour sampling period. Blood levels of t-butanol peaked at 0.23  $\mu\text{mol/L}$  at the 45-minute sampling period and did not return to pre-exposure levels by the 24-hour sampling period. Elimination of MTBE from the blood was best characterized by a three-compartment model. The mean half-life for MTBE elimination from the blood in the first, second, and third phases was 14.9, 102.0, and 417.3 minutes, respectively. The mean area under the plasma concentration versus time curve was estimated to be 1,682  $\mu\text{mol/hr/L}$  for MTBE alone, 20,025  $\mu\text{mol/hr/L}$  for t-butanol, and 10,854  $\mu\text{mol/hr/L}$  for MTBE and t-butanol combined. The mean area under the curve ratio of t-butanol to MTBE was 13.1 in the blood. Since this study also included the dermal and inhalation routes of exposure, the study authors suggested that these pharmacokinetic estimates

were useful in constructing a physiologically-based pharmacokinetic model for MTBE in humans across different routes of administration.

Three human volunteers per sex and dose ingested 0, 5, or 15 mg <sup>13</sup>C-MTBE in 100 mL water (Amberg et al., 2001; Dekant et al., 2001). Blood samples were collected at 60-minute intervals for the first four hours and at 120-minute intervals thereafter until 12 hours. A final blood sample was collected 24 hours after administration.

At 5 mg, the maximum concentration in the blood averaged 0.10 μM, and these concentrations were obtained with the first blood samples, which were taken after one hour. Elimination of MTBE from the blood occurred in three phases, and the mean half-life of each phase was 0.8, 1.8, and 8.1 hours. Mean blood concentrations of t-butanol were 1.82 μM. The mean terminal half-life of t-butanol clearance from the blood was 8.1 hours. Levels of MTBE and t-butanol in blood declined to at or near the limit of detection at the 12- and 24-hour sampling times, respectively.

At 15 mg, the maximum concentration in the blood, which was reached after one hour, averaged 0.69 μM. Elimination of MTBE from the blood occurred in three phases, and the mean half-life of each phase was 0.7, 1.2, and 3.7 hours. Mean blood concentrations of t-butanol were 0.45 μM. The mean terminal half-life of t-butanol clearance from the blood was 8.5 hours.

### **5.3 Metabolism**

#### **5.3.1 Humans**

The metabolism of MTBE was studied in three human volunteers per sex and dose after ingestion of 0, 5, or 15 mg <sup>13</sup>C-MTBE in 100 mL water (Amberg et al., 2001; Dekant et al., 2001). Mass spectrometry was used to identify urinary metabolites in urine samples collected at 6-hour intervals for 96 hours. At 5 and 15 mg, 46% and 49%, respectively, of the administered dose was eliminated in the urine as unchanged MTBE, t-butanol, 2-methyl-1,2-propanediol, and 2-hydroxyisobutyrate. At 5 mg, unchanged MTBE, t-butanol, 2-methyl-1,2-propanediol, and 2-hydroxyisobutyrate comprised 0.01, 1, 9, and 36% of the administered dose, respectively. At 15 mg, unchanged MTBE, t-butanol, 2-methyl-1,2-propanediol, and 2-hydroxyisobutyrate comprised 0.1, 1, 8, and 40% of the administered dose, respectively. Hepatic first-pass metabolism was not observed. The authors concluded that the metabolic pathway for MTBE after oral exposure was identical to concurrently conducted inhalation exposure studies.

The metabolism of MTBE was studied in a panel of 12 human liver microsomes isolated from nine male and two female donors (Le Gal et al., 2001). The human liver microsomes metabolized MTBE into t-butanol and formaldehyde. The mean Michaelis-Menten constant (K<sub>m</sub>), which describes the catalytic power of an enzyme or rate of a reaction catalyzed by an enzyme, was determined. The mean apparent K<sub>m</sub>(1) was determined to be 0.25 mM, which was considered low by the study authors, and the mean apparent K<sub>m</sub>(2) was 2.9 mM, which was considered high. The study authors concluded

that kinetic data, along with the results from correlation studies and chemical inhibition studies, support the assertion that the major enzyme involved in MTBE metabolism is CYP2A6, with a minor contribution of CYP3A4 at low substrate concentration.

### 5.3.2 Laboratory Animals

Williams and Borghoff (2000) investigated the hypothesis that MTBE-induced decrease in serum testosterone levels in male rats may be due in part to the ability of MTBE to induce the metabolism of endogenous testosterone and, hence, enhance its clearance. Fifteen male Sprague-Dawley rats per dose were administered 0 or 1,500 mg/kg-day MTBE (> 99.9% purity in corn oil) via gavage for 15 days. In a second experiment, fifteen male Sprague-Dawley rats per dose were administered 0, 250, 500, 1,000, or 1,500 mg/kg-day MTBE (> 99.9% purity in corn oil) via gavage for 28 days. At study termination, the rats were sacrificed, body and liver weights were determined, and hepatic microsomes were isolated for measurement of CYP450 activity. Testosterone hydroxylase activities of hepatic microsomes, which were used as markers for CYP450 enzyme activities, were also assessed. These enzymes included 2- $\alpha$ -, 2- $\beta$ -, 6- $\beta$ -, 7- $\alpha$ -, 16- $\alpha$ -, and 17- $\beta$ -hydroxytestosterone. The activities of p-nitrophenol and UDP-glucuronosyltransferase were also assessed to evaluate the mechanism of centrilobular hypertrophy observed in rodents after repeated MTBE exposures. The formation of formaldehyde, a metabolite of MTBE, was also measured.

After 15 days, total hepatic microsomal cytochrome CYP450 was increased 1.3-fold in rats treated with 1,500 mg/kg-day MTBE. CYP1A1/2, CYP2A1, CYP2E1, and CYP2B1/2 activities were increased 1.5-, 2.4-, 2.3-, and 6.5-fold, respectively, at 1,500 mg/kg-day after 15 days. 7- $\alpha$ -hydroxytestosterone was statistically increased by 2.4-fold compared to controls.

After 28 days, total hepatic microsomal cytochrome CYP450 was not statistically different compared to control. At 1,000 mg/kg-day after 28 days, a statistical increase in mean relative liver weight (10-14%, not further specified) and a 2.0-fold increase in CYP2B1/2 were observed compared to controls.

After 28 days at 1,500 mg/kg-day, a statistical increase in mean relative liver weight (10-14%, not further specified) was observed. CYP 2B1/2, CYP2E1, CYP3A1/2, and UDP-glucuronosyltransferase activities were statistically increased by 2.9-, 2.0-, 2.1-, and 1.7-fold respectively, compared to controls. 6- $\beta$ -hydroxytestosterone was statistically increased by 2.1-fold compared to controls. UDP-glucuronosyltransferase was statistically increased compared to controls. Formaldehyde production was statistically increased compared to controls at 1,500 mg/kg-day after 28 days. MTBE also induced its own metabolism 2.1-fold at 1,500 mg/kg-day after 28 days, and the authors noted that this effect was consistent with the induction of CYP2E1 and CYP2B1. It should be noted that mean body weight was reduced by 12% compared to controls at 1,500 mg/kg-day after 28 days.

The study authors concluded that MTBE induced mild increases in testosterone hydroxylase enzymes. Further, the increase in UDP-glucuronosyltransferase was consistent with the centrilobular hypertrophy observed in rodents after repeated MTBE exposures. The decrease in serum testosterone observed following MTBE administration may be the result of enhanced testosterone metabolism and subsequent clearance. However, the authors stated that the most pronounced effects were observed at the high dose of 1,500 mg/kg-day, at which clinical signs of toxicity and reduced body weight (12%) were also observed. The authors further noted that since the increases in testosterone hydroxylase enzyme activities were generally mild, the hypothalamus-pituitary hormonal feedback loop could be expected to compensate for mild reductions in circulating testosterone *in vivo*.

Eight female B6C3F<sub>1</sub> mice per dose were given MTBE (> 99.95% purity in corn oil) by gavage at 0 or 1,800 mg/kg-day for three days (Moser et al., 1996). Food and water were available *ad libitum*. Eighteen hours after the last dose, the mice were sacrificed and hepatocyte cytochromes were isolated. MTBE induced a statistical increase (37%) in total hepatic cytochrome P450 content, a 9-fold increase in hepatic 7-pentoxo-resorufin-*O*-dealkylase activity (a CYP2B marker) and a 2-fold increase in hepatic 7-ethoxy-resorufin-*O*-deethylase activity compared to controls.

#### 5.4 Elimination/Excretion

The elimination of MTBE and t-butanol in expired air was investigated in seven healthy male volunteers who ingested 2.8 mg MTBE (unspecified purity) in 250 mL Gatorade (Prah et al., 2004). Gatorade was used as the vehicle to mask the unpleasant taste of MTBE. Exhaled air samples were collected after 0, 5, 15, 30, 45, 60, 75, 90, 105, 120, 180, 240, 360, and 1,440 minutes. Mean levels of MTBE and t-butanol in exhaled air were determined using gas chromatography/mass spectrometry.

Elimination of MTBE from expired air was best characterized by a three-compartment model. The mean half-life for MTBE in expired air in the first, second, and third phases was 13.0, 63.1, and 254.0 minutes, respectively. The mean area under the curve ratio of t-butanol to MTBE was 0.175 in exhaled air. Since this study also included the dermal and inhalation routes of exposure, the study authors suggested that these pharmacokinetic estimates were useful in constructing a physiologically-based pharmacokinetic model for MTBE in humans across different routes of administration.

The urinary elimination of MTBE was examined in three healthy human volunteers per sex administered 5 and 15 mg <sup>13</sup>C-MTBE (> 98% purity) in spiked tap water samples (Amberg et al., 2001). The different doses were administered four weeks apart. Urine samples were collected for 96 hours after administration in six hour intervals, and blood samples were taken in 60-minute intervals up to four hours, then at 120-minute intervals up to 12 hours, and ultimately at 24 hours. MTBE and t-butanol concentrations in blood were determined. Urine metabolites, including the parent compound, t-butanol, 2-methyl-1,2-propanediol, and 2-hydroxyisobutyrate were quantified.

At 5 and 15 mg/kg, 46% and 49%, respectively, of the administered dose was eliminated in the urine as unchanged MTBE, t-butanol, 2-methyl-1,2-propanediol, and 2-hydroxyisobutyrate. The authors concluded that the kinetics of excretion after oral exposure were identical to concurrently conducted inhalation exposure studies.

In the same experiment, the respiratory elimination of MTBE was examined in three healthy male volunteers administered 15 mg <sup>13</sup>C-MTBE (> 98% purity) in 100 mL tap water samples (Amberg et al., 2001). Approximately 30% of the MTBE dose was cleared by exhalation as unchanged MTBE and as t-butanol. MTBE exhalation was rapid and maximum concentrations of 100 nM in exhaled air were achieved within 10-20 minutes. Less than 0.1% of the administered dose was recovered in expired air as <sup>13</sup>C-acetone. The study authors concluded that the results indicate that the biotransformation and excretion of MTBE after oral exposure is similar to inhalation exposure and suggested the absence of a significant first-pass metabolism of MTBE in the liver after oral administration.

## **5.5 Physiologically-based pharmacokinetic models**

Although several physiologically-based pharmacokinetic models have been constructed to model the behavior of inhaled MTBE, models describing the behavior of MTBE after oral exposure are limited and usually include multiple exposure routes. Kim et al. (2007) developed a multiple-route (oral, inhalation and dermal) nine-compartment model of MTBE and t-butanol in humans based on blood measurements of these compounds. Borghoff et al. (1996) developed a multiple-route (oral, inhalation and intravenous) seven-compartment model of MTBE and t-butanol in F344 rats. A model describing MTBE-binding to  $\alpha$ -2 $\mu$ -globulin in the kidneys of male rats that inhaled MTBE has also been developed (Leavens and Borghoff, 2009).

## **6.0 EFFECTS ON HUMANS**

### **6.1 Case Reports**

No recent case reports regarding oral exposure to MTBE were identified.

### **6.2 Epidemiological Studies**

Epidemiological studies of human populations exposed under occupational as well as non-occupational conditions, and experimental studies of human volunteers exposed under controlled conditions, have not been able to identify a basis for headache, eye and nose irritation, cough, nausea, dizziness, and disorientation reported by consumers in some areas as a result of fueling with gasoline (IPCS, 1998). Although results are mixed, IPCS (1998) suggested that community studies conducted in Alaska, New Jersey, Connecticut, and Wisconsin provided limited or no evidence of an association between MTBE exposure and the prevalence of health complaints. A review of these epidemiology studies by Phillips et al. (2008) reached a similar conclusion based primarily on the limitations of the study designs.

In controlled experimental studies on adult volunteers exposed in inhalation chambers to MTBE at concentrations ranging from 5.0 mg/m<sup>3</sup> (1.4 ppm) to 270 mg/m<sup>3</sup> (75 ppm), there were no evident effects on either subjective reports of symptoms or objective indicators of irritation or other effects up to 180 mg/m<sup>3</sup> (50 ppm) for up to two hours (IPCS, 1998). Thus, it appears unlikely that MTBE alone induces adverse acute health effects in the general population after inhalation exposure. However, the potential effects of mixtures of gasoline and MTBE, and the manner in which most persons are exposed to MTBE in conjunction with the use of oxygenated fuels, have not been examined experimentally or through prospective epidemiological methods.

Occupational exposure to MTBE (96 Chinese petroleum factory workers aged 20 to 49, mean age 29) compared to 102 controls was investigated by Zhou and Ye (1999). Based on self-reported responses to a questionnaire, occupationally exposed workers reported health complaints (62 cases, 65%) significantly more than controls (16 cases, 17%). Data were analyzed with an Epi Info 6 and SAS statistical package and logistical regression was used to identify confounding factors. The most frequently reported symptoms in occupationally exposed workers were eye irritation (20%), dizziness (19%), burning sensation in the nose or throat (18%), insomnia (14%), nausea or vomiting (14%), headache (13%), fatigue (13%), poor memory (13%), irritability (6%) and skin irritation or redness (5%). Among these workers, 65 were male and 31 were female; 40 were smokers and 56 were nonsmokers, and among the 56 nonsmokers 22 were negative smokers; 9 workers drank and 87 did not drink. The duration of exposure was 1 to 10 years. The TWA (time weighted average) concentrations of MTBE in workplaces ranged from 10 ppm to 56 ppm (36 mg/m<sup>3</sup> to 202 mg/m<sup>3</sup>). The 102 unexposed controls (aged from 20 to 49, mean age 28) were from the same factory. Of these controls, 69 were male and 33 were female; 45 were smokers and 57 were nonsmokers, and among the 57 nonsmokers 20 were negative smokers; 6 workers drank and 96 did not drink. The list of symptoms including dizziness, headache, eye irritation, burning sensation in the nose or throat, anxiety, “spaciness” or disorientation, insomnia, fever, sweats or chills, inability to concentrate, irritability, fatigue, poor memory, skin irritation or redness, muscle aches, nausea or vomiting, fatigue, fever, diarrhea, cough, difficulty in breathing, sneezing, bronchitis, rashes and others. Gender, age, exposure duration/day, length of service, or drinking or smoking habits did not statistically influence the prevalence of symptoms. The study authors did not indicate whether they accounted for potential concurrent exposures to other chemicals in the occupationally-exposed individuals.

## **7.0 EFFECTS ON LABORATORY ANIMALS AND *IN VITRO* TEST SYSTEMS**

Numerous regulatory organizations, including European Chemicals Bureau (ECB, 2002), Office of Environmental Health Hazard Assessment of the California EPA (OEHHA, 1999), the World Health Organization (WHO, 2005; IPCS, 1998), the European Center for Ecotoxicology and Toxicology of Chemicals (ECETOC, 1997), U.S. EPA (1997), the Agency for Toxic Substances and Disease Registry (ATSDR, 1996), and Health Canada (1992) have critically reviewed the studies in laboratory animals for MTBE. This section

includes only the oral studies for MTBE, due to their significance in the development of lifetime drinking water levels for MTBE, since studies by the inhalation and/or dermal routes have been critically reviewed elsewhere.

In addition to the previously reported gavage toxicity studies identified by NSF International in the February 2008 draft assessment for MTBE, recently published short-term drinking water studies in adult and juvenile CD-1 or BALB/c mice (de Peyster et al., 2008), short-term gavage studies in SD rats (Dongmei et al., 2009) as well as preliminary details for unpublished short- and long-term drinking water studies in Wistar rats (Bermudez et al., 2007, 2008, 2009) have become available.

No evidence of hepatic peroxisome proliferation was observed in male rats administered MTBE via gavage at 800 mg/kg-day for 14 days. Increased mean relative liver weight, cholesterol levels, and/or minimal-to-moderate centrilobular hypertrophy were observed in rats administered MTBE via gavage at 1,000 mg/kg-day and above for 28 days. Subchronic gavage and drinking water exposures to MTBE were associated with increased mean absolute and relative kidney weights in male rats accompanied by hyaline droplet formation, renal tubular cell regeneration, and/or  $\alpha$ -2 $\mu$ -globulin immunoreactivity in the proximal tubules. Gavage but not drinking water exposure to MTBE for 90 days was associated with increased mean liver weights, liver-associated blood effects (aspartate aminotransferase, blood urea nitrogen, and/or cholesterol) and/or centrilobular hepatocyte hypertrophy in rats. Mean relative testes weights were reduced in the absence of associated histopathology in male rats that received MTBE at 384 mg/kg-day in their drinking water for one-year. Chronic gavage exposure to MTBE was associated with an increase in Leydig cell tumors in male rats and leukemias/lymphomas (combined) in female rats.

## **7.1 Limited-Exposure Effects**

MTBE was found to be irritating to the eyes and skin of rabbits, but did not induce skin sensitization in guinea pigs.

### **7.1.1 Irritation and Sensitization Studies**

Following the application of 0.5 mL of neat MTBE to the intact and abraded skin of six rabbits for 24 hours, a primary irritation index of 3.36 was reported, which was considered "moderately" irritating to skin (IPCS, 1998). Moderate erythema and edema were observed. Effects were slightly more pronounced on abraded skin. In mice, MTBE can induce slight to severe respiratory irritation following inhalation of 300 to 30,000 mg/m<sup>3</sup>, respectively. A 1% induction and challenge concentration of MTBE did not induce skin sensitization in twenty guinea pigs (IPCS, 1998).

### 7.1.2 Ocular Exposure Studies

MTBE was irritating to the eyes of rabbits and caused mild, but reversible, changes (IPCS, 1998).

## 7.2 Single-Exposure Studies

The oral (gavage) LD<sub>50</sub> for MTBE is approximately 3,800 mg/kg in rats (IPCS, 1998) and 4,000 in mice (OEHHA, 1999). An LD<sub>50</sub> of 3,433 mg/kg in SD rats has also been reported (Dongmei et al., 2008). Signs of intoxication after a single oral lethal dose consisted of central nervous system depression, ataxia, labored respiration, and death.

## 7.3 Short-Term Exposure Studies

### 7.3.1 Three-Day Gavage Study in Female B6C3F<sub>1</sub> Mice

Eight female B6C3F<sub>1</sub> mice per dose were given MTBE (> 99.95% purity in corn oil) by gavage at 0 or 1,800 mg/kg-day for three days (Moser et al., 1996). Food and water were available *ad libitum*. Eighteen hours after the last dose, the mice were sacrificed and hepatocytes were isolated for measurement of hepatocyte proliferation *in vitro*, expressed as the amount of 5-bromo-2'-deoxyuridine incorporation into hepatocyte nuclei. The hepatic labeling index was calculated by dividing the number of labeled nuclei by the total number of nuclei and multiplying by 100. Body weight and absolute and relative liver weights were also measured. Body and liver weights were not affected by treatment, but MTBE induced a statistical increase in the hepatocyte labeling index of 6.5% compared to 2.5% in controls.

### 7.3.2 Fourteen-Day Drinking Water Study in Wistar Rats

Wistar rats were administered MTBE via the drinking water at 0, 3, 7, or 15 mg/mL for 14 days (Bermudez et al., 2007). The mean received doses were 0, 371, 799, or 1,624 mg/kg-day in males and 0, 363, 843, or 1,839 mg/kg in females. Body weights, clinical signs, and food and water consumption were monitored daily. Kidneys and testes weights were recorded. Hematology included hematocrit, blood urea nitrogen, serum creatinine, and blood levels of MTBE and t-butanol. Airborne concentrations of MTBE averaged ≤0.2 ppm in ambient air and control cages and ≤33 ppm in high-dose cages throughout the study. Consumption of water was significantly reduced in treated compared to control rats by approximately 20-30% and 35-39%, in males and females, respectively, in the absence of an impact on mean body weight or food consumption. MTBE blood levels averaged ≤2.1 μM, while t-butanol blood concentrations ranged from 38-116 μM. Kidney weights were increased in high-dose male rats. The study authors considered the MTBE exposure to be associated with increased blood t-butanol levels, increased kidney weights in males, and reduced blood urea nitrogen and hematocrit levels in females. Complete study details are not available at this time.

### 7.3.3 Fourteen-Day Gavage Study in Sprague-Dawley Rats

Ten Sprague-Dawley rats per sex and dose were administered 0, 357, 714, 1,071, or 1,428 mg/kg-day MTBE (99.95% purity in corn oil) by gavage for 14 days (Robinson et al., 1990). The high dose was selected because it was 37% of the LD<sub>50</sub>. Rats were housed separately by sex and food and water were available *ad libitum*. Mortality and clinical signs were monitored daily. Food and water consumption were measured throughout the study at unspecified intervals. Body weight was measured on Days 0, 4, 6, and 14. Hematology parameters and clinical chemistry were conducted on all rats at study termination. Hematology included hematocrit, hemoglobin, mean corpuscular volume, erythrocytes, leukocytes, differential leukocytes, and reticulocytes. Clinical chemistry included glucose, blood urea nitrogen, creatinine, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, cholesterol, calcium, and phosphorus. Brain, liver, spleen, lung, thymus, kidney, adrenal, heart, and “gonads” weights were measured at study termination, and relative organ-to-body-weight ratios were calculated. Gross necropsies were conducted on all rats at study termination. Histological examinations were conducted on all control and high-dose rats at study termination, and included unspecified “major organs”. If target histopathological organs were identified, these organs were also examined histologically in the remaining dose groups.

At 357 mg/kg-day, two males died, but the deaths were attributed to the gavage treatment. Diarrhea was observed in treated rats. Mean creatinine was statistically increased by 16% in males compared to controls. Mean absolute (15%) and relative (16%) lung weights were statistically lower in females compared to controls.

At 714 mg/kg-day, diarrhea and statistically reduced food intake (unspecified magnitude) were observed in males compared to controls. Mean hemoglobin (6%), hematocrit (4%), differential lymphocytes (6%), and creatinine (16%) were statistically increased in males compared to controls. Mean alanine aminotransferase (21%) and cholesterol (22%) were statistically increased and mean serum calcium (6%) was statistically decreased in females compared to controls. Mean absolute (11%) and relative (11%) lung weights were statistically lower in females compared to controls. Mean absolute (12%) and relative (9%) lung weights were statistically lower in males compared to controls.

At 1,071 mg/kg-day, diarrhea was observed in treated rats. Mean erythrocytes (6%), hemoglobin (6%), aspartate aminotransferase (43%), and lactate dehydrogenase (78%) were statistically increased, and mean differential monocytes (33%) were statistically decreased in males compared to controls. Mean cholesterol (34%) was statistically increased in females compared to controls. Mean absolute (14%) and relative (11%) lung weights were statistically lower in females compared to controls.

At 1,428 mg/kg-day, two males and two females died, but the deaths were attributed to gavage. Diarrhea and profound but transient (< two hours) anesthesia were observed after dosing in male and female rats. Statistically reduced food intake (unspecified magnitude) was observed in females compared to controls. Statistically reduced mean terminal body weight of 10% was observed in females compared to controls. Mean

erythrocytes (7%), blood urea nitrogen (14%), aspartate aminotransferase (38%), cholesterol (37%), and lactate dehydrogenase (63%) were statistically increased, and mean differential monocytes (33%) were statistically decreased in males compared to controls. Mean glucose (15%) was statistically increased and mean blood urea nitrogen (27%) and creatinine (20%) were statistically decreased in females compared to controls. Mean absolute (22%) and relative (15%) lung weights were statistically lower in females compared to controls. Mean absolute spleen (18%) and mean absolute (20%) and relative thymus (27%) weights were statistically lower in females compared to controls. Mean relative kidney (8%) and brain (9%) weights were statistically higher in females compared to controls. The incidence of hyaline droplet nephropathy in the renal tubules was “moderately” increased in dosed male rats, but no further details were provided, with the exception that increased hyaline droplets within the cytoplasm of proximal tubular epithelial cells were noted in 7/8 (88%) high-dose males compared with 2/5 (40%) controls.

#### **7.3.4 Fourteen-Day Gavage Studies in Male Sprague-Dawley Rats**

In a 14-day gavage study, de Peyster et al. (2003) examined whether MTBE exposure could induce hepatic peroxisome proliferation, since other chemicals that cause Leydig cell tumors in rats were also shown to induce peroxisome proliferation. Six male Sprague-Dawley rats per dose were administered MTBE (> 99.8% purity in corn oil) via gavage at 0 or 800 mg/kg-day via gavage for 14 days. Positive control rats were administered gemfibrozil via the diet. Hepatic peroxisomes were isolated from liver sections and processed for peroxisomal  $\beta$ -oxidation and examined with an electron microscope. Terminal blood samples were collected for measurement of cholesterol, triglyceride, alanine aminotransferase, and aspartate aminotransferase. Liver weights were measured, and relative liver-to-body-weight ratios were calculated. According to the study authors, there were no statistical differences between treated and vehicle control rats, but not all of the data were provided. It should be noted that although the methodology stated that MTBE doses of 800 mg/kg-day were administered, the results section indicated that MTBE doses were 1,000 mg/kg-day.

Ten male Sprague-Dawley rats per dose were administered MTBE (> 99.8% purity in corn oil) via gavage at 0 or 1,200 mg/kg-day for 14 days (de Peyster et al., 2003). This dose was determined in previous experiments to lower circulating testosterone levels without affecting body weight. Liver, testes, accessory sex organs (unspecified), and brain weights were measured. Total protein content and P450 content in hepatic microsomes was determined, and hepatic microsomal aromatase activity was measured.

In rats treated with 1,200 mg/kg-day MTBE, a statistical increase in mean relative liver weight of 15% was observed compared to controls. Although hepatic P450 content was comparable to controls, hepatic microsomal aromatase activity was decreased by 36% compared to controls.

### 7.3.5 Fifteen-Day Gavage Study in Male Sprague-Dawley Rats

Fifteen male Sprague-Dawley rats per dose were administered 0 or 1,500 mg/kg-day MTBE (> 99.9% purity in corn oil) via gavage for 15 days (Williams and Borghoff, 2000; Williams et al., 2000). At study termination, the rats were sacrificed, body, adrenal, kidney, epididymides, testes, prostate, pituitary gland, seminal vesicle, and liver weights were determined, and histopathological examination of the liver, kidneys, testes, and adrenals was conducted.

There were no treatment-related deaths. Deaths attributed to gavage, which were confirmed at necropsy, were primarily limited to the high-dose rats. Statistically increased mean absolute and relative adrenal weights of 15% and 17%, respectively, were observed at 1,500 mg/kg-day compared to controls. Minimal-to-moderate centrilobular hypertrophy was observed in 8/12 treated rats, but not in controls. The hypertrophy was characterized as increased size and cytoplasmic eosinophilia of hepatocytes that were oriented around central veins, which at times extended into the midzonal region of the lobule. The severity was dose-related and ranged from minimal to moderate, and the authors suggested that the effect was similar to that observed with phenobarbital administration. Protein droplet nephropathy of the kidney was observed in 11/12 treated rats and 1/15 controls.

### 7.3.6 Two- and Four-Week Gavage Studies in SD rats

Ten male SD rats per dose and exposure duration received MTBE (99.8% purity in peanut oil) via gavage at 0, 400, 800, or 1,600 mg/kg-day for two or four weeks (Dongmei et al., 2009a). The basis of the dose selection was not specified. Mortality, body weight, and food consumption were recorded daily. Terminal hematology (total and differential leukocytes, erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red blood cell volume distribution width, and platelets) and clinical chemistry (alanine aminotransferase, aspartate aminotransferase, total protein, albumin, globulin, alkaline phosphatase, urea, creatinine, cholesterol, triglyceride, high- and low-density lipoprotein cholesterol) parameters were included for all dose groups. Brain, heart, liver, spleen, lung, kidneys, testes, epididymis, thymus, and prostate weights were recorded at study termination.

In both the two- and four-week studies, transient signs of central nervous system depression were observed in treated rats at an unspecified incidence, particularly at the high-dose.

In the two-week study, one low- and mid-dose rat each died. Mean creatinine level was reduced ( $p < 0.01$ ) at all doses compared to controls. The decrease was 22%, 28%, and 33% lower than controls at 400, 800, and 1,600 mg/kg-day, respectively. Mean relative testes to body weight ratios were statistically ( $p < 0.05$ ) decreased in a non-dose-related manner in all treated groups. The decrease was 12%, 11%, and 12% lower than controls at 400, 800, and 1,600 mg/kg-day, respectively. At the mid-dose and higher, mean alanine aminotransferase was reduced ( $p < 0.01$ ) compared to controls. The decrease was

31% and 40% lower than controls at 800 and 1,600 mg/kg-day, respectively. Mean relative thymus weight was 24% and 20% lower ( $p < 0.05$ ) than controls at 800 and 1,600 mg/kg-day, respectively. At the high-dose after four weeks, mean cholesterol was increased (9%) compared to controls and an increase ( $p < 0.001$ ) in mean leukocytes counts (55%) was accompanied by a shift in differential counts of various populations. Mean relative heart (17%) and liver (11%) weights were increased in high-dose rats compared to controls.

In the four-week study, one rat each in the control, mid- and high-dose group died. Three low-dose rats died but the cause of death was not reported. Mean creatinine level was reduced at all doses compared to controls. The decrease was 19% ( $p < 0.05$ ), 23% ( $p < 0.01$ ), and 30% ( $p < 0.01$ ) lower than controls at 400, 800, and 1,600 mg/kg-day, respectively. Mean low-density lipoprotein was reduced at the mid- and high-dose compared to controls. The decrease was 13% ( $p < 0.05$ ) and 31% ( $p < 0.01$ ) lower than controls at 800 and 1,600 mg/kg-day, respectively. Mean globulin was increased (14%,  $p < 0.01$ ) and alkaline phosphatase (36%,  $p < 0.01$ ) and triglycerides (22%,  $p < 0.05$ ) were decreased at the high-dose. Other sporadic statistical differences in various clinical chemistry parameters, including alanine aminotransferase, aspartate aminotransferase, cholesterol and high-density lipoprotein, were not considered biologically-significant due to the lack of a dose response. Mean eosinophil counts were increased ( $p < 0.05$ ) in mid- (50%) but not high-dose rats and mean hemoglobin was increased ( $p < 0.05$ ) in high-dose rats. Mean relative liver (15%) and kidney (7%) weights were increased ( $p < 0.05$ ) in mid-dose rats but not high-dose rats. Mean relative prostate weights (33%) were increased ( $p < 0.05$ ) in high-dose rats. The study authors considered the possibility that MTBE may be associated with testicular atrophy or necrosis. However, the authors of the present assessment consider the decrease in mean relative testes weights in treated males after two-weeks to likely be due to an unusually high control mean relative weight since there was no dose-response and the effect was not seen after four weeks of exposure to the same doses or in a separate study conducted under the same protocol (two-week exposure with the same doses; Dongmei et al., 2008). The study authors did not identify a NOAEL.

### **7.3.7 Three-Week Gavage Study in CD-1 Mice**

CD-1 mice were administered MTBE via gavage five days per week for three weeks (Ward et al., 1994). This study was not available, but OEHHA (1999) and ATSDR (1996) indicated that no effects on body weight or unspecified reproductive parameters were observed at doses up to 1,000 mg/kg, and thus identified the NOAEL as 1,000 mg/kg (or 714 mg/kg-day).

## 7.4 Long-Term and Chronic Exposure Studies

### 7.4.1 Subchronic Studies

#### 7.4.1.1 Four-Week Gavage Studies In Sprague-Dawley Rats

Ten Sprague-Dawley rats per sex and dose were administered MTBE (unspecified purity unspecified in water vehicle) via gavage at 0, 90, 440, or 1,750 mg/kg for five days per week for four weeks (Johnson et al., 1992; Klan et al., 1992). These doses were approximately equivalent to 0, 64, 314, or 1,250 mg/kg-day. Rats were housed individually, and food and water were available *ad libitum*. Mortality and clinical signs were monitored daily. Body weights were measured weekly. Hematology and clinical chemistry were conducted on all rats at study termination. Hematology included erythrocytes, platelets, leukocytes, differential leukocytes, hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular volume, globulin, and albumin/globulin ratio. Clinical chemistry included glucose, creatine kinase, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, blood urea nitrogen, creatinine, sodium, potassium, calcium, chloride, total protein and bilirubin, albumin, cholesterol and triglycerides. Adrenal, brain, ovary, testes, heart, kidney, liver, and spleen weights were measured, and relative organ-to-body-weight ratios were calculated. Gross necropsies were performed on all rats at study termination. Histological examinations were conducted on all control and high-dose rats at study termination, and included the adrenals, aorta, brain, cecum, colon, duodenum, epididymides, esophagus, eye, heart, ileum, jejunum, kidneys, liver, lung, mammary glands, muscle, nerve, ovaries, pancreas, pituitary, prostate, rectum, salivary gland, seminal vesicle, skin, spleen, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder, and uterus. If effects were noted, the same organs were examined in the lower doses as well.

No non-gavage-related deaths occurred at any dose. At 64 mg/kg-day, transitory (<one hour after dosing) salivation was observed in several rats. Mean corpuscular hemoglobin was statistically increased in females by 4% compared to controls. Mean alkaline phosphatase was statistically increased in males by 15% compared to controls. Mean relative kidney weights were increased in females by 6% compared to controls.

At 314 mg/kg-day, transitory (<one hour after dosing) salivation was observed in all rats, and hypoactivity and/or ataxia was observed in several rats. Mean erythrocytes were statistically increased in males by 6% compared to controls. Mean relative kidney weights were statistically increased in males by 8% compared to controls. Hyaline droplet formation in the proximal convoluted tubules was observed in 7/10 males.

At 1,250 mg/kg-day, transitory (<one hour after dosing) salivation was observed in all rats, and hypoactivity and/or ataxia was observed in several rats. Mean corpuscular hemoglobin was statistically increased in females by 3% compared to controls. Mean total protein was statistically increased by 8% in females compared to controls, and cholesterol was statistically increased in males by 20% and females by 26% compared to controls. Mean relative kidney weights were increased in males by 13% and females by

17% compared to controls. Mean relative liver weights were increased in males by 8% and females by 12% compared to controls. Mean relative adrenal weights were increased in males by 19% compared to controls. Hyaline droplet formation in the proximal convoluted tubules was observed in 9/10 males. Various effects in the stomach, including submucosal edema, subacute inflammation, epithelial hyperplasia, and ulceration were observed in up to 4/7 males and 5/10 females. The effects were largely confined to the forestomach.

The study authors concluded that the hyaline droplet formation in the proximal tubules in males was attributable to  $\alpha$ -2 $\mu$ -globulin nephropathy, which was not relevant to humans. Further, the stomach lesions were attributable to local irritation, which was not considered a direct result of systemic toxicity.

Fifteen male Sprague-Dawley rats per dose were administered 0, 250, 500, 1,000, or 1,500 mg/kg-day MTBE (> 99.9% purity in corn oil) via gavage for 28 days (Williams and Borghoff, 2000; Williams et al., 2000). At study termination, the rats were sacrificed, body, adrenal, kidney, epididymides, testes, prostate, pituitary gland, seminal vesicle, and liver weights were determined, and histopathological examination of the liver, kidneys, testes, and adrenals was conducted.

There were no treatment-related deaths. Deaths attributed to gavage, which were confirmed at necropsy, were primarily limited to the high-dose rats. At 250 mg/kg-day, statistically increased mean relative kidney weights of 10% were observed compared to controls. Minimal-to-moderate centrilobular hypertrophy was observed in 1/15 treated rats, but not in controls. The hypertrophy was characterized as increased size and cytoplasmic eosinophilia of hepatocytes that were oriented around central veins, which at times extended into the midzonal region of the lobule. The severity was dose-related and ranged from minimal to moderate, and the authors suggested that the effect was similar to that observed with phenobarbital administration. Protein droplet nephropathy of the kidney was observed in 12/15 treated rats, but not in controls.

At 500 mg/kg-day, statistically increased mean relative kidney weights of 9% were observed compared to controls. Minimal-to-moderate centrilobular hypertrophy was observed in 10/15 treated rats, but not in controls. Protein droplet nephropathy of the kidney was observed in 15/15 treated rats, but not in controls.

At 1,000 mg/kg-day, statistically increased mean absolute and relative kidney weights of 10% and 16%, respectively, were observed compared to controls. Statistically increased mean relative liver weights of 10% were observed compared to controls. The increased relative liver weight was accompanied by minimal-to-moderate centrilobular hypertrophy in 11/13 treated rats, but not in controls. Protein droplet nephropathy of the kidney was observed in 12/13 treated rats, but not in controls.

At 1,500 mg/kg-day, mean body weight was reduced by 12% compared to controls. Statistically increased mean relative kidney weights of 18% were observed compared to controls. Statistically increased mean relative liver weights of 14% were observed

compared to controls. Statistically increased mean relative testes weights of 15% were observed compared to controls. The increased relative liver weight was accompanied by minimal-to-moderate centrilobular hypertrophy in 11/11 treated rats, but not in controls. Increased mean relative kidney weights, accompanied by protein droplet nephropathy of the kidney, were observed in 10/11 treated rats, but not in controls.

#### **7.4.1.2 13-Week Drinking Water Study in Wistar Rats**

In the range finding study for a chronic study (Bermudez et al., 2009), Wistar rats were administered MTBE via drinking water at 0, 0.5, 3, 7.5, or 15 mg/mL for 13 wks (Bermudez et al., 2008). The mean received doses were 0, 37, 209, 514, or 972 mg/kg-day in male rats and 0, 50, 272, 650, or 1,153 mg/kg-day in females. Body weights, clinical signs, and food and water consumption were monitored weekly. Urine was collected from males and analyzed at Day 4 and 21. Cell replication in the kidney was assessed at Week 1, 4, and 13. Complete histological examinations were performed on all rats at study termination. Serum hormone levels were assayed after 28 days of exposure. Mean terminal body weights in males at 514 and 972 mg/kg-day were less than controls. MTBE exposure resulted in decreased water consumption in both sexes of all treated groups in the absence of an impact on food consumption. Males had elevated urine specific gravity and osmolality. Serum hormone levels were unchanged by treatment in either sex. Kidney wet weights were elevated in males and females that received 7.5 and 15 mg/mL. Cell replication of kidney cortical epithelial cells was unchanged in females but was elevated in males of the 15mg/mL group at Week 4. Renal tubular cell regeneration was noted in males exposed to 15 mg/mL at 13 wks of exposure. Quantitative  $\alpha$ -2 $\mu$  globulin levels in kidney were elevated in high-dose males at Week 1 and 4. The study authors considered the reduction in water consumption in male and female rats of all MTBE dose groups to be treatment-related and attributed the reduction in mean body weights and renal tubule effects in males receiving the two highest dose levels to  $\alpha$ -2 $\mu$ - nephropathy. Complete study details are not available at this time.

#### **7.4.1.3 Thirteen-Week or Longer Gavage Studies In Sprague-Dawley Rats**

Ten Sprague-Dawley rats per sex and dose were administered MTBE ( $\geq$  99.95% purity in corn oil) via gavage at 0, 100, 300, 900, or 1,200 mg/kg-day for 90 days (Robinson et al., 1990). Rats were housed separately by sex and food, and water was available *ad libitum*. Mortality and clinical signs were monitored daily. Food consumption was measured once a week and water consumption was measured three times a week. Body weight was measured twice a week. Hematology and clinical chemistry were conducted on all rats at study termination. Hematology included hematocrit, hemoglobin, mean corpuscular volume, erythrocytes, leukocytes, differential leukocytes, and reticulocytes. Clinical chemistry included glucose, blood urea nitrogen, creatinine, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, cholesterol, calcium, and phosphorus. Brain, liver, spleen, lung, thymus, kidney, adrenal, heart, and “gonads” weights were recorded at study termination, and relative organ-to-body-weight ratios were calculated. Gross necropsies were conducted on all rats at study termination. Histological examinations were conducted on all control and high-dose rats at study termination, and

included unspecified “major organs”. If target histopathological organs were identified, these organs were also examined histologically in the remaining dose groups.

This study was not designed to meet current U.S. EPA (2009) Health Effects Testing Guidelines, since hematology did not include a measure of clotting potential, and clinical chemistry did not include albumin, alkaline phosphatase, gamma glutamyl transferase, globulin, sorbitol dehydrogenase, bilirubin, protein, or serum chloride, magnesium, potassium, or sodium. Further, urinalysis was not conducted, and organs examined histologically were specified only as including “major organs”. Although Robinson et al. (1990) noted that the weight of the “gonads” were reported, data for the testes were not reported.

At 100 mg/kg-day, one male died. Diarrhea was observed in male and female rats. Water consumption (unspecified magnitude) was statistically increased in females compared to controls. A statistical decrease in blood urea nitrogen was observed in males (15%) and females (20%).

At 300 mg/kg-day, one female died. Diarrhea was observed in male and female rats. A statistical decrease in blood urea nitrogen was observed in males (20%) and females (33%). A statistical decrease in glucose (17%) and lactate dehydrogenase (62%) and an increase in cholesterol (11%) were observed in females. A statistical decrease in creatinine (15%) and an increase in aspartate aminotransferase (34%) were observed in males. Mean absolute (4%) and relative (4%) brain weights were statistically increased in males compared to controls. Mean relative kidney weights (10%) were statistically increased in females compared to controls.

At 900 mg/kg-day, two females and one male died. Diarrhea was observed in male and female rats. Food consumption (unspecified magnitude) was statistically increased in females compared to controls. A statistical decrease in blood urea nitrogen was observed in males (18%) and females (35%). A statistical decrease in mean glucose (13%) and lactate dehydrogenase (16%) and an increase in cholesterol (31%) were observed in females compared to controls. A statistical decrease in mean creatinine (26%) and an increase in cholesterol (22%) and lactate dehydrogenase (5%) were observed in males compared to controls along with a statistical increase in mean relative liver weights (13%). Mean absolute (14%) and relative (15%) kidney weights and relative liver weights (13%) were statistically increased in males compared to controls. Mean relative heart (11%), liver (12%), kidney (13%), and thymus (33%) weights were statistically increased in females compared to controls.

At 1,200 mg/kg-day, four females and one male died. Diarrhea and a profound but transient (<two hours) anesthetic effect were observed in male and female rats. Water consumption (unspecified magnitude) was statistically increased in males and females compared to controls. A statistical decrease in blood urea nitrogen was observed in males (18%) and females (17%). A statistical decrease in mean glucose (24%) and lactate dehydrogenase (16%) and an increase in cholesterol (20%) were observed in females compared to controls. A statistical decrease in mean creatinine (19%) and an increase in

aspartate aminotransferase (33%) were observed in males compared to controls. Terminal mean body weight was statistically reduced by 9% in males compared to controls. Mean absolute (18%) and relative (21%) kidney weights, absolute (9%) and relative (13%) lung weights, and relative liver weights (13%) were statistically increased in male rats and mean relative kidney (12%) and adrenal (25%) weights were statistically increased in female rats compared to controls. Microscopic findings included chronic nephropathy in control and high-dose male rats. These changes, such as renal tubular degeneration, were more severe in treated rats than controls. Renal tubules plugged with granular casts were found in 5/10 high-dose males, and 10/10 males exhibited slight increases in cytoplasmic hyaline droplets in proximal tubular epithelial cells. No further details regarding the renal changes were provided. The study authors attributed the early deaths in treated rats to dosing error since macroscopic findings in the lungs of most rats that died included “lungs that were mottled to uniformly red, fluid-filled, and often exhibited foreign material in airways”.

Ten male Sprague-Dawley rats per dose were administered 0, 200, 600, and 1,000 mg/kg MTBE (98.8% purity in soybean oil) by gavage for five days per week for 13 weeks (Zhou and Ye, 1999). These doses were equivalent to 0, 143, 428, or 857 mg/kg-day, respectively. Body weight and food and water consumption were measured weekly. Clinical chemistry was conducted at study termination and included aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, total protein, albumin, globulin, albumin/globulin ratio, blood urea nitrogen, and creatinine. Liver, kidney, testes, and lung weights were measured at study termination. Gross necropsies and histopathological examinations were conducted at study termination, and included the liver, kidney, testes, and lung. Liver sections were also examined under an electron microscope.

This study was not designed to meet current U.S. EPA (2009) Health Effects Testing Guidelines, since only males were evaluated, hematology was not conducted, and clinical chemistry did not include alkaline phosphatase, gamma glutamyl transferase, glucose, sorbitol dehydrogenase, total bilirubin, total cholesterol, or serum electrolytes. Further, urinalysis was not conducted; spleen, heart, ovary, and brain weights were not measured; and histopathology included only the liver, kidney, testes, and lung.

At 143 mg/kg-day, mean absolute and relative liver weights were statistically increased by 12% and 14%, respectively, compared to controls. Lactate dehydrogenase was statistically decreased (32%) at the low, but not mid or high doses compared to controls. Aspartate aminotransferase was statistically increased by 31% compared to controls, but within historical control ranges. Histopathological examination in treated rats was comparable to controls. Electron microscopy of the liver revealed nuclear condensation, fat droplets, lysosome appearance in cells, and smooth endoplasmic reticulum disintegration, but the magnitude and number of animals affected was not specified. The study authors did, however, indicate that more severe changes were observed at higher doses.

At 428 mg/kg-day, mean absolute and relative liver weights were statistically increased by 18% and 15%, respectively, compared to controls. Mean relative kidney weight was statistically increased by 6% compared to controls, but no accompanying renal pathology was observed. Aspartate aminotransferase was statistically increased by 29% compared to controls, but within historical control ranges. Histopathological examination in treated rats was comparable to controls. Electron microscopy of the liver revealed nuclear condensation, fat droplets, lysosome appearance in cells, and smooth endoplasmic reticulum disintegration, but the magnitude and number of animals affected was not specified. However, the study authors indicated that more severe changes were observed at higher doses.

At 857 mg/kg-day, mean absolute and relative liver weights were statistically increased by 21% and 22%, respectively, compared to controls. Mean absolute and relative kidney weights were statistically increased by 12% and 13%, respectively, compared to controls, but no accompanying renal pathology was observed. Aspartate aminotransferase was statistically increased by 27% compared to controls, but within historical control ranges. Histopathological examination in treated rats was comparable to controls. Electron microscopy of the liver revealed nuclear condensation, fat droplets, lysosome appearance in cells, and smooth endoplasmic reticulum disintegration, but the magnitude and number of animals affected was not specified. However, the study authors indicated that more severe changes were observed at higher doses.

## **7.4.2 Chronic Studies**

### **7.4.2.1 One-year Drinking Water Study in Wistar Rats**

As part of a two-year bioassay, Wistar rats received MTBE (>99% purity) in their drinking water at 0, 0.5, 3, or 7.5 mg/mL in males and 0, 0.5, 3, or 15 mg/mL in females for 52 weeks (Bermudez et al., 2009). Dose selection was based on the results of a 90-day study (Bermudez et al., 2008). The mean received doses ranged from 30 to 384 mg/kg-day in males and 56 to 1,147 mg/kg-day in females. The mid-dose was not reported and can not be calculated due to the lack of body weight and water consumption data. Body weights, clinical signs, and food and water consumption were monitored regularly. Interim and terminal hematology, clinical chemistry, and urinalyses parameters were included at various intervals. A complete necropsy was performed at six months (males) and 12 months (males and females). Blood levels of t-butanol (TBA) were determined for males and females at 12-months. Food consumption was comparable to controls, while water consumption was less than control ( $p<0.01$ ) in treated males and females. Mean body weight in male rats was 9%, 11%, and 7% less than controls in the low-, mid- and high-dose groups, respectively. There were no significant changes in hematology or serum chemistry parameters for males or females. Blood levels of TBA increased with dose in males and females. Urine osmolality and specific gravity were increased in males (7.5 mg/mL MTBE) and females (15 and 3 mg/mL MTBE) and urine creatinine was increased in males (3 mg/mL MTBE) and females (3 and 15 mg/mL MTBE). There was a trend in males and females of increasing urine protein with dose. Increases in osmolality and specific gravity suggest increased concentration of urine as a response to reduced

intake of water and suggest that the apparent increase in protein is due to a concentration effect. The statistical increase in mean relative kidney weights in treated male rats at 12 months of exposure was accompanied by an increase in nephropathy of minimal to mild severity. Nephropathy was observed in 3/10, 8/9, 9/10, and 9/10 male rats in the control, low-, mid-, and high-dose groups, respectively. Nephropathy was observed in 2/9, 2/10, 1/10, and 2/10 female rats in the control, low-, mid-, and high-dose groups, respectively. A non-dose-related but statistical increase in mean relative testes weights was observed in mid- (20%, right testes) and high-dose (13%, right; 15% left) male rats in the absence of testicular histopathology. The study authors considered the treatment-related effects to be limited to reduced water intake in males and females and an increase in nephropathy in males. Complete study details are not available.

#### 7.4.2.2 Two-year Gavage Study in SD rats

Sixty Sprague-Dawley rats per sex and dose were administered 0, 250, or 1,000 mg/kg MTBE (> 99% purity in extra virgin olive oil) by gavage four times a week for 104 weeks on a weekly schedule of two days dosing, one day without dosing, two days dosing, and two days without dosing (Belpoggi et al., 1995; 1997). These doses were approximately equivalent to daily doses of 0, 143, or 571 mg/kg-day. The animals were housed five per cage and kept under observation until natural death. Food and water were available *ad libitum*. Mortality and clinical signs were monitored daily. Food and water consumption and body weight were measured weekly for the first 13 weeks and twice monthly thereafter until 112 weeks. Thereafter, body weights were measured every eight weeks until death. Gross necropsies were performed on all rats after natural death. Histopathological examinations, which were performed on all rats after natural death, included the aorta, adrenals, bone, bone marrow, brain, bronchi, cecum, colon, diaphragm, duodenum, esophagus, eye, Harderian gland, heart, ileum, jejunum, kidneys, liver, lung, lymph nodes (mediastinal, subcutaneous, mesenteric), mammary glands, muscles, nerve, ovaries, pancreas, pharynx, larynx, pituitary, prostate, salivary gland, seminal vesicle, subcutaneous tissue, skin, subcutaneous tissue, spinal cord, spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus, Zymbal gland, and gross lesions.

This study was not designed to meet current U.S. EPA (2009) Health Effects Testing Guidelines, since the dosing occurred on a four-day per week schedule, with two days dosing, one day without dosing, two days dosing, and two days without dosing. Further, since no results for hematology, clinical chemistry, urinalysis, or organ weights were reported, it was presumed that these parameters were not examined. Histology did not include the aorta, bone, bone marrow, eye, mammary glands, muscles, nerve, seminal vesicle, or spinal cord. The tumor incidences reported in this study were reviewed by Belpoggi et al. (1998) after a re-evaluation of the histopathology slides.

At the low dose, survival at the end of the treatment period (104 weeks) was 35% in treated females compared to 48% in controls. Survival at the end of the treatment period (104 weeks) was 30% in low-dose males compared to 30% in controls. There was a statistical increase in lymphomas and leukemias combined (7/51) in female rats

compared to controls (2/58). The individual incidence of lymphomas or leukemias was not indicated. The lymphatic tumors were accompanied by an increase in dysplastic proliferation of lymphoreticular tissue, which was characterized as hyperplastic lymphoid tissues at various sites, in which atypical lymphoid cells, usually lymphoimmunoblasts, isolated and/or aggregated in small clusters, were observed. An increased incidence of uterine sarcomas was observed in low-dose females, but not high-dose females, compared to controls.

At the high-dose, survival at the end of the treatment period (104 weeks) was 28% in treated females compared to 48% in controls. Survival at the end of the treatment period (104 weeks) was 42% in high-dose males compared to 30% in controls. There was a statistical increase in the incidence of testicular Leydig cell (interstitial cell) tumors in male rats compared to controls. The incidence was 3/26, 5/25, and 11/32 in control, low-, and high-dose males (based on the number of rats surviving at the occurrence of the first Leydig tumor, which was 96 weeks). In female rats, there was a dose-related statistical increase in lymphomas and leukemias combined (12/47) compared to controls (2/58), and an increase in dysplastic proliferation of lymphoreticular tissue. The study authors reported that the range of the lymphatic tumors in females in this study was within the historical control incidence for these tumors in female Sprague-Dawley rats from studies in their laboratory (below 10%).

The study authors reported that “no treatment-related non-oncological pathological changes were detected by gross inspection and histological examination”, but the data were not provided.

## **7.5 Reproductive and Developmental Toxicity Studies**

No *in vivo* oral two-generation reproduction or developmental studies were identified for MTBE. One- and two-generation inhalation reproductive studies in rats and four inhalation developmental studies in rats, mice, and rabbits are available for MTBE. These studies have been reviewed by ECB (2002), OEHHA (1999), IPCS (1998), ECETOC (1997), and ATSDR (1996). Specific reproductive effects were not observed in rats at concentrations up to 28,800 mg/m<sup>3</sup>. MTBE was not associated with developmental effects at concentrations below those associated with maternal toxicity. Decreases in uterine weight and increases in estrogen metabolism in mice have been observed at 28,800 mg/m<sup>3</sup> (IPCS, 1998). Since the reproductive and developmental studies for MTBE have been extensively reviewed by several other regulatory agencies, this section includes only the oral reproductive and developmental studies for MTBE, including those published since the various regulatory reviews. None of the oral studies identified were standardized two-generation reproduction or developmental studies.

### 7.5.1 Reproduction Studies

No oral one- or two-generation reproduction studies were identified for MTBE, but effects on reproductive hormone levels, reproductive organ weights and/or histology has been investigated following oral exposure to MTBE in non-standardized reproduction studies.

#### *In vivo studies*

Since MTBE exposure has previously been linked to some limited evidence of seminiferous tubule degeneration in CD-1 or BALB/c mice (Billiti et al., 1999; Almeida et al., 2004), de Peyster et al. (2008) conducted a series of experiments to replicate or further characterize these effects. Six adult male CD-1 mice per dose received 0, 400, 1,000, or 2,000 mg/kg MTBE (unspecified purity in corn oil) via gavage on Days 1, 3, and 5 (de Peyster et al., 2008). After an intraperitoneal injection of human Chorionic Gonadotropin on Day 6 to stimulate testosterone production, rats were sacrificed on Day 7. Body, testes, epididymides, seminal vesicle, liver and brain weights were recorded along with testicular histology and testosterone levels. No examined parameter was in treated mice was statistically different than controls. Mild unilateral seminiferous tubule degeneration was observed in one control mouse and an abscess of the preputial gland was observed in one mid-dose mouse.

In a second experiment, six male BALB/c mice per dose received MTBE in their drinking water at 0, 80, 800, or 8,000 µg/L for 28 days (de Peyster et al., 2008). Mean received doses were calculated by the study authors to be 0, 305, 3,180, or 31,920 mg/mouse/day. Based on mean terminal body weight for each respective group, the approximate received doses can be considered 0, 11, 111, or 1,178 mg/kg-day. Dose selection was based on a two-week palatability study which found no difference in water intake in treated mice compared to controls. Mean terminal serum testosterone or number of sperm/ mg cauda in treated mice was not statistically different than controls. Mild or minimal unilateral seminiferous tubule degeneration was observed in 2/6, 1/6, 1/6, and 2/6 mice in the control, low-, mid-, and high-dose groups, respectively. All mice had a Grade 1 (minimal) severity score with exception of one control male that had a Grade 2 (mild) severity score.

In a third experiment, ten male juvenile (22-day old) BALB/c mice per dose received MTBE in their drinking water at 0, 80, 800, or 8,000 µg/L for 51 days through PND 77 (de Peyster et al., 2008). Mean received doses were calculated by the study authors to be 0, 381, 3,900, or 39,170 mg/mouse/day. Based on mean terminal body weight for each respective group, the approximate received doses can be considered 0, 15, 155, or 1,536 mg/kg-day. Mean relative (but not absolute) seminal vesicle (low-dose) and lung weights (mid-dose) were statistically increased compared to controls. The increase in mean lung weight was attributed to one mid-dose mouse that had a large lung mass. However, since bloody lungs were noted upon necropsy in several treated mice (incidence/group unspecified but including the one with the mass) and one control mouse, the possibility that the effect was treatment-related could not be discounted by the study authors,

particularly considering the increasing trend (non-statistical) in lung weights. Mean serum estradiol or testosterone concentrations or mean seminiferous tubule diameter in treated mice were not statistically different from controls.

No evidence of oxidative stress in liver homogenates from juvenile mice was observed based on malondialdehyde, Trolox equivalent antioxidant capacity (TEAC) and 8-hydroxy-2'-deoxyguanosine adduct formation as endpoints. Collectively, the study authors concluded that drinking water exposure to MTBE at up to 8,000 µg/L for up to 51 days was not associated with adverse effects on reproductive hormones, organ weights, or histology under the conditions of their studies.

Ten male SD rats per dose and exposure duration received MTBE (99.8% purity in peanut oil) via gavage at 0, 400, 800, or 1,600 mg/kg-day for two or four weeks (Dongmei et al., 2009b). The basis of the dose selection was not specified. Mortality, body weight, and food consumption were recorded daily. Serum testosterone, leutenizing hormone, and follicle stimulating hormone were measured at study termination along with serum total antioxidant ability and peroxide levels (serum maleic dialdehyde levels), and the mRNA expressions of androgen binding protein, 8-oxoguanine DNA glycosidase, and extracellular superoxide dismutase. Epididymides sperm counts and abnormal sperm were recorded by one technician blinded to the treatment group. Liver, kidneys, testes, and epididymis weights were recorded at study termination and testicular histology was included on all rats.

Although the authors indicated that liver, kidneys, and testes weights were recorded at study termination, these data were not reported. After two weeks of exposure, mean serum leutenizing hormone was statistically increased in a non-dose-related manner in all dosed groups (~25-30% estimated from graph). Mean serum follicle stimulating hormone was statistically increased in mid- (~30%) and high-dose rats (~40%). Mean serum testosterone was reduced in mid- (~70%) and high-dose rats (~60%). High-dose rats had “less compact cells” in the testes upon histological examination compared to controls. The mRNA level of androgen binding protein was decreased at the high-dose (33%).

After four weeks of exposure, no effects on reproductive organ weights were observed. The percent of abnormal sperm was statistically increased in treated rats in a dose-related fashion. The mean “semina deformity ratio” estimated from the graph was ~12%, 18% ( $p < 0.05$ ), 19% ( $p < 0.05$ ), and 28% ( $p < 0.01$ ) in rats from the control, 400, 800, or 1,600 mg/kg-day groups, respectively. Mid-dose rats had increased (as opposed to reduced after two weeks) serum testosterone levels (~35%, estimated). Mid- and high-dose rats had “irregular and disordered arrangement, with the shedding of cellular material from the seminiferous epithelium” in the testes upon histological examination compared to controls. The mRNA level of androgen binding protein was decreased at the mid- (~20%) and high-dose (~22%).

Some other parameters (serum maleic dialdehyde total serum antioxidant ability, 8-oxoguanine DNA glycosidase and extracellular superoxide dismutase) were statistically significant compared to controls but due to the lack of a dose- or temporally-related

pattern, they were not considered biologically-significant by the authors of the present assessment. The study authors concluded that high-doses of MTBE could disrupt spermatogenesis but did not identify a NOAEL. The authors of the present assessment consider the four-week NOAEL to be 400 mg/kg-day since the non-dose related reduction in serum leutenizing hormone observed after two weeks was not seen after four weeks as well and the minimal magnitude and lack of dose-response of the increase in sperm deformity ratio at the low- and mid-dose after four weeks. The LOAEL can be considered 800 mg/kg-day based on the alterations in serum testosterone and histopathology in the testes.

Potential testicular toxicity associated with MTBE was assessed in five male CD-1 mice per dose that received MTBE (unspecified purity in canola oil) via gavage on Days 1, 3, and 5 at 0, 400, 1,000 or 2,000 mg/kg (Billitti et al., 2005). Testosterone levels were measured on Day 6 fecal samples collected from all mice. Thereafter, mice were injected with human chorionic gonadotrophin to stimulate maximum testosterone production and fecal samples were collected after one day. Body weight and serum testosterone were measured and histological examination of the testes was included at study termination. Two high-dose mice died as a result of dosing error. All examined parameters in the treated mice that survived were comparable and/or not statistically different compared to controls.

Eight female B6C3F<sub>1</sub> mice per dose were given MTBE (> 99.95% purity in corn oil) by gavage at 0 or 1,800 mg/kg-day for three days (Moser et al., 1996). Food and water were available *ad libitum*. Twenty-four hours after the last dose, the mice were sacrificed and hepatocytes were isolated for measurement of estrogen metabolism *in vitro*, which was expressed as the amount (nM) of 17- $\beta$ -estradiol metabolized/ mg protein/ minute. MTBE induced a two-fold statistical increase in the rate of estrogen metabolism *in vitro* compared to controls.

Six to eleven female CD-1 mice per dose were administered MTBE via gavage at 0, 600, or 1,500 mg/kg-day for five days either with or without subcutaneous administration of 1 ug estradiol on Days 3-5 (Okahara et al., 1998). The authors reported that MTBE had some mild, but in some cases, seemingly opposite, activity under these conditions, but no further details were provided. At 1,500 mg/kg-day, delayed vaginal opening by Postnatal Day 26 was observed in half of the treated females. Mean relative uterine weights were statistically increased in the MTBE/estradiol group compared to the estradiol alone control group, but the dose level or magnitude was not specified. According to the authors, no clear or consistent effect was observed in uterine peroxidase activity or in ovarian, liver, or kidney weights compared to controls. No further details were available in this abstract, and a full publication was not located.

Twelve male Sprague-Dawley rats per dose were administered MTBE (> 99.8% purity in corn oil) via gavage at 0, 1,000, or 1,500 mg/kg every other day for a total of 14 doses over 28 days (de Peyster et al., 2003). The 1,000 mg/kg dose was selected since it was the highest dose in the Belpoggi et al. (1995) chronic gavage study, and since this dose induced a statistical increase in Leydig cell tumors in male rats compared to controls.

The 1,500 mg/kg dose was chosen since it was approximately the highest dose from a 90-day gavage study for MTBE by Robinson et al. (1990). The experiment originally included an untreated and a vehicle-treated control group, but the results were ultimately combined into one control group. Due to excess weight loss and one death, the 1,000 and 1,500 mg/kg doses were reduced to 500 and 750 mg/kg, respectively, starting on Day 13. The terminal doses were approximately equivalent to 0, 357, or 536 mg/kg-day. This study was conducted to investigate the mechanism of Leydig cell tumors induced in male rats after chronic gavage exposure to MTBE in a study by Belpoggi et al. (1995). It has been suggested that increased hepatic metabolism through P450 enzymes results in increased steroid catabolism, resulting in reduced testosterone circulation.

Testosterone concentrations were measured on Day 1 and 14 (tail blood) and 28 (cardiac puncture). If the serum sample volume was sufficient, terminal corticosterone was also measured to determine whether the Leydig tumors were induced through an increased stimulation of testicular glucocorticoid receptors, which can impair testosterone production. Liver, kidney, testes, seminal vesicles, and epididymides weights were measured, and mean organ-to-body weight ratios were calculated. Total protein and total P450 were measured from isolated liver microsomes.

At study termination, mean body weight gain was 8, 3, 1, and 0% in the negative control, vehicle control, 357 mg/kg-day, and 536 mg/kg-day groups, respectively. The Day 1 testosterone concentration in rats administered 537 mg/kg-day MTBE was statistically reduced by approximately 70% compared to pooled controls (vehicle and negative, n=4 only). The Day 14 and 28 testosterone concentrations in treated rats were not statistically different compared to controls. At study termination, mean absolute liver weight and total microsomal protein in treated rats were comparable to controls, but mean liver P450 content (mmol/mg protein and nmol/g liver weight) was slightly, but statistically, increased in rats administered 537 mg/kg-day compared to controls. There was a 24% increase in mmol/mg P450 protein and a 35% increase in nmol P450/g liver weight compared to pooled controls. Mean corticosterone levels on Day 1, 14, and 28 were not statistically different compared to pooled controls, but the sample size was only about 4-5 rats per dose. The authors concluded that high gavage doses of MTBE result in reduced circulating testosterone in rats during the hours immediately following dosing (4-5 hours). However, the increase in hepatic P450 content did not result in reduced circulating testosterone, as originally hypothesized by the study authors, but the authors could not rule out other hormonal or metabolic compensatory mechanisms.

Twelve male Sprague-Dawley rats per dose were administered MTBE (> 99.8% purity in corn oil) via gavage at 0, 40, 400, or 800 mg/kg-day for 28 days (de Peyster et al., 2003; Day et al., 1998). This study was conducted to further investigate the mechanism of Leydig cell tumors induced in male rats after chronic gavage exposure to MTBE in a study by Belpoggi et al. (1995). Luteinizing hormone, prolactin, testosterone, and corticosterone concentrations were measured on Day 1 and 14 (tail blood) and 28 (trunk). Liver, pituitary, testes, epididymides, thyroid, adrenal, prostate, and brain weights were measured, and mean organ-to-body and brain weight ratios were calculated.

At 40 mg/kg-day, mean plasma corticosterone was statistically reduced by 28% compared to controls on Day 14, but the corticosterone was not statistically different from controls at study termination.

At 400 mg/kg-day, terminal mean body weight was statistically reduced by 7% compared to controls. Mean plasma corticosterone was statistically reduced by 42% compared to controls on Day 14, but the corticosterone was not statistically different from controls at study termination. Mean pituitary weight was statistically reduced by 23% compared to controls.

At 800 mg/kg-day, terminal mean body weight was statistically reduced by 13% compared to controls. Mean plasma corticosterone was statistically reduced by 43% compared to controls on Day 14. At study termination, mean plasma testosterone was statistically reduced by 35% and mean plasma corticosterone was statistically reduced by 44% compared to controls. The mean adrenal-to-body-weight ratio was statistically reduced by 20% compared to controls. The mean thyroid-to-body-weight ratio was statistically reduced by 29% compared to controls.

Six male Sprague-Dawley rats per dose were administered MTBE (> 99.8% purity in corn oil) via gavage at 0 or 800 mg/kg-day for five days (de Peyster et al., 2003). This study was conducted to further investigate the mechanism of Leydig cell tumors induced in male rats after chronic gavage exposure to MTBE in a study by Belpoggi et al. (1995). The effect of castration on the hypothalamic-pituitary axis was investigated using testosterone implants in phosphate buffered saline (PBS) and four experimental groups of male rats. The four groups consisted of sham implant (PBS) and 800 mg/kg-day MTBE via gavage, sham implant (PBS) and corn oil vehicle gavage, testosterone implant and 800 mg/kg-day MTBE via gavage, and testosterone implant and corn oil vehicle gavage. The amount of testosterone in each implant was intended to result in average circulating testosterone as in normal non-castrated rats. Lutenizing hormone, prolactin, and testosterone concentrations from the tail vein were measured four hours after the initial dose (Day 1) and two hours after the final dose (Day 5). Terminal prostate and seminal vesicle weights were measured. The experiment was repeated with a younger set of animals, reportedly to reduce the amount of body weight variation, since each testosterone implant contained a standard amount of testosterone.

In the first experiment, the authors found that circulating testosterone was higher and lutenizing hormone was lower in rats with testosterone implants compared to controls, but the differences were not statistically significant. Since each testosterone implant contained a standard amount of testosterone, the authors suggested that the results were confounded by the difference in body weights between the rats after the 3-day recovery period from the surgical implant, even though prior to surgery, the rats were of comparable body weights. Thus, the experiment was repeated with a younger set of animals, but the results of the first experiment could not be duplicated and may have been confounded by a small sample size, since one control rat gained a large amount of body weight. Recognizing confounding factors, the authors concluded that there was no clear evidence of an effect on the hypothalamic-pituitary axis in either experiment.

Ten male Sprague-Dawley rats per dose were administered MTBE (> 99.8% purity in corn oil) via gavage at 0 or 1,200 mg/kg-day for 14 days (de Peyster et al., 2003). This dose was determined in previous experiments to lower circulating testosterone levels without affecting body weight. Terminal plasma estradiol, luteinizing hormone, and testosterone concentrations were measured from trunk blood samples. Testes and accessory sex organs (unspecified) weights were measured. Total protein content in testicular microsomes was determined, and testicular microsomal aromatase activity was also measured.

In rats treated with 1,200 mg/kg-day MTBE, a statistical decrease in mean testosterone and luteinizing hormone of 51% and 10%, respectively, was observed compared to controls, and a statistical increase in mean estradiol of 26% was observed compared to controls. Testicular microsomal aromatase activity was decreased by 55% compared to controls.

Williams and Borghoff (2000) and Williams et al. (2000) investigated the hypothesis that MTBE-induced decrease in serum testosterone levels in male rats may be due in part to the ability of MTBE to induce the metabolism of endogenous testosterone and, hence, enhance its clearance. Male Sprague-Dawley rats were administered 0, 250, 500, 1,000, or 1,500 mg/kg-day MTBE (> 99.9% purity in corn oil) via gavage for 15 or 28 days. Rats were sacrificed one hour following the last dose, and serum and interstitial fluid testosterone, and serum dihydrotestosterone, 17- $\beta$ -estradiol, prolactin, triiodothyronine (T3), thyroxin (T4), thyroid stimulating hormone, follicle stimulating hormone, and luteinizing hormone levels were measured. Histopathology of the testes was performed in all rats.

After 15 days at 1,500 mg/kg-day, interstitial fluid and serum testosterone levels (approximately 60% each, estimated from graph) and serum prolactin levels (56%) were statistically decreased compared to controls.

After 28 days at 1,000 mg/kg-day, serum triiodothyronine (T3) was statistically decreased by 19% compared to controls.

After 28 days at 1,500 mg/kg-day, serum triiodothyronine (T3; 19%), luteinizing hormone (approximately 20%, estimated from graph), and dihydrotestosterone (45%) were statistically decreased compared to controls.

No testicular lesions were observed at any dose level. The authors concluded that MTBE causes mild perturbations in T3 and prolactin; however, the short-term (15-day), but not longer-term (28-day), decrease in testosterone and the mild increase in luteinizing hormone levels did not fit the pattern caused by known Leydig cell tumorigens, since larger increases in luteinizing hormone have been caused by chemicals known to cause Leydig cell tumors.

Ten CD-1 mice per sex and dose were given 0, 1, 10, 100 or 1,000 mg/kg MTBE (purity unspecified in corn oil) by gavage for five days per week for three weeks (Ward et al., 1994). As this study was not available, this summary was based on IPCS (1998). These doses were approximately equivalent to 0, 0.7, 7, 71, or 714 mg/kg-day. At study termination, the mice were sacrificed and one testis from each male and both ovaries from each female were sectioned for cytological evaluation. In males, sperm number, Sertoli cells, spermatogonia, spermatocytes, and capped spermatids were evaluated. In females, oocyte quality was assessed. There were no effects of MTBE on any of the cell types examined, but no further details were provided. OEHHA (1999) and ATSDR (1996) indicated that the reproductive NOAEL for this study was 1,000 mg/kg-day, but no further details were available. It should be noted that OEHHA (1999) and ATSDR (1996) likely did not adjust for the less than daily dosing regimen, and likely should have indicated the reproductive NOAEL as 714 mg/kg-day.

### *In vitro* assays

High concentrations of MTBE were cytotoxic to cultured Sertoli seminiferous epithelium cells possibly through an oxidative stress-mediated pathway (Dongmei et al., 2008). Cytotoxicity and oxidative stress were measured in cultured SD rat Sertoli cells exposed to MTBE (99.8% purity) concentrations at 0, 0.005, 0.5 or 50 mM (Dongmei et al., 2008). The production of reactive oxygen species, maleic dialdehyde content and the level of superoxide dismutase activity in cell supernatants were measured along with the expression of 8-oxoguanine DNA glycosidase and extracellular superoxide dismutase in Sertoli cells. Effects at the low-dose (0.005 mM) were limited to an increase in reactive oxygen species after three and 48 hours of exposure but not after two, six or 24 hours. High concentrations (0.5 mM and higher) were associated with cytotoxicity, induced lactate dehydrogenase leakage, and increased plasma membrane damage in Sertoli cells. The relevance of these effects at lower, more environmentally-relevant exposure concentrations was not discussed.

The effect of MTBE on the testosterone production of Leydig cells in culture was examined *in vitro* by de Peyster et al. (2003). Leydig cells were isolated from adult male Sprague-Dawley rats and incubated for three hours with 0, 50, or 100 mM MTBE (> 99.8% purity) or t-butanol, a major metabolite of MTBE. The same concentrations were also tested with human Chorionic Gonadotropin (hCG), added to stimulate testosterone production. Cell viability at the tested concentrations was at least 85%. Testosterone production after the three-hour exposure was measured by radioimmunoassay. Aminoglutethimide was used as a positive control, and the experiment was conducted in triplicate.

A statistical reduction in basal testosterone production of 56% and 76%, compared to controls, was observed at 50 and 100 mM MTBE, respectively. A statistical reduction in human Chorionic Gonadotropin-stimulated testosterone production of 51% and 60%, compared to controls, was observed at 50 and 100 mM MTBE, respectively. T-butanol induced a statistical reduction in basal testosterone production of 72% and 66% at 50 mM and 100 mM compared to controls, respectively. T-butanol induced a statistical

reduction in human Chorionic Gonadotropin-stimulated testosterone production of 73% and 83% at 50 mM and 100 mM compared to controls, respectively. The positive control, aminoglutethimide (5 mM) induced a statistical reduction of basal and human Chorionic Gonadotropin-stimulated testosterone production of 80% and 75% compared to controls, respectively.

In a 14- and 90-day systemic gavage study in Sprague-Dawley rats by Robinson et al. (1990), effects on ovary weight and histology and testes weight and histology were examined, and no effects were reported.

### **7.5.2 Developmental Toxicity Studies**

No oral developmental studies were identified for MTBE.

## **7.6 Studies of Immunological and Neurological Effects**

No standardized immunological or neurological assays were identified for MTBE, but some immunological or neurological effects have been reported in systemic studies for MTBE. Reported immunological effects were limited to reduced circulating corticosterone levels and thyroid weights in rats after short-term gavage exposures. Reported neurological effects were limited to transitory post-dosing salivation after gavage doses of 64 mg/kg-day and higher and transitory hypoactivity and/or ataxia at higher doses in rats. However, the transient salivation may reflect the irritating properties of methyl *tert*-butyl ether rather than a neurological effect.

### **7.6.1 Immunological Effects**

Twelve male Sprague-Dawley rats per dose were administered MTBE (> 99.8% purity in corn oil) via gavage at 0, 1,000, or 1,500 mg/kg every other day for a total of 14 doses over 28 days (de Peyster et al., 2003). After an adjustment of doses due to excess weight loss, the terminal doses were approximately equivalent to 0, 357, or 536 mg/kg-day. Terminal corticosterone was measured on Day 1, 14, and 28. Mean corticosterone levels on Day 1, 14, and 28 were not statistically different compared to controls, but the sample size was only about 4-5 rats per dose, due to other analyses concurrently requiring blood volume.

Twelve male Sprague-Dawley rats per dose were administered MTBE (> 99.8% purity in corn oil) via gavage at 0, 40, 400, or 800 mg/kg-day for 28 days (de Peyster et al., 2003). Corticosterone concentrations were measured on Day 1 and 14 (tail blood) and 28 (trunk). Thyroid weights were measured, and mean organ-to-body and brain weight ratios were calculated.

At 40 mg/kg-day, mean plasma corticosterone was statistically reduced by 28% compared to controls on Day 14, but the corticosterone was not statistically different from controls at study termination.

At 400 mg/kg-day, mean plasma corticosterone was statistically reduced by 42% compared to controls on Day 14, but the corticosterone was not statistically different from controls at study termination.

At 800 mg/kg-day, mean plasma corticosterone was statistically reduced by 43% compared to controls on Day 14. At study termination, mean plasma corticosterone was statistically reduced by 44% compared to controls. The mean thyroid-to-body-weight ratio was statistically reduced by 29% compared to controls.

In a 14-day and 90-day systemic gavage study in Sprague-Dawley rats by Robinson et al. (1990), effects on spleen and thymus weight and histology were examined, and no effects were reported. Although some statistical reductions in monocyte differential counts were observed, the effect was not dose- or duration-related and did not occur in both sexes.

In a 28-day gavage study by Lee et al. (1998), MTBE (unspecified purity in corn oil) was administered to male Sprague-Dawley rats at 0, 40, 400, or 800 mg/kg-day via gavage. At 800 mg/kg-day, high corticosterone levels were observed, but the magnitude and statistical significance were not specified. Limited details were available in this published abstract and a full publication was not located.

### 7.6.2 Neurological Effects

Neurological effects in rats were limited to transitory salivation reported after a single gavage dose of 90 mg/kg-day and higher and transitory hypoactivity and/or ataxia at higher doses (Johnson et al., 1992).

Intraventricular injection of high doses of MTBE impaired the performance of rats in a Morris water maze task, significantly increased the expression of GABA(A) receptor alpha1 subunit in the hippocampus, and reduced phosphorylation of ERK1/2 (Zheng et al., 2009). The biological significance of this effect in humans was not proposed by the authors.

Martin et al. (2002) studied the effect of 200 and 400 mM MTBE (unspecified purity) on binding at the gamma-aminobutyric acid receptor site in cerebral cortex membrane preparations isolated from male Sprague-Dawley rats. The gamma-aminobutyric acid receptor was probed using the <sup>3</sup>H-t-butylbicycloorthobenzoate, which binds to the convulsant recognition site of the receptor. The experiment was conducted in triplicate.

The 50% inhibitory concentration (IC<sub>50</sub>) of MTBE and its metabolite, t-butanol, on the binding of <sup>3</sup>H-t-butylbicycloorthobenzoate at the gamma-aminobutyric acid(A) receptor site was 120 and 69 mM, respectively. In additional saturation binding assays, 200 and 400 mM MTBE statistically reduced apparent density of convulsant binding, or B<sub>max</sub>, to 36 and 17% of the control value, respectively. The study authors suggested that their results indicate that direct effects on the gamma-aminobutyric acid(A) receptor site by MTBE or its metabolite t-butanol could explain some of the neurotoxicological or

neurobehavioral effects observed after MTBE exposures in humans and laboratory animals.

## **8.0 RISK CHARACTERIZATION**

### **8.1 Hazard Identification**

#### **8.1.1 Major Non-Cancer Effects**

The scientific literature for MTBE in humans and laboratory animals has been reviewed extensively by several national and international regulatory agencies, including the World Health Organization (WHO, 2005; IPCS, 1998), the Netherlands (Baars et al. 2004); European Chemicals Bureau (ECB, 2002), California EPA (OEHHA, 1999), International Agency for Research on Cancer (IARC, 1999), U.S. EPA (1997), European Center for Ecotoxicology and Toxicology of Chemicals (ECETOC, 1997), Agency for Toxic Substances and Disease Registry (ATSDR, 1996), and Health Canada (1996; 1992). Thus, this risk assessment to determine a non-cancer RfD for MTBE focuses mainly on the oral exposure studies included in these reviews or that have been published since these reviews. Oral LOEL and NOEL values from the animal studies reviewed are shown in the Appendix. Although inhalation toxicity studies are available for MTBE, oral studies were preferred due to the lack of a physiologically-based pharmacokinetic model to reliably extrapolate inhaled doses in rodents to human equivalent oral doses.

Drinking water exposure to MTBE for up to one year was associated with reduced water intake likely secondary to palatability (Bermudez et al., 2007; 2008, 2009). As a result, the increases in urine osmolality, specific gravity, and creatinine levels were attributed to a “concentration effect” secondary to reduced water intake (Bermudez et al., 2009). Statistical increases in mean relative kidney weights in treated male rats at 12 months were accompanied by nephropathy of minimal to mild severity. A statistical increase in mean relative testes weights was also observed in treated male rats in the absence of testicular histopathology. The LOAEL for one-year drinking water exposure to MTBE can be considered 384 mg/kg-day based on the increase in mean relative testes weights at the high-dose. However, the received mg/kg-day dose at the mid-dose was not reported and thus the NOAEL is not known for this effect (Bermudez et al., 2009). Interpretation of this effect in high-dose males may be impacted by concurrent reductions in mean body weight (7%). Full details of these unpublished drinking water studies are not available.

Gavage (Dongmei et al., 2009a; Johnson et al., 1992; Robinson et al., 1990; Williams and Borghoff, 2000; Williams et al., 2000) but not drinking water exposure (Bermudez et al., 2008, 2009) to MTBE was associated with increased mean liver weights, liver-associated blood effects (aspartate aminotransferase, blood urea nitrogen, and/or cholesterol) and minimal-to-moderate centrilobular hepatocyte hypertrophy in SD rats. “No treatment-related nononcological pathological changes were detected by gross inspection and histological examination” after two-years of gavage exposure to MTBE at adjusted doses

up to 571 mg/kg-day (1,000 mg/kg-day adjusted for daily dosing; Belpoggi et al., 1995), but the data were not reported. Similar to drinking water exposure, gavage exposure to MTBE was associated with increased mean absolute and relative kidney weights in male rats accompanied by hyaline droplet formation in the renal proximal tubules (Johnson et al., 1992; Robinson et al., 1990; Williams and Borghoff, 2000; Williams et al., 2000).

### 8.1.2 Mode of Action (Non-Cancer Effects)

The effects seen in the kidneys of male rats that received MTBE via drinking water or gavage exposures for up to one year (Bermudez et al., 2007, 2008, 2009; Johnson et al., 1992; Klan et al., 1992; Williams and Borghoff, 2000; Williams et al., 2000) were consistent with  $\alpha$ -2 $\mu$ -globulin nephropathy. These effects are specific to male rats and of questionable relevance to humans (Meek et al., 2003).

The liver effects associated with four or 13 weeks of gavage exposure to MTBE are likely due to an adaptive mechanism to metabolize bolus doses of MTBE at 250 mg/kg-day and above (Johnson et al., 1992; Robinson et al., 1990; Williams and Borghoff, 2000; Williams et al., 2000) since they were not observed after drinking water exposure to doses up to 972 mg/kg-day in male rats and 1,153 mg/kg-day in females for the equivalent exposure duration or longer (Bermudez et al., 2008, 2009). Limited *in vivo* metabolism data suggest that oral exposure to MTBE induces various CYP450 isozymes (Le Gal et al., 2001; Williams and Borghoff, 2000), and MTBE has been shown to induce its own metabolism by 2.1-fold beginning after 15 days of gavage exposure to 1,500 mg/kg-day MTBE (> 99.9% purity in corn oil; Williams and Borghoff, 2000). The lack of multiple examined doses in the study precludes an assessment of dose-response. These adaptive mechanisms likely lead to the centrilobular hepatocyte hypertrophy observed after gavage exposure but that appear to be lacking after drinking water exposure (Bermudez et al., 2008), recognizing that the latter study is not available for review. The lack of hepatic tumors from the Belpoggi et al. (1995) chronic gavage study is reassuring.

The lack of reported thyroid or liver tumors in rats from the Belpoggi et al. (1995) study at adjusted doses of 571 mg/kg-day (1,000 mg/kg-day adjusted for less than seven day dosing) supports a mode of action independent of a disruption of the thyroid/pituitary axis (as seen with phenobarbital), a mode of action that has unclear relevance to humans (Meek et al., 2003). Studies examining the activation of the constitutive androstane receptor (CAR) or thyroid stimulating hormone (TSH) levels after oral exposure to MTBE were not identified in the public literature.

The potential for MTBE to induce peroxisome proliferation, which is mediated through a series of events that are unlikely to be relevant to humans (Cohen et al., 2003; Klaunig et al., 2003), has been examined in one study (de Peyster et al., 2003). There were no statistical differences in liver weights, liver-associated blood effects (cholesterol, triglyceride, alanine aminotransferase, and aspartate aminotransferase), or peroxisomal  $\beta$ -oxidation in hepatic peroxisomes from male rats that received MTBE via gavage at 800 mg/kg-day for 14 days compared to controls (de Peyster et al., 2003). In addition to liver

tumors, a peroxisome proliferation-mediated mode of action may also result in Leydig cell tumors and/or pancreatic cell tumors (Klaunig et al., 2003). An increase in Leydig cell tumors were observed in male rats that received MTBE for two years (Belpoggi et al., 1995). The mode of action of the Leydig cell tumors observed in male rats after chronic gavage dosing (Belpoggi et al., 1995) is unclear and may be mediated through mechanisms considered to have a threshold (ie. may be potentially relevant to the derivation of a RfD for MTBE). Reproductive hormone levels and reproductive organ weights and histology have been examined after single and repeated gavage or drinking water exposure in rats and mice. Single or repeated gavage dosing (up to 28 days) of MTBE in corn oil at  $\geq 800$  mg/kg-day was associated with reductions in mean serum testosterone (de Peyster et al., 2003). Increased mean relative testes weights (15%) and estradiol levels along with reduced serum luteinizing hormone and testicular microsomal aromatase levels were observed in SD rats after high gavage doses of MTBE ( $\geq 1,200$  mg/kg-day) for 28 days (de Peyster et al., 2003; Williams and Borghoff, 2000; Williams et al., 2000). The decrease in mean relative testes weights in male SD rats that received MTBE via gavage in peanut oil at 400 mg/kg-day and higher for two weeks was likely due to an unusually high control mean relative testes weight since there was no dose-response and the effect was not seen after four weeks of exposure to the same doses or in a separate study conducted under the same protocol (two-week exposure with the same doses; Dongmei et al., 2008). In this latter study, the NOAEL was considered 400 mg/kg-day based on the alterations in serum testosterone and histopathology in the testes observed at 800 mg/kg-day after four weeks of gavage exposure (Dongmei et al., 2008). No effects on testes weights were reported in SD rats that received MTBE in soybean oil via gavage at adjusted doses up to 857 mg/kg-day (1,000 mg/kg-day adjusted for less than daily dosing) for 13 weeks (Zhou and Ye, 1999). Although Robinson et al. (1990) noted that the weight of the “gonads” were reported, data for the testes were not reported. A statistical increase in mean relative testes weights was observed in the absence of testicular histopathology after one year of drinking water exposure to MTBE at 384 mg/kg-day (Bermudez et al., 2009), but the received mg/kg-day dose at the mid-dose was not reported and thus the NOAEL is not known for this effect. High concentrations ( $> 50$  mM) of MTBE were also found to reduce basal and human Chorionic Gonadotropin (hCG)-stimulated testosterone production in cultured rat Leydig cells (de Peyster et al., 2003). All investigations in male mice failed to find a clear or consistent effect on reproductive hormone levels, organ weights or histology at gavage doses up to 2,000 mg/kg-day for three days (Billitti et al., 2005); gavage doses of 714 mg/kg-day (adjusted for less than daily dosing) for three weeks (Ward et al., 1994), or drinking water exposure at up to  $\sim 1,500$  mg/kg-day for up to 51 days (de Peyster et al., 2008).

Collectively, studies examining reproductive hormone levels or reproductive tissue responses in male rats or mice after oral exposure to MTBE failed to find a clear or consistent pattern. Some studies reported effects on testes weights or histology in rats at high gavage doses (800 mg/kg-day) for 28 days (de Peyster et al., 2003; Dongmei et al., 2008) while other studies reported no effects in rats at higher exposure doses ( $\geq 857$  mg/kg-day) for longer (13 weeks) exposure periods (Robinson et al., 1990; Zhou and Ye, 1999). Nonetheless, in studies reporting an effect, the gavage NOAEL and LOAEL for alterations in serum testosterone accompanied by testicular histopathology in male SD

rats may be ~400 mg/kg-day and 800 mg/kg-day, respectively (de Peyster et al., 2003; Dongmei et al., 2008). The drinking water NOAEL is unknown for the increase in relative testes weights observed in the absence of testicular histopathology in Wistar rats after one year of drinking water exposure to MTBE at 384 mg/kg-day (Bermudez et al., 2009).

### **8.1.3 Key Study and Critical Effect for RfD**

The liver effects associated with subchronic gavage exposure to MTBE in rats (Robinson et al., 1990) were attributed to adaptive mechanisms responding to bolus dosing since they do not appear to have been observed after drinking water exposure to higher doses for longer exposure periods (Bermudez et al., 2008, 2009), recognizing that these latter studies are not available. The highest dose administered in the 13-week gavage study was 1,200 mg/kg-day (Robinson et al., 1990) and the highest dose received in the 13-week drinking water study was 972 mg/kg-day in male rats and 1,153 mg/kg-day in females. (Bermudez et al., 2008)

Since full study details are not available for the unpublished 13-week or one-year drinking water studies, the key study for the RfD is therefore considered the subchronic gavage study in rats (Robinson et al., 1990). No critical effects could be identified since the liver effects, which include increased mean liver weights and liver-associated blood effects (aspartate aminotransferase, blood urea nitrogen, and cholesterol) were attributed to bolus dosing and the kidney effects were attributed to  $\alpha$ -2 $\mu$ -globulin nephropathy.

Although the two-year gavage study (Belpoggi et al., 1995) was perhaps conducted for a more appropriate exposure duration to serve as the basis of a lifetime drinking water level, the non-neoplastic data were not available for review. Recognizing that standardized chronic inhalation studies in rats and mice are available (Bird et al., 1997), insufficient kinetics data are available to reliably extrapolate an inhalation concentration in rats to human equivalent oral doses.

### **8.1.4 Identification of Susceptible Populations**

Individuals with reduced ability to metabolize MTBE may potentially be more sensitive to adverse health outcomes resulting from MTBE exposure. Some human variants of CYP2A6, obtained from people who claimed to be sensitive to MTBE had 33% less activity than the wild type in oxidizing MTBE (Hong et al., 2001; as cited by McGregor, 2006). There are no other data by which to identify any subpopulations (e.g., the elderly, pregnant women, children, or people with allergies or asthma) that might be at special risk to MTBE exposure (IPCS, 1998).

### **8.1.5 Dose-Response Assessment**

The liver effects observed after gavage dosing in the Robinson et al. (1990) study include increased mean liver weights and liver-associated blood effects (aspartate aminotransferase, blood urea nitrogen, and cholesterol), with the most sensitive effect

being the statistical reduction in mean blood urea that occurred at all doses (250 mg/kg-day and above) in both sexes (Table 2). While centrilobular hepatocyte hypertrophy was not specifically reported in the Robinson et al. (1990) study, there is dose-related evidence of this observation in another study administering lower doses (250 mg/kg-day) for a shorter exposure duration (28 days; Williams and Borghoff, 2000). While adaptive mechanisms to metabolizing bolus or high-dose chemical exposures are usually reversible upon cessation of treatment, these mechanisms, if provoked for a sufficiently prolonged duration, may result in irreversible changes that are considered adverse and potentially relevant to humans.

**Table 2. Decreased mean blood urea nitrogen in SD rats that received MTBE via gavage (Robinson et al., 1990)**

Dose (mg/kg-day)	Mean blood urea nitrogen (mg/dL)		Mean terminal relative liver weight (g) <sup>1</sup>		Mean terminal body weight (g)	
	Males	Females	Males	Females	Males	Females
0	22.5	24.8	3.13	3.83	477.1	330.2
100	17.9 (15%)**	21.1 (20%)**	3.12 (0%)	3.78 (↓1%)	427.7 (↓1%)	330.1 (0%)
300	18.1 (20%)*	16.7 (33%)**	3.08 (↓2%)	4.03 (↑5%)	475.5 (0%)	312.5 (↓5%)
900	18.4 (18%)*	16.2 (35%)**	3.60 (↑13%)**	4.39 (↑13%)*	470.1 (↓2%)	311.3 (↓6%)
1,200	18.5 (18%)*	20.6 (17%)*	3.54 (↑13%)**	4.07 (↑6%)	458.8 (↓4%)	302.1 (↓9%)*

\* p<0.05 compared to controls  
\*\* p<0.001  
<sup>1</sup> Relative to mean terminal body weight; mean terminal absolute liver weights were not statistically different from controls at any dose

The plateau in the reduction of blood urea nitrogen and the increase in mean relative liver weights in male rats observed at 900 mg/kg-day and above after gavage dosing (Robinson et al., 1990) suggests that the threshold for inducing adaptive metabolic pathways after bolus dosing may be around 900 mg/kg-day (Table 1). Although environmentally-relevant exposure concentrations of MTBE are unknown, they are likely to be much lower than those associated with an adaptive response. Williams and Borghoff (2000) demonstrated that MTBE induced its own metabolism 2.1-fold beginning after 15 days of gavage exposure to 1,500 mg/kg-day MTBE. The effect of long-term drinking water exposure to MTBE at levels below the threshold that would elicit such adaptive responses is unknown since data from the two-year drinking water study are not available (Bermudez et al., 2009). Therefore, the NOAEL can be considered 300 mg/kg-day based on the induction of adaptive responses observed at 900 mg/kg-day. The lack of a critical effect precludes dose response assessment for MTBE and thus, the NOAEL/uncertainty factor approach can be used to calculate the RfD for MTBE.

There are insufficient details available from the drinking water studies to compare to the results of gavage dosing (Bermudez et al., 2008). Other than reduced water intake and  $\alpha_2\mu$ -globulin associated renal effects, mean body weight was reduced by an unspecified magnitude in males that received 514 mg/kg-day for 13 weeks (Bermudez et al., 2008). Mean relative testes weights were reduced in the absence of associated histopathology in male rats that received MTBE at 384 mg/kg-day in their drinking water for one-year (Bermudez et al., 2009).

### 8.1.6 Uncertainty Factor Selection

- **Interspecies Extrapolation = 10x**

Chemical-specific adjustment of either portion of the interspecies uncertainty factor can be made using chemical-specific data if the conditions described in WHO/IPCS (2005) can be satisfied. For the toxicokinetic portion of the factor, these conditions include:

1. Identification of the active chemical moiety.

There are insufficient data to clearly establish a mode of action or identify the active chemical moiety since a critical effect could not be identified for MTBE based on the available studies.

2. Determination of whether the toxicity depends on the area under the concentration-time curve (AUC) or the maximum concentration ( $C_{max}$ ).

The liver changes likely depend on the maximum concentration ( $C_{max}$ ) since they are observed after bolus dosing but not drinking water exposure to comparable doses.

3. The availability of a physiologically based pharmacokinetic (PBPK) model to describe target organ dosimetry, or comparable animal and human data.

A physiologically-based model extrapolating from oral dosing in rats to humans was not identified. Preliminary details from the unpublished drinking water studies (Bermudez et al., 2007, 2008, 2009) indicate that blood levels of MTBE and t-butanol were included. These data may be useful in constructing such a model.

4. The route of administration to laboratory animals must be relevant to human exposure. The animal doses must approximate to the expected human exposure, and an adequate number of subjects and samples should be included.

Although the drinking water route of exposure is preferred, these studies are not finalized and not available at this time (Bermudez et al., 2007, 2008, 2009).

There is insufficient information for chemical-specific adjustment of the interspecies uncertainty factor based on WHO/IPCS (2005) criteria. Thus, the default 3x factor was considered appropriate to address these toxicokinetic differences between humans and rats.

With respect to the toxicodynamic portion of the interspecies uncertainty factor, the adjustment factor for interspecies toxicodynamics will usually be based on results of *in*

*vitro* studies comparing animal and human tissue (WHO/IPCS, 2005). The active chemical moiety should be identified and *in vitro* data examining the critical effects or key events should serve as the basis for quantitatively defining interspecies toxicodynamic differences. The default 3x factor was considered appropriate to address potential toxicodynamic differences between humans and rats, since the active chemical moiety is unclear and there are no *in vitro* studies comparing animal and human tissue responses at target tissues. Thus, the interspecies uncertainty factor for MTBE is 10x.

- **Intraspecies Extrapolation = 10x**

Individuals with reduced ability to metabolize MTBE may potentially be more sensitive to adverse health outcomes resulting from MTBE exposure. Some human variants of CYP2A6, obtained from people who claimed to be sensitive to MTBE had 33% less activity than the wild type in oxidizing MTBE (Hong et al., 2001). Therefore, the 10x default intraspecies uncertainty factor is appropriate.

- **LOAEL to NOAEL Extrapolation = 1x**

The NOAEL from the key Robinson et al. (1990) study was considered 300 mg/kg-day. This study is supported by a 13-week drinking water study that administered comparable doses (Bermudez et al., 2008).

- **Extrapolation from Subchronic to Chronic Exposure = 10x**

Details for the two-year drinking water study currently in progress are not available (Bermudez et al., 2009). “No treatment-related nononcological pathological changes were detected by gross inspection and histological examination” after two-years of gavage exposure to MTBE at adjusted doses up to 571 mg/kg-day (1,000 mg/kg-day adjusted for daily dosing; Belpoggi et al., 1995), but the non-neoplastic data were not available for review. The LOAEL for one-year drinking water exposure to MTBE can be considered 384 mg/kg-day based on the increase in mean relative testes weights. However, the received mg/kg-day dose at the mid-dose was not reported and thus the NOAEL is not known for this effect (Bermudez et al., 2009). Although Robinson et al. (1990) reported that “gonads” were weighed, testes weights were not reported. Since the NOAEL of 900 mg/kg-day from the key Robinson et al. (1990) study used as the point of departure is higher than the one-year LOAEL (Bermudez et al., 2009), a departure from the default subchronic to chronic uncertainty factor can not be justified. The lack of associated testicular histopathology after one-year is reassuring (Bermudez et al., 2009), recognizing that chronic gavage exposure to MTBE at 571 mg/kg-day (1,000 mg/kg-day adjusted for daily dosing) was associated with a statistical increase in Leydig cell tumors (Belpoggi et al., 1995).

- **Incomplete Database = 3x**

When considering only the oral toxicity data for MTBE, data are available to satisfy only one of the five core areas due to the lack of a second-species systemic bioassay of at least

13 weeks in duration as well as the lack of a two-generation reproduction study. A second-species chronic bioassay in CD-1 mice (Bird et al., 1997), a two-generation reproduction study in SD rats (Bevan et al., 1997a), and developmental toxicity data in New Zealand white rabbits and CD-1 mice (Bevan et al., 1997b) are available when the inhalation data are also considered. Thus, based on both routes of exposure, standardized studies are available in all five core areas. Complete study details are not available for the unpublished drinking water studies in rats (Bermudez et al., 2007, 2008, 2009)

The Total Uncertainty Factor is therefore, 3000x (10 x 10 x 1 x 10 x 3).

### 8.1.7 RfD Calculation

The NOAEL of 300 mg/kg-day from the Robinson et al. (1990) subchronic gavage study can be used as the point of departure for the RfD.

$$\begin{aligned}\text{RfD} &= \frac{\text{NOAEL}}{\text{Total UF}} \\ &= \frac{300 \text{ mg/kg-day}}{3000} \\ &= \mathbf{0.1 \text{ mg/kg-day}}\end{aligned}$$

### 8.2 TAC Derivation

The Total Allowable Concentration (TAC) is used to evaluate the results of extraction testing normalized to static at-the-tap conditions and 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), applied when calculating a TAC for non-carcinogens, is used to ensure that the RfD is not exceeded when food and other non-water sources of exposure to the chemical are considered. In the absence of data to estimate the contribution from food or other non-water sources of exposure to the chemical of concern, a default of 20% can be used (EPA, 1991).

The RfD of 0.1 mg/kg-day can be used to calculate the TAC for MTBE. Due to the lack of data estimating potential environmental exposure levels for MTBE, a default of 20% RSC was used to calculate the TAC.

$$\begin{aligned}\text{TAC} &= \frac{\text{RfD} \times 70 \text{ kg} \times 20\% \text{ RSC}}{2 \text{ L/day}} \\ &= 3.5 \text{ mg/L} \times 0.2 \\ &= \mathbf{0.7 \text{ mg/L (or } 700 \text{ } \mu\text{g/L)}}\end{aligned}$$

Taste and odor thresholds for MTBE in water have been reported to be between 2.5 and 680 µg/L (IPCS, 1998; OEHHA, 1999; Suffet et al., 2007; U.S. EPA, 1997).

### 8.3 SPAC Derivation

The SPAC (single product allowable level) is the TAC action level divided by the estimated number of drinking water sources for MTBE. Since there were insufficient data to quantify the number of drinking water sources of MTBE, a default standard multiple source factor of 10 was used.

$$\begin{aligned}\text{SPAC} &= \frac{\text{TAC}}{10} = \frac{0.7 \text{ mg/L}}{10} \\ &= \mathbf{0.07 \text{ mg/L (or } 70 \text{ } \mu\text{g/L)}}\end{aligned}$$

## 9.0 RISK COMPARISONS AND CONCLUSIONS

One of the primary uncertainties associated with the present assessment is the lack of complete study details for the drinking water studies (Bermudez et al., 2007, 2008, 2009) thus necessitating the use of a gavage study as the key study (Robinson et al., 199). Health Canada (1992) and the Netherlands (Baars et al., 2004) have derived non-cancer oral risk levels for MTBE. Health Canada (1991) derived a tolerable daily intake (TDI) of 0.01 mg/kg-day based on a NOAEL of 100 mg/kg-day from Robinson et al. (1990). An uncertainty factor of 10,000 was applied (10 for intraspecies variation, 10 for interspecies variation, and 100 for a less-than-chronic study, lack of data on carcinogenicity and minimal effects observed at the NOAEL). Using the same study as Health Canada (1992), the Netherlands (Baars et al., 2004) derived a tolerable daily intake (TDI) of 0.3 mg/kg-day based on a NOAEL of 300 mg/kg-day for liver and kidney toxicity in rats from the Robinson et al. (1990) study. The Netherlands (Baars et al., 2004) applied an uncertainty factor of 1000 (10 each for intra- and interspecies differences, and an additional 10 for limited duration of the study and database deficiencies). The RfD of 0.1 mg/kg-day derived herein is based on the same key Robinson et al. (1990) study and NOAEL of 300 mg/kg-day as the Netherlands (Baars et al., 2004). However, NSF International did not depart from the default study duration extrapolation factor.

The effect of long-term drinking water exposure to MTBE at levels below the threshold that would elicit an adaptive liver response is unknown since data from the two-year drinking water study are not available (Bermudez et al., 2009). While adaptive mechanisms to metabolizing high-dose chemical exposures are usually reversible upon cessation of treatment, these mechanisms, if provoked for a sufficiently prolonged duration, may result in irreversible changes that are considered adverse and potentially relevant to humans. A physiologically-based pharmacokinetic model extrapolating oral rat doses to humans and additional studies examining potential modes of action would increase the confidence and reduce the uncertainty associated with the non-cancer risk levels derived herein. The relevance of the drinking water levels derived herein should be

re-evaluated when the results of the two-year drinking water study are available (Bermudez et al., 2009).

## 10.0 ADDENDUM REFERENCES:

Almeida, L., C. Pascale, and E. Hall. 2004. The effects of methyl tertiary-butyl ether on mouse testis, Abstract #914. *Toxicol.* 78(S-1):188.

Amberg, A., E. Rosner, and W. Dekant. 2001. Toxicokinetics of methyl tert-butyl ether and its metabolites in humans after oral exposure. *Toxicol Sci.* 61(1):62-7.

ATSDR (Agency for Toxic Substances and Disease Registry). 1996. Toxicological Profile for MTBE. U.S. Department of Health and Human Services. Public Health Service. Agency on Toxic Substances and Disease Registry. August.

Baars, A.J., P.J.C.M. Janssen, and W.C. Mennes. 2004. Human-Toxicological Maximum Permissible Risk Levels for Methyl-tertiary-butylether (MTBE). Unpublished report. National Institute of Public Health and the Environment, Bilthoven, The Netherlands. June. Available at <http://www.tera.org/iter/rivm/mtbereport.htm>.

Barnes, D.G., and M. Dourson. 1988. Reference Dose (RfD): Description and use in health risk assessments. *Regul Toxicol Pharmacol* 8:471-486.

Belpoggi, F., M. Soffritti, and C. Maltoni. 1998. Pathological characterization of testicular tumors and lymphomas-leukaemias and of their precursors observed in Sprague-Dawley rats exposed to methyl tertiary-butyl ether (MTBE) *Eur. J. Oncol.* 3(3):201-206.

Belpoggi, F., M. Soffritti, and C. Maltoni. 1995. Methyl-tertiary-butyl ether (MTBE) - a gasoline additive - causes testicular and lympho-haematopoietic cancers in rats. *Toxicol. Ind. Health.* 11(2):119-149.

Belpoggi, F., M. Soffritti, F. Filippini, and C. Maltoni. 1997. Results of long-term experimental studies on the carcinogenicity of methyl tert-butyl ether. *Ann. N.Y. Acad. Sci.* 837:77-95.

Bermudez, E., H. Parkinson, and S.J. Borghoff. 2007. Methyl Tertiary Butyl Ether (MTBE) 14-Day Drinking Water Study In Wistar Rats. Abstract #1515. *Toxicol Sci.* 96(S-1):313.

Bermudez, E., H. Parkinson and D.E. Dodd. 2008. Methyl Tertiary Butyl Ether (MTBE) 13-Week Drinking Water Study In Wistar Rats. Abstract #508. *Toxicol Sci.* 102(S-1):104.

Bermudez, E., H. Parkinson and D.E. Dodd. 2009. Methyl Tertiary Butyl Ether (MTBE): One-year Toxicity Drinking Water Study In Wistar Rats. Abstract #1481. *Toxicol Sci.* 108(S-1):307.

Bevan, C., T.L. Neeper-Bradley, R.W. Tyl, L.C. Fisher, R.D. Panson, J.J. Kneiss and L.S. Andrews. 1997a. Two-generation reproductive toxicity study of methyl tertiary-butyl ether (MTBE) in rats. *J Appl Toxicol.* 17(Suppl 1):S13-9.

Bevan, C., R.W. Tyl, T.L. Neeper-Bradley, L.C. Fisher, R.D. Panson, J.F. Douglas and L.C. Andrews. 1997b. Developmental toxicity evaluation of methyl tertiary-butyl ether (MTBE) by inhalation in mice and rabbits. *J Appl Toxicol.* 17:S21-9.

Billitti, J.E., B.C. Faulkner, and B.W. Wilson. 1999. Acute testicular toxicity of MTBE and breakdown products in lab mice, Abstract #1255. *Toxicol.* 48(S-1).

Billitti, J.E., B.C. Faulkner, and B.W. Wilson. 2005. Absence of acute testicular toxicity of methyl-tert butyl ether and breakdown products in mice. *Bull Environ Contam Toxicol.* 75(2):228-35.

Bird, M.G., H.D. Burleigh-Flayer, J.S. Chun, J.F. Douglas, J.J. Kneiss and L.S. Andrews. 1997. Oncogenicity studies of inhaled methyl tertiary-butyl ether (MTBE) in CD-1 mice and F-344 rats. *J Appl Toxicol.* 17(Suppl 1):S45-55.

Borghoff, S.J., J.E. Murphy, and M.A. Medinsky. 1996. Development of physiologically based pharmacokinetic model for methyl tertiarybutyl ether and tertiary-butanol in male Fisher-344 rats. *Fundam Appl. Toxicol.* 30:264–275.

Cohen, S.M., M.E. Meek, J.E. Klaunig, D.E. Patton, and P.A. Fenner-Crisp. 2003. The human relevance of information on carcinogenic modes of action: overview. *Crit Rev Toxicol.* 33(6):581-9.

Day, K.J., A. de Peyster, B.S. Allgaier, A. Luong, J.A. MacGregor. 1998. MTBE (MTBE) effects on the male rat reproductive endocrine axis [abstract]. *Toxicol.* 42(1-S):174.

Dekant, W., U. Bernauer, E. Rosner, and A. Amberg. 2001. Biotransformation of MTBE, ETBE, and TAME after inhalation or ingestion in rats and humans. *Res. Rep. Health Eff. Inst.* 102:29-71; discussion 95-109.

de Peyster, A., K.J. MacLean, B.A. Stephens, L.D. Ahern, C.M. Westover, and D. Rozenshteyn. 2003. Subchronic studies in Sprague-Dawley rats to investigate mechanisms of MTBE-induced Leydig cell cancer. *Toxicol Sci.* 72(1):31-42.

de Peyster, A., Y. Rodriguez, R. Shuto, B. Goldberg, F. Gonzales, X. Pu, and J.E. Klaunig. 2008. Effect of oral methyl-t-butyl ether (MTBE) on the male mouse reproductive tract and oxidative stress in liver. *Reprod Toxicol.* 26(3-4):246-53.

Dongmei, L., Y. Chuntao, G. Yi, H. Yufeng, and H. Xiaodong. 2008. The effects of methyl tert-butyl ether (MTBE) on the male rat reproductive system. *Food Chem Toxicol.* 46(7):2402-8.

Dong-mei, L., G. Yi, Y. Chun-Tao, H. Yu-feng, and H. Xiao-dong. 2009a. Effects of subchronic methyl tert-butyl ether exposure on male Sprague-Dawley rats. *Toxicol Ind Health.* 25(1):15-23.

Dongmei, L., L. Quin, G. Yi, H. Yufeng, and H. Xiaodong. 2009b. Cytotoxicity and oxidative stress study in cultured rat Sertoli cells with methyl tert-butyl ether (MTBE) exposure. *Reprod Toxicol.* 27(2):170-6.

European Center for Ecotoxicology and Toxicology of Chemicals (ECETOC). 1997. Technical Report No. 72. Methyl tert-Butyl Ether (MTBE) Health Risk Characterisation CAS No.1634-4-4 (EINECS No. 216.653.1). ISSN-0773-8072-72. June.

European Chemicals Bureau (ECB). 2002. European Union Risk Assessment Report. Tert-Butyl Methyl Ether. CAS No. 1634-04-4. EINECS No. 216-653-1. 3rd Priority List. Volume 19. Institute for Health and Consumer Protection, European Chemicals Bureau, European Commission. EUR 20417 EN. September 9.

Health Canada. 1992. Canadian Environmental Protection Act. Priority Substances List Assessment Report No. 5. Methyl tertiary-butyl ether. Government Canada, Environment Canada, Health and Welfare Canada, Health Canada. ISBN 0-662-19941-3.

Health Canada. 1996. Health-Based Tolerable Daily Intakes/Concentrations and Tumorigenic Doses/Concentrations for Priority Substances. Environmental Health Directorate, Health Protection Branch, Health Canada. ISBN 0-662-24858-9.

Hong, J.Y., Y.Y. Wang, S.N. Mohr, F.Y. Bondoc, and C. Deng. 2001. Human cytochrome P450 isozymes in metabolism and health effects of gasoline ethers. *Health Effects Institute Research Report.* 102:7–27. As cited by McGregor, 2006.

IARC (International Agency for Research on Cancer). 1999. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. Geneva: World Health Organization, International Agency for Research on Cancer, 1972-PRESENT. (Multivolume work). Vol. 73, pp 339. September 30.

IPCS (International Programme On Chemical Safety). 1998. Environmental Health Criteria 206. Methyl Tertiary-Butyl Ether. United Nations Environment Programme. International Labour Organisation. World Health Organization. Available from: <http://www.inchem.org/documents/ehc/ehc/ehc206.htm#PartNumber:1>.

Johnson, W.D., J. Findlay, and R.A. Boyne. 1992. 28-day oral (gavage) toxicity study of MTBE in rats. Prepared for Amoco. Illinois Institute of Technology Research. ITT Research Institute Project No. L08100. Chicago, Illinois, 48 pp.

Kim, D., M. E. Andersen, J.D. Pleil, L.A. Nylander-French, and J.D. Prah. 2007. Refined PBPK model of aggregate exposure to methyl tertiary-butyl ether. *Toxicol Lett.* 169(3):222-35.

Klan, M.J. W. Johnson, N.S. Hatoum, and J.K. Yermakoff. 1992. 28-day oral (gavage) toxicity study of MTBE in rats [abstract]. *Toxicol.* 12(1):117.

Klaunig, J.E., M.A. Babich, K.P. Baetcke, J.C. Cook, J.C. Corton, R.M. David, J.G. DeLuca, D.Y. Lai, R.H. McKee, J.M. Peters, R.A. Roberts, P.A. Fenner-Crisp. 2003. PPARalpha agonist-induced rodent tumors: modes of action and human relevance. *Crit. Rev. Toxicol.* 33(6):655-780.

Leavens TL and S.J. Borghoff. 2009. Physiologically based pharmacokinetic model of methyl tertiary butyl ether and tertiary butyl alcohol dosimetry in male rats based on binding to alpha2u-globulin. *Toxicol Sci.* 109(2):321-35.

Le Gal, A., Y. Dreano, P.G. Gervasi, and F. Berthou. 2001. Human cytochrome P450 2A6 is the major enzyme involved in the metabolism of three alkoxyethers used as oxyfuels. *Toxicol Lett.* 124(1-3):47-58.

Martin, J.V., N.M. Bilgin, and M.M. Iba. 2002. Influence of oxygenated fuel additives and their metabolites on the binding of a convulsant ligand of the gamma-aminobutyric acid(A) (GABA(A)) receptor in rat brain membrane preparations. *Toxicol Lett.* 129(3):219-26.

McGregor, D. 2006. Methyl *tertiary*-Butyl Ether: Studies for Potential Human Health Hazards. *Crit Rev Toxicol.* 36(4):319-358.

Meek, M.E., J.R. Bucher, S.M. Cohen, V. Dellarco, R.N. Hill, L.D. Lehman-McKeeman, D.G. Longfellow, T. Pastoor, J. Seed, and D.E. Patton. 2003. A framework for human relevance analysis of information on carcinogenic modes of action. *Crit Rev Toxicol.* 33(6):591-653.

Moser, G.J., B.A. Wong, D.C. Wolf, O.R. Moss, and T.L. Goldsworthy. 1996. Comparative short-term effects of methyl tertiary butyl ether and unleaded gasoline vapor in female B6C3P1 mice. *Fundam Appl Toxicol.* 31:173-183.

NSF/ANSI 61. 2009. Drinking Water System Components - Health Effects. NSF International, Ann Arbor, MI.

OECD (Organisation for Economic Co-Operation and Development). 2004. The 2004 OECD list of high production volume chemicals. OECD. Paris, 2004. Available at : <http://www.oecd.org/dataoecd/55/38/33883530.pdf>.

OEHHA (Office of Environmental Health Hazard Assessment). 1999. Public Health Goal for Methyl Tertiary-Butyl Ether (MTBE) in Drinking Water. Pesticide and Environmental Toxicology Section. Office of Environmental Health Hazard Assessment. California Environmental Protection Agency. March.

Okahara, N. A. de Peyster, S.E. McPherson, and J.A. MacGregor. 1998. Effect of MTBE on estrogen-sensitive tissues of immature female CD-1 mice [abstract]. *Toxicol.* 42(1-S):174-175.

Phillips, S., R.B. Palmer, and A. Brody. 2008. Epidemiology, toxicokinetics, and health effects of methyl tert-butyl ether (MTBE). *J Med Toxicol.* 4(2):115-26.

Prah, J., D. Ashley, B. Blount, M. Case, T. Leavens, J. Pleil, and F. Cardinali. 2004. Dermal, Oral, and Inhalation Pharmacokinetics of Methyl Tertiary Butyl Ether (MTBE) in Human Volunteers. *Toxicol. Sci.* 77:195-205.

Robinson, M., R.H. Bruner, and G.R. Olson. 1990. Fourteen- and ninety-day oral toxicity studies of methyl tertiary-butyl ether in Sprague-Dawley rats. *J. Am. Coll. Toxicol.* 9(5):525-540.

Suffet, I.H. 2007. A re-evaluation of the taste and odour of methyl tertiary butyl ether (MTBE) in drinking water. *Water Sci Technol.* 55(5):265-73.

U.S. Environmental Protection Agency. 1991. National primary drinking water regulations: Final Rule. *Federal Register* 56(20):3526-3614.

U.S. Environmental Protection Agency. 1993. Reference Dose (RfD): Description and use in health risk assessment. Integrated Risk Information System (IRIS) background document 1A. <http://www.epa.gov/ngispgm3/iris/rfd.htm>

U.S. Environmental Protection Agency. 1995. Methods for the Determination of Organic Compounds in Drinking Water-Supplement III. Method 502.2. VOCs by Purge and Trap Capillary GC with Photoionization and Electrolytic Conductivity Detectors in Series. EPA/600/R-95-131, NTIS Pub. No. PB95-261616.

U.S. Environmental Protection Agency. 1997. Drinking Water Advisory: Consumer Acceptability Advice and Health Effects Analysis on Methyl tertiary-Butyl Ether (MtBE). EPA 822-F-97-008. December.

U.S. Environmental Protection Agency. 2002. A Review of the Reference Dose and Reference Concentration Process. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC, EPA/630/P-02/002F. December 2002. Available at: <http://www.epa.gov/iris/backgrd.html>.

U.S. Environmental Protection Agency. 2007. ChemRTK HPV Challenge Program. U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics. Available at: <http://www.epa.gov/chemrtk/pubs/general/sponsorship.htm>. Last Updated November 27, 2007.

U.S. EPA (U.S. Environmental Protection Agency). 2009. Health effects testing guidelines. U.S. Code of Federal Regulations, Title 40, Part 798. U.S. Environmental Protection Agency. Last Updated July 1, 2009.

U.S. Environmental Protection Agency. 2010. Glossary of IRIS terms. Integrated Risk Information System (IRIS). Last Updated March 16, 2010. Available at: [http://www.epa.gov/iris/help\\_gloss.htm](http://www.epa.gov/iris/help_gloss.htm).

U.S. FDA (U.S. Food and Drug Administration). 2010. U.S. Code of Federal Regulations, Title 21 (Food and Drugs). Available at: <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=176.170>. Last updated April 1, 2010.

Ward Jr., J.B., W.W. Au, E.B. Whorton, et al. 1994. Genetic toxicity of methyl tertiary-butyl ether. Division of Environmental Toxicology, Department of Preventive Medicine and Community Health. Galveston, Texas: University of Texas Medical Branch. As cited in IPCS (1998).

Ward Jr., J.B., D.H. Daiker, D.A. Hastings, M.M. Ammenheuser, and M.S. Legator. 1995. Assessment of the mutagenicity of methyl tertiary-butyl ether at the HPRT gene in CD-1 mice [abstract]. *Toxicol.* 15:79.

WHO (World Health Organization). 2005. Methyl tertiary-Butyl Ether (MTBE) in Drinking-water, Background document for development of WHO Guidelines for Drinking-water Quality. WHO/SDE/WSH/05.08/122. Available at: [http://www.who.int/water\\_sanitation\\_health/dwq/chemicals/MTBE200605.pdf](http://www.who.int/water_sanitation_health/dwq/chemicals/MTBE200605.pdf). Accessed on April 5, 2010.

Williams, T.M. and S.J. Borghoff. 2000. Induction of testosterone biotransformation enzymes following oral administration of methyl tert-butyl ether to male Sprague-Dawley rats. *Toxicol. Sci.* 57(1):147-55.

Williams, T.M., R.C. Cattley, and S.J. Borghoff. 2000. Alterations in endocrine responses in male Sprague-Dawley rats following oral administration of methyl tert-butyl ether. *Toxicol. Sci.* 54(1):168-76.

Zheng, G., W. Zhang, Y. Zhang, Y. Chen, M. Liu, T. Yao, Y. Yang, F. Zhao, J. Li, C. Huang, W. Luo, and J. Chen. 2009. gamma-Aminobutyric acid(A) (GABA(A)) receptor regulates ERK1/2 phosphorylation in rat hippocampus in high doses of methyl tert-butyl ether (MTBE)-induced impairment of spatial memory. *Toxicol Appl Pharmacol.* 236(2):239-45.

Zhou, W. and S. Ye. 1999. Subchronic oral methyl tertiary-butyl ether exposure (MTBE) in male Sprague-Dawley rats and effects on health of MTBE exposed workers. *J. Occup. Health.* 41:33-38.

## APPENDIX

Summary of non-cancer LOEL and NOEL values from repeated-dose oral studies with MTBE <sup>a</sup>

Study Type (Species)	Route of Exposure	NOEL mg/kg-day <sup>a</sup>	LOEL mg/kg-day <sup>a</sup>	Non-Cancer Biological Effect(s)	Reference
Two-Week (Wistar rat)	Drinking Water	Insufficient details to determine		↑ kidney weights in males, ↓ blood urea nitrogen and hematocrit in females. Study not available.	Bermudez et al., 2007
Two-Week (SD Rat)	Gavage	None (♀) 357 (♂)	357 (♀) 714 (♂)	↓ mean absolute and relative lung weights in male and female rats and altered clinical parameters in male rats. Limited endpoints evaluated.	Robinson et al., 1990
Two-Week (SD Rat)	Gavage	800 (♂ only)	None (♂ only)	No effect on hepatic clinical chemistry or peroxisomal proliferation. Limited endpoints evaluated.	DePeyster et al., 2003
Up to Four-Week (SD rat)	Gavage	None	400 (♂ only)	Reduced serum creatinine. Limited endpoints evaluated.	Dongmei et al., 2009a
Three-Week (CD-1 Mouse)	Gavage	714 <sup>b</sup>	None	No effects on body weight or reproductive parameters (sperm number, Sertoli cells, spermatogonia, spermatocytes, and capped spermatids in males and oocyte quality in females). Limited endpoints evaluated.	Ward et al., 1994 <sup>c</sup> ; 1995 <sup>c</sup>
Four-Week (CD-1 mouse)	Drinking Water	1,178 (♂ only)	None	No effects on testosterone level, testes weight or histology. Limited endpoints evaluated	De Peyster et al., 2008
Four-Week (SD Rat)	Gavage	250 (♂ only)	500 (♂ only)	↑ mean relative liver weight and minimal-to-moderate centrilobular hypertrophy. ↑ mean relative kidney weight and protein droplet nephropathy in renal tubules. Limited endpoints evaluated.	Williams and Borghoff, 2000; Williams et al., 2000
Four-Week (SD Rat)	Gavage	314 (♂ and ♀) <sup>b</sup>	1,250 (♂ and ♀) <sup>b</sup>	↑ mean cholesterol and relative liver weight. Gastric inflammation, edema, hyperplasia, and ulcers. ↑ mean relative kidney weight and hyaline droplet formation in renal tubules of males. Limited endpoints evaluated.	Johnson et al., 1992; Klan et al., 1992
Four-Week (SD Rat)	Gavage	357 (♂ only)	536 (♂ only)	↓ circulating testosterone concentration immediately following dosing; ↑ mean liver P450 content. Limited endpoints evaluated.	DePeyster et al., 2003
Four-Week (SD Rat)	Gavage	400 (♂ only)	800 (♂ only)	↓ body weight and ↓ plasma testosterone and corticosterone. Limited endpoints evaluated.	DePeyster et al., 2003; Day et al., 1998

Study Type (Species)	Route of Exposure	NOEL mg/kg-day <sup>a</sup>	LOEL mg/kg-day <sup>a</sup>	Non-Cancer Biological Effect(s)	Reference
Four-Week (SD Rat)	Gavage	None (♂ only)	1,200 (♂ only)	↑ mean relative liver weight, ↓ mean testosterone and luteinizing hormone, ↑ mean estradiol, ↓ hepatic and testicular microsomal aromatase activity. Limited endpoints evaluated.	DePeyster et al., 2003
Up to Four-Week (SD Rat)	Gavage	400 (♂ only)	800 (♂ only)	Alterations in serum testosterone and histopathology in the testes. Limited endpoints evaluated.	Dongmei et al., 2008
Seven-Week (CD-1 mouse)	Drinking Water	1,536 (juvenile ♂ only)	None	No effect on serum estradiol or testosterone concentrations or mean seminiferous tubule diameter or reproductive organ histology. No evidence of oxidative stress in liver homogenates. Limited endpoints evaluated.	De Peyster et al., 2008
13-week (Wistar rat)	Drinking Water	Insufficient details to determine		↓ mean body weights (unspecified magnitude) and α-2μ-globulin-associated renal effects in males at 514 and 972 mg/kg-day. Study not available.	Bermudez et al., 2008
13-Week (SD Rat)	Gavage	None (♂ only)	143 (♂ only) <sup>b</sup>	↑ liver weights and aspartate aminotransferase, hepatic nuclear condensation, fat droplets, lysosome appearance in cells, and smooth endoplasmic reticulum disintegration. Limited endpoints evaluated.	Zhou and Ye, 1999
13-Week (SD Rat)	Gavage	None (♂ and ♀)	100 (♂ and ♀)	↓ blood urea nitrogen in males and females. Limited endpoints evaluated.	Robinson et al., 1990
One-year (Wistar rat)	Drinking Water	Insufficient details to determine		Study not available. Part of a two-year study.	Bermudez et al., 2009
104-Week (SD Rat)	Gavage	Not determined		Although study authors reported “no treatment-related nononcological pathological changes were detected by gross inspection and histological examination,” data were not provided. Limited endpoints evaluated (no hematology, clinical chemistry, or urinalysis).	Belpoggi et al., 1995; 1997; 1998

<sup>a</sup> Biologically-observed effects not necessarily considered adverse (see text)  
<sup>b</sup> Doses were adjusted to account for a less than 7-day dosing regimen.  
<sup>c</sup> Study not available, and thus as cited in OEHHA (1999) and ATSDR (1996).