



**EPA**

**Drinking Water Advisory: Consumer  
Acceptability Advice and Health Effects  
Analysis on Methyl Tertiary-Butyl Ether  
(MtBE)**

## TABLE OF CONTENTS

LIST OF ABBREVIATIONS.....	I
FOREWORD.....	ii
EXECUTIVE SUMMARY.....	1
1.0. INTRODUCTION.....	5
2.0. MtBE IN THE ENVIRONMENT.....	5
3.0. CHEMICAL AND PHYSICAL PROPERTIES.....	6
4.0. TOXICOKINETICS.....	7
4.1. Dosimetry: Route-to-Route Extrapolation.....	7
4.2. NSTC's Extrapolation of Dose from Inhalation Exposure.....	9
5.0. HEALTH EFFECTS DATA.....	10
5.1. Human Studies.....	10
5.2. Animal Studies.....	11
5.2.1. Noncancer Effects.....	11
5.2.1.1. Acute and Subchronic.....	11
5.2.1.2. Reproductive and Developmental Studies.....	13
Reproductive Studies.....	13
Developmental Studies.....	14
5.2.1.3. Neurotoxicity Studies.....	15
5.2.1.4. Mutagenicity Studies.....	16
5.2.2. Cancer Effects.....	16
5.2.2.1. Studies of the Carcinogenicity of the Parent Compound (MtBE).....	16
Gavage Study.....	17
Inhalation Studies.....	17
5.2.2.2. Studies of the Carcinogenicity of MtBE Metabolites.....	21
<i>tertiary</i> -Butyl Alcohol.....	21
Formaldehyde.....	21
6.0. ORGANOLEPTIC PROPERTIES.....	22
7.0. CHARACTERIZATION OF HAZARD AND DOSE RESPONSE.....	23
7.1. Hazard Characterization.....	23
7.2. Characterization of Organoleptic Effects.....	26

7.3.	Dose Response Characterization.....	26
7.4.	Comparison of Margins of Exposure with Potential Environmental Concentrations and Guidance on Taste and Odor.....	28
8.0.	REFERENCES.....	30

## LIST OF ABBREVIATIONS

DWEL	Drinking-Water-Equivalent-Level
HA	Health Advisory
kg	kilogram
L	liter
LOAEL	lowest-observed-adverse-effect level
MoE	margin of exposure
mg	milligram
MtBE	Methyl <i>tertiary</i> -butyl ether
MTD	Maximum Tolerated Dose
NOAEL	no-observed-adverse-effect level
OFW	odor free water
ppm	parts per million
$\mu\text{g}$	microgram
TBA	<i>tertiary</i> -butyl alcohol
VOC	volatile organic compound

## FOREWORD

EPA's Human Health and Criteria Division (HECD) of the Office of Water developed an Advisory document for methyl *tertiary*-butyl ether (MtBE). This document is a non-regulatory document that analyses the currently available cancer and non-cancer data on this contaminant, as well as studies on its organoleptic (taste and odor) effects. The document is not a mandatory standard for action; however, this Advisory supersedes any previous drafts of drinking water advisories for this chemical.

There are many uncertainties and limitations associated with the toxicity data base for this chemical. The animal tests available to date (1997) were not conducted by exposing the animals to MtBE in drinking water, but rather by inhalation exposure or by introducing MtBE in oil directly to the stomach several times a week. Although useful for identifying potential hazards, limitations of the reported studies do not allow confident estimates of the degree of risk MtBE may pose to humans from low-level drinking water contamination. The toxicokinetic models are also limited in helping to perform an adequate extrapolation from the inhalation data to actual oral exposure from drinking water intake. Additional research is needed to resolve these issues before a more complete health advisory can be issued. Therefore, given the needs of the States and Regions for an Office of Water (OW) position on MtBE contamination of drinking water, HECD developed this "Drinking Water Advisory: Consumer Acceptability Advice and Health Effects Analysis on Methyl tertiary-Butyl Ether (MtBE)".

MtBE is generally unpleasant in taste and odor. Studies have been conducted on the concentrations of MtBE in drinking water at which individuals can detect the odor or taste of the chemical. This Advisory recommends that keeping levels of contamination in the range of 20 to 40  $\mu\text{g/L}$  or below to protect consumer acceptance of the water resource would also provide a large margin of exposure (safety) from toxic effects.

The Advisory discusses the limitations of the current database for estimating a risk level for this contaminant in drinking water and characterizes the hazards associated with this route of exposure. This document has been peer reviewed both internally in the Agency and externally by experts in the field before its release to the public.

Note: In this Advisory, we use a risk characterization method called "Margin of Exposure (or safety)" which is different from traditional slope factors and reference doses (RfDs) as estimates of response to defined exposures. The "margin" is how far the environmental exposure of interest is from the lower end of the exposures at

which animals or humans have shown some toxicity effect. The use of the margin of exposure approach is helpful in the following ways: 1. It allows for comparison of exposures associated with carcinogenic potential to those associated with non cancer health effects; 2. It provides the risk manager with a quick check to decide if the margin of exposure (safety) appears to be adequate even when mathematical extrapolation of data from high to low dose cannot be done; and 3. It gives a better understanding of the degree of risk associated with extrapolation of exposure data from animal studies to humans. For example, given the limited number of animals that usually can be used in experiments, they, at best, would detect a one in ten response ( $1 \times 10^{-1}$ ). A common procedure for carcinogens is to mathematically extrapolate from the exposure levels of animal tests to estimate risk at lower, environmental exposure levels. If the extrapolation is done as a straight line, a risk estimate of  $1 \times 10^{-6}$  generally corresponds to a margin of exposure of 100,000. If the true, but unknown, relationship is downward sloping, not a straight line, the risk at a 100,000 margin of exposure would be less than  $1 \times 10^{-6}$  and might be zero.

Health and Ecological Criteria Division  
Office of Science and Technology  
Office of Water

**DRINKING WATER ADVISORY: CONSUMER ACCEPTABILITY  
ADVICE AND HEALTH EFFECTS ANALYSIS ON  
METHYL TERTIARY-BUTYL ETHER (MtBE)**

**EXECUTIVE SUMMARY**

**MtBE**

MtBE is a volatile, organic chemical. Since the late 1970's, MtBE has been used as an octane enhancer in gasoline. MtBE promotes more complete burning of gasoline, thereby reducing carbon monoxide and ozone levels. Hence, MtBE is commonly used as a gasoline additive in localities that participate in the Winter Oxygenated Fuels program and/or the Reformulated Gasoline program to achieve or maintain compliance with the National Ambient Air Quality Standards. A limited number of instances of significant contamination of drinking water with MtBE have occurred due to leaks from underground and above ground petroleum storage tank systems and pipelines. MtBE, due to its small molecular size and solubility in water, moves rapidly into groundwater, faster than other constituents of gasoline. Public and private wells have been contaminated in this manner. Non-point sources, such as recreational watercraft, are most likely to be the cause of small amounts of contamination of surface waters. Air deposition through precipitation of industrial or vehicular emissions may also contribute to surface and ground water contamination. The extent of any potential for build-up in the environment from such deposition is uncertain.

**This Advisory**

The EPA Office of Water is issuing this Advisory to provide guidance for communities that may be exposed to drinking water contaminated with MtBE. The Advisory provides an analysis of current health hazard information and an evaluation of currently available data on taste and odor problems associated with MtBE contamination of water, as the latter affect consumer acceptance of the water resource. This Advisory does not recommend either a low-dose oral cancer risk number or a reference dose (RfD)<sup>1</sup> due to certain limitations of available data

---

<sup>1</sup>Reference Dose is defined as “an estimate (with uncertainty spanning approximately an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious effects over a lifetime” (U.S. EPA, 1987).

December 1997

for quantifying risk. Guidance is given on the concentrations at which taste and odor problems likely would be averted, and how far these are from MtBE concentrations at which toxic effects have been seen in test animals. (The measure used is called a "margin of exposure" or MoE. For instance, if a measured concentration is 100,000 times less than the range of observation of effects in test animals, the margin of exposure is 100,000.

### **Conclusion and Recommendation**

This Advisory recommends that keeping levels of contamination in the range of 20 to 40  $\mu\text{g/L}$  or below to protect consumer acceptance of the water resource would also provide a large margin of exposure (safety) from toxic effects.

Taste and odor values are presented as a range, since human responses vary depending upon the sensitivities of the particular individual and the site-specific water quality conditions. These values are provided as guidance recognizing that water suppliers determine the level of treatment required for aesthetics based upon the customers they serve and the particular site-specific water quality conditions.

There are over four to five orders of magnitude between the 20 to 40  $\mu\text{g/L}$  range and concentrations associated with observed cancer and noncancer effects in animals. There is little likelihood that an MtBE concentration of 20 to 40  $\mu\text{g/L}$  in drinking water would cause adverse health effects in humans, recognizing that some people may detect the chemical below this range. It can be noted that at this range of concentrations, the margins of exposure are about 10 to 100 times greater than would be provided by an EPA reference dose (RfD) for noncancer effects. Additionally, they are in the range of margins of exposure typically provided by National Primary Drinking Water Standards under the Federal Safe Drinking Water Act to protect people from potential carcinogenic effects.

When adequate data become available, the Office of Water will publish another Advisory that includes quantitative estimates for health risks. This Advisory gives practical guidelines for addressing contamination problems and supersedes previous draft advisories. An Advisory does not mandate a standard for action.

### **Studies of MtBE Effects**

There are no studies of effects on humans of long-term exposure to MtBE. All of the studies available for hazard assessment are laboratory animal studies.

**Cancer effects.** There are studies in rodents of the carcinogenicity of MtBE, as well as its metabolites, *tertiary*-butyl alcohol (TBA) and formaldehyde. The only oral cancer exposure study was conducted by Belpoggi and coworkers (1995).

They gave MtBE to Sprague-Dawley rats (gavage in olive oil, at doses up to 1,000 mg/kg/day, 4 days per week for two years). Exposure caused a dose-related increase in the incidence of combined leukemia and lymphomas in the female rats and an increase in Leydig cell adenomas (benign testicular tumors) in the high-dose male rats. Use of this study to quantitatively assess risks from drinking water exposure has limitations. There are potential differences in bolus versus drinking water exposures and possible vehicle (olive oil) effects. Moreover, there are few details on the actual reported tumor response data provided in the report. The lack of histopathological diagnoses and of individual animal data were reasons that the National Research Council panel recommended not using these tumor data in risk estimation until after a thorough peer review of this study.

There are two studies on the potential carcinogenicity of MtBE after inhalation exposure.

Chun et al. (1992) administered MtBE to F344 rats at concentrations up to 8,000 ppm for 2 years. Exposure to MtBE caused an increase in the incidence of combined renal tubular adenomas and carcinomas, as well as Leydig cell adenomas of the testes in the male rats. The mild induction of  $\alpha$ -2u-globulin by MtBE suggested that this protein may have played a role in male rat kidney tumorigenesis. The increase in the incidence of Leydig cell adenomas of the male rats in this study was not significantly different from the historical control value, although the difference from the concurrent controls was significant. Induction of Leydig cell tumors was also observed in Sprague-Dawley rats after oral exposure by gavage (Belpoggi et al., 1995) and lends support to the conclusion that the appearance of the tumor in both studies is treatment-related.

In the other inhalation study, Burleigh-Flayer et al. (1992) gave MtBE to CD-1 mice at concentrations up to 8,000 ppm for 18 months. This exposure was associated with a statistically significant increase in the incidence of hepatocellular carcinomas in male mice and of hepatocellular adenomas in female mice. The Chun et al. (1992) and the Burleigh-Flayer et al. (1992) studies currently cannot be used to calculate adequate hazard advisory values since we have no well-developed pharmacokinetic model for converting a chronic inhalation exposure of MtBE to an equivalent oral exposure. On-going work may support route-to-route extrapolation in the future.

The potential carcinogenicity of two metabolites of MtBE, TBA and formaldehyde has also been examined. In F344 rats, TBA has provided some evidence of carcinogenic activity in the males (but not in the female rats). In B6C3F1 mice, TBA exposure gave equivocal evidence of carcinogenic activity in male mice based on marginally increased incidence of thyroid tumors, and some evidence of carcinogenicity in female mice, based on an increased incidence of follicular cell hyperplasia and follicular cell adenomas of the thyroid gland. Data for

carcinogenic activity is ambiguous for drinking water exposure to formaldehyde. A study by Soffritti et al. (1989) reported a dose-related increase in the incidence of leukemia and intestinal tumors in Sprague-Dawley rats. However, the experimental data presented in this publication was limited. Another drinking water study on formaldehyde by Til and coworkers (1989), using Wistar rats, found no evidence of carcinogenicity.

The carcinogenicity data support a conclusion that MtBE poses a potential for carcinogenicity to humans at high doses. The data do not support confident, quantitative estimation of risk at low exposure due to the limitations described above.

**Noncancer toxicity.** The collective evaluation of the reproductive and developmental studies of MtBE in animals indicate that inhalation exposure can result in maternal toxicity and adverse effects on the developing fetus (Bushy Run Research Center, 1991, 1989a, 1989b; Conaway et al., 1985). The fetal toxicity in the mouse developmental studies indicate that it may be more sensitive to inhalation of MtBE vapors than the rat or rabbit during gestation. However, it is possible to conclude that, at low concentrations, MtBE does not cause a developmental or reproductive hazard by inhalation in three different animal species. This also suggests that humans may not be at risk when exposed to very low concentrations of MtBE.

Effects on the kidney were observed in rats after oral and inhalation exposure to MtBE. The most pertinent noncancer toxicity data come from a 90-day oral exposure study in rats. The authors reported minimal effects on the kidneys at doses of 300 mg/kg/day and above (Robinson et al., 1990). In these animals, the MtBE was given once a day, as a bolus dose in corn oil. A single oral dose of MtBE in corn oil would not be considered representative of an intermittent exposure to MtBE that one would normally obtain from drinking water containing MtBE. In a longer term inhalation study, histopathological abnormalities were apparent (Chun et al., 1992). Uncertainties exist in quantifying risk from the oral data in the short-term study because of the bolus gavage dosing regime and the less-than-lifetime duration of the study. The uncertainty in extrapolating between routes affects the interpretation of the inhalation data.

The studies support a conclusion that MtBE can pose a hazard of noncancer effects to humans at high doses. The data do not support confident quantitative estimation of risk at low exposure.

**Taste and Odor.** Studies were conducted on the concentrations of MtBE in drinking water at which individuals respond to the odor or taste of the chemical. Human responses vary widely in this respect. Some who are sensitive can detect

very low concentrations, others do not taste or smell the chemical even at much higher concentrations. Moreover, the presence or absence of other natural or water treatment chemicals can mask or reveal the taste or odor effects. Thus, variable preexisting water conditions around the country will increase variability in the acceptability of MtBE's presence in drinking water.

The studies have not been extensive enough to completely describe the extent of human variability, or to establish a population threshold of response. Nevertheless, the available studies allow a conclusion that keeping concentrations in the range of 20 to 40 micrograms per liter ( $\mu\text{g/L}$ ) of water or below will likely avert unpleasant taste and odor effects, recognizing that some people may detect the chemical below this range.

### **Characterization Summary**

Section 7.0 on hazard and dose response characterization summarizes the MtBE data. In this section, a table (Table 1) presents the margins of exposure comparing animal effects and human taste and odor data.

## 1.0 INTRODUCTION

The purpose of this Advisory is to support immediate needs for information by State and local drinking water facilities and public health personnel due to MtBE contamination of potable water. Ongoing research is anticipated to decrease some of the uncertainties in the current toxicity data as applied to the drinking water route of exposure. A Health Hazard Advisory value will be issued when the data base is improved to allow greater confidence in the toxicity conclusions.

Nevertheless, there are sufficient data to give a general picture of the ranges of exposure that may raise concerns for people. In addition, the taste and odor of MtBE affect the potability of water at levels that provide an additional basis for assessment of quality and usability of water resources.

## 2.0 MtBE IN THE ENVIRONMENT

MtBE is used as an octane enhancer to replace lead in gasoline. It also promotes more complete burning of gasoline, thereby reducing carbon monoxide and ozone levels in localities which do not meet National Ambient Air Quality Standards (ATSDR, 1996; USGS, 1996). Almost all of the MtBE produced is used as a gasoline additive; small amounts are used by laboratory scientists (ATSDR, 1996). When used as a gasoline additive, MtBE may constitute up to 15% (v/v) of the gasoline mixture. MtBE production in the United States was estimated at 6.2 billion kilograms in 1994 and 21 billion kilograms in 1995 (NSTC, 1997 and 1996).

In the Clean Air Act of 1990 (Act), Congress mandated the use of reformulated gasoline (RFG) in those areas of the country with the worst ozone or smog problems. RFG must meet certain technical specifications set forth in the Act, including a specific oxygenate content. Ethanol and MtBE are the primary oxygenates used in the RFG program to meet the oxygen content requirement. MtBE is used in about 84% of RFG supplies. Currently, 32 areas in a total of 18 states are participating in the RFG program, and RFG accounts for about 30% of the gasoline nationwide. Studies have identified significant air quality and public health benefits that directly result from the use of oxygenated fuels. The refiners' 1995/96 fuel data submitted to EPA indicate that the national emissions benefits exceeded the required reductions. The 1996 Air Quality Trends Report showed that toxic air pollutants, such as benzene, a known carcinogen, declined significantly between 1994 and 1995. Early analysis indicates this progress may be attributable to the use of RFG. Starting in the year 2000, the required emission reductions are substantially greater, at about 27% for VOCs, 22% for toxics, and 7% for NOX.

December 1997

About 40% of the U.S. population live in areas where MtBE is used (USGS, 1996). MtBE is a volatile chemical; therefore, in most areas, the major exposure to MtBE is from air. In some instances, drinking water sources may be contaminated. Leaking underground storage tank systems and pipelines for gasoline products are the cause of reported ground water contamination. According to the Toxic Chemical Release Inventory published in 1995, approximately 3% of the MtBE released from industrial sources enters surface water or publicly-owned treatment plants (ATSDR, 1996). Surface waters can also become contaminated as noncombusted MtBE in gasoline is released into air and precipitated by rain and snow.

Unlike most gasoline components, MtBE is a small, highly water-soluble molecule. Therefore, it does not bind strongly to soils, but travels relatively rapidly to and through surface and underground water. In addition, MtBE appears to be resistant to chemical and microbial decomposition in water (ATSDR, 1996).

MtBE has been reported in ground water and drinking water derived from ground water. Based on monitoring data collected by the U.S. Geological Survey (USGS), it appears that wells most susceptible to contamination are shallow ground water wells in urban areas (USGS, 1996). There is limited MtBE drinking water occurrence information. The information available is insufficient to characterize the extent of drinking water contamination on a nationwide basis, because the samples collected are generally from locations with known or suspected contamination (NSTC, 1996).

In air, MtBE may represent 5-10% of the volatile organic compounds that are emitted from gasoline-burning vehicles, particularly in areas where MtBE is added to fuels as part of an oxygenated fuel program (ARCO, 1995). There are no reliable data on MtBE levels in food, but food should not be a significant source of exposure to MtBE. Limited data suggest that MtBE will not bioaccumulate in fish or food chains (ATSDR, 1996).

The recent report of the National Science and Technology Council (NSTC, 1997) provides extensive occurrence data for MtBE and other fuel oxygenates, as well as information on applicable treatment technologies. For additional information concerning MtBE in the environment, this report can be accessed through the NSTC Home Page via link from the Office of Science and Technical Policy (OSTP) at the following address:

Home Page at:  
[http://www.whitehouse.gov/WH/EOP/OSP/html/OSTP\\_Home.html](http://www.whitehouse.gov/WH/EOP/OSP/html/OSTP_Home.html).

Information on analytical methods for determining MtBE in environmental media are compiled in the ATSDR Toxicological Profile (1996) for this chemical.

### **3.0 CHEMICAL AND PHYSICAL PROPERTIES**

MtBE is an aliphatic ether. It is a colorless liquid with a characteristic odor. It has a low molecular weight (88.15 g/mole), high volatility (vapor pressure 245 mm Hg at 25° C), and high water solubility (40-50 g/L; ATSDR, 1996). In its liquid or gaseous state, it is expected to be readily absorbed into the blood stream. It is moderately lipophilic with a log  $K_{ow}$  of 1.24 (ATSDR, 1996), which will facilitate its absorption across the lipid matrix of cell membranes.

### **4.0 TOXICOKINETICS**

There are no data on the absorption of MtBE in humans after ingestion; the uptake of MtBE via inhalation has been reported to be rapid (Cain et al., 1994; Prah et al., 1994; Johanson et al., 1995). In animals, absorption of MtBE administered by oral, intraperitoneal, or inhalation routes is rapid and extensive (Industrial Bio-Test Laboratories Inc., 1972a,b; Bio/dynamics, 1984; Savolainen et al., 1985; Bio-Res Lab., 1990a,b,c,d; Miller et al., 1997). The extent of dermal absorption in rats is slow and limited, but increases with increasing dose levels (Bio-Res Lab., 1990a,b).

The metabolism and elimination of MtBE and its metabolites also proceed rapidly regardless of the route of administration. After absorption, MtBE is demethylated to form TBA and formaldehyde by the *O*-demethylase of the microsomal cytochrome P-450 system (Brady et al., 1990). TBA is further metabolized to formaldehyde (in rodents) or conjugated with glucuronic acid to form TBA-glucuronide, which is excreted in urine (Cederbaum and Cohen, 1980; Williams, 1959). Other oxidative metabolites of TBA include 2-methyl-1,2-propanediol and alpha-hydroxy isobutyric acid (Bio-Res Lab., 1990b; Miller et al., 1997). Formaldehyde may be reduced to methanol or oxidized to formic acid, which is further biotransformed to carbon dioxide.

Since MtBE is rapidly absorbed into the circulation from inhalation and ingestion exposures, it is expected that MtBE is distributed to all major tissues. A large fraction of the MtBE in blood has a very short half-life of 10-30 minutes. The minor long-term exponential decay component in humans exposed to MtBE via inhalation suggests that a small amount of MtBE can deposit in the tissues (Prah et al., 1994; Johanson et al., 1995). Animal studies showed that 24-96 hours after single short-term exposures, the total residual levels in various tissues (brain, muscle, skin, fat, liver, and kidney) were, in general, low regardless of route of exposure (Industrial Bio-Test Laboratories Inc., 1972a, 1972b; Bio/dynamics,

1984; Savolainen et al., 1985; Bio-Res Lab., 1990a,b,c,d; Miller et al., 1997). Several investigators (Borghoff et al., 1996; Rao and Ginsberg, in press) are developing toxicokinetic models to derive concentrations in blood and brain for rodents and humans after short-term exposure. These models will be reviewed before being used for route-to-route extrapolation, especially when exposures are repeated or continuous.

#### **4.1 Dosimetry: Route-to-Route Extrapolation**

While there are few reports available on the effect of MtBE via ingestion, there are many on inhalation exposure. Attempts have been made to crudely extrapolate inhalation dose-response to an equivalent oral dose-response to offer a perspective on the possible oral hazard/risk suggested by the inhalation data given that the available direct oral data are so limited. In so doing, one must convert the inhalation dose to units of mg/kg-day, determine what assumptions are reasonable for extrapolating this to an equivalent oral exposure in mg/kg-day, and then calculate a related oral potency (slope factor) using the calculated oral dose and the inhalation response.

There are several inherent uncertainties or limitations involved in the estimation of human equivalent oral dose from animal inhalation data. Factors that impact absorption from the lungs and thus dose include: 1) the physical properties of the chemical (e.g., aerosol or gas, including the particle size), 2) respiration rate and minute volume of the experimental animal, and 3) exposure conditions (continuous vs. intermittent exposures). Factors that impact the interspecies aspects of the conversion are: 1) allometric scaling between species to compensate for different body sizes, 2) differences in respiratory system structure and physiology, and 3) the qualitative and quantitative differences in absorption and biotransformation between species.

Another important uncertainty in the extrapolation is in establishing whether the parent toxicant or its metabolite(s) is responsible for the biological activity. The absorbed dose via inhalation exposure does not go through the same liver metabolism (the first-pass effect) as that via ingestion. Many chemicals (e.g. formaldehyde) produce different toxic and carcinogenic effects via different routes of exposure. This means that it is important to determine whether it is the parent compound or a metabolite that is responsible for the observed effects. Specific uncertainties and limitations in the toxicokinetic data for MtBE are discussed below.

Most of the absorption data on MtBE were collected following short-term inhalation exposure. Duration of exposure and the rate of respiration are two very important parameters which control the absorption of MtBE. During the exposure

period, a state of equilibrium is established between the inhaled and exhaled air; therefore, the percent absorbed dose by inhalation is influenced by the pharmacological properties of the toxicant. For example, substances like MtBE with an anesthetic effect at higher dose will slow down the respiratory rate and, thereby, slow down the rate of absorption via the lungs into the blood. Accordingly, overall absorption of MtBE would be anticipated to be lower at a higher dose because of its effect on the central nervous system. There is not enough information to estimate the exact absorbed dose in long-term inhalation or oral exposure.

As already mentioned, via the inhalation route, MtBE enters the blood without passing through the gastrointestinal tract and the liver which is responsible for most of MtBE metabolism by way of the hepatic cytochrome P-450 system. To what extent MtBE metabolism is influenced by the gastrointestinal tract is not known. It is likely that differences in the metabolism between exposure routes do occur and affect toxicity. Using inhalation exposure to estimate the oral dose ignores potential first-pass effects in the liver. However, the uncertainties in the route-to-route extrapolation of dose for MtBE are mitigated by the fact that the metabolites qualitatively appear to be the same by differing routes, the distribution and excretion patterns are the same and the tissues in which toxicity, including carcinogenicity, have been reported overlap between routes.

#### **4.2 NSTC's Extrapolation of Dose from Inhalation Exposure**

A number of the studies utilized for this Advisory involved the inhalation route of exposure. At present, there is no appropriate toxicokinetic model to convert an applied inhalation exposure concentration to a dose in the target organ, although models are under development at CIIT (Borghoff et al., 1996) and the University of Connecticut (Rao and Ginsberg, in press). In the absence of a well-developed toxicokinetic model, the inhalation exposure concentrations were converted to dose values following the method used by the interagency task force on MtBE (NSTC, 1996; 1997). The NSTC (1996) conversion method assumes that for a given exposure concentration of MtBE, the adjusted external human equivalent dose would be the same from studies of any kind of animals, regardless of the species used. The calculation also assumes 100% absorption of MtBE, and appears to be a default value in the absence of reliable inhalation and absorption data.

The equation used for the dose conversion by the NSTC (1997) is presented as follows:

$$\text{Human Equivalent Dose (HED)} = \frac{\text{C ppm} \times 10^{-6} \text{ ppm}^{-1} \times \text{MM} \times \text{RR}}{\text{MV} \times \text{BW}} \times \text{EC}$$

Where:

- C = Atmospheric concentration
- MM = Molar mass expressed in milligrams (88,150 mg for MtBE)
- MV = Molar volume at 20°C (24.04 L)
- RR = Human respiration rate (20,000 L/day)
- EC = Exposure condition (# hrs/24 hr) x (# days/week)
- BW = Average human body weight (70 kg)

The value of  $10^{-6} \text{ ppm}^{-1}$  in the equation is a unit adjustment factor that expresses the amount of the contaminant that is present in each unit of inspired air.

When the concentration of MtBE is 1 ppm, the exposure condition is continuous (24 hrs/day and 7 days per week), the EC is 1 and the HED is calculated as 1.05 mg/kg-day as follows:

$$\text{HED} = \frac{1 \text{ ppm} \times 10^{-6} \text{ ppm}^{-1} \times 88,150 \text{ mg} \times 20,000 \text{ L/day}}{24.04 \text{ L} \times 70 \text{ kg}} = 1.05 \text{ mg/kg/day}$$

In cases where exposures are conducted for 6 hrs/day and 5 days per week, the EC is equal to  $(6/24) (5/7)$  or 0.1786. Consequently, 1 ppm of MtBE is equivalent to 0.1875 mg/kg-day.

The Office of Water has presented the NSTC (1997) methodology for extrapolation of the inhalation exposure doses to oral doses in studies with MtBE in order to be consistent with the risk assessment values of those provided in the NSTC (1997) report. The limitations of the methodology generate significant uncertainties.

## 5.0 HEALTH EFFECTS DATA

### 5.1 Human Studies

There are very limited data on the effects of MtBE in humans by any route of exposure and no data are available for the oral route. In cases where 37 or 43 human volunteers were exposed to low levels of MtBE in air (1.39 or 1.7 ppm) for 1 hour (Cain et al., 1994; Prah et al., 1994), there was no significant increase in

symptoms of eye, nasal, or pulmonary irritation when the results for periods of exposure to MtBE were compared to results from exposure to ambient air. There were also no significant effects on mood (determined by the Profile of Mood States test) or in the results from several performance-based neurobehavioral tests. In both studies, the females ranked the quality of the air containing MtBE lower than the control atmosphere. However, in the study by Cain et al. (1994), where the subjects were also exposed to an atmosphere containing a 7.1 ppm mixture of 17 volatile organic compounds (VOCs) that are frequent air contaminants in areas around gasoline stations, the air quality of the MtBE-containing atmosphere ranked higher than that with the VOC mixture.

The results from studies of neurological effects (headache, dizziness, disorientation, fatigue, emotional distress, etc.), gastrointestinal problems (nausea, diarrhea), and symptoms of respiratory irritation in individuals exposed to MtBE vapors through MTBE-containing fuels are inconclusive (Hakkola et al., 1996; Moolenaar et al., 1994; White et al., 1995). The three studies cited were different in their design and utilized slightly different parameters for monitoring effects. All studies evaluated exposure to a MtBE-gasoline mixture and not MtBE.

The studies by Hakkola et al. (1996) and White et al. (1995) compared the effects in two groups exposed to different concentrations of MtBE from treated gasoline because of their lifestyles. The moderately-exposed individuals either drove a gasoline delivery truck, worked in a gasoline station or worked on car repairs. The minimally-exposed individuals merely used a gasoline-powered vehicle to go to and from work or as part of their job. Hakkola et al. (1996) found that there were no statistically-significant differences between the signs and symptoms reported by 101 drivers of tanker trucks in Finland (where the gasoline contains 10% MtBE) and 100 milk truck drivers. Blood concentrations of MtBE or its metabolites were not monitored. In the study by White et al. (1995), the odds ratio was 8.9 (95% CI = 1.2-75.6) for the reporting of one or more symptoms when 11 individuals with blood MtBE levels of  $>2.4 \mu\text{g/L}$  were compared with 33 individuals with lower levels. The odds ratio increased to 21 (95% CI = 1.8-539) when commuters were excluded from the population studied and 8 workers with blood levels of  $>3.8 \mu\text{g/L}$  were compared to 22 individuals with lower blood MtBE levels. All individuals lived and worked in the area around Stamford, Connecticut.

A study in Alaska (Moolenaar et al., 1994) compared effects and blood levels of MtBE from a time period when oxygenated fuels were in use (Phase I) to those after the oxygenated fuels use had stopped (Phase II). The subjects were volunteers who were occupationally exposed to motor vehicle exhaust or gasoline fumes. Eighteen workers participated in Phase I and 22 in Phase II. Twelve of those that participated in Phase I of the study also participated in Phase II. A

questionnaire was used to gather information on signs and symptoms and blood samples were collected for measurement of MtBE at the beginning and end of a typical work day. In Phase I, the median post-shift MtBE level was higher than the pre-shift value (1.80 vs. 1.15  $\mu\text{g/L}$ ). During Phase II, the values were more comparable (0.25 vs. 0.21  $\mu\text{g/L}$ ). Median post-shift blood measurements of TBA were higher during Phase I than in Phase II (5.6 vs. 3.9  $\mu\text{g/L}$ ).

Signs and symptoms that could be associated with MtBE exposure were reported more frequently during Phase I than Phase II (Moolenaar et al., 1994). During Phase I, 50% or more of the participants reported headaches, eye irritations and nose and throat irritations. Reporting of these symptoms occurred in less than 10% of the participants during Phase II. However, it is difficult to evaluate if psychosomatic factors and individual sensitivity had influenced these results. The volunteers may have chosen to participate because of their sensitivity to contaminants in the atmosphere.

Perfusion of MtBE through the bile duct and gallbladder was once used as a medical treatment for gallstones. During this procedure, some of the MtBE enters the blood stream and is distributed systemically. Effects reported in patients treated by this procedure included sedation, perspiration, bradycardia (slow heart beat) and elevation of liver enzymes (Allen et al., 1985; Juliani et al., 1985, and Wyngaarden, 1986). These signs cannot be attributed totally to MtBE because of the confounding effects of anesthesia and the infusion process itself.

## **5.2 Animal Studies**

### **5.2.1 Noncancer Effects**

#### **5.2.1.1 Acute and Subchronic**

Studies of the systemic effects of MtBE have been conducted in animals, but the majority involve inhalation exposure. Since this Provisional HA is mainly concerned with the effects of MtBE in drinking water, it will focus on oral toxicity studies. From an acute standpoint, MtBE is not very toxic. The oral  $\text{LD}_{50}$  in rats is 3.9 g/kg (3,900 mg/kg). Treated animals exhibit central nervous system depression, ataxia and labored breathing (ARCO, 1980).

In a two-week study, Sprague-Dawley rats (10/sex/dose) were dosed daily with MtBE in corn oil by gavage at 0, 537, 714, 1,071 or 1,428 mg/kg/day. At the highest dose, anesthesia was immediate, but recovery was complete within two hours. Although there was a dose-related decrease in body weight gain, it was significant only in females at the highest treatment regimen. Increases in relative

kidney weights were noted in the males at 1,071 and at 1,428 mg/kg/day and in females at the 1,428 mg/kg/day dose. There were no gross lesions seen at any treatment level. Based on the increases in relative kidney weight, a No-Observed-Adverse-Effect-Level (NOAEL) of 714 mg/kg/day and a Lowest-Observed-Adverse-Effect-Level (LOAEL) of 1,071 mg/kg/day are established by these experiments (Robinson et al., 1990).

Sprague-Dawley rats (10/sex/dose) were treated orally with MtBE in corn oil for 90-days at 0, 100, 300, 900 or 1,200 mg/kg/day. Anesthesia was evident at the highest dose, but as in the 14-day study, full recovery occurred in two hours. There was a significant decrease in final body weight of females only at the highest level of treatment. The diarrhea seen in the treated animals was considered to be the consequence of the bolus dosing regime. In females, there were increases in relative kidney weights at 300, 900 and 1,200 mg/kg/day, while in males, increases were noted only at the two highest treatment levels. Reductions in blood urea nitrogen, serum calcium and creatinine were observed in males and a reduction in cholesterol in females was reported, but there were no clear dose-dependent results. Based on the alterations in kidney weights, a NOAEL and LOAEL of 100 and 300 mg/kg/day, respectively, are identified by this study (Robinson et al., 1990).

Sprague-Dawley rats (60 animals per sex, per dose group) were given 0, 250 or 1,000 mg/kg/day MtBE in olive oil via gavage, 4 days per week, for 104 weeks. This dosing regimen gives a 7-day time-weighted average daily dose of 0, 143 and 571 mg/kg/day. Survival appeared to be decreased in female rats after 16 weeks, but no statistical treatments on data were reported. There was no reporting of hematological, clinical chemistry or urinalysis parameters, or any indication as to whether or not these endpoints were evaluated. The authors did not observe any differences in food consumption or final body weights in the various groups. In addition, they did not report any noncancer histopathological changes (Belpoggi et al., 1995). Due to the limited scope, intermittent treatment schedule and scant data reporting in this study, it is not possible to set a NOAEL or LOAEL.

The subchronic data from the study by Robinson et al. (1990) were used to develop a DWEL for kidney effects from MtBE. The increase in kidney weights at doses of 300 mg/kg/day and higher was considered to be an adverse effect, since increases in organ weights are a marker for adverse organ effects (Weil, 1970). The diarrhea observed was considered to be a gastrointestinal complication of the gavage dosing. Based on the NOAEL of 100 mg/kg/day, a DWEL for kidney effects of 3,500  $\mu\text{g/L}$  can be derived for a 70 kg adult drinking 2 L of water per day, using an uncertainty factor of 1,000. The uncertainty factor reflects a 10 for

the less-than-lifetime duration of the study, a 10 for interspecies variability and a 10 for intraspecies variability.

Kidney toxicity was also observed in both males and females in the 2-year inhalation study in F344 rats by Chun et al. (1992) discussed in the section on cancer effects. In fact, EPA derived a Reference Concentration of 3 mg/m<sup>3</sup>, based on the kidney and liver effects of MtBE (U.S. EPA, 1993). These data support the conclusion that, after MtBE exposure, kidney toxicity is of concern. However, the use of the Robinson et al. (1990) study for evaluation of kidney effects has two significant uncertainties. One is that the study was for 90 days and not for a lifetime, and the second is the extrapolation of dose from a single daily bolus dose in corn oil to the continuous small doses from drinking water exposure. In general, it would be anticipated that a 90-day exposure period would tend to underestimate the toxicity, while the bolus dose would be more likely to overestimate the toxic response. However, the relative effects of these two factors are uncertain.

### **5.2.1.2 Reproductive and Developmental Studies**

#### **Reproductive Studies**

Two inhalation studies in rats were available on the reproductive effects of MtBE. A two-generation reproduction study was conducted in Sprague-Dawley CD rats using target concentrations of 0, 400, 3,000 or 8,000 ppm of MtBE for 6 hours/day, 5 days/week for 10 weeks before mating, during mating, gestation and lactation days 5-21 (Bushy Run Research Center, 1991; Bevan et al., 1997b). Statistically-significant reductions in body weight and body weight gains in male and female F<sub>1</sub> and F<sub>2</sub> pups were noted with the 3,000 ppm and 8,000 ppm exposures during the latter periods of lactation. At 3,000 ppm, only transient body weight reductions were noted in F<sub>1</sub> males and females during their pre-mating period. At 8,000 ppm, pup survival was significantly reduced ( $p < 0.01$ ) in the F<sub>1</sub> litters on lactation days 0-4 and in F<sub>2</sub> litters on postnatal day 4. Clinical signs of toxicity were noted in both generations at 3,000 and 8,000 ppm; this included hypoactivity and lack of startle reflex. Ataxia and blepharospasm (eyelid twitching) were observed at 8,000 ppm. At necropsy, increased liver weights were reported in the F<sub>1</sub> generation at 3,000 and 8,000 ppm in both sexes, although no histopathological effects were noted. The NOAEL and LOAEL for both parental and pup toxicity were 400 and 3,000 ppm, respectively.

A one-generation study (Biles et al., 1987) in Charles River CD rats was carried out with two matings, using target concentrations of 0, 300, 1,300 or 3,400 ppm of MtBE vapor for 6 hours/day, 5 days/week, prior to and during mating. Exposure was continued during 5-day mating intervals. In males, exposure

continued until the end of the second mating to produce the F<sub>1b</sub> litters. In females, exposure continued during the gestation period and lactation days 5 to 21, but not during the first 4 days of the lactation period. A NOAEL and a LOAEL may be identified at 300 ppm and 1,300 ppm, respectively, based on pup viability in the F<sub>1b</sub> litters. However, this study has limited usefulness in the evaluation of reproductive toxicity because of some noted flaws (e.g., the loss of one entire litter of 12 pups at birth in the mid-dose group remains unexplained).

### **Developmental Studies**

Four inhalation studies were evaluated: one in rats (Conaway et al., 1985), two in mice (Conaway et al., 1985; Bushy Run Research Center, 1989a; Bevan et al., 1997a) and one in rabbits (Bushy Run Research Center, 1989b; Bevan et al., 1997a). The Conaway et al. studies in the rat and mouse were performed at target concentrations of 0, 250, 1,000 or 2,500 ppm of MtBE for 6 hrs per day on days 6 to 15 of gestation. Dams were sacrificed at gestation day 20 for rats and gestation day 18 for mice. The concentrations for the Bushy Run studies in mice and rabbits were 0, 1,000, 4,000 ppm or 8,000 ppm. Mice were exposed on days 6 to 15 of gestation and rabbits were exposed on days 6 to 18 of gestation. Mice dams were sacrificed on gestation day 18 and rabbits on gestation day 28.

In the rat study (Conaway et al., 1985), no effects were noted in rats at the highest dose tested, 2,500 ppm. Also, in the rabbit study (Bushy Run Research Center, 1989b; Bevan et al., 1997a), no developmental toxicity was noted at the highest dose tested, 8,000 ppm, but maternal toxicity was noted at 4,000 ppm and above.

For mice, in the Bushy Run study, maternal toxicity was noted at the two higher concentrations (4,000 ppm and 8,000 ppm). Also, fetal skeletal variations and reduction in fetal weight were noted at the higher doses. In the Conaway et al. (1985) mouse study, the most noted developmental effect was a dose-related increase in the incidence of skeletal malformations per litter with incidence of 7.4 percent in the control group compared to 11.5 percent, 16 percent and 22.2 percent in the 250, 1,000 and 2,500 ppm groups, respectively. These malformations included cleft palate, scrambled and fused sternebra and angulated ribs. Cleft palate occurred in two fetuses of one litter in the control group; one fetus in the 1,000 ppm group; two fetuses, each in a different litter of the 2,500 ppm group; and none in the 250 ppm group. There were also 17, 11 and 17.3 percent resorptions in the 250, 1,000 and 2,500 ppm groups, respectively, compared to 9 percent in control. Based on the incidence of skeletal malformations in these two mice studies, a developmental NOAEL in mice can be projected in the range of 250 ppm to 1,000 ppm.

The collective evaluation of the two developmental mouse studies discussed above reflects a NOAEL in the range of 250 to 1,000 ppm for developmental toxicity. The NOAEL of 400 ppm for parental toxicity in the rat two-generation reproductive study falls within the NOAEL range for developmental effects. These values are projected as equivalent to doses of 65.6 mg/kg/day to 262.5 mg/kg/day, respectively. Using these two values, the projected, no-effect-concentration in drinking water for humans is in the range of 2.3 to 9.2 mg/L (2,300 to 9,200  $\mu\text{g/L}$ ). Since the NOAEL in the reproductive study is also 400 ppm, exposure to MtBE in drinking water within this concentration range should not cause reproductive or developmental toxicity in humans. This health range assumes that a 70 kg adult consumes 2 L of water per day. An uncertainty factor of 1,000 was applied to the NOAEL. This factor includes a 10-fold factor for interspecies variability, 10 for intraspecies variability, and 10 to account for acute exposure and the limitation associated with the conversion of the inhaled dose to an oral dose in the absence of adequate pharmacokinetic models. The conservative use of the 10-fold factor for acute exposure should provide an additional margin of protection for potential effects on the developing fetus.

### 5.2.1.3 Neurotoxicity Studies

Inhalation exposure of animals to high levels of MtBE is associated with depression of the central nervous system in the period immediately after exposure (Daughtrey et al., 1997). Symptoms observed in groups of 22 male and 22 female F344 rats in the hour after a 6-hour exposure to an atmosphere containing 4,000 or 8,000 ppm MtBE included labored respiration, ataxia, decreased muscle tone, abnormal gait, impaired treadmill performance and decreased hind-limb grip strength. These effects were not noted 6 and 24 hours after the cessation of exposure. There were no apparent effects from a single 6-hour exposure to 800 ppm MtBE.

Subchronic exposures of groups of 15 male and 15 female rats under the same daily exposure conditions used for the acute study gave no indication that the repetition of exposure exacerbated the acute central nervous system response (Daughtrey et al., 1997). There was a significant decrease in the absolute, but not the relative, brain weight in the high-dose group at the end of the 13-week exposure period. However, there were no significant changes in brain or peripheral nervous system histopathology that could be related to MtBE. These studies identified 800 ppm as a NOAEL and 4,000 ppm as a LOAEL for acute effects of MtBE on the central nervous system.

The 800 ppm NOAEL for acute neurotoxic effects is projected to be equivalent to a dose of 210 mg/kg/day. Using this value, the projected no-effect concentrations

December 1997

in humans is 7.35 mg/L (7,350  $\mu$ g/L) for a 70 kg adult drinking 2 L/day water. An uncertainty factor of 1,000 was used for this calculation. The uncertainty factor includes a 10 for use of a frank effect, 10 for interspecies variability and 10 for intraspecies variability. The uncertainty factor does not include an adjustment for the short-term duration, because the daily repetition of exposure had no influence on the effects observed.

#### 5.2.1.4 Mutagenicity Studies

Several studies were available to assess the mutagenicity of MtBE. With one exception, this chemical has not exhibited genetic toxicity in a variety of *in vitro* and *in vivo* mammalian and non-mammalian test systems. Positive results were noted in a mouse lymphoma assay in the presence of microsomal enzymes (ARCO, 1980). The only positive response is due to the formaldehyde produced from *in vitro* metabolism (Stoneybrook Laboratories Inc., 1993). The objective of the mutagenicity studies is to determine whether MtBE's carcinogenic activity is associated with positive *in vivo* genetic activity (Mckee et al., 1997). The weight of evidence from the mutagenicity data summarized below indicated that MtBE is not mutagenic.

MtBE was negative in sex-linked recessive lethal test in the *Drosophila melanogaster* (Hazelton, 1989). It was also negative in the Ames assays using *Salmonella*, both with and without metabolic activation (ARCO, 1980; Life Science Research, 1989a).

Chromosome aberrations (ABS) or sister chromatid exchange (SCE) induction tests in Chinese hamster ovary cells were negative with or without activation (ARCO, 1980). MtBE did not cause mutations in cultured Chinese hamster V79 cells (Life Science Research, 1989b). Inhalation of MtBE at dose levels up to 8,000 ppm did not cause chromosomal aberrations in bone marrow cells of F344 rats exposed 6 hours/day for 5 days (Bushy Run Research Center, 1989c) or micronuclei in bone marrow cells of CD-1 mice exposed for 6 hours/day for 2 days (Bushy Run Research Center, 1993). MtBE was also negative for mutations at the *hprt* locus in lymphocytes of CD-1 mice (Ward et al., 1995).

No increase in unscheduled DNA synthesis was observed in the hepatocytes of CD-1 mice that were exposed to MtBE vapor concentrations of up to 8,000 ppm for 6 hours /day for two consecutive days (Bushy Run Research Center, 1994). It did not cause DNA damage in the primary rat hepatocyte culture test (Life Science Research, 1989c), nor was it clastogenic in a rat *in vivo* cytogenetic assay (ARCO, 1980).

### 5.2.2 Cancer Effects

#### 5.2.2.1 Studies of the Carcinogenicity of the Parent Compound (MtBE)

There are three chronic/cancer studies of MtBE in two rodent species (two inhalation studies, one in mice and one in rats, and one gavage study in rats). High

doses of MtBE were used in all of the carcinogenicity studies and in some cases they have exceeded the Maximum Tolerated Dose (MTD).

### **Gavage Study**

When MtBE (99% pure) was administered orally to Sprague-Dawley rats (gavage in olive oil, at doses of 0, 250 or 1,000 mg/kg-day, 4 days/week for two years), no significant differences in food/water consumption or body weight gain were observed. The chemical caused a dose-related increase in the incidence of leukemia and lymphomas in females (2/58 in the controls, 6/51 in the low-dose group and 12/47 in the high-dose group) and an increase in the testicular interstitial Leydig cell adenomas in the high-dose males (18.3% vs. 3.3% in the controls and/or low-dose animals). Survival was decreased 15 and 20% in the low- and high-dose females, respectively after 9 to 12 months of treatment (Belpoggi et al., 1995). There are some limitations in the reporting of the data as discussed below (quoted from NSTC, 1997):

The Belpoggi et al. study was published in the peer-reviewed literature. However, no detailed technical report of the bioassay is available. Lacking a detailed report about the bioassay, the NRC panel (NRC, 1996) identified a number of issues and questions which reflects upon the risk assessment use of these data. The NRC noted that the morphological criteria used to classify histopathological findings for both the lymphoma-leukemia and interstitial cell tumor responses were not adequately described and that the study did not adequately address the impact on tumor outcomes or differences in survival between controls and dosed groups. NRC went on to say that 'because of the importance of this study for eventual use in risk assessment, the superficial reporting of the data and the nature of the observed lesions, the committee felt strongly that an independent in-depth review of the data, especially the pathology (microscopic slides) of the critical lesions is warranted (as was done with the inhalation studies) before the data are used for risk assessment'. While the NRC raised questions about survival differences and the tumor outcome, it should be noted that Belpoggi et al. included statistical analyses that adjusted for intercurrent mortality. Several attempts by the Interagency Oxygenated Fuels Assessment Steering Committee to arrange for a pathology review of the Belpoggi et al. study have not been

successful, hence, the underlying concerns raised by NRC review cannot yet be resolved.

**Inhalation Studies**

In a report by Chun et al. (1992), F344 rats were exposed to 0, 400, 3,000, or 8,000 ppm MtBE by inhalation, 6 hrs/day, 5 days/week for 2 years. This study was recently published as Bird et al. (1997). Survival time was statistically and significantly reduced in the exposed male rats in a dose-related manner. The mean body weights of the 8,000 ppm group (both sexes) were reduced throughout the experiment. (The mean body weight was decreased 19% in the males at week 82 and 13% in the females at the end of the experiment). An increase in chronic, progressive nephropathy was observed in the exposed male and female rats. The combined incidence of renal tubular adenomas and carcinomas<sup>2</sup> was increased significantly in the male rats exposed to the mid-dose (controls, 1/35; low-dose, 0/32; mid-dose, 8/31; high-dose, 3/20). The reduced survival rate of the high-dose group may have decreased the sensitivity of the test to produce a dose-related increase in tumors.

A study by CIIT (Prescott-Mathews et al., 1997) shows that MtBE caused a mild induction of  $\alpha$ -2u-globulin nephropathy and enhanced renal cell proliferation in F344 male rats, suggesting that  $\alpha$ -2u-globulin nephropathy may potentially play a role in male rat kidney tumorigenesis.

EPA (U. S. EPA, 1991) published three criteria for establishing whether  $\alpha$ -2u-globulin is responsible for the kidney tumor in male rats: 1) increased number and size of hyaline droplets in renal proximal tubule cells of treated rats, 2) accumulating protein in the hyaline droplets is  $\alpha$ -2u-globulin, and 3) additional aspects of the pathological sequence of lesions associated with  $\alpha$ -2u-globulin

---

<sup>2</sup>Renal Tumor Incidence of F344 Male Rats After Inhalation Exposure to MtBE (Chun et al., 1992)

Administered exposure (ppm)	Human equiv. Dose* (mg/kg-day)	Tumor incidence+	Survival-adjusted Tumor incidence
0	0	1/50	1/35
400	75	0/50	0/32
3000	562.5	8/50	8/31
8000	1500	3/50	3/20

+tumor type: combined renal tubular cell adenomas and carcinomas

\* See section 4.2 NSTC's Extrapolation of Dose from Inhalation Exposure

nephropathy are present. EPA's policy states that if experimental data do not meet the criteria in any one of the three categories, the  $\alpha$ -2u-globulin alone is not considered responsible for the renal tumor formation and the renal tumor may be used for risk assessment, both qualitatively and quantitatively. Based on the available data, EPA concludes that the first criteria has been met, but the second and third criteria have not been adequately satisfied.

The mechanism of action of MtBE kidney carcinogenesis in male rats is not fully understood at the present time. In this case, the identification of the full spectrum of  $\alpha$ -2u-globulin-specific nephropathy is complicated by a background of chronic progressive nephropathy (CPN) in both male and female rats and the apparent absence of one or more key  $\alpha$ -2u-globulin pathological factors. The apparent absence may be a true non  $\alpha$ -2u-globulin consequence, it may be masked by CPN, or it may be that the mild induction is insufficient to elicit the full  $\alpha$ -2u-globulin response. It is possible that other proteins related to  $\alpha$ -2u-globulin may also be involved (HEI, 1996). Ongoing research on the potential role of  $\alpha$ -2u-globulin accumulation in male rat kidney may improve our understanding of the carcinogenesis of MtBE and its metabolite, TBA, in the kidney.

A statistically significant increased incidence of the interstitial testicular Leydig cell adenomas of the treated rats was detected in the Chun et al. (1992) study (32 in the controls, 35 in the low-dose, 41 in the mid-dose, and 47 in the high-dose). The increase in the incidence of Leydig cell adenomas of the male rats in this study (Chun et al., 1992; Bird et al., 1997) was not significantly different from the historical control value, although the difference from the concurrent controls was significant. The concurrent control incidence was 64% and the historical control values ranged from 64 to 98% in the same laboratory (Bird et al., 1997). (Leydig cell adenomas occur at a high spontaneous rate in the F344 strain of rats.) However, this type of tumor was also observed in another strain of rats, the Sprague-Dawley, upon oral exposure by gavage (Belpoggi et al., 1995). Since the Sprague-Dawley rat does not have a significant spontaneous background incidence for this type of tumor, the conclusion that the appearance of the tumor in both studies is MtBE treatment-related is more confident.

In a report by Burleigh-Flayer et al. (1992), CD-1 mice were exposed to 0, 400, 3,000 or 8,000 ppm MtBE by inhalation, 6 hrs/day, 5 days/week for 18 months. Mortality was increased and the mean survival time was decreased in the high-dose mice compared to controls. The body weight gain was also decreased in the 8,000 ppm group compared to the controls (a decrease of 16% and 24% for male and female mice, respectively), indicating that the high dose exceeded the MTD. A statistically-significant increase was found in the incidence of hepatocellular carcinomas in male mice and of hepatocellular adenomas in female mice exposed to

8,000 ppm of MtBE<sup>3</sup>. The hepatic tumors were only evidenced at the high dose. Since MtBE is generally negative in mutagenicity tests, and the hepatocellular tumors induced by MtBE in CD-1 mice were detected only in the high-dose animals where the dose exceeded the MTD, the authors of the study (Burleigh-Flayer et al., 1992; Bird et al., 1997) considered the mouse liver tumor finding not likely to be due to a direct-DNA acting phenomenon. The NAS panel (NRC, 1996) also suggested that the non genotoxic, hormonally-related mechanisms are the most plausible explanation for the development of mouse liver tumors”

Based on short-term studies in mice at CIIT, Moser et al. (1996) speculated that endocrine modulations may play a role in the hepatocarcinogenic effect of MtBE. The CIIT studies include: a) inhalation exposure (approximately 8,000 ppm, 6 hrs per day, 5 days per week) of female B6C3F1 mice to MtBE for 3 or 21 days, resulting in an increased relative liver weight, increased P450 content and its activity, as well as a decreased relative uterus weight; b) gavage treatment of B6C3F1 mice with MtBE (1,800 mg MtBE/kg body weight/day for 3 days) resulting in increased estrogen metabolism in isolated mouse hepatocytes (Moser et al. 1996).

EPA has calculated three slope factors from the cancer studies which appeared in the NSTC (1997) document. These estimates of slope factors are not likely to underestimate risk for the general population. The ability to calculate such an estimate does not imply greater confidence in potential cancer hazard. True risk for most individuals in the population is likely to be lower and for some may even be nearly zero. Because there are uncertainties inherent in these values, they should be used cautiously.

The first slope factor is based on the Belpoggi et al. (1995) gavage study. Using the combined tumor incidence of lymphoma and leukemia in the female rats and a

---

<sup>3</sup>Hepatocellular Tumors in Female Mice After Inhalation Exposure to MtBE (Burleigh-Flayer et al., 1992)

Administered exposure (ppm)	Human equiv. Dose (mg/kg-day)	Tumor incidence		
		Adenoma	Carcinoma	combined
0	0	2/50	0/50	2/50
400	75	1/50	1/50	2/50
3000	562.5	2/50	0/50	2/50
8000	1500	10/50	1/50	11/50

In the male mice, the combined hepatocellular tumor incidence for the control, low-, mid-, and high-dose groups are 12/47, 12/47, 12/46 and 16/37, respectively.

scaling factor of body weight raised to the 2/3 power, a slope factor of  $4 \times 10^{-3}$  (mg/kg/day)<sup>-1</sup> can be calculated by the linearized, multistage model<sup>4</sup>.

The second slope factor is based on the Chun et al. (1992) data. Based on the combined renal tubular cell adenomas and carcinomas in the male F344 rats, using a scaling factor of body weight raised to the 2/3 power, a slope factor of  $6 \times 10^{-4}$  per ppm can be calculated by the linearized, multistage model. Additional understanding of the mode of action of this response could substantially alter these estimates or make them irrelevant.

The third slope factor is based on the Burleigh-Flayer et al. (1992) data. Based on the liver tumor incidence in the female CD-1 mice, using a scaling factor of body weight raised to the 2/3 power, a slope factor of  $3 \times 10^{-4}$  per ppm was calculated by the linearized, multistage model.

### 5.2.2.2 Studies of the Carcinogenicity of MtBE Metabolites

#### *tertiary-Butyl Alcohol*

F344 rats were exposed to TBA via drinking water at concentrations of 0, 1.25, 2.5 or 5 mg/mL for 2 years (the average delivered, daily doses of TBA were approximately 0, 85, 195, and 420 mg/kg-day for males and 0, 175, 330 and 650 mg/kg-day for females). There was some evidence of carcinogenic activity in male rats based on an increased incidence of renal tubular hyperplasia and renal tubular adenomas or carcinomas, and no evidence of carcinogenic activity in female rats (Cirvello et al., 1995; NTP, 1995). Compared to controls, the survival was significantly lower for the high-dose animals, especially in the males. Increased nephropathy was also noted in all treated animals.

B6C3F1 mice were exposed to TBA in drinking water at concentrations of 0, 5, 10 or 20 mg/mL for 130 weeks (the average daily delivered doses were 0, 535, 1035 or 2065 mg/kg-day for males and 0, 510, 1015 or 2105 mg/kg-day for females). There was equivocal evidence of carcinogenic activity in male mice, based on

---

<sup>4</sup>Based on the Proposed Guidelines for Carcinogen Risk Assessment (FR 61, 17960, April 23, 1996), with the same tumor data, using a scaling factor of body weight raised to the 3/4 power, an LED<sub>10</sub> of 35.6 mg/kg-day and a slope factor of  $2.8 \times 10^{-3}$  (mg/kg-day)<sup>-1</sup> are obtained. The drinking water concentration will be 12 µg/L for a risk of one in a million using this slope factor.

marginally increased incidence of thyroid tumors and some evidence of carcinogenicity in female mice, based on an increased incidence of follicular cell hyperplasia and follicular cell adenomas of the thyroid gland. Survival of males in the high-dose group was significantly lower than that of the control group. Thus, the National Toxicology Program (NTP) studies of TBA show no clear evidence of carcinogenicity in either species.

### **Formaldehyde**

There is sufficient evidence of carcinogenicity in animals by the inhalation route (IARC, 1995). Inhalation exposure of F344 rats to formaldehyde for 2 years at 14.3 ppm induced squamous cell carcinomas of the nasal cavity in both male and female F344 rats (the doses were: 0, 2, 5.6 or 14.3 ppm, 6 hours per day, 5 days per week), but not in female B6C3F1 mice (same doses and exposure conditions) (Kerns et al., 1983). Lifetime inhalation studies of formaldehyde in Sprague-Dawley rats at 14 ppm (Sellakumar et al., 1985), and Wistar rats at 10 ppm (Woutersen et al., 1989) also produced nasal tumors.

By the drinking water route of exposure, the evidence of carcinogenic activity for formaldehyde is somewhat ambiguous. One lifetime drinking water study of formaldehyde in Sprague-Dawley rats at concentrations of 0, 10, 50, 100, 1,000 or 1,500 ppm showed a dose-related increase in the incidence of leukemia and intestinal tumors (Soffritti et al., 1989). Similar to the Belpoggi et al. (1995) study of MtBE (which was conducted by the same laboratory), the reporting of the study is somewhat limited and the pathology also lacks an independent review. Another 2-year drinking water study of formaldehyde using Wistar rats at doses ranging from 0, 1.2, 15 to 82 mg/kg/day for males and 0, 1.8, 21, to 109 mg/kg/day for females showed no evidence of carcinogenicity (Til et al., 1989).

## **6.0 ORGANOLEPTIC PROPERTIES**

Water contaminated with MtBE may have an unpleasant taste or odor. These characteristics, often referred to as “organoleptic properties,” cannot be used by EPA for developing primary drinking water standards, but are of concern and do play a role in the production of finished drinking water, as most U.S. citizens would not drink “unpleasing” water. Taste and odor may also alert consumers to the fact that the water is contaminated with MtBE and, therefore, were considered in the development of this Advisory.

Not all individuals respond equally to taste and odor because of differences in individual sensitivity. The taste and odor responses reported in observed individuals for MtBE are in the 15 to 180  $\mu\text{g/L}$  range for odor and the 24 to 135  $\mu\text{g/L}$  range

for taste (NSTC, 1997, Young et al., 1996; API, 1993; Prah et al., 1994; Dale et al., 1997). The ranges are indicative of the variability in individual response. The lower ends of the range for both taste and odor are the lowest concentrations eliciting a response among 7 of 9 participants in a study by Young et al. (1996). In this study, the geometric mean for taste was 48  $\mu\text{g/L}$  and that for odor was 34  $\mu\text{g/L}$ . Participants in this study were selected for their above average sensitivity to basic tastes and odors. In fact, 3 of the 7 participants detected the lowest odor concentration, while 4 of 9 participants detected the lowest taste concentration. The homogeneity in the response among the small group of female subjects, along with the geometric mean values support classification of the subjects as sensitive.

A study commissioned by the American Petroleum Institute (API, 1993) and conducted by TRC Environmental Corp. used 6-7 individuals "chosen to represent a normal distribution of olfactory sensitivity" to measure taste and odor thresholds of 97% MtBE in distilled water. Calculated threshold values were 39  $\mu\text{g/L}$  for taste, 45  $\mu\text{g/L}$  for odor detection, and 55  $\mu\text{g/L}$  for odor recognition. The intensity of the odor of MtBE was also reported to be greater in water than in air. The subjects described the taste of MtBE in water as "nasty", bitter, nauseating, and similar to rubbing alcohol.

In a study by Prah et al. (1994), the concentration of MtBE in distilled water that was identified as having an odor by 50% of the study participants (19 males and 18 females) was 180  $\mu\text{g/L}$ . This value is regarded as the high end of the odor range even though it is a median response concentration. There were undoubtedly individuals who could only detect the odor of MtBE at even higher concentrations.

The Metropolitan Water District of Southern California recently conducted a study on the taste and odor thresholds and other characteristics of MtBE (Dale et al., 1997). They found that the range for the 60% probability ( $\pm 1$  SD) of correct taste detection of MtBE in odor-free water (OFW) and untreated Colorado River water was 24 to 37 and 26 to 58  $\mu\text{g/L}$ , respectively. The corresponding range for detecting the odor of MtBE in OFW was 43 to 71  $\mu\text{g/L}$ . These tests were conducted by having nine trained analysts undergo six "triangle tests" for several concentrations, in which each analyst determined the odd case when blindly presented with either two blanks and one spiked sample or one blank and two spiked samples. It cannot be determined from this small, non-representative sample what percentage of the general population would be able to detect MtBE in their drinking water at these concentrations. However, these taste and odor threshold data are consistent with those reported by Young et al. (1996) and API (1993). This study by Dale et al. (1997) found people more sensitive to taste than odor, which is consistent with the API (1993) findings for MtBE taste and odor thresholds, but in the opposite order to that found by Young et al. (1996).

Collectively, these data support a range of 20 to 40 µg/L as an approximate "threshold" for organoleptic responses. However, some subjects in this study were able to detect MtBE at much lower concentrations; thus, in a general population, some unknown percentage of people will be likely to detect the taste and odor of MtBE in drinking water at concentrations below 20 µg/L.

The study by Dale et al. (1997) went beyond simply measuring taste and odor thresholds. The investigators also asked four panelists to describe the taste and odor of MtBE in OFW at concentrations ranging from 2 µg/L to 190 µg/L. At concentrations of 2-5 µg/L, the consensus judgment of the panelists was that the taste of MtBE in OFW could be described as "sweet." At concentrations of 21-190 µg/L, the characterization was either "solvent" or "sweet solvent." Similar characteristics applied at concentrations of 21-190 µg/L for the odor of MtBE in OFW. The panelists were also asked to rate the intensity of the taste and odor, which can become "objectionable" at a sufficiently high intensity. The panelists considered the taste of MtBE in OFW objectionable at a concentration of approximately 50 µg/L and the odor objectionable at approximately 90-100 µg/L. It is noted that these tests were conducted with non-chlorinated water at 25 degrees C. Chlorination would likely raise the thresholds for the taste and odor of MtBE in water, and higher temperatures (e.g., for showering) would likely reduce these thresholds.

It is not possible to identify point threshold values for the taste and odor of MtBE in drinking water, as the concentration will vary for different individuals, for the same individuals at different times, for different populations, and for different water matrices, temperatures, and many other variables. Nevertheless, it seems reasonable to offer a range of 20-40 µg/L as advisory guidance for helping to ensure consumer acceptance of the taste and odor of MtBE in drinking water.

## **7.0 CHARACTERIZATION OF HAZARD AND DOSE RESPONSE**

### **7.1 Hazard Characterization**

There are very few data on human responses to MtBE. In controlled studies, there were no observable responses to short-term (1 hour) exposures to low concentrations of MtBE in air, although women felt the air quality was substandard (Cain et al., 1994; Prah et al., 1994). Other short-term human studies of MtBE are of limited value, because they evaluated effects under conditions where MtBE was combined with simultaneous exposures to other chemicals, such as gasoline, medicines and/or anesthesia (Allen et al., 1985; Hakkola et al., 1996; Juliani et al., 1985; Moolenaar et al., 1994; White et al., 1995; Wyngaarden 1986). Studies of gasoline/MtBE mixtures are inconclusive, but suggest that MtBE-containing

gasoline vapors may be irritating to eyes, the respiratory system and the nervous system (Hakkola et al., 1996; Moolenaar et al., 1994; White et al., 1995). There have been no long-term studies of human exposure to MtBE.

Rodent studies identify the kidneys, brain and developing fetus as sensitive to MtBE. The neurotoxicity data from inhalation exposures in rats (Daughtrey et al., 1997) showed transient CNS depression and decreased motor activity at high levels of MtBE (8,000 ppm). However, there are no data to support the hypothesis that MtBE dissolved in drinking water has adverse effects on the nervous system in humans.

The collective evaluation of the reproductive and developmental studies of MtBE in animals indicate that inhalation exposure can result in maternal toxicity and adverse effects on the developing fetus (Bushy Run Research Center, 1991, 1989a, 1989b; Conaway et al., 1985). The fetal toxicity in the mouse developmental studies indicate that it may be more sensitive to inhalation of MtBE vapors than the rat or rabbit during gestation. However, it is possible to conclude that, at low concentrations, MtBE does not cause a developmental or reproductive hazard by inhalation in three different animal species. This also suggests that humans may not be at risk when exposed to very low concentrations of MtBE.

Effects on the kidney were observed in rats after oral and inhalation exposure to MtBE. After short-term oral exposure, increases in kidney weights were noted (Robinson et al., 1990), while in a longer term inhalation study, histopathological abnormalities were apparent (Chun et al., 1992). The oral data from the short-term study are confounded by the bolus gavage dosing regime and the less-than-lifetime duration of the study, while the uncertainty in extrapolating between routes affects the interpretation of the inhalation data.

The use of inhalation data to project effects from the oral exposures is generally not desirable but, in the case of MtBE, there is qualitative similarity in the effects observed with both routes. However, when using the inhalation data to calculate a human equivalent dose for the risk assessment calculations, additional uncertainty is introduced by the mathematical conversion.

In animals, there are two chronic inhalation studies available, one in rats causing increased incidence of renal and testicular tumors (Chun et al., 1992) and one in mice inducing liver tumors (Burleigh-Flayer et al., 1992). By the oral route, there is one gavage study in rats producing a dose-related increase in leukemia and lymphoma in the females and an increase in testicular tumors in the males (Belpoggi et al., 1995). In addition, formaldehyde, a metabolite of MtBE, is an animal carcinogen. By inhalation exposure, it induces nasal tumors in rats (Kerns

et al., 1983). By the drinking water route of exposure, one study shows a dose-related increase in leukemia (Soffritti et al., 1989) and another study shows no evidence of carcinogenicity (Til et al., 1989). In addition, there is some suggestive evidence of carcinogenicity of TBA (another MtBE metabolite) -- an increased incidence of renal tumors in rats and an increase in thyroid tumors in the female mice after drinking water exposure.

Most of the cancer studies of MtBE and its metabolites have limitations, such as high mortality among the treated animals, limited reporting of pathology and of historical tumor incidence, etc. In spite of the limitations, there are some consistent tumor findings for MtBE and its metabolites. This consistency contributes to the overall weight of evidence. A statistically-significant increase in interstitial Leydig cell adenomas of the testes was detected in the exposed rats after both inhalation (Chun et al., 1992; Bird et al., 1997) and gavage exposures (Belpoggi et al., 1995). In addition, the elevation of kidney tumors in male F344 rats treated with TBA (a metabolite of MtBE), via drinking water (Cirvello et al., 1995; NTP, 1995) supports the increase of similar tumors in male rats after exposure to MtBE by inhalation (Chun et al., 1992; Bird et al., 1997). The similarity in the finding of a dose-related increase in leukemia of rats (Sprague-Dawley, male and female combined) after exposure to formaldehyde (also a metabolite of MtBE) via drinking water (Soffritti et al., 1989) and the increase of leukemia/lymphomas in female rats (same strain) after exposure to MtBE via gavage (Belpoggi et al., 1995), suggests a possible involvement of formaldehyde in the leukemogenic effect of MtBE. However, issues remain unresolved related to these studies, which were conducted by the same laboratory. Both studies provided limited reporting and no information on historical incidence of leukemia<sup>5</sup>.

MtBE does not appear to be DNA reactive. The chemical has been tested in an array of both *in vitro* and *in vivo* systems, and the results have been negative overall. The possibility that the genesis of the rat kidney tumors involves the  $\alpha$ -2u-globulin mechanism is being investigated, but, as yet, the evidence does not show that the mechanism accounts for the tumors satisfying all the EPA criteria (U.S. EPA, 1991). The observation of nephropathy and toxicity in association with tumorigenicity in the rat kidney suggests that a number of factors, possibly

---

<sup>5</sup>Unlike NTP carcinogenicity studies, the histopathology diagnoses from the inhalation studies of MtBE in rats and mice have not been subject to a full peer-review. Also, there is a major difference between the oral and inhalation carcinogenicity studies of MtBE. Lengthy reports of the inhalation studies of MtBE in rats and mice were submitted to EPA. These reports (Burleigh-Flayer et al., 1990; Chun et al., 1992) provide significantly more information than what is contained in the published peer-reviewed literature (Belpoggi et al., 1995; Bird et al., 1997). Based on these reports, we can conclude that the inhalation studies were conducted in conformance with Good Laboratory Practices, while there is a lack of evidence to back up that the gavage study is also conducted in conformance with Good Laboratory Practices .

including the  $\alpha$ -2u-globulin mechanism, may be at work. The observation of testicular tumors from MtBE and thyroid tumors from TBA suggest the need for examination of disruption of pituitary and thyroid hormone function, as such disruption is not uncommon with these tumors (Hill et al., 1989; USEPA, 1997). It has been suggested that MtBE-induced mouse liver tumors also may be hormone-related (Moser et al., 1996; Bird et al, 1997).

Although MtBE is not mutagenic, a nonlinear mode of action has not been established for MtBE. In the absence of sufficient mode of action information at the present time, it is prudent for EPA to assume a linear dose-response for MtBE. Although there are no studies on the carcinogenicity of MtBE in humans, there are multiple animal studies (by inhalation and gavage routes in two rodent species) showing carcinogenic activity and there is supporting animal carcinogenicity data for the metabolites. The weight of evidence indicates that MTBE is an animal carcinogen, and the chemical poses a carcinogenic potential to humans (NSTC, 1997, page 4-26).

## **7.2 Characterization of Organoleptic Effects**

There have been several studies of taste and odor response by humans. There is typically variation among individuals in these responses to a chemical, and this is the case for MtBE. The studies on MtBE have been of a few individuals each. Larger numbers of individuals might show the full distribution of sensitivity of humans which remains uncharacterized. Nevertheless, the existing studies were performed independently and show distributions that are consistent with one another. This lends confidence to the conclusion that sensitive individuals respond to odor and taste at about 20 to 40  $\mu\text{g/L}$ .

Other influences on consumer perception and acceptance of the taste and odor of MtBE contamination of water are as yet uncharacterized. These include development of tolerance, exposure through food and beverage preparation or showering, and reaction to published reports of contamination. Moreover, the presence or absence of other natural or water treatment chemicals can mask or reveal the taste or odor effects. Thus, variable preexisting water conditions around the country will increase variability in the acceptability of MtBE's presence in drinking water.

## **7.3 Dose Response Characterization**

There are no studies of long-term human exposure to MtBE; the pertinent data on potential adverse effects are from rodent studies. The available data do not provide sufficient information on the potential toxic effects from drinking water exposure

and support only an uncertain view of the quantitative dose and response relationship. For quantitative assessment of adverse health effects from drinking water exposure, the preferred data would be from studies of effects of episodic oral exposure through water or food. For MtBE, the data are either from inhalation studies or from daily, high dose (bolus), gavage studies, using vegetable oil as a vehicle. Estimating drinking water dose equivalents based on inhalation studies or on bolus dosing studies introduces significant uncertainties.

The results of the Robinson et al. (1990) study, supported by the inhalation exposure data of Chun et al. (1992) provide adequate support for the conclusion that MtBE may exert adverse effects on the kidney. However, EPA does not have high confidence in the use of the Robinson et al. (1990) study, nor any other study presently available for quantitation of the potential noncancer or cancer effects of MtBE. Because of the lack of confidence in quantitative estimation of drinking water risk, this Advisory does not recommend either a low-dose oral cancer risk number or a low end RfD. Instead, the Advisory provides perspective by showing the margins of exposure between observations of the range of animal effects and water concentrations. Table 1 summarizes this margin of exposure information. A final health advisory will be written when the data base is improved sufficiently to allow greater confidence in the integration of data. Since the production of potable water is a prerequisite for its use, it is evident that the organoleptic (taste and odor) effects of MtBE should be considered. The available data (Prah et al. 1994; Young et al., 1996; Dale et al., 1997; NSTC, 1997) suggest that the lower range for the organoleptic effects of MtBE is 20 to 40 µg/L.

The values in Table 1 show the lower end of ranges of observation of effects in animals tested for cancer and noncancer responses. Table 1 also shows the MoEs (i.e., the ratios of the observed numbers to the sensitive range of human response to odor and taste (20 to 40 µg/L). The cancer LED<sub>10</sub> are based on analyses of the Belpoggi et al. (1995), Chun et al. (1992), and Burleigh-Flayer et al. (1992) studies as described in section 5.2.2.1 above. The noncancer NOAEL values are based on analyses recounted in section 5.2.1.: kidney effects in a subchronic gavage study on rats, reproductive/developmental effects from inhalation studies in rodents, neurotoxicity for frank, reversible effects in rats observed after short-term inhalation exposures. The ranges given for taste and odor represent the low ends of the reported values for organoleptic responses to MtBE in water discussed in section 6.0. These available data provide an estimate that the lower range for the organoleptic effects of MtBE is about 15 to 39 µg/L (taste and odor) from an empirical observation.

Values are rounded to one significant number, 20 and 40 (odor and taste), to avoid the appearance of precision that use of two significant numbers would give. Since

characterization of the full distribution of sensitivity is not provided by available data, the numbers should be regarded as approximate, not precise. For the same reason, a range is presented. The data are used only to estimate sensitive range and should not be mistaken as defining thresholds of human response. In practice, the efforts of water suppliers to satisfy consumers on the acceptability of taste and odor of water, also will be influenced by considering the effects of other chemical in local waters.

#### 7.4 Comparison of Margins of Exposure with Potential Environmental Concentrations and Guidance on Taste and Odor

Table 1 permits comparison of an observed environmental concentration with the observed effects levels for test animals to calculate a margin of exposure by dividing the environmental concentration into the value at the low end of the range for an effect displayed.

If the objective is to avoid unpleasant taste and odor, this Advisory recommends that a concentration in the range of 20 to 40  $\mu\text{g/L}$  likely will protect sensitive members of a population.

At 20  $\mu\text{g/L}$ , the margin of exposure is approximately forty thousand (40,000) for cancer effects and over one hundred thousand (100,000) for some noncancer effects. At 40  $\mu\text{g/L}$ , the MoE is approximately twenty thousand (20,000) for cancer effects and sixty thousand (60,000) for some noncancer effects. In the case of noncancer critical effects, the lower end of the developmental NOAEL-range was used as the minimum effect level in the MoE calculation; the cancer value was calculated using the LED<sub>10</sub> (95% lower bound of the dose for a 10% extra risk)<sup>6</sup>.

---

<sup>6</sup>Based on the USEPA's recently proposed guidelines for carcinogen risk assessment (U.S. EPA, 1996), the rationale supporting the use of the LED<sub>10</sub> is that a 10% response is at or just below the limit of sensitivity for discerning a significant difference in most long-term rodent studies. The NOAEL in most study protocols is about the same as an LED<sub>5</sub> or LED<sub>10</sub> -- the lower 95% confidence limit on a dose associated with a 5% or 10% increased effect. The MoE value for cancer was obtained by dividing the concentration equivalent to the LED<sub>10</sub> (23 mg/kg/day equivalent to 805,000  $\mu\text{g/L}$ ) by 20  $\mu\text{g/L}$  to obtain a MoE of 40,200. The MoE for noncancer effects was obtained by dividing the concentration equivalent to the lower end of the NOAEL for the developmental toxicity range (65.6 mg/kg/day equivalent to 2,292,500  $\mu\text{g/L}$ ) by the environmental water concentration of 20  $\mu\text{g/L}$  to obtain an MoE of 114,625. The calculations assume a 70 kg body weight and 2 L/day water consumption.

December 1997

Comparison indicates that there are over four to five orders of magnitude between the 20 to 40  $\mu\text{g/L}$  range and concentrations associated with observed ranges of effects in animals. There is little likelihood that an MtBE concentration of 20 to 40  $\mu\text{g/L}$  in drinking water would cause adverse effects in humans, recognizing that some people may detect the chemical below this range. It can be noted that at this range of concentrations, the margins of exposure are about 10 to 100 times greater than would be provided by an EPA reference dose (RfD) for noncancer effects. Additionally, they are in the range of margins of exposure typically provided by National Primary Drinking Water Standards under the Federal Safe Drinking Water Act to protect people from potential carcinogenic effects.

<i>Table 1. Estimation of Margin of Exposure for MtBE on Water Concentration, 20-40 µg/l<sup>1</sup></i>				
<i>Endpoint</i>	<i>Parameter</i>	<i>Concentration<sup>4</sup></i> µg/L	<i>MoE compared to 40 µg/L</i>	<i>MoE compared to 20 µg/L</i>
<b>Noncancer</b>	<i>NOAEL</i>			
<i>Kidney</i>		3,500,000	90,000	180,000
<i>Neurological</i>		7,400,000	185,000	370,000
<i>Reproductive/Developmental</i>		2,300,000 - 9,200,000	≥60,000	≥120,000
<b>Cancer<sup>2</sup></b>	<i>LED<sub>10</sub><sup>3</sup></i>			
<i>Rat Lymphoma and Leukemia (gavage) in females</i>		805,000	20,000	40,000
<i>Rat Kidney Tumor (inhalation) in males</i>		6,230,000	160,000	320,000
<i>Mouse Liver Tumor (inhalation) in females</i>		11,025,000	280,000	550,000

<sup>1</sup> The margins of exposure is calculated by dividing the NOAELs for noncancer endpoints or LED<sub>10</sub> for cancer effects by 40 µg/L or 20 µg/L which is the low end of the taste and odor threshold, respectively.

<sup>2</sup> The data from Belpoggi gavage study and the Chun and Burleigh-Flayer inhalation studies were used in the calculation. Air concentration of MtBE in ppm was converted to mg/kg-day by the NSTC method: 1 ppm = 1.05 mg/kg-day (NSTC, 1996, See also 4.2).

<sup>3</sup> The LED<sub>10</sub> is defined as the 95% lower bound on dose for a 10% extra risk which was calculated by applying the tumor incidence data to the multistage model. As indicated by the NSTC (1996), a lifetime adjustment factor of 2.37 [i.e., (24/18)<sup>3</sup>] was applied to the mouse liver tumor data to account for the short duration of the study (18 months instead of 24 months). In addition, as done by NTIS, the rat kidney tumor incidence in the highest exposure group was excluded from the risk analysis because this exposure group was terminated at 82 weeks (not 102 weeks) due to extremely high mortality.

<sup>4</sup> The NOAEL and LED<sub>10</sub> were initially calculated in mg/kg-day and then converted to µg/L, assuming a body weight of 70 kg and a water consumption rate of 2 liters per day.

## 8.0 REFERENCES

- Allen, M.J., Borody, T.J., Bugliosi, T.F., May, GR., LaRusso, N.F., and J.L. Thistle. 1985. Cholelitholysis using methyl tertiary-butyl ether. *Gastroenterology* 88:122-125.
- API. 1993. American Petroleum Institute. Odor threshold studies performed with gasoline and gasoline combined with MtBE, EtBE and TAME. Washington, DC: API # 4592
- ARCO. 1980. ARCO Chemical Company. Methyl tertiary-butyl ether: acute toxicological studies. Unpublished study for ARCO Research and Development, Glenolden, PA.
- ARCO. 1995. ARCO Chemical Company. Methyl t-Butyl Ether (MtBE): A status report of its presence and significance in US drinking water. Presented to the Office of Water, U.S. Environmental Protection Agency. Presented by ARCO Chemical Company. June 8, 1995.
- ATSDR. 1996. U.S. Department of Health and Human Services. Toxicological profile for methyl *tert*-butyl ether.
- Belpoggi, F., Soffritt M., and C. Maltoni. 1995. Methyl-tertiary-butyl ether (MtBE) — a gasoline additive — causes testicular and lymphohaematopoietic cancers in rats. *Toxicol. Ind. Health* 11:1-31.
- Bevan, C., Tyl, R.W., Neeper-Bradley, T.L., Fischer, L.C., Panson, R.D., Kneiss, J.J., and L.S. Andrews. 1997a. Developmental toxicity evaluation of methyl tertiary-butyl ether (MTBE) by inhalation in mice and rabbits. *J. Appl. Toxicol.* 17(S1):S21-S30.
- Bevan, C., Neeper-Bradley, T.L., Tyl, R.W., Fischer, L.C., Panson, R.D., Kneiss, J.J., and L.S. Andrews. 1997b. Two-generation reproductive study of methyl tertiary-butyl ether (MTBE) in rats. *J. Appl. Toxicol.* 17(S1):S13-S20.
- Biles, R.W., Schroeder, R.E., and C.E. Holdsworth. 1987. Methyl tert-butyl ether inhalation in rats: a single generation reproduction study. *Toxicol. Ind. Health.* 34:519-534.
- Bio/dynamics, Inc. 1984. The metabolic fate of methyl tertiary-butyl ether (MtBE) following acute intraperitoneal injection. Project No. 80089. Unpublished report submitted to American Petroleum Institute, Washington, DC. 150 pp.

December 1997

Bio-Res. Lab. 1990a. Bio-Research Laboratories. Pharmacokinetics of methyl tertiary-butyl ether (MtBE) and tert-butyl alcohol (TBA) in male and female Fischer-344 rats after administration of  $^{14}\text{C}$ -MtBE by iv, oral, and dermal routes. Report #38842. Senneville, Quebec, Canada: Bio-Research Laboratories.

Bio-Res. Lab. 1990b. Bio-Research Laboratories. Mass balance of radioactivity and metabolism of methyl tert-butyl ether (MtBE) in male and female Fischer-344 rats after administration of  $^{14}\text{C}$  MtBE by iv, oral, and dermal routes. Report #38843. Senneville, Quebec, Canada: Bio-Research Laboratories.

Bio-Res. Lab. 1990c. Bio-Research Laboratories. Pharmacokinetics of methyl tert-butyl ether (MtBE) and tert-butyl alcohol (TBA) in male and female Fischer-344 rats after single and repeat inhalation nose-only exposure to  $^{14}\text{C}$ -MtBE. Report #38844. Senneville, Quebec, Canada: Bio-Research Laboratories.

Bio-Res. Lab. 1990d. Bio-Research Laboratories. Disposition of radioactivity of methyl tertiary-butyl ether (MtBE) in male and female Fischer-344 rats after nose-only inhalation exposure to  $^{14}\text{C}$ -MtBE. Report #38845. Senneville, Quebec, Canada: Bio-Research Laboratories.

Bird, M.G., Burleigh-Flayer, H.D., Chun, J.S., Douglas, J.F., Kneiss, J.J. 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:(S 1): S45-S56.

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

Brady, J.F., Xiao, F., Ning, W.J., and C.S. Yang. 1990. Metabolism of methyl tertiary-butyl ether by rat hepatic microsomes. *Arch. Toxicol.* 64:157-160.

Burleigh-Flayer, H.D., Chun, J.S., and W.J. Kintigh. 1992. Methyl tertiary butyl ether: vapor inhalation oncogenicity study in CD-1 mice. Report 91N0013A. Export, PA: Bushy Run Research Center.

Bushy Run Research Center. 1994. Methyl tertiary-butyl ether: *in vivo- in vitro* hepatocyte unscheduled DNA synthesis assay in mice. Project ID 93N1316. Export, PA.

Bushy Run Research Center. 1993. Methyl tertiary-butyl ether: bone marrow micronucleus test in mice. Project ID 93N1244. Export, PA.

December 1997

Bushy Run Research Center. 1991. Two-generation reproduction study of inhaled methyl tert-butyl ether in CD Sprague-Dawley rats. Final Report, August 13. Project ID 53-594. Export, PA.

Bushy Run Research Center. 1989a. Developmental toxicity study of inhaled methyl tertiary butyl ether in CD-1 mice. Final Report, July 20. TSCATS/403186. EPS/OTS No. FYI-OTS-0889-0689. Export, PA.

Bushy Run Research Center. 1989b. Developmental toxicity study of inhaled methyl tertiary butyl ether in New Zealand white rabbits. Final Report, May 12. EPA/OTS No. FYI-OTS-0889-0689. Export, PA.

Bushy Run Research Center. 1989c. Methyl tertiary butyl ether repeated exposure vapor inhalation study in rats: in vivo cytogenetic evaluation. Project Report 51-635. Export, PA.

Cain, W.S., Leaderer, B.P., Ginsberg, G.L., Andrews, L.S., Cometto-Muniz, J.E., Gent, J.F., Buck, M., Berglund, L.G., Mohsenin, V., Monhan, E., and S. Kjaergaard. 1994. Human reactions to brief exposures to methyl tertiary-butyl ether. (Unpublished data from John B. Pierce Laboratory, New Haven, Connecticut).

Cederbaum, A.I. and G. Cohen. 1980. Oxidative demethylation of t-butyl alcohol by rat liver microsomes. *Biochem. Biophys. Res. Comm.* 97:730-736.

Chun J.S., Burleigh Flayer, H.D., and W.J. Kintigh. 1992. Methyl tertiary ether: vapor inhalation oncogenicity study in Fisher 344 rats. Export, PA; Bushy Run Research Center; report 91N0013B.

Cirvello, J.D., Radovsky, J.E. Heath, D.R. Farnell and C. Lindamood, III. 1995. Toxicity and carcinogenicity of t-butyl alcohol in rats and mice following chronic exposure in drinking water. *Toxicol. and Ind. Health.* 11:151-165.

Conaway, C.C., Schroeder, R.E., and N.K. Snyder. 1985. Teratology evaluation of methyl tertiary-butyl ether in rats and mice. *J. Toxicol. Environ. Health.* 166:797-809.

Dale, M.S., Moylan, M.S., Koch, B., and Davis, M.K. 1997. MTBE: Taste and odor threshold determinations using the flavor profile method. Presented at the Water Quality Technology Conference, November 9-13, 1997. Denver, CO.

Daughtrey, W.C., Gill, M.W., Pritts, I.M., Fielding Douglas, J., Kneiss, J.J., and L.S. Andrews. 1997. Neurotoxicological evaluation of methyl tertiary-butyl ether in rats. *J. of Appl. Toxicol.* 17 (S1):S57-S64.

Hakkola, M., Honkasalo, M.L., and P. Pulkkinen. 1996. Neuropsychological symptoms among tanker drivers exposed to gasoline. *Occup. Med.* 46:125-130.

Hazelton. 1989. Hazelton Laboratories America, Inc. Mutagenicity test on methyl tertiary-butyl ether. *Drosophila melanogaster* sex-linked recessive lethal test. Study No. 1484-0-461. Kensington, MD.

Health Effect Institute report (HEI), 1996. The potential health effects of oxygenates added to gasoline. A review of the current literature. A special report of the Institute's oxygenates evaluation committee. Health Effects Institute, Cambridge, MA. In *Interagency Oxygenated Fuel Assessment*. 1996. Office of Science and Technology (OSTP) through the committee on Environment and Natural Science and Technology Council (NSTC).

Hill, R.N., Erdreich, L.S., Paynter, O.E., Roberts, P.A., Rosenthal, S.L., and Wilkinson, C.F. 1989. Thyroid follicular cell carcinogenesis. *Fundam. Appl. Toxicol.* 12:629-697.

Industrial Bio-Test Laboratories Inc. 1972a. Absorption, distribution, and excretion study with 2,2-MMOP in albino rats. (Unpublished data from Industrial Bio-Test Laboratories Inc., Northbrook, Illinois, to Sun Oil Company). IBT No. E200 (A).

Industrial Bio-Test Laboratories. 1972b. Absorption, distribution, and excretion study with 2,2-MMOP in monkeys. (Unpublished data from Industrial Bio-Test Laboratories Inc., Northbrook, Illinois, to Sun Oil Company). IBT No. E200 (B).

IARC. 1995. International Agency for Research on Cancer. Formaldehyde. In: *IARC monographs on the evaluation of carcinogenic risks to humans: wood dust and formaldehyde*. Lyon, France: IARC. 62:217-362.

Johanson, G., Nihlen, A., and A. Lof. 1995. Toxicokinetics and acute effects of MtBE and EtBE in male volunteers. *Toxicol. Lett.* 82/83:713-718.

Juliani, G., Gandini, G., Gabasio, S., Bonardi, L., Fascetti, E., and L. Gremon. 1985. Colelitolisi chimica transcutanea con metil-ter-butyl etere (MtBE). *La Radiol. Med.* 71:569-574.

Kerns K.D., Pavkov K.L., Donofrio D.J., Gralla E.J., and J.A.Swenberg. 1983. Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure. *Cancer Res.* 43:4382-4392.

Life Science Research, Roma Toxicology Centre S.P.A. 1989a. Reverse mutation in *Salmonella typhimurium*, test substance: MtBE. Report No. 216001-M-03489. Rome, Italy.

Life Science Research, Roma Toxicology Centre S.P.A. 1989b. Gene mutation in Chinese hamster V79 cells, test substance: MtBE. Report No. 216002-M-03589. Rome, Italy.

Life Science Research, Roma Toxicology Centre S.P.A. 1989c. Unscheduled DNA synthesis (UDS) in primary rat hepatocytes (autoradiographic method), test substance: MtBE. Report No. 216003-M-03689. Rome, Italy.

McKee, R.H., Vergnes, J.S., Galvin, J.B., Douglas, J.F., Kneiss, J.J., and L.S. Andrews. 1997. Assessment of the *in vivo* mutagenic potential of methyl tertiary-butyl ether. *J. Appl. Toxicol.* 17 (S1):S31-S36.

Miller, M.J., Ferdinandi, E.S., Klan, M., Andrews, L.S., Douglas, J.F., and J.J. Kneiss. 1997. Pharmacokinetics and disposition of methyl t-butyl ether in Fischer-344 rats. *J. Appl. Toxicol.* 17(S1):S3-S13.

Moolenaar, R.L., Hefflin, B.J., Ashley, D.L., Middaugh, J.P., and R.A. Etzel. 1994. Methyl tertiary butyl ether in human blood after exposure to oxygenated fuel in Fairbanks, Alaska. *Arch. Environ. Health* 49(5):402-409. (also CDC 1993a. Centers for Disease Control and Prevention. An investigation of exposure to methyl tertiary-butyl ether in Fairbanks, Alaska. Atlanta, GA: U.S. Department of Health and Human Services, National Center for Environmental Health. October 22, 1993.)

Moser, G.J., Wong, B.A., Wolf, D.C., Moss, O.R., and T.L. Goldsworthy. 1996. Comparative short-term effects of methyl tertiary-butyl ether and unleaded gasoline vapor in female B6C3F1 mice. *Fundam. Appl. Toxicol.* 31:173-183.

NRC. 1996. National Research Council. Toxicological and performance aspects of oxygenated motor vehicle fuels. Washington, DC: National Academy Press.

NSTC. 1996. National Science and Technology Council. Interagency assessment of potential health risks associated with oxygenated gasoline. National Science and

Technology Council Committee on Environment and Natural Resources and Interagency Oxygenated Fuels Assessment Steering Committee.

NSTC. 1997. National Science and Technology Council Committee on Environment and Natural Resources. Interagency Assessment of Oxygenated Fuels.

NTP. 1995. National Toxicology Program. Toxicology and carcinogenesis studies of t-butyl alcohol (CAS No. 76-65-0) in F344/N rats and B6C3F1 mice (drinking water studies). Research Triangle Park, NC: National Institute of Health; Technical Report Series No. 436, NIH Publication No. 94-3167.

Prah, J.D., Goldstein, G.M., Devlin, R., Otto, D., Ashley, D., House, S., Cohen, K.L., and T. Gerrity. 1994. Sensory, symptomatic, inflammatory, and ocular responses to and the metabolism of methyl tertiary-butyl ether in a controlled human exposure experiment. *Inhal. Toxicol.* 6:521-538.

Prescott-Mathews, J.S., Wolf, D.C., Wong, B.A., and S.J. Borghoff. 1997. Methyl tert-Butyl Ether Causes  $\alpha$ 2u-globulin Nephropathy and Enhanced Renal Cell Proliferation in Male F344 Rats. *Toxicol. Appl. Pharm.* 143:301-314.

Rao, H.V. and G.L. Ginsberg. A Physiologically-Based Pharmacokinetic Model Assessment of Methyl t-Butyl Ether in Groundwater for a Bathing and Showering Determination. *Risk Anal.* (In press)

Robinson, M., Bruner, R.H., 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.* 9:525-540.

Savolainen, H., Pfaffli, P., and E. Elovaara. 1985. Biochemical effects of methyl tertiary-butyl ether in extended vapor exposure in rats. *Arch. Toxicol.* 57:285-288.

Sellakumar A.R., Snyder C.A., Solomon J.J., and R.E. Albert. 1985. Carcinogenicity of formaldehyde and hydrogen chloride in rats. *Toxicol. Appl. Pharmacol.* 81:401-406.

Soffritti M., Maltoni C., Maffei, F., and R. Biagi. 1989. Formaldehyde: an experimental multipotential carcinogen. *Toxicol. Ind. Health.* 5:699-730.

Stoneybrook Laboratories Inc. 1993. Activated mouse lymphoma (L5178Y/TK/+) mutagenicity assay supplemented with formaldehyde dehydrogenase for methyl tertiary butyl ether. Status Report 65579. Princeton, NJ.

Til, H.P., Woutersen R.A., Feron, V.J., Hollanders, V.M.H., and H.E. Falke. 1989. Two-year drinking-water study for formaldehyde in rats. *Food Chem. Toxicol.* 27:77-87.

U.S. EPA. 1987. Reference Dose (RfD): Description and use in health risk assessments. Integrated risk information system (IRIS): Appendix A. United States Environmental Protection Agency. Washington, D.C. Integrated risk information system documentation, vol. 1. EPA/600/8-66/032a.

U.S. EPA. 1991. Alpha 2 $\mu$ -globulin association with chemically induced renal toxicity and neoplasia in the male rat. Risk Assessment Forum. United States Environmental Protection Agency. Washington, D.C. EPA/625/3-91/019F.

U.S. EPA. 1993. Assessment of potential health risks of gasoline oxygenated with methyl tertiary-butyl ether (MtBE). Office of Research and Development. United States Environmental Protection Agency. EPA/600/R.93/206.

U.S. EPA. 1996. Proposed guidelines for carcinogen risk assessment. United States Environmental Protection Agency. Federal Register 61(79):17960-18011.

U.S. EPA. 1997. Assessment of thyroid follicular cell tumors. Risk Assessment Forum. United States Environmental Protection Agency. Washington, D.C. EPA/630/R-97/002.

USGS. 1996. Occurrence of the gasoline additive MtBE in shallow ground water in urban and agricultural areas. United States Geological Survey Fact Sheet 114.95. October.

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

Weil, C.S. 1970. Significance of organ-weight changes in food safety evaluation. In: Roe, F.J., ed., *Metabolic Aspects of Food Safety*. New York, NY, Academic Press, pp. 419-454.

White, M.C., Johnson, C.A., Ashley, D.L., Buchta, T.M. and D.J. Pelletier. 1995. Exposure to methyl tertiary-butyl ether from oxygenated gasoline in Stamford,

December 1997

Connecticut. Arch. Environ. Health 50(3):183-189. (also CDC 1993b. Centers for Disease Control and Prevention. An investigation of exposure to methyl tertiary-butyl ether among motorists and exposed workers in Stamford, Connecticut. Atlanta, GA: U.S. Department of Health and Human Services, National Center for Environmental Health. September 14, 1993.)

Williams, R.T. 1959. Detoxication Mechanisms, 2nd Ed., p. 67, John Wiley and Sons, Inc., New York. (As cited in Cederrbaum, A.I, G. Cohen. 1980. Oxidative demethylation of t-butyl alcohol by rat liver microsomes. Biochem. Biophys. Res. Comm. 97: 730-736.)

Woutersen R.A., van Garderen-Hoetmer A., Bruijntjes J.P., Swart A., and V.J. Feron. 1989. Nasal tumors in rats after severe injury to the nasal mucosa and prolonged exposure to 10 ppm formaldehyde. J. Appl. Toxicol. 9: 39-46.

Wyngaarden, J.B. 1986. New nonsurgical treatment removes gallstones. JAMA. 256:1692.

Young, W.F., Horth, H., Crane, R., Ogden, T., and M. Arnott. 1996. Taste and odor threshold concentrations of potable water contaminants. Water Res. 30:331-340.