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REVISED AND UPDATED DRINKING WATER QUANTIFICATION OF TOXICOLOGIC EFFECTS FOR METHYL TERT-BUTYL ETHER (MTBE)

Prepared for

Office of Water

Prepared by

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DISCLAIMER

This report is an external draft for review purposes only and does not constitute Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

PREFACE

Section 1412 (b)(3)(A) of the Safe Drinking Water Act, as amended in 1986, requires the Administrator of the U.S. Environmental Protection Agency to publish maximum contaminant level goals (MCLGs) and promulgate National Primary Drinking Water Regulations for each contaminant, which, in the judgment of the Administrator, may have an adverse effect on public health and that is known or anticipated to occur in public water systems. The MCLG is nonenforceable and is set at a level at which no known or anticipated adverse health effects in humans occur and that allows for an adequate margin of safety. Factors considered in setting the MCLG include health effects data and sources of exposure other than drinking water.

This document provides the health effects basis to be considered in establishing the MCLG. To achieve this objective, data on toxicokinetics and acute, subchronic and chronic toxicity to animals and humans are evaluated. Specific emphasis is placed on data published in peer-reviewed literature providing dose-response information. Thus, while the literature search and evaluation performed in the development of this document have been comprehensive, only the reports considered most pertinent in the derivation of the MCLG are cited in the document. The comprehensive literature data base in support of this document includes information published up to 1992; however, more recent data may have been added during the review process.

When adequate health effects data exist, Health Advisory (HA) values for less-than-lifetime exposures (1-day, 10-day and longer-term, i.e., ~10% of an individual's lifetime) are included in this document. These values are not used in setting the MCLG, but serve as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur. With adequate data, a Reference Dose (RfD) is derived to be utilized in the derivation of a Drinking Water Equivalent Level (DWEL) on which the MCLG is based. Also provided is the U.S. EPA's determination of the contaminant's carcinogenic potential. When the contaminant has been determined to be a probable or possible human carcinogen, the estimated excess cancer risk associated with ingestion of contaminated water is included.

This document was prepared for the Office of Water by the Office of Health and Environmental Assessment (Environmental Criteria and Assessment Office, Cincinnati, Ohio) to provide the scientific support for the human health-based risk assessment used in the determination of the drinking water MCLG. For more information, contact the Human Risk Assessment Branch of the Office of Water at (202)260-7571.

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LIST OF ABBREVIATIONS

AST	Aspartate aminotransferase
AUC	Area under curve
BUN	Blood urea nitrogen
CAS	Chemical Abstract Service
CNS	Central nervous system
DWEL	Drinking water equivalent level
FEL	Frank effect level
HA	Health advisory
HCT	Hemocrit
LDH	Lactate dehydrogenase
LOAEL	Lowest-observed-adverse-effect level
LOEL	Lowest-observed-effect level
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MTD	Maximum tolerated dose
NOAEL	No-observed-adverse-effect level
RBC	Red blood cell
RfD	Reference dose

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INTRODUCTION

BACKGROUND

The quantification of toxicologic effects of a chemical consists of separate assessments of noncarcinogenic and carcinogenic health effects. Chemicals that do not produce carcinogenic effects are believed to have a threshold dose below which no adverse, noncarcinogenic health effects occur, while carcinogens are assumed to act without a threshold.

In the quantification of noncarcinogenic effects, a Reference Dose (RfD), [formerly termed the Acceptable Daily Intake (ADI)] is calculated. The RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious health effects during a lifetime. The RfD is derived from a no-observed-adverse-effect level (NOAEL), or lowest-observed-adverse-effect level (LOAEL), identified from a subchronic or chronic study, and divided by an uncertainty factor(s) times a modifying factor. The RfD is calculated as follows:

$$= \quad RfD \quad \frac{(NOAEL \text{ or } LOAEL)}{[Uncertainty\ Factor(s) \times Modifying\ Factor]} = ___\ mg/kg\ bw/day$$

Selection of the uncertainty factor to be employed in the calculation of the RfD is based upon professional judgment, while considering the entire data base of toxicologic

effects for the chemical. In order to ensure that uncertainty factors are selected and applied in a consistent manner, the U.S. EPA (1993) employs a modification to the guidelines proposed by the NAS (1977, 1980) as follows:

Standard Uncertainty Factors (UFs)

- Use a 10-fold factor when extrapolating from valid experimental results from studies using prolonged exposure to average healthy humans. This factor is intended to account for the variation in sensitivity among the members of the human population. [10H]

- Use an additional 10-fold factor when extrapolating from valid results of long-term studies on experimental animals when results of studies of human exposure are not available or are inadequate. This factor is intended to account for the uncertainty in extrapolating animal data to the case of humans. [10A]

- Use an additional 10-fold factor when extrapolating from less than chronic results on experimental animals when there are no useful long-term human data. This factor is intended to account for the uncertainty in extrapolating from less than chronic NOAELs to chronic NOAELs. [10S]

- Use an additional 10-fold factor when deriving an RfD from a LOAEL instead of a NOAEL. This factor is intended to account for the uncertainty in extrapolating from LOAELs to NOAELs. [10L]

Modifying Factor (MF)

- Use professional judgment to determine another uncertainty factor (MF) that is greater than zero and less than or equal to 10. The magnitude of the MF depends upon the professional assessment of scientific uncertainties of the study and data base not explicitly treated above, e.g., the completeness of the overall data base and the number of species tested. The default value for the MF is 1.

The uncertainty factor used for a specific risk assessment is based principally upon scientific judgment rather than scientific fact and accounts for possible intra- and interspecies differences. Additional considerations not incorporated in the NAS/ODW guidelines for selection of an uncertainty factor include the use of a less-than-lifetime study for deriving an RfD, the significance of the adverse health effects and the counter-balancing of beneficial effects.

From the RfD, a Drinking Water Equivalent Level (DWEL) can be calculated. The DWEL represents a medium specific (i.e., drinking water) lifetime exposure at which adverse, noncarcinogenic health effects are not anticipated to occur. The DWEL assumes 100% exposure from drinking water. The DWEL provides the noncarcinogenic health effects basis for establishing a drinking water standard. For ingestion data, the DWEL is derived as follows:

$$DWEL = \frac{(RfD) \times (Body\ weight\ in\ kg)}{Drinking\ Water\ Volume\ in\ L/day} = ___\ mg/L$$

where:

Body weight = assumed to be 70 kg for an adult

Drinking water volume = assumed to be 2 L/day for an adult

In addition to the RfD and the DWEL, Health Advisories (HAs) for exposures of shorter duration (1-day, 10-day and longer-term) are determined. The HA values are used as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur. The HAs are calculated using an equation similar to the RfD and DWEL; however, the NOAELs or LOAELs are identified from acute or subchronic studies. The HAs are derived as follows:

$$HA = \frac{(NOAEL\ or\ LOAEL) \times (bw)}{(UF) \times (___\ L/day)} = ___\ mg/L$$

Using the above equation, the following drinking water HAs are developed for noncarcinogenic effects:

1. 1-day HA for a 10 kg child ingesting 1 L water per day.
2. 10-day HA for a 10 kg child ingesting 1 L water per day.
3. Longer-term HA for a 10 kg child ingesting 1 L water per day.
4. Longer-term HA for a 70 kg adult ingesting 2 L water per day.

The 1-day HA calculated for a 10 kg child assumes a single acute exposure to the chemical and is generally derived from a study of <7 days duration. The 10-day HA assumes a limited exposure period of 1-2 weeks and is generally derived from a study of <30 days duration. The longer-term HA is derived for both the 10 kg child and a 70 kg adult and assumes an exposure period of ~7 years (or 10% of an individual's lifetime).

The longer-term HA is generally derived from a study of subchronic duration (exposure for 10% of animal's lifetime).

The U.S. EPA categorizes the carcinogenic potential of a chemical, based on the overall weight-of-evidence, according to the following scheme:

Group A: Human Carcinogen. Sufficient evidence exists from epidemiology studies to support a causal association between exposure to the chemical and human cancer.

Group B: Probable Human Carcinogen. Sufficient evidence of carcinogenicity in animals with limited (Group B1) or inadequate (Group B2) evidence in humans.

Group C: Possible Human Carcinogen. Limited evidence of carcinogenicity in animals in the absence of human data.

Group D: Not Classified as to Human Carcinogenicity. Inadequate human and animal evidence of carcinogenicity or for which no data are available.

Group E: Evidence of Noncarcinogenicity for Humans. No evidence of carcinogenicity in at least two adequate animal tests in different species or in both adequate epidemiologic and animal studies.

If toxicologic evidence leads to the classification of the contaminant as a known, probable or possible human carcinogen, mathematical models are used to calculate the estimated excess cancer risk associated with the ingestion of the contaminant in drinking water. The data used in these estimates usually come from lifetime exposure studies using animals. In order to predict the risk for humans from animal data, the animal dose-response is converted, using experimentally derived or default assumptions, to equivalent

human values. The conversion includes correction for noncontinuous exposure, less-than-lifetime exposure and differences in pharmacologic effects. The default factor that compensates for the differences in pharmacologic effects is related to body surface area, which is proportional to body weight to the two-thirds power. It is assumed that the average adult human body weight is 70 kg and that the average water consumption of an adult human is 2 L water per day.

For contaminants with a carcinogenic potential, chemical levels are correlated with a carcinogenic risk estimate by employing a cancer unit risk (sometimes referred to as a cancer potency) value together with the assumption for lifetime exposure from ingestion of water. The cancer unit risk is usually derived from a linearized multistage model with a 95% upper confidence limit, thus providing a low dose upper bound estimate of risk; that is, the true risk to humans, while not identifiable, is not likely to exceed the upper bound estimate and, in fact, may be lower. Excess cancer risk estimates may also be calculated using other models such as the one-hit, Weibull, logit and probit. There is little basis in the current understanding of the biologic mechanisms involved in cancer to suggest that any one of these models is able to predict the true risk more accurately than any other. Because each model is based upon differing assumptions, the estimates derived for each model can differ by several orders of magnitude.

The scientific data base used to calculate and support the setting of cancer risk rate levels has an inherent uncertainty that is due to the systematic and random errors in

scientific measurement. In most cases, only studies using experimental animals have been performed. Thus, there is uncertainty when the data are extrapolated to humans. When developing a cancer risk characterization, several other areas of uncertainty exist, such as the usual incomplete knowledge about interrelated health effects of contaminants in drinking water, the impact on the observed response of the experimental animal's age, sex and species compared with humans, and the actual dose received by the internal organs in experimental animals or expected in humans. Dose-response data usually are available only for high levels of exposure and not for the lower levels of exposure closer to where a standard may be set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects.

PHYSICAL AND CHEMICAL PROPERTIES, USES AND OCCURRENCE

The saturated aliphatic ether methyl tertiary- butyl ether, commonly called MTBE, is known as 2-methoxy-2-methylpropane by the Chemical Abstracts Service (CAS). The CAS Registry number for MTBE is 1634-04-4. It has a molecular formula of $C_5H_{12}O$ and a molecular weight of 88.15. MTBE is a colorless liquid that freezes at -109°C and boils at 55.2°C (Weast, 1985). It has a density of 0.74 at 20°C (Weast, 1985) and a water solubility of 51,260 mg/L at 25°C (Yalkowsky, 1989). It is soluble in ethanol and ethyl ether (Weast, 1985). The vapor pressure of MTBE is 249.3 mm Hg at 25°C (Daubert and Danner, 1992). It is used as an octane booster for unleaded gasoline, in the manufacture

of isobutene and recently in the non-surgical treatment of gallbladders (Hawley, 1981; Bruckstein, 1990).

The annual mean concentration of MTBE in the atmosphere in the United States during 1987-1988 was <0.2 ppb (v/v) (LaGrone, 1991). Potable water from a few private wells from the state of Connecticut contained MTBE in the concentration range of 1-7750 µg/L (U.S. EPA, 1987). The concentration of MTBE in potable well water in Raynham, MA, ranged from not detected to 22.0 µg/L, with a mean value of 7.8 µg/L (U.S. EPA, 1987). Three percent of potable well water samples in the state of Maine contained MTBE in the concentration range of 20-236,000 µg/L (U.S. EPA, 1987). Potable water from two private wells in New Mexico contained 70 and 350 µg/L of MTBE (U.S. EPA, 1987). In New Hampshire, MTBE was detected at concentrations ranging from not detected to 100 µg/L in 145 potable well water samples, at concentrations ranging from 101-1000 µg/L in 20 samples and at concentrations ranging from 1001-10,000 µg/L in 11 samples (U.S. EPA, 1987).

TOXICOKINETICS

The toxicokinetics of MTBE (purity 99%) were evaluated in rats after oral administration by Bioresearch Laboratories (1990a). Groups of 10, 40 and 40 Fischer 344 rats (59-61 days old, fasted) of each sex were administered single doses of 0, 40 or 400 mg/kg, respectively, by gavage in saline vehicle. Plasma concentrations of MTBE and t-butanol (a major circulating metabolite) were determined in four males and four females in each treated group at various times up to 36 hours after dosing. Area under curve

in each treated group at various times up to 36 hours after dosing. Area under curve (AUC) comparisons showed that levels of MTBE and t-butanol were similar in both sexes, indicating no gender-related pharmacokinetic differences. Gastrointestinal absorption of MTBE was rapid as shown by maximum plasma concentration 15 minutes post-exposure (the first sampling time) at both low and high doses. t-Butanol was rapidly formed with peak plasma concentrations occurring 2 hours post-exposure. Relative to the increase in dose (from 40-400 mg/kg), the high dose of MTBE gave a greater than proportional increase in MTBE AUC and a less than proportional increase in t-butanol AUC, suggesting a saturation of the enzymes catalyzing the formation of t-butanol from MTBE. The plasma elimination half-life of MTBE, based on a one compartment model for the plasma concentration/time curve, was approximately 0.45-0.62 and 0.79-0.93 hours after the low and high doses, respectively. The plasma elimination half-life of t-butanol was approximately twice as long as that of MTBE.

In a related mass balance and metabolism experiment, groups of six male and six female Fischer 344 rats (fasted) were administered single 0, 40 or 400 mg/kg doses of ^{14}C-MTBE in saline vehicle by gavage (Bioresearch Laboratories, 1990b). Urine, feces and expired air were collected at various times ≤48 hours post-treatment for determination of ^{14}C-MTBE, $^{14}CO_2$ and other ^{14}C-metabolites (e.g., t-butanol and acetone). There was no further collection of excreta and expired air because, based on the amount of radioactivity remaining in the tissues and carcasses at 48 hours (<2% of the dose), it was unlikely that overall recoveries would have significantly increased.

The total recovery of radioactivity after 48 hours was 85.2±2.9% and 86.3±5.5% of the 40 mg/kg dose in the males and females, respectively, and 81.8±6.3% and 80.2±10.7% of the 400 mg/kg dose in the males and females, respectively. The two major routes of excretion were the lungs and kidneys. At 40 mg/kg, the mean recovery of radioactivity was 36.2% (males) and 29.0% (females) in the urine and 45.8% (males) and 54.4% (females) in the expired air. At 400 mg/kg, a larger proportion of the dose was exhaled from the lungs [65.3% (males) and 68.7% (females)] than at the lower dose, possibly due to saturation of MTBE metabolizing enzymes. At the high dose, a lower proportion of the dose was eliminated by urinary excretion [16.0% (males) and 10.8% (females)]. Approximately 55.6% and 59.2% of the 400 mg/kg dose was exhaled by the males and females, respectively, within the first 3 hours after dosing. The clearance from the lungs was thought to be a function of the blood/air partition coefficient of MTBE. The rats in the 400 mg/kg dose group having the highest total radioactivity recoveries (88-92% of the dose) were those with the highest recoveries in the expired air. Therefore, it is likely that the relatively lower total recoveries at 400 mg/kg (compared to the low dose), and incomplete recoveries of radioactivity at both doses, was due to a rapid exhalation of MTBE between treatment and the start of expired air collection. The amount of radioactivity recovered in the feces of both sexes was ~1% of the low dose and 0.3% of the high dose. The amount of radioactivity combined in the tissues and carcass at 48 hours post-treatment ranged from 1.94-2.00% of the low dose and 0.67-1.02% of the high dose. Therefore, although total recoveries were incomplete, the low recoveries of

radioactivity in the feces, tissues and carcass and other data suggest that the absorption and elimination of MTBE were rapid and virtually complete.

The major biotransformation pathway appeared to be oxidative: demethylation to t-butanol, oxidation of t-butanol to 2-methyl-1,2-propanediol, and further oxidation to α-hydroxyisobutyric acid (Bioresearch Laboratories, 1990b). The radioactivity in urine was composed mainly of 2-methyl-1,2-propanediol and α-hydroxyisobutyric acid. Pilot studies indicated that glucuronide conjugation was not a significant pathway for any of these metabolites. Most of the radioactivity in the expired air was present as MTBE with a small amount (~1-3%) occurring as t-butanol.

The toxicokinetics of MTBE following inhalation exposure was studied by Savolainen et al. (1985). In this study, male Wistar rats were exposed to 0, 50, 100 or 300 ppm MTBE 6 hours/day, 5 days/week for 2, 6, 10 or 15 weeks. MTBE and t-butanol, a metabolite of MTBE, were detected in the blood, brain and perirenal fat of the animals at all time points, indicating that MTBE was absorbed from the lungs, distributed systemically and metabolized to t-butanol. The levels of MTBE and t-butanol in the blood were related to the levels of inhaled MTBE; the level of MTBE in the blood tended to decrease slightly (at 50 ppm) or remain fairly constant (at higher exposures) after 6 weeks of exposure, while the level of t-butanol increased considerably at all exposure levels after 6 weeks of exposure, and subsequently decreased. Levels of MTBE and t-butanol in the brain showed a similar pattern. Only MTBE was detected in the perirenal fat and the levels tended to decrease following 2 weeks of exposure. Exposure resulted in a transient but

significant dose-dependent increase in UDP-gluconosyltransferase in liver and kidney microsomes and a slight increase in renal, but not hepatic, cytochrome P-450 activity.

The toxicokinetics of MTBE following intraperitoneal administration were studied by Bio/dynamics, Inc. (1984). Charles River CD rats were injected intraperitoneally with ^{14}C-MTBE (60 µCi/rat, average dose of 232 mg MTBE/kg bw) and the animals were sacrificed at 5, 15, 30 or 45 minutes, or 1, 2, 3, 6, 12, 24 or 48 hours after treatment. Samples of blood, tissues, urine, feces and expired air were taken at various intervals of exposure, and the radioactive content of the samples was determined. In selected samples, the quantitation of ^{14}C-MTBE and ^{14}C-metabolites was also determined, along with a total ^{14}C-content. Peak blood levels occurred 5 minutes after injection, and plasma levels peaked 5 and 15 minutes after injection for male and female rats, respectively. The half-lives of MTBE in the blood were 59.8 minutes (males) and 49 minutes (females) and in the plasma were 2.3 hours (males) and 1.3 hours (females). Blood and plasma levels of ^{14}C-MTBE indicated rapid absorption and elimination. Forty-eight hours after administration, an average of 103.83% of the injected radioactive dose was recovered, 99.86% of ^{14}C in expired air (91.75% as ^{14}C-MTBE, 7.45% as $^{14}CO_2$ and the remainder not quantitated), 2.95% in urine and 1.02% in the feces (3.08% as formic acid and the remainder not quantitated). Evidence of very low levels of methanol and formic acid were found in the plasma, liver and kidneys at 15 minutes, 6 hours and 24 hours after treatment. Radioactivity was found in the urine, feces, blood, liver, kidney and expired

air, indicating that absorption from the peritoneum, distribution to the tissues of the body and excretion from the body had occurred.

NONCARCINOGENIC EFFECTS IN HUMANS

MTBE has been used in humans as a cholelitholytic agent (to dissolve gallstones) (Allen et al., 1985a,b; Gonzaga et al., 1985; Juliani et al., 1985; Wyngaarden, 1986). This process involves perfusion of MTBE through a catheter directly into the gallbladder. Most reports have indicated that side effects were not seen, but one case of acute renal failure was described and attributed to hemolysis due to leakage of MTBE alongside the catheter during a 7-hour infusion (Ponchon et al., 1988). Clinical evaluations have not been sufficient to rule out the possibility of other acute or long-term side effects.

NONCARCINOGENIC EFFECTS IN LABORATORY ANIMALS

SHORT-TERM EXPOSURE

In an oral lethality study, six groups of five male and five female Sprague-Dawley rats were administered MTBE by gavage at 1900, 2450, 3160, 4080, 5270 or 6810 mg/kg bw. The rats were observed for immediate effects after dosing, at 1 and 4 hours, and then daily for 14 days. The LD_{50} of MTBE was determined to be 3866 mg/kg bw with 95% confidence limits of 3327 and 4492 mg/kg (Arco Chemical Company, 1980) following administration by gavage. At doses ≥4080 mg/kg, the animals experienced "marked central nervous system (CNS) depression, ataxia, tremors, labored respiration and loss of the righting reflex." Ataxia was observed at 2450 and 3160 mg/kg, and slight to

marked CNS depression was observed at 1900 mg/kg, the lowest dose tested. Deaths were observed at all but the lowest dose. A reduction or absence of clinical signs of toxicity was observed in surviving rats at 24 hours after dosing. No grossly observable lesions were seen in examinations of major organ systems at 1900 mg/kg; at higher doses, the few grossly observable lesions "could be attributed to the irritating nature of the ether." No additional information regarding necropsy results was reported.

In a 14-day oral toxicity study, groups of 10 male and 10 female Sprague-Dawley rats (age ~10 weeks) were administered MTBE (purity ≥99.95%) in corn oil vehicle by gavage in doses of 0, 357, 714, 1071 and 1428 mg/kg/day for 14 consecutive days (Robinson et al., 1990). Clinical signs (daily), body weight (initial, days 4 and 6 and terminal), food and water consumption (throughout study), hematology and clinical chemistry (prior to termination) and pathology (scheduled and unscheduled deaths) were evaluated in all rats. The pathology evaluations included organ weight measurements (brain, liver, spleen, lungs, thymus, kidneys, adrenals, heart, gonads) and gross examinations of all major organs. Histology was evaluated in the major organs of the control and high-dose groups and in the target tissue(s) (if identified) in the remaining dose groups.

Treated rats of both sexes in all dose groups developed loose stools by the third day of treatment, and continuing throughout the study. Profound anesthesia occurred immediately after dosing in the male and female rats treated with 1428 mg/kg/day.

Normal motor and sensory functions returned within ~2 hours of treatment and anesthetic effects were not observed in other dosed or control groups. Early deaths (two males at 357 mg/kg/day, two males and two females at 1428 mg/kg/day) were attributed to gavage errors, but these errors may have been secondary to MTBE-induced irritation of pharyngeal mucous membranes which contributed to difficulty in passing dosing needles. Average food consumption, water consumption, total weight gain and final body weight were generally reduced in both sexes at the higher doses, but the only decreases that were statistically significant ($p \leq 0.05$) were decreased food intake in males at 714 mg/kg/day and females at 1428 mg/kg/day, and decreased weight gain in males at ≥714 mg/kg/day and females at ≥1071 mg/kg/day.

Red blood cells (RBC) and hemoglobin were significantly increased in males at ≥714 mg/kg/day. Increases in these hematologic indices in treated females were not statistically significant. Clinical chemistry alterations generally occurred at the high dose or inconsistently at lower doses, including significantly increased serum cholesterol in females at 714 and 1071 mg/kg/day and males at 1428 mg/kg/day, increased serum aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) in males at ≥1071 mg/kg/day, decreased serum creatinine and increased serum glucose in females at 1428 mg/kg/day, and decreased blood urea nitrogen (BUN) in females and males at 1428 mg/kg/day.

Statistically significant ($p \leq 0.05$) changes in organ weight included reduced absolute and relative lung weights in females at ≥357 mg/kg/day, decreased absolute and relative thymus weights and absolute spleen weight in females at 1428 mg/kg/day, increased relative kidney weight in males at ≥1071 mg/kg/day and females at 1428 mg/kg/day, and decreased brain weight in males (absolute) and females (relative) at 1428 mg/kg/day. Relative lung weights were also reduced in treated males, but differences were significantly different from controls only at 714 mg/kg/day.

The only pathologic finding considered to be treatment-related was renal tubular disease in male rats receiving 1428 mg/kg/day. The renal lesions, characterized by increased hyaline droplets within the cytoplasm of proximal tubular epithelial cells, occurred in seven of eight (88%) treated males and two of five (40%) controls. These changes were consistent with male rat hyaline droplet (α_{2u}-globulin) nephropathy. No information on kidney histology in the lower dose groups was reported.

In male Sprague-Dawley rats, the inhalation LC_{50} for a single 4-hour exposure to Arco MTBE (96.2% pure) was determined to be 142 mg/L (39,400 ppm) with 95% confidence limits of 120 mg/L (33,300 ppm) and 168 mg/L (46,600 ppm). After exposure to commercial MTBE (99.1% pure), the LC_{50} was 120 mg/L (33,300 ppm) with 95% confidence limits of 104 mg/L (28,900 ppm) and 139 mg/L (38,600 ppm) (Arco Chemical Company, 1980). The concentrations tested ranged from ~70 (~19,400 ppm) to ~230 mg/L (~63,800 ppm). The observation period was 14 days. During this period, the

animals experienced eye irritation, irregular respiration, incoordination, ataxia, loss of righting reflex and prostration. The intensity of the effects increased with increasing exposure level.

No deaths occurred in 10 New Zealand white rabbits treated with occlusive dermal applications of 10 mg/kg of Arco MTBE or commercial MTBE, indicating that the dermal LD_{50} for MTBE is >10 mg/kg bw for the rabbit (Arco Chemical Company, 1980). Epidermal scaling and thickening were found at the site of administration.

In a repeated-exposure inhalation study, Sprague-Dawley rats (20 animals/sex/group) were exposed to 0, 101, 300, 1020 and 2970 ppm MTBE (>98% pure) 6 hours/day, 5 days/week for 9 exposure days (Bio/dynamics, Inc., 1981) to determine appropriate dose levels for later reproduction studies. Most of the animals were fasted prior to sacrifice, but five animals/sex/dose were not fasted due to technician error. Survival, urinalysis and hematologic and clinical chemistry parameters were monitored for all of the animals. Gross necropsy and organ weight determinations were performed on all animals; extensive histologic examinations were performed on all animals in the control and the 2970 ppm group. The trachea, nasal turbinates, kidney and liver were examined histologically in animals of the 1020 ppm group. No deaths, changes in "physical observations" (including righting reflex), body weight changes or abnormalities in urinalysis were attributed to exposure to MTBE. Clinical chemistry analyses revealed a statistically significant ($p \leq 0.05$) increase in phosphorus levels in blood from fasted female

animals exposed to the two highest concentrations. An increase in relative liver weights was found in the high dose groups of both the fasted males and the females, and a similar trend was found in the high dose unfasted male animals. No compound-related effects were found upon gross necropsy of all animals, but an increased incidence and severity of chronic inflammation was found in the nasal mucosa and the trachea of animals exposed to 1020 ppm (27/40) and 2970 ppm (27/40), compared with controls.

In a inhalation range-finding study (Dodd and Kintigh, 1989), Fischer 344 rats and CD-1 mice (5/sex/species/exposure-level) were exposed to target levels of 0, 2000, 4000 or 8000 ppm for 6 hours/day for 13 consecutive days. The animals were observed during exposure for neurotoxic or other effects and the rats received a neurobehavioral observational battery immediately after the 13th exposure. Hypoactivity was observed during exposure in both species at 2000 ppm on days 2 and 3; hypoactivity and ataxia were observed in both species during exposure to 4000 and 8000 ppm on most exposure days and prostration of two of five female mice occurred at 8000 ppm on day 9. The neurobehavioral observational battery administered to the rats immediately after the 13th exposure, however, revealed effects only at 8000 ppm. These effects included ataxia, decreased startle and pain reflexes, or decreased muscle tone in all of the male and most of the female rats. The effects were reversible (i.e., not detectable 1 hour after the initial testing). Additional significant findings included increased relative liver weights in rats at ≥4000 ppm and increased relative liver weights in all MTBE-treated groups of mice. No compound-related macroscopic lesions were detected during necropsy and no compound-

related histologic effects were found, but only grossly observable lesions were examined histologically.

LONG-TERM EXPOSURE

In the only subchronic oral study, groups of 10 male and 10 female Sprague-Dawley rats (age ~10 weeks) were administered MTBE (purity ≥99.95%) in corn oil vehicle by gavage in doses of 0, 100, 300, 900 or 1200 mg/kg/day for 90 consecutive days (Robinson et al., 1990). Evaluations included clinical signs (daily), body weight (initial and biweekly) and food and water consumption (once and three times weekly, respectively). Hematology, clinical chemistry, organ weights and pathology were evaluated as in the 14-day study described in the preceding section on short-term exposure. It is implied, but not specifically stated, that the histology examinations were performed for the control and high-dose groups, but were limited to the target tissue(s) in the other dose groups.

Rats in the 1200 mg/kg/day dose group exhibited profound anesthesia immediately following treatment but recovered within 2 hours. Mortality occurred in some of the treated males (one, two and one deaths at 100, 900 and 1200 mg/kg/day, respectively) and females (one, two and four deaths at 300, 900 and 1200 mg/kg/day, respectively), but was not clearly related to dose in the males and, based on pathology findings and observations during dosing, was attributed to gavage error secondary to pharyngeal mucous membrane irritation.

Changes in daily average water consumption were generally inconclusive. Female rats treated with 100 or 1200 mg/kg/day had significantly increased water consumption compared with controls, and male rats treated with 1200 mg/kg/day had significantly increased water consumption compared with those treated with 100 and 900 mg/kg/day. Average daily food consumption in treated males and females did not differ significantly from controls. Actual data and p values were not reported for water and food consumption. Treated rats of all groups had diarrhea throughout the study, but it did not appear to be more severe with increasing dose, and it was not accompanied by histopathologic effects (Olson, 1992).

Average final body weight was decreased in a dose-related manner in both male and female treated groups, but the only statistically significant ($p \leq 0.05$) difference was between the female 1200 mg/kg/day and control groups. Organ weight measurements showed significantly ($p \leq 0.05$) increased relative kidney weight in females at ≥300 mg/kg/day, increased absolute and relative kidney weights in males at ≥900 mg/kg/day, increased relative liver weight in males at ≥900 mg/kg/day, increased relative liver, heart and thymus weights in females at 900 mg/kg/day, and increased absolute and relative lung weights in males at 1200 mg/kg/day. The increases are generally dose-related but the inconsistent pattern of statistical significance ($p > 0.05$) for some of the organ weights (e.g., significant changes at 900 mg/kg/day but not 1200 mg/kg/day) may be related to smaller numbers of surviving animals at the higher doses (8 and 6 females at 900 and 1200 mg/kg/day, respectively).

There were no distinct dose-related variations in hematology values in either sex, although several parameters were significantly ($p \leq 0.05$) altered at 1200 mg/kg/day. These changes included decreased white blood cell count and increased erythrocyte count, hemoglobin concentration and hematocrit in females, and decreased mean corpuscular volume in males. The clinical chemistry evaluations showed dose-related changes in several parameters, consisting of statistically significant decreased BUN in both sexes, increased serum cholesterol in females and decreased serum creatine in males at ≥100 mg/kg/day, and decreased serum glucose and calcium in females and increased AST in males at ≥300 mg/kg/day.

Treatment-related pathologic changes were observed only in the kidneys of the male rats treated with 1200 mg/kg/day. Chronic nephropathy, characterized by tubular degenerative changes, was common in both the control and treated male rats. These degenerative changes, however, were more severe in the treated male rats than in control males. In addition, 50% (5/10) of the treated male rats had small numbers of tubules that were plugged with granular cysts, and all of the treated males had slightly increased numbers of cytoplasmic hyaline droplets in proximal tubular epithelial cells. These changes appeared to be consistent with male rat hyaline droplet (α_{2u}-globulin) nephropathy. No information on kidney histology in the lower dose groups was reported.

In a subchronic inhalation study, groups of 10 male and 10 female CD rats (Sprague-Dawley derived) were exposed by inhalation to MTBE (purity not reported) at

concentrations of 0, 250, 500 and 1000 ppm for 6 hours/day, 5 days/week for 13 weeks (Greenough et al., 1980). No effects on survival, body weight, food or water consumption, hematology, clinical chemistry or urinalysis were found following exposure. An increasing depth of anesthesia was found with increasing exposure concentration; no additional information regarding this effect was reported. No compound-related effects were found following gross necropsy or histologic examination of tissues and organs from all animals. A slight reduction in absolute and relative lung weight was found in female rats exposed to 1000 ppm MTBE.

In another subchronic inhalation study (Dodd and Kintigh, 1989), groups of 25 male and 25 female Fischer 344 rats were exposed to target concentrations of 0, 800, 4000 or 8000 ppm MTBE 6 hours/day, 5 days/week for 13 weeks. Evaluations included clinical and ophthalmic observations, food consumption, body weight and hematology (five rats/sex/group). Pathology (comprehensive organ weight and histology) was evaluated in 15 rats/sex/group and nervous system pathology (nervous system histology, brain weight, brain measurements) was evaluated in 6 or 10 rats/sex/group. Behavioral evaluations (functional observational battery) were performed on 10 rats/sex/group prior to the first exposure and at exposure weeks 1, 2, 4, 8 and 13, and motor activity was assessed in 15 rats/sex/group prior to the first exposure and at exposure weeks 4, 8 and 13.

Ataxia was observed immediately following exposure for the first 25 days of the study in all of the rats exposed to 8000 ppm. No other clinical signs of toxicity were reported for this or any other exposure group. Slight hematologic alterations occurred in males including significantly ($p<0.01$) reduced mean corpuscular hemoglobin concentration (MCHC) (week 14) and increased mean corpuscular volume (MCV) (weeks 5 and 14), mean corpuscular hemoglobin (MCH) (week 5 and week 14 at 8000 ppm) and reticulocytes (week 14) at ≥4000 ppm. At 8000 ppm, the following parameters were significantly reduced ($p<0.01$): leukocytes (week 5), lymphocytes (weeks 5 and 14), hematocrit (HCT) (week 14), reticulocytes (week 14) and segmented neutrophils (week 14). Segmented neutrophils were also significantly increased in females at week 14 at 8000 ppm. The only significant ($p<0.05$) biochemical finding was increased cortisone levels in both sexes at 8000 ppm. Other effects included reduced body weight gain in both sexes during the first 1-4 weeks of exposure at ≥4000 ppm, concentration-related increased relative weights of liver, kidney and adrenals in both sexes at ≥4000 ppm, and increased degree but not frequency of hemosiderosis in spleen and number and/or size of hyaline droplets in renal proximal tubules in males at 8000 ppm.

The nervous system necropsies showed significantly ($p>0.05$) decreased brain length in male rats at ≥4000 ppm (concentration-related) and reduced absolute brain weight in both sexes at 8000 ppm (no changes in relative brain weight or brain width were observed). The neurobehavioral assessments showed some statistically significant findings (e.g., elevated body temperature, decreased latency to rotate on an inclined

findings (e.g, elevated body temperature, decreased latency to rotate on an inclined screen, decreased hindlimb grip strength, decreased motor activity) primarily at 4000 and 8000 ppm that did not show clear dose-response relationships or occurred sporadically. Although the methods section of the report stated that the animals were monitored during exposure for signs of toxicity, the same "boilerplate" was used in the methods section of the 13-day range-finding study, which did report effects during exposure, whereas no mention of the results of any such monitoring is made in the results section of the subchronic study. Because the range-finding study detected signs of central nervous system (CNS) depression in the same strain and size rats during exposure at 2000 and 4000 ppm, but did not detect neurotoxic effects in the neurobehavioral observational battery administered after the 13th exposure to 2000 or 4000 ppm, it would appear that observation during exposure is essential to detect the threshold for MTBE neurotoxicity, and this monitoring during exposure may not have been performed or reported in the subchronic study.

In a chronic study, four groups of CD-1 mice (50/sex) were exposed to target concentrations of 0, 400, 3000 or 8000 ppm MTBE for 6 hours/day, 5 days/week for 18 months (Burleigh-Flayer et al., 1992). Evaluations included clinical observations, body and organ weights, hematologic evaluations, urinalysis, gross necropsy, and histopathology.

observed in both sexes in the mid- and high-dose groups, including blepharospasm (spasmodic contraction of the orbicular muscle of the eye), hypoactivity, ataxia, stereotypy (3000 ppm only), prostration (8000 ppm only), and lack of startle reflex. The only clinical effect reported in both sexes of the 8000 ppm group was ataxia. Other effects reported in both sexes of the high-dose group included a decreased body weight gain and absolute body weight, and a slight decrease in urinary pH. No hematologic effects were reported.

Dose-related increases in liver weight (absolute and relative to body and brain weights) were reported in both male and female mice; with only minimal effects in the 400 ppm group. Increases in kidney weight were reported for high-dose female mice and all groups of exposed male mice, but not in a dose-related manner. Decreases in absolute brain and spleen weight were also reported in both sexes of the high-dose group. Histopathologic evaluation revealed no lesions in any of these organs except for the liver. An increased incidence of hepatocellular hypertrophy was seen in males in the 3000 ppm group and both sexes in the 8000 ppm group. The only neoplastic lesion reported was an increased number of hepatocellular adenomas in female mice in the 8000 ppm group; a dose-related response was not apparent.

The increased incidence of early mortality, anesthetic effects, and the significant (as much as 24%) decrease in body weight suggests that the highest concentration of 8000 ppm exceeded the maximum tolerated dose (MTD) for this study. The lowest

concentration (400 ppm) was a NOAEL, and the 3000 ppm group represented a LOAEL based on clinical effects and organ weight changes in the mice.

The same laboratory performed a study using F344 rats (50/sex/group) that were exposed to target concentrations of 0, 400, 3000 or 8000 ppm MTBE for 6 hours/day, 5 days/week for 24 months (Chun et al., 1992). Effects were more severe in the rats than in mice, with increased mortality reported in males from both the 3000 and 8000 ppm groups, leading to earlier sacrifice times at 97 and 82 weeks, respectively. Chronic, progressive nephropathy was indicated to be the main cause of death in the mid- and high-dose groups, and also contributed to a slight increase in mortality in the 400 ppm group. Mortality and survival time for females were not significantly different between exposed and control rats.

Clinical signs reported in rats exposed to 3000 and 8000 ppm included blepharospasm, hypoactivity, ataxia, lack of startle reflex, and swollen periocular tissue and/or salivation (males only).

Body weight gain and absolute body weight were decreased in both sexes of the high-dose group. No hematologic effects were reported in any group. Dose-related increases in kidney and liver weights (absolute and relative to body and brain weights) were reported in females in the mid- and high-dose groups. No histopathologic findings were reported in the liver. Increases in gross and microscopic kidney changes indicative

of nephropathy were seen in a dose-related manner in all groups of exposed male rats and in females exposed to 3000 and 8000 ppm MTBE.

The only neoplastic finding in rats was an increased number of renal tubular cell tumors in males exposed to 3000 and 8000 ppm MTBE, originally suggested to have resulted from the accumulation of α_{2u}-globulin. Whether the etiology of these tumors can be fully attributed to the accumulation of α_{2u}-globulin has since been questioned (Garman, 1993). This issue requires further investigation before any conclusions can be drawn.

Based on nephropathy that was reported in all groups of exposed male rats, a true NOAEL could not be identified for this study. Both the 3000 and 8000 ppm exposures exceeded the MTD, as evidenced by increased mortality.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

A two-litter, one-generation inhalation reproduction study was performed in which groups of 15 male CD rats (Sprague-Dawley derived) were exposed to actual concentrations of 0, 290, 1180 or 2860 ppm MTBE (95-96% pure) for 6 hours/day, 5 days/week for a premating interval of 12 weeks, and groups of 30 female rats were similarly exposed to 0, 300, 1240 or 2980 ppm for a premating interval of 3 weeks (Biles et al., 1987). The exposures continued in the males during mating intervals. In the females, exposure increased to 6 hours/day, 7 days/week during gestation days 0-20 and from days 5-21 of lactation following delivery of the F_{1a} litters. The litters were not exposed. Both the

male and female animals underwent a 2-week resting period and then produced a second litter (F_{1b}) under the same regimen described above. In the parental animals, no adverse effects were observed on mortality, weight gain, "in-life observations" for gross signs of toxicity, or on the macro- or microscopic appearance of male or female reproductive organs. In females, no effects on the pregnancy rate were found in the breeding for the F_{1a} litters. Slightly lower pregnancy rates were found in the breeding for the second (F_{1b}) litters in all treated groups, but these findings were not statistically significant or dose-related. For males, it was reported that no adverse effects were found on reproductive function, but it is not possible to distinguish between male and female reproductive function when the endpoint is pregnancy rates. A nonsignificant increase in the incidence of dilated renal pelves was found in the dams exposed to 300 and 2980 ppm, but not 1240 ppm MTBE, and hence did not appear to be related to treatment. Histologic examination of tissues other than the testes, epididymis and ovaries was not performed.

In both the F_{1a} and F_{1b} generations, slightly lower body weights (not statistically significant) were found in the pups nursing from dams exposed to 1240 and 2980 ppm MTBE at days 14 and 21 of lactation. Pup survival indices were significantly lower during lactation days 0-4 in the F_{1b} litter in the low- and middle-dose groups, but since the high-dose group was not affected and no decrease in survival was found in the F_{1a} litter, this finding was not believed to be treatment-related. No abnormalities in the pups of either litter were found following gross external or internal examination. Skeletal examinations were not performed on the pups from either litter.

A two-generation inhalation reproduction study was conducted in which CD (Sprague-Dawley) rats were exposed to mean MTBE concentrations of 0, 400, 3000 and 8000 ppm (Neeper-Bradley, 1991). Groups of 25 male and 25 female P_0 rats (age approximately 6 weeks) per concentration were exposed for 10 weeks and then bred once to produce the F_1 generation. Groups of 25 male and 25 female randomly selected F_1 pups per concentration were exposed for at least 8 weeks and bred to produce F_2 litters. In both parental generations, the exposures continued through mating, gestation and lactation. The rats were exposed for 6 hours/day, 5 days/week prior to mating and for 6 hours/day, 7 days/week during mating, gestation and postnatal periods. The prebreeding exposures for the selected F_1 pups began after weaning (age 29-31 days). Parental evaluations included clinical signs, food consumption, body weight, liver weight (F_1 generation only), gross pathology and histology (respiratory tract, reproductive tissues and tissues with gross lesions in control and high dose groups). Viability, survival, body weight and sex distribution were evaluated in offspring.

Parental effects observed during the prebreeding exposures included hypoactivity, lack of startle reflex, blepharospasm and increased relative liver weight (F_1 generation) at ≥3000 ppm; and perioral wetness, ataxia, reduced food consumption during the first 2-3 weeks (P_0 and F_1 males) and reduced body weight and body weight gain throughout the exposure period (P_0 and F_1 males and F_1 females) at 8000 ppm. Histopathologic examinations did not reveal any treatment-related lesions in any of the three generations. There were also no treatment-related effects on mating, fertility and gestational indices

in either parental generation. Postnatal effects included significantly reduced body weight and body weight gain in F_1 and F_2 pups (principally during the latter part of lactation period) at ≥3000 ppm, and reduced F_2 pup survival on postnatal day 4 (93.5% compared to 98.1% in controls) at 8000 ppm. Therefore, the LOAEL is the same (3000 ppm) for parental and offspring effects.

The developmental effects of inhaled MTBE were determined in rats and mice by Conaway et al. (1985). Groups of 25 female CD rats (Sprague-Dawley derived) and 30 female CD-1 mice were exposed to target concentrations of 0, 250, 1000 or 2500 ppm MTBE (95-99% pure) for 6 hours/day during gestation days 6-15. Actual levels were somewhat higher, ~0, 260, 1100 and 3300 ppm, and there were some problems in vapor generation (the mid- and high-exposure levels included a substantial amount of aerosol, most of which had a particle diameter less than 1-2 μm) and with leakage during sampling/analyzing. No treatment-related effects on mortality, overt signs of toxicity (checked after exposure), maternal body weight, water consumption, liver weight, pregnancy rate, number of implants, resorptions and live fetuses, sex ratios or gross pathology were found in either the rats or mice. Food consumption was significantly decreased in all three groups of treated rats during gestation days 9-12 and was slightly, but not significantly, decreased in all three groups of treated mice during gestation days 12-15. Histopathologic examinations were not performed in the dams or fetuses of the rats or mice. No treatment-related fetal abnormalities (external, soft-tissue or skeletal malformations) were found in rats. In mice, a slight (nonsignificant) increase in the

incidence of fused sternebrae was found in fetuses and litters exposed to 3300 ppm MTBE. Fetal body weights in both the rats and mice were comparable to controls.

More recent developmental toxicity studies in mice (Tyl and Neeper-Bradley, 1989) and rabbits (Tyl, 1989) reported maternal and fetal effects at higher inhalation exposure levels than those tested in the study by (Conaway et al., 1985). In these studies, groups of 30 female CD-1 mice and 15 female New Zealand rabbits were exposed to target concentrations of 0, 1000, 4000 or 8000 ppm MTBE for 6 hours/day on days 6-15 (mice) or 6-18 (rabbits) of gestation. Actual monitored exposure levels were very close to target levels (1035, 4076 and 8153 ppm in mice, and 1021, 4058 and 8021 ppm in rabbits). The lowest target exposure level, 1000 ppm, was a NOAEL for both species. In mice, 4000 ppm produced hypoactivity, ataxia, a decrease in maternal body weight, which was not significant but was part of a dose-related trend, and a significant decrease in fetal body weight and significant reduction in fetal ossification. Hypoactivity and ataxia in this group were observed during exposure only and not after exposure when the individual animals were observed to determine incidence of clinical signs. The effects in mice at 8000 ppm were more severe and included not only hypoactivity and ataxia, but also prostration, labored respiration and lacrimation, observed both during exposure and after exposure. Additional findings at 8000 ppm were significantly reduced maternal body weight relative to controls and reduced body weight gain and food consumption, adverse effects on gestational indices (e.g., decreased viable implantations, increased resorptions and dead fetuses), decreased fetal body weights and ossification and an increase in the

incidence of cleft palate. In rabbits, 4000 ppm produced a decrease in maternal body weight gain and food consumption. These effects were also seen at 8000 ppm, plus a significant increase (14%) in relative liver weight that the authors suggested could be due to enzyme induction. Additional effects at 8000 ppm were hypoactivity and ataxia, observed only during exposure. There were no compound-related effects on gestational or fetal indices in the rabbits.

QUANTIFICATION OF NONCARCINOGENIC EFFECTS

The development of a quantitative risk assessment based on the toxicologic data on MTBE following oral exposure is currently under Agency review.

CARCINOGENIC EFFECTS

HUMAN DATA

Pertinent data regarding the potential carcinogenic effects of MTBE in humans were not located in the available literature.

LABORATORY ANIMAL DATA

Oral Studies

Pertinent data regarding the potential carcinogenic effects of MTBE in animals after oral exposure were not located in the available literature.

Inhalation Studies

In an inhalation bioassay, four groups of CD-1 mice and Fischer 344 rats (50/sex/species) were exposed to target concentrations of 0, 400, 3000 or 8000 ppm MTBE for 6 hours/day, 5 days/week for 18 months in mice (Burleigh-Flayer et al., 1992) and for 24 months in rats (Chun et al., 1992). Evaluations included clinical observations, body and organ weights, hematologic evaluations, urinalysis, gross necropsy, and histopathology. These inhalation bioassays are currently (July, 1993) being reviewed by the Human Health Assessment Group of the U.S. EPA.

Non-neoplastic findings in mice, reported earlier in this document, suggested that 400 ppm was a NOAEL for this study and 3000 ppm was the LOAEL, based on clinical effects and body and organ weight effects. The increased incidence of early mortality, anesthetic effects, and the significant decrease in body weight (as much as 24%) suggest that the highest concentration of 8000 ppm exceeded the MTD for this study. The only neoplastic lesion reported in mice was an increased number of hepatocellular adenomas in females in the 8000 ppm group, but a dose-related response was not apparent (2/50, 1/50, 2/50 and 10/50 for control, low-, mid- and high-dose groups, respectively). In females, only one hepatocellular carcinoma was seen at the low and high doses; none were observed in controls or the mid-dose group. In male mice, the incidence of hepatocellular carcinomas was statistically significantly increased in the high-dose group when an adjustment was made for mortality (2/42, 4/45, 3/41 and 8/34 for the control, low-, mid- and high-dose groups, respectively); the Cochran-Armitage trend test was also

significant for carcinomas (p=0.004) (Jinot, 1993). There was no increase in the frequency of hepatocellular adenomas in male mice, but the combined incidence of adenomas and carcinomas was significantly increased for high-dose males, and the Cochran-Armitage trend test on the combined tumors was significant (p=0.025) (Jinot, 1993). The investigators noted that the combined frequency of hepatic tumors in high-dose males is within the range of historical controls for 24-month studies in CD-1 mice. This bioassay was terminated at 18 months, however, making the validity of this comparison questionable. Statistical analysis of the tumors in the animals that died during the study (0/18, 2/12, 2/19 and 6/24 for control, low-, mid- and high-dose groups, respectively) also demonstrated a significant difference (p=0.026) for the pairwise comparison between controls and high-dose males, suggesting a decreased latency time for tumors developing in mice exposed to MTBE.

Rats were more sensitive to MTBE, with increased mortality reported in both the mid-dose (3000 ppm) and high-dose (8000 ppm) groups. The MTD was exceeded for both of these concentrations. The primary neoplastic finding in rats was an increase in the incidence of renal tubular cell tumors in males exposed to 3000 and 8000 ppm. The investigators reported that these lesions were considered to result from the accumulation of α_{2u}-globulin, a protein specific to male rats. The report on the anatomic pathology study, however, indicates that the renal tumors cannot be solely attributed to the accumulation of α_{2u}-globulin because an increased incidence of nephropathy was seen in female rats as well as male rats (Garman, 1993). An analysis of α_{2u}-globulin in the

13-week inhalation study (Dodd and Kintigh, 1989) also yielded equivocal results. The percentage of renal cortex staining for α_{2u}-globulin was approximately doubled in male rats exposed to MTBE, but the increase was not concentration-related (Garman, 1993). It was further noted that the nephropathy present in the 24-month study was reported to be the same for male and female rats and did not differ histologically from the spontaneous nephropathy that is common in older rats (Garman, 1993). It was suggested that the increased nephropathy seen in the MTBE-exposed rats was more likely an exacerbation of the spontaneous disease rather than being a direct effect of MTBE exposure (Garman, 1993). The only other neoplastic lesion reported was an increased incidence of interstitial cell adenomas of testes in male rats. The incidence of these adenomas was 32/50 (controls), 35/50 (400 ppm), 41/50 (3000 ppm) and 47/50 (8000 ppm). Statistical significance was demonstrated for both the 3000 and 8000 ppm groups ($p=0.035$ and $p=0.0002$, respectively), and the Cochran-Armitage trend test ($p=0.0001$) (Jinot, 1993). The study report suggests that the increased incidences in the 3000 and 8000 ppm groups may be the consequence of an unusually low incidence in the controls (64%, compared with 86 and 91% in controls from two other studies conducted at the same laboratory). The strong trend, however, suggests that the testicular adenomas do represent a treatment-related effect.

Injection Studies

Pertinent data regarding the potential carcinogenic effects of MTBE in animals after administration by injection were not located in the available literature.

SHORT-TERM STUDIES

No evidence of mutagenicity was found in the Ames *Salmonella/Saccharomyces* assay both with and without metabolic activation (<10 µL/plate) (Arco Chemical Company, 1980). In a mouse lymphoma forward mutation assay, no mutagenic activity was found without metabolic activation, but activity was found in the rat liver S9-activated assay system (Arco Chemical Company, 1980). In the presence of S9, MTBE consistently produced a dose-related increase in mutation frequency in four replicates using Arco MTBE (96.2% purity) and two replicates using a commercial MTBE (99.1% purity). MTBE did not induce sex-linked recessive lethal mutations in a feeding study using male *Drosophila melanogaster* (Semau, 1989).

MTBE did not induce sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells *in vitro* with either activated or nonactivated media (<5.0 µL/mL) (Arco Chemical Company, 1980). No evidence of a clastogenic effect was found in bone marrow of rats administered MTBE orally at single or repeated daily doses (for 5 days) of 0, 0.04, 0.13 and 0.4 mL/kg or mL/kg/day (Arco Chemical Company, 1980). MTBE also did not induce bone marrow chromosomal aberrations in rats that inhaled measured concentrations of 776, 4098 or 8086 ppm for 6 hours/day on 5 consecutive days (Vergnes and Morabit, 1989).

QUANTIFICATION OF CARCINOGENIC EFFECTS

WEIGHT-OF-EVIDENCE FOR CLASSIFICATION

No human data are available on the carcinogenicity of MTBE. Inhalation bioassays have been conducted in CD-1 mice and Fischer 344 rats; these studies are currently (July, 1993) under Agency review. The CRAVE Work Group of the U.S. EPA has not yet evaluated the carcinogenicity data on MTBE.

QUANTITATIVE ESTIMATE

A quantitative estimate of carcinogenicity for MTBE is not available.

EXISTING GUIDELINES, RECOMMENDATIONS AND STANDARDS

Not available at this time.

SPECIAL GROUPS AT RISK

No especially sensitive high risk human population has been identified for MTBE.

REFERENCES

Allen, M.J., T.J. Borody, T.F. Bugliosi, G.R. May, N.F. LaRusso and J.L. Thistle. 1985a. Cholelitholysis using methyl tertiary-butyl ether. Gastroenterology. 88: 122-125.

Allen, M.J., T.J. Borody, T.F. Bugliosi, G.R. May, N.F. LaRusso and J.L. Thistle. 1985b. Rapid dissolution of gallstones by methyl tertiary-butyl ether. N. Engl. J. Med. 312: 217-220.

Arco Chemical Company. 1980. Methyl tertiary-butyl ether: Acute toxicological studies. Unpublished study from Arco Research and Development, Glenolden, PA.

Biles, R.W., R.E. Schroeder and C.E. Holdsworth. 1987. Methyl tertiary butyl ether inhalation in rats: A single generation reproduction study. Toxicol. Ind. Health. 3: 519-534.

Bio/dynamics, Inc. 1981. A Nine-day inhalation toxicity study of MTBE in the rat. Project No. 80-7452. Unpublished report submitted to American Petroleum Institute, Washington, DC. 352 p.

Bio/dynamics, Inc. 1984. The metabolic fate of methyl-t-butyl ether (MtBE) following an acute intraperitoneal injection. Project No. 80089. Unpublished report submitted to American Petroleum Institute, Washington, DC. 150 p.

Bioresearch Laboratories. 1990a. Pharmacokinetics of methyl tert-butyl ether (MTBE) and tert-butyl alcohol (TBA) in male and female Fischer-344 rats after administration of MTBE by the intravenous, oral and dermal routes. Report no. 38842. Prepared by Bushy Run Research Center, Union Carbide Corporation, for the Methyl Tertiary Butyl Ether Committee, Washington, DC. Microfiche No. OTS0528044.

Bioresearch Laboratories. 1990b. Mass balance of radioactivity and metabolism of methyl tert-butyl ether (MTBE) in male and female Fischer-344 rats after intravenous, oral and dermal administration of ^{14}MTBE. Report no. 38843. Prepared by Bushy Run Research Center, Union Carbide Corporation, for the Methyl Tertiary Butyl Ether Committee, Washington, DC. Microfiche No. OTS0528044.

Bruckstein, A.H. 1990. Nonsurgical management of cholelithiasis. Arch. Intern. Med. 150: 960-964.

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

Chun, J.S., H.D. Burleigh-Flayer and W.J. Kintigh. 1992. Methyl tertiary butyl ether: Vapor inhalation oncogenicity study in Fischer 344 rats. Bushy Run Research Center, Export, PA. Laboratory Project ID# 91N0013B.

Conaway, C.C., R.E. Schroeder and N.K. Snyder. 1985. Teratology evaluation of methyl tertiary-butyl ether in rats and mice. J. Toxicol. Environ. Health. 16: 797-809.

Daubert, T.E. and R.P. Danner. 1992. Physical and Thermodynamic Properties of Pure Chemicals: Data Compilation. Part I. Design Institute for Physical Property Data. American Institute of Chemical Engineers. Hemispheric Publishing Corp., Washington, D.C.

Dodd, D.E. and W.J. Kintigh. 1989. Methyl tertiary butyl ether (MTBE): Repeated (13-week) vapor inhalation study in rats with neurotoxicity evaluation (unpublished study). Union Carbide, Bushy Run Research Center for MTBE Committee. TSCATS 403189. EPA/OTS #FYI-OTS-0889-0689.

Garman, R.H. 1993. DVM Consultants in Veterinary Pathology, Murrysville, PA. Letter to L.S. Andrews, ARCO Chemical Co., Newtown Square, PA. May 3.

Gonzaga, R.A.F., F.R. Lima and S. Carneiro. 1985. Dissolution of gallstones by methyl tertiary-butyl ether. N. Engl. J. Med. 313: 385.

Goth, A. 1974. Medical Pharmacology: Principles and Concepts, 7th ed. C.V. Mosby Company, St. Louis, MO.

Greenough, R.J., P. McDonald, P. Robinson et al. 1980. Methyl Tertiary-Butyl Ether (Driveron) Three Month Inhalation Toxicity in Rats. Project No. 413038. Unpublished report submitted to Chemische Werke Höls AG, Marl, West Germany. 230 p.

Hawley, G.G., Ed. 1981. The Condensed Chemical Dictionary, 10th ed. Van Nostrand Reinhold Co, New York, NY. p. 761-772.

Jinot, J. 1993. Statistical analysis of tumors from MTBE studies. Internal memorandum to J. Parker, Chief, Carcinogen Assessment Toxicology Branch, U.S. EPA, Washington DC. May 17.

Juliani, G., G. Gandini, S. Gabasio, L. Bonardi, E. Fascetti and L. Gremo. 1985. Colelitolisi chimica transcutanea con metil-ter-butil etere (MTBE). Radiol. Med. (Torino) 71: 569-574. (Ital.)

LaGrone, F.C. 1991. Potential community exposure to toxic chemicals. Environ. Sci. Technol. 25: 366-368.

NAS (National Academy of Science). 1977. Drinking Water and Health. Vol. 1. p. 19-63.

NAS (National Academy of Science). 1980. Drinking Water and Health. Vol. 3. p. 25-67.

Neeper-Bradley, T.L. 1991. Two-generation reproduction study of inhaled methyl tert-butyl ether in CD Sprague-Dawley rats (unpublished study). Union Carbide, Bushy Run Research Center.

Olson, G.R. 1992. Pathologist, Pathology Associates, Inc. Personal Communication to S.F. Velazquez, U.S. EPA, Cincinnati, OH. September 30.

Ponchon, T., J. Baroud, B. Pujol, P.J. Valette and D. Perrot. 1988. Renal failure during dissolution of gallstone by methyl tert-butyl ether. Lancet. 2: 276-277.

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

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

Sernau, R.C. 1989. Mutagenicity test on methyl tertiary butyl ether *Drosophila melanogaster* sex-linked recessive lethal test. Prepared by Hazelton Laboratories America, Inc., for the Methyl Tertiary Butyl Ether Committee, Washington, DC. Microfiche No. OTS0528039.

Tyl, R.W. 1989. Developmental toxicity study of inhaled methyl tertiary butyl ether in New Zealand white rabbits. Project Report 51-268. Prepared by Bushy Run Research Center, Union Carbide Corporation for the Methyl Tertiary Butyl Ether Committee, Washington, DC. Microfiche No. OTS0000689-1. (Cited as Union Carbide, 1989b in U.S. EPA, 1993)

Tyl, R.W. and T.L. Neeper-Bradley. 1989. Developmental toxicity study of inhaled methyl tertiary butyl ether in CD-1 mice. Project Report 52-526. Prepared by Bushy Run Research Center, Union Carbide Corporation for the Methyl Tertiary Butyl Ether Committee, Washington, DC. Microfiche No. OTS0000689-1. (Cited as Union Carbide, 1989a in U.S. EPA, 1993)

U.S. EPA. 1980. Guidelines and Methodology Used in the Preparation of Health Effect Assessment Chapters of the Consent Decree Water Criteria Documents. Federal Register. 45 (231): 79347-79357.

U.S. EPA. 1986. Guidelines for Carcinogen Risk Assessment. Federal Register. 51(185): 33992-34003.

U.S. EPA. 1987. Information on the Incidence of Methyl tert-Butyl Ether in Groundwater from Different States. Submitted to OTS. Microfiche No. OTS 0000574-0. Document No. FYI-OTS-0987-0574.

U.S. EPA. 1991. Alpha$_{2u}$-globulin: Association with Chemically Induced Renal Toxicity and Neoplasm in the Male Rat. Prepared for the Risk Assessment Forum, U.S. EPA, Washington, DC. EPA/625/3-91/019F.

U.S. EPA. 1993. Integrated Risk Information System (IRIS). Online. Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH.

Vergnes, J.S. and E.R. Morabit. 1989. Methyl tertiary butyl ether repeated exposure vapor inhalation study in rats: *In vivo* cytogenetic evaluation. Project No. 51-635. Prepared by Bushy Run Research Center, Union Carbide Corporation for the Methyl Tertiary Butyl Ether Committee, Washington, DC. Microfiche No. OTS0528040.

Weast, R.C., Ed. 1985. CRC Handbook of Chemistry and Physics, 66th ed. CRC Press, Boca Raton, FL. p. C-177.

Wyngaarden, J.B. 1986. New nonsurgical treatment removes gallstones. J. Am. Med. Assoc. 256: 1692.

Yalkowsky, S.H. 1989. Arizona Database of Aqueous Solubility. College of Pharmacy, University of Arizona, Tucson, AZ.