FINAL

Report on Carcinogens Background Document for

Methyl tertiary-Butyl Ether

December 2 - 3, 1998

Meeting of the
NTP Board of Scientific Counselors
Report on Carcinogens Subcommittee

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Summary of NIEHS Report on Carcinogens Review Group (RG1) and NTP Executive Committee Interagency Working Group for the Report on Carcinogens (RG2) actions for the nomination of Methyl-tert-Butyl Ether for listing in the 9th RoC.

The RG1 reviewed the available carcinogenicity data for the nomination of methyl tertiary-butyl ether (MtBE). After applying the current criteria for listing substances in the RoC, the RG1 passed a motion, by a vote of 4 in favor to 3 opposed, to recommended that MtBE should be listed in the 9th RoC as reasonably anticipated to be a human carcinogen based on evidence of benign and malignant tumor induction at multiple organ sites in long-term studies in two animal species. Inhalation exposure to MtBE produced increased incidences of kidney and testicular tumors in male rats and liver tumors in mice (Bird *et al*. 1997). Oral administration of MtBE produced increased incidences of leukemias and lymphomas (combined) in female rats and testicular tumors in male rats (Belpoggi *et al.* 1995). The Summary Statement contained in the MtBE draft background document for the December 2 & 3, 1998 NTP Board RoC Subcommittee meeting summarizes all the relevant information used by the RG1 to support their recommendation to list MtBE in the 9th RoC.

The RG2 reviewed the available carcinogenicity data for the nomination of MtBE. After applying the current criteria for listing substances in the RoC, a motion recommending MtBE be listed in the 9th RoC as reasonably anticipated to be a human carcinogen was defeated by a vote of 3 in favor to 4 opposed. Reasons given by the RG2 members voting against the motion included the perception that the rodent cancer data are not strong enough to list the chemical in the RoC as "reasonably anticipated to be a human carcinogen", uncertainty that the formation of the observed kidney tumors in rats and liver tumors in mice may be arising by mechanisms not relevant to humans, and the lack of any supporting human data. Therefore, the recommendation going forward from the RG2 is that MtBE should not be listed in the Report on Carcinogens at this time.

Summary Statement

Proposed RoC Listing for Methyl tertiary-Butyl Ether

Carcinogenicity

Methyl tertiary-butyl ether (MtBE) is reasonably anticipated to be a human carcinogen based on evidence of benign and malignant tumor induction at multiple organ sites in long-term studies in two animal species. Inhalation exposure to MtBE produced increased incidences of kidney and testicular tumors in male rats and liver tumors in mice (Bird *et al*. 1997). Oral administration of MtBE produced increased incidences of leukemias and lymphomas (combined) in female rats and testicular tumors in male rats (Belpoggi *et al.* 1995).

No studies on the potential carcinogenicity of MtBE in humans have been reported.

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

The initial step in the biotransformation of MtBE involves oxidative demethylation via cytochrome P450 enzymes to tertiary-butyl alcohol (TBA) and formaldehyde. Genotoxicity tests on MtBE and TBA are, for the most part, negative. Formaldehyde, however, is genotoxic in a variety of experimental systems. MtBE was mutagenic in mouse lymphoma cells in the presence of rat liver activating enzymes (Mackerer *et al.* 1996); this effect was considered to be due to metabolism of MtBE to formaldehyde, because induction of mutants was inhibited by formaldehyde dehydrogenase. Incubation of mouse or rat hepatocytes with MtBE produced low levels of DNA-protein cross links (DPX) or RNA-formaldehyde adducts (RFA) with no apparent concentration dependence. Incubation with formaldehyde produced concentration-dependent increases in both DPX and RFA (Casanova and Heck 1997). Both TBA and formaldehyde are carcinogenic in experimental animals, with some responses being similar to those seen with MtBE (*e.g*., TBA induced kidney tumors in male rats). Epidemiological studies suggest a causal relationship between exposure to formaldehyde and nasopharyngeal cancer (IARC 1995).

The mechanisms by which MtBE causes cancer in rodents are not understood, nor is the relative role of the parent compound and its metabolites known. Leydig cell tumors of the testes were induced in male rats after inhalation (Bird *et al*. 1997) or oral exposure (Belpoggi *et al*. 1995) to MtBE. The exposure-related increase in testicular tumors in the inhalation study was attributed to a low incidence of these tumors in the control group (Bird *et al*. 1997); however, the incidence observed in the control male F344 rats in that study was the same as the historical control rate for National Toxicology Program (NTP) inhalation studies in this strain of rat (Nyska *et al*. 1998). The increased incidence of testicular tumors in the oral study in Sprague-Dawley rats, a strain with a low spontaneous incidence of testicular tumors, was attributed to increased survival of the high-dose group compared to controls (NRC 1996); however, the statistical method used to analyze this tumor response adjusts for differences in survival between control and treatment groups. No data are available showing that the testicular tumor response in MtBE-exposed rats involves an endocrine disturbance.

The induction of liver tumors in female mice exposed to MtBE occurred at an exposure that did not affect survival or liver toxicity, and resulted in only a 7% reduction in final mean body weight relative to controls (Bird *et al.* 1997). Subsequent studies showed that exposure concentrations used in the cancer bioassay of MtBE did not promote the development of liver tumors in N-nitrosodiethylamine-initiated female mice (Moser *et al*. 1996a), that MtBE does not alter serum estrogen levels, and that effects of MtBE in endocrine-sensitive tissues in female mice are not mediated through the estrogen receptor (Moser *et al*. 1998). Hence, the hypothesis that liver tumors induced in mice exposed to MtBE result from an alteration in estrogen homeostasis has not been demonstrated.

The α_{2u}-globulin-associated mechanistic hypothesis of kidney tumor induction in male rats was not considered to be applicable for MtBE because: a) nephrotoxicity induced by MtBE or TBA was not specific for male rats (both compounds increased the severity of nephropathy in female rats); b) the binding affinity of MtBE for α_{2u}-globulin is very weak; c) the modest increases in concentration of α_{2u}-globulin in the male rat kidney are insufficient to explain the increases in protein droplets, renal tubular cell proliferation, or severity of chronic nephropathy observed in male rats exposed to MtBE; and d) MtBE is metabolized to a genotoxic intermediate (formaldehyde).

In a subsequent pathology review of the oral study of MtBE in rats, Belpoggi *et al.* (1998) reported that all of the hematopoietic neoplasms (lymphomas and leukemias) observed in the original study (Belpoggi *et al.* 1995) were of lymphoid cell origin, supporting the conclusion that the combination of hemolymphoreticular neoplasms diagnosed in this study were lymphomas. This review also found a dose-related increase in interstitial cell hyperplasia of the testis and multiplicity of interstitial cell adenomas in only the high-dose group.

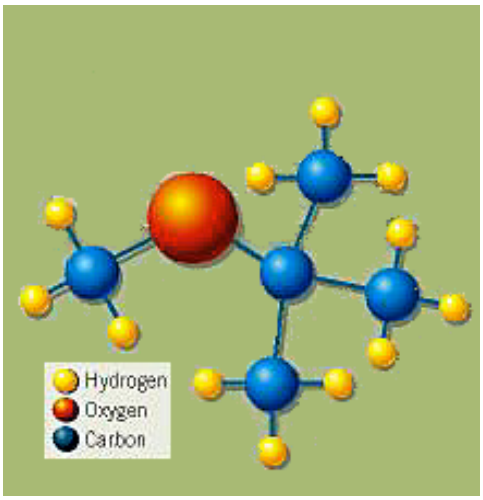
For chemicals, such as MtBE, that induce tumors in animals and for which there are no human data and limited mechanistic information, there remain uncertainties in quantitative estimates of human cancer risk at environmental or occupational exposure levels. One factor is whether the mechanisms that caused tumors in rodents under bioassay conditions operate in humans at ambient exposures (NSTC 1997). Because cancer can have a long latency period and use of MtBE has increased dramatically over the past ten years, epidemiological studies at this time may not provide adequate information on human cancer risk. A better understanding of the mechanisms by which MtBE causes cancer in animals could help characterize dose-response relationships. No data are available that would support the conclusion that the mechanisms thought to account for tumor induction by MtBE in experimental animals would not also operate in humans.

1 Physical and Chemical Properties

1.1 Chemical Identification

Methyl tertiary-butyl ether $(CH_3)_3C(OCH_3)$

Figure 1. Chemical stucture of MtBE



(structure adapted from http://www.oxybusters.com/MTBE.htm)

Methyl tertiary-butyl ether ($C_5H_{12}O$, CASRN 1634-04-4, Mol. Wt. = 88.149) is also known as:

MtBE

2-Methoxy-2-methylpropane

2-Methyl-2-methoxypropane

Methoxy-2-methylpropane

tertiary-Butyl methyl ether

Methyl 1,1-dimethylethyl ether

MtBE's RCRA waste number is D001 (ignitable waste) and, in shipping, its UN number is 2398.

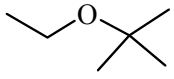
1.2 Physical and Chemical Properties

Property	Information	Reference
Molecular Weight	88.149	Budavari *et al.* (1996)
Color	Colorless	U.S. EPA (1993a)
Physical State	Volatile, flammable liquid	HSDB (1994)
Melting Point at, ^{o}C	-109	Budavari *et al.* (1996)
Boiling Point at 760 mm, ^{o}C	55.2	Budavari *et al.* (1996)
Density at 20^{o}C/4^{o}C, g/mL	0.7404	Budavari *et al.* (1996)
Odor Threshold	0.32-0.47 mg/m^3	U.S. EPA (1993a)
Solubility Water at 25^{o}C Organic Solvents Ethanol Ether	 51.26 g/L soluble soluble	 U.S. EPA (1993a) EHIS (1998)
Vapor pressure at 25^{o}C (mm Hg)	245	Budavari *et al.* (1996)
Relative Vapor Density (air=1)	3.1	U.S. EPA (1993a)
Flash Point ^{o}C	-28, flammable	HSDB (1994)

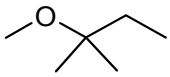
1.3 Identification of Structural Analogs and Metabolites

Structural analogs and metabolites discussed here include: ethyl t-butyl ether, t-amyl methyl ether, t-butyl alcohol, tertiary-butyl formate, and formaldehyde.

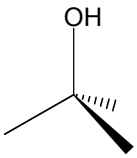
Ethyl t-butyl ether ($C_6H_{14}O$, CASRN 637-92-3, Mol. Wt. = 102.13) is also known as: EtBE; 2-methyl-2-ethoxypropane; tertiary-butyl ethyl ether; ethyl tertiary-butyl oxide, and 2-ethoxy-2-methylpropane. It is a clear, colorless, flammable liquid at room temperature. Its structure is as follows:



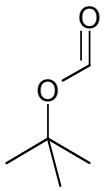
tertiary-Amyl methyl ether ($C_6H_{14}O$, CASRN 994-05-8, Mol. Wt. = 102.18) is also known as: Tame; tertiary-methyl amyl ether; tertiary pentyl methyl ether; butane; 2-methoxy-2-methyl-; 2-methyl-2-methoxybutane; methyl 1,1-dimethylpropyl ether; methyl tertiary-amyl ether; and methyl 2-methyl-2-butyl ether. A clear, colorless, flammable liquid at room temperature, its structure is illustrated below:



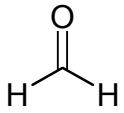
t-Butyl alcohol ($C_4H_{10}O$, CASRN 75-65-0, Mol. Wt. = 74.122) is also known as 2-methyl-2-propanol; 1,1-dimethylethanol; trimethylcarbinol; 2-methylpropan-2-ol; tertiary-butanol; TBA; t-butyl hydroxide; trimethyl methanol; dimethylethanol; methyl-2-propanol; and t-butyl alcohol. It is a colorless, flammable liquid at room temperature with a camphor-like odor. Its structure is as follows:



tertiary-Butyl formate ($C_5H_{10}O_2$, CASRN 762-75-4, Mol. Wt. = 102.13) is also known as formic acid 1,1-dimethylethyl ester. It is a flammable liquid. The structure is as follows:



Formaldehyde (CH_2O, CASRN 50-00-0, Mol. Wt. = 30.026) is also known as formalin; methylene oxide; methyl aldehyde; methanol; HCHO; formic aldehyde; oxomethane; formol; oxymethylene; morbicid; veracur; formalin 40; BFV; fannoform; formalith; FYDE; HOCH; karsan; lysoform; superlysoform; oxomethylene; and methan 21. It is a colorless, combustible liquid or gas with a pungent odor. Its chemical structure is as follows:



2 Human Exposure

Human contact with methyl tertiary-butyl ether (MtBE) occurs both in the workplace and through environmental contamination. Occupational exposures to MtBE mainly result from inhalation of vapors in the work environment. Consumers who pump their own gasoline are exposed to a lesser extent. Oral and dermal exposure may also result from drinking and bathing in water contaminated by seepage of MtBE into groundwater (ATSDR 1996).

2.1 Use

With the passing of the Clean Air Act of 1970, the United States enacted various regulations to control levels of air pollutants. These regulations were updated by the Clean Air Act Amendments of 1990. To decrease levels of carbon monoxide (CO), the Amendments required the use of oxygenated fuel (at least 2.7% oxygen by weight) in regions of the country that failed to attain the National Ambient Air Quality Standard for CO. The oxygenated gasoline program was introduced nationally in the winter of 1992-1993. To achieve the required 2.7% oxygen content, up to 15% (by volume) MtBE must be added to fuels (HEI 1996). Oxygenates are added to over 30% of all gasoline consumed in the United States (Stern and Kneiss 1997). MtBE has been used as an octane enhancer since the late 1970s and is used in the reformulated gasoline program (2% oxygen, 11% MtBE) to reduce motor vehicle emission of hydrocarbons.

MtBE is sometimes administered medically for contact dissolution of cholesterol gallstones in gallbladder or bile ducts. This approach is used electively when surgical and endoscopic methods of treatment are ruled out or refused. As of 1993, worldwide, more than 500 patients have been treated successfully (Brown 1997).

2.2 Production

Production of MtBE has slowly increased as local, state, and federal governments have emphasized cleaner air standards. The Federal Clean Air Act (FCAA) required that MtBE be added to gasoline, especially during the winter, to meet CO emission standards and during the summer to reduce motor vehicle emissions of hydrocarbons that lead to higher ozone levels. In 1992, 27 companies (32 facilities) produced 9.1 billion pounds of MtBE (OFA 1998). Currently, 23.7 billion pounds (250,000 barrels/day) are used in United States, with 3.8 billion pounds (40,000 barrels/day) being imported. Texas produces about 80%, Louisiana 10%, and California 5% of the MtBE used in the United States (CEPA 1998).

2.3 Biomarkers

Elimination of MtBE from the human body is multiexponential with $t_{1/2}$ = 10 min. 1.5 h, and 19 h. MtBE is transformed in the body by microsomal oxidative demethylation to tertiary butyl-alcohol (TBA) and formaldehyde. MtBE and TBA levels can be analyzed in human blood samples by a modified gas chromatography/mass spectrometry technique. Statistical analysis (such as exposure modeling) can then be used to estimate MtBE exposure levels. Other methods for evaluating MtBE exposure include measuring MtBE and TBA in urine and MtBE in exhaled breath (Hutcheon *et al.* 1996).

2.4 Environmental Exposure

Industrial emissions of MtBE into the atmosphere are, for the most part, attributable to petroleum refineries. Though other industries contribute to the atmospheric emissions, petroleum refineries emit more MtBE than all other industries combined. Emissions from gasoline stations (refueling) are on the same order as the industries (other than petroleum refining). Leaking underground storage tanks (USTs) and motor boat use are additional sources of MtBE exposure, contributing to groundwater levels of MtBE. Vehicles are, however, the leading source of MtBE emissions at 149-179 million pounds per year. Tailpipe emissions cause 73% of this release, evaporation (12%), running losses (12%), and heat soak losses (15%) account for the remainder (Brown 1997). These atmospheric emission values are summarized in Table 2-1 and Table 2-2 summarizes the Toxic Release Inventory (TRI) data, showing sources of MtBE release.

Table 2-1. Atmospheric emissions of MtBE

Source Type	Number Reporting	Total Emissions (million lb/yr)	Reference [a]
Petroleum Refineries	81	3.0	U.S. EPA (1995)
Industrial Organic Chemicals	17	0.34	U.S. EPA (1995)
Motor Vehicles and Car Bodies	9	0.013	U.S. EPA (1995)
Pharmaceutical Preparations	5	0.068	U.S. EPA (1995)
Internal Combustion Engines	3	0.058	U.S. EPA (1995)
Petroleum Bulk Stations	2	0.028	U.S. EPA (1995)
Other Industries	24	0.162	U.S. EPA (1995)
Gasoline Service Stations (Refueling)	-	1.3	Brown (1995)
Evaporative Emissions	-	39	Brown (1995)
Tailpipe Emissions	-	110	Brown (1995)

[a] Cited by Brown (1997)

Table 2-2. Estimated release of MtBE for the United States for Report Year 1993

Release To:	Reported Releases (kg/yr) by:		
	Petroleum Refineries	All Other Facilities	Total
Atmosphere	1,398,026	274,571	1,672,597
Underground Injection	288	3,979	4,267
Land	184	2	186
Water	41,799	74	41,873
TOTAL RELEASES	1,440,297	278,626	1,718,923

NSTC (1997)

2.4.1 Air Exposure

The geometric mean of environmental exposure to MtBE in air is estimated to be 1 μg/m^3 (SD = 4.0, arithmetic mean 2.6 μg/m^3). The estimated geometric lifetime dose of MtBE in air is 4.00 x 10^{-4} mg/kg/day (geometric mean, range 2.0 x 10^{-5}–7.8 x 10^{-4} mg/kg/day, SD = 4.0). The arithmetic mean is 1.05x10^{-3} mg/kg/day (Brown 1997).

Table 2-3 outlines concentration estimates for MtBE exposure in air for the general public. The time-weighted average (TWA) exposures (during an oxyfuel season) were determined for two hypothetical scenarios, with varying activity patterns and oxyfuel exposure potentials. The TWA for the lower exposure scenario was calculated to be 0.018 ppm/h (0.065 mg/m^3/h) while the TWA for the higher exposure scenario was 0.035 ppm/h (0.126 mg/m^3/h). Oxyfuel seasons were assumed to run either four or six months long (NSTC 1996).

Table 2-3. MtBE air exposure estimates

Activity	Occurrence	Time/year (h)	Concentration in mg/m^3 (ppm)	Exposure in mg/m^3-h (ppm-h)
Gas Fill-up	1.5 occurences/week at 2 minutes Other at 10 minutes [a] Total	2.6 13.0 15.6	36.0 (10) 3.6 (1) 39.6 (11)	93.6 (26) 46.8 (13) 140.4 (39)
Commute/in vehicle	10 h/week	520	0.36 (0.1)	187.2 (52)
Auto shop	4 occurences/year (15 minutes)	1.0	1.8 (0.5)	1.8 (0.5)
Public garage	10 min/day	60.83	1.8 (0.5)	109.5 (30.4)
Residential garage	2 min/day	12.16	3.6 (H) (1) 0.018 (L) (0.005)	13.8 (3.8) 0.22 (0.06)
Residence	10 h/day + weekend	4,160	0.036 (H) (0.01) 0.018 (L) (0.005)	149.8 (41.6) 74.9 (20.8)
Office	40 h/week	2,080	0.036 (0.01)	74.9 (20.8)
School/public buildings	17 h/week	884	0.036 (0.01)	31.8 (8.8)
Outdoors	20 h/week	1,040	0.36 (H) (0.1) 0.036 (L) (0.01)	374.4 (104) 37.4 (10.4)

[a] Gas fill-up is divided into two parts, the 1st is actual time pumping gas, and the 2nd is time spent at the station.

H = High; L = Low

Huber (1993; cited by U.S. EPA 1993a)

2.4.2 Groundwater

MtBE exposure occurs after its discharge into groundwater, which may then be used in public water supplies. Of the estimated 3.8 million pounds released from 141 facilities, 2.4% of the MtBE was likely to have been released directly to water. Industrial contributions to MtBE levels are minimal, however, compared to leaks from underground storage tanks (USTs) (NSTC 1997). Many USTs are located in sites with unused, shallow groundwater where MtBE concentrations are exceptionally high (CEPA 1998).

2.4.3 Water Exposure

MtBE levels in lakes and reservoirs have also been studied as there is potential for both dermal and oral exposure. Concentrations of MtBE in reservoirs with recreational boating range from <1-15 ppb (1-15 μg/L). In areas of high boating activity such as at marinas and boating events, MtBE levels are much higher. MtBE levels in a small holding basin reached levels of 40 ppb (40 μg/L) a few days after an organized jet ski event. Possible sources of MtBE contamination were droplets from exhaust, leaks, and spillage while fueling vehicles, and leaks in piping and marina storage tanks. Classic gasoline contamination markers in these areas were found at lower concentrations than MtBE (CEPA 1998).

2.4.4 Drinking Water

The National Water Quality Assessment (NAWQA) Program has studied drinking water by sampling both private and public wells. Although the U.S. EPA does not have the authority to require monitoring of MtBE in drinking water supplies, individual states have monitored their drinking water, as summarized in Table 2-4. The average exposure for a 70 kg man who drinks on average 2 L/day is 0.57 μg/kg/day based on EPA's draft health advisory lower limit for daily MtBE exposure in drinking water of 20 μg/L. In a high dose scenario of 100 μg/L MtBE contamination in drinking water, average exposure would be 2.86 μg/kg/day.

Table 2-4. MtBE levels in drinking water supplies summarized by state

State	N	Arithmetic Mean in μg/L	Geometric Mean in μg/L	Median in μg/L	Range in μg/L
Colorado (monitoring)	17	1408	4.4	1.2	0.2–23000
Colorado (other)	5	0.74	0.56	0.5	0.2–1.4
Connecticut (domestic)	5	1.14	0.93	0.9	0.3–2.2
Connecticut (monitoring)	7	0.41	0.37	0.4	0.2–0.7
Illinois (public wells)	29	131.5	22.8	20	0.4–770
Iowa (public wells)	3	31.7	25.2	17	15–63
Massachusetts (monitoring)	5	0.54	0.48	0.4	0.3–1.1
Missouri (private wells)	133	2399	–	1400	8.8–17000
Nevada (monitoring)	3	0.5	0.48	0.4	0.4–0.7
Nevada (other)	7	54.5	7.0	6.2	0.3–220
New Jersey (public wells)	82	2.6	1.3	1.1	0.1–33.6
New York (monitoring)	3	2.9	1.0	0.6	0.2–7.9
Rhode Island (private wells)	119	156.4	4.1	4	0.29–17800
Rhode Island (public wells)	536	83.7	–	16	1–3000
Texas (public wells)	7	10.9	7.8	7.15	3.6–42
Texas (private wells)	8	379.3	9.6	5	2–3360

Taken from NSTC (1997)

With increased use of MtBE in the U.S. gasoline supply, increases in the MtBE levels in drinking water have been observed. Nationwide MtBE drinking water concentrations, however, have not been adequately identified. It is important to note that in Table 2-4, many of the samples were taken after spills were identified. National averages, therefore, cannot be determined from the data (NSTC 1997).

2.4.5 Dermal Exposure

Skin exposure occurs when baths or showers contain MtBE-contaminated water. Bath water contact is estimated at 2.6×10^{-8} mg/kg/day (geometric mean; arithmetic mean = 4.6×10^{-6} mg/kg/day). Dermal exposure was estimated by factoring MtBE concentrations in water, total skin area, fraction of skin area exposed, exposure time, and frequency of baths per year. Exposure by showering was not determined because immersion models include many uncertainties (Brown 1997).

2.4.6 Medical Exposure

Patients with cholesterol gallstones electing to use medical exposure are generally dosed with 1-15 mL MtBE administered over an average of 5.1 hours. Blood levels of MtBE averaged 40

µg/mL immediately after infusion, 18 µg/mL at five hours, and <10 µg/mL after 12-18 hours. TBA levels were higher for each of these time periods (approx. 40, 42, and 25 µg/mL respectively) trailing off at a slower rate than MtBE. Fatty tissue concentrations of MtBE from the abdominal wall, in nine patients studied, ranged from 68-303 µg/g MtBE (Hutcheon *et al.* 1996).

2.5 Occupational Exposure

MtBE exposures are predominately atmospheric. Exposure comes from manufacturing MtBE, blending gasoline, transporting MtBE, distributing gasoline, dispensing gasoline, vehicle repair, and driving vehicles fueled with the additive. Water exposure can occur from drinking potable water that has been contaminated with MtBE at work sites. Table 2-5 summarizes atmospheric exposure concentrations of MtBE for various occupations.

Table 2-5. MtBE atmospheric concentration distributions for exposed populations

Population	Number of Data Sets	Geometric mean concentration in µg/m^3 (ppb)	Geometric Standard Deviation	Arithmetic mean concentration in µg/m^3 (ppb)
Manufacturing workers	2	300 (83.2)	6.0	1500 (416)
Blending workers	2	1000 (277)	6.0	5000 (1387)
Transportation workers	1	870 (241)	10.6	14000 (3883)
Distribution workers	1	470 (130)	6.3	2600 (721)
Gasoline station workers	7	2000 (555)	4.0	5200 (1442)
Mechanics, etc.	11	300 (83.2)	3.5	660 (183)

Values were based on accepted risk assessment assumptions and additional situation-specific data sources. Brown (1997)

2.5.1 Service Station Attendants and Operators

Environmental air samples via personal pumps were taken in two cities: Los Angeles, California and Phoenix, Arizona. Approximately 60 eight-hour shifts were sampled. Using this data (2 samples/shift), geometric means and ranges of MtBE were determined on sites where service station attendants and operators pumped gasoline containing 12 to 15% MtBE, as summarized in Table 2-6.

Table 2-6. MtBE exposure data for Phoenix and Los Angeles

Location	N[a]	Geometric Mean, ppm (mg/m^3)	Minimum, ppm (mg/m^3)	Maximum, ppm (mg/m^3)
Phoenix, site 1	19/20	0.53 (1.91)	0.11 (0.40)	3.88 (14.0)
Phoenix, site 2	21/21	0.17 (0.61)	0.04 (0.14)	2.12 (7.6)

Location	N[a]	Geometric Mean, ppm (mg/m^3)	Minimum, ppm (mg/m^3)	Maximum, ppm (mg/m^3)
Phoenix, site 1 and 2	40/41	0.30 (1.08)	0.04 (0.14)	3.88 (14.0)
Los Angeles [b], site 1	6/38	0.04 (0.14)	0.02 (0.07)	0.11 (0.40)
Los Angeles [b], site 2	9/10	0.21 (0.76)	0.21 (0.76)	0.73 (2.63)
Los Angeles [b], site 1 and 2	15/48	0.14 (0.50)	0.02 (0.07)	0.73 (2.63)

[a] Number of detectable samples/Total samples
[b] Los Angeles sites were using the phase II vapor recovery system.
Cited by Hartle (1993)

Average occupational exposure to MtBE among service station attendants and operators in Phoenix and Los Angeles was less than 1 ppm. The study was carried out during a shortage of Persian Gulf oil and the results may be ambiguous. Gasoline prices were steadily increasing at this time and many users purchased self-served fuel, and less of it than during a stable fuel market. The Los Angeles area gas stations were using a phase II vapor recovery system, decreasing the amount of MtBE released into the air (Hartle 1993). A comprehensive survey of various service attendants and operators showed that during the summer time, long-term exposure (averaging over eight hours) to MtBE was $\leq$ 0.5 ppm (areas studied were New York, Arizona, Minnesota, and Oregon). MtBE was collected for these studies either by absorption through carboxen or through direct sampling in evacuated containers. During winter, exposure averages increased to $\leq$ 0.7 ppm MtBE, since gasoline MtBE levels are increased in winter months (HEI 1996).

2.5.2 Mechanics

Motor vehicle mechanics are exposed to MtBE at work. The American Petroeum Institute (API) (1996; cited by HEI 1996), found that in the Northeast and Southwest (Connecticut, New Jersey, and Arizona), MtBE exposure levels ranged from 0.3 ppm, the minimum detectable concentration (MDC), to 32 ppm. While the high was 32 ppm, nine of 13 values measured were below the MDC. The median exposure value for mechanics in an eight-hour sample period was 0.09 ppm. This value appears reasonable in light of similar observations from other studies: for Stamford, CT (0.11 ppm; range <0.03-12.04 ppm) and Fairbanks, AK (0.10 ppm; range 0.01-0.81 ppm) (HEI 1996).

2.5.3 Manufacturing and Distribution Workers

The API study (1996; cited by HEI 1996), also conducted a survey to determine MtBE in air exposure among petroleum manufacturers and distributors from 1982-1993. The results are summarized in Table 2-7. Particularly high values for some of the ranges were caused by fuel spills or overflows; dispensing rates exceeding 325 gal/h and unusually large numbers of automobiles were fueled within a brief period. Exposure concentrations also increased when wind speeds were low (< 3 mph) and street traffic was very heavy (API 1993; cited by API 1996).

Table 2-7. Occupational exposure with airborne MtBE in the petroleum industry [a]

Industry Category	Occupation	Sampling Time	Samples >MDC/Total	MtBE (ppm)	MtBE (mg/m^3)

				Range	Median	Range	Median
MtBE Manufacturing							
Routine Operations	Oil refinery and chemical plant manufacturing personnel handling neat MtBE.	< 30 min 6-9 h TWA > 9 h TWA	14/27 38/76 2/2	0.2-7.8 0.01-248.7 0.16-0.17	1.0 0.03 0.2	0.06-2.16 0.003-69 0.04-0.05	.28 0.008 0.06
Routine maintenance		< 30 min 30 min–6 h 6-9 h TWA >9 h TWA	7/8 1/1 4/4 2/2	0.5-7.2 0.2 0.0-0.7 0.16-0.2	0.9 0.2 0.1 0.2	0.14-2.00 0.06 0-0.20 0.04-0.06	0.25 0.06 0.03 0.06
MtBE Blending							
Neat MtBE	Personnel involved in fuel-blending activities.	< 30 min 30 min-6 h 6-9 h TWA > 9 h TWA	34/35 12/13 7/12 0/9	0.0-97.0 0.2-72.0 0.0-88.0 0.2-0.3	2.9 1.03 2.2 0.3	0-26.9 0.06-20.0 0-24.4 0.06-0.08	0.80 0.29 0.61 0.08
Fuel mixtures		< 30 min 30 min-6 h 6-9 hr TWA >9 h TWA	51/98 5/19 34/112 9/22	0.02-100.0 0.03-2.0 0.02-14.0 0.0-0.3	0.3 0.1 0.04 0.02	0.006-27.7 0.008-0.55 0.006-3.88 0-0.08	0.08 0.03 0.01 0.005
MtBE Transport							
Neat MtBE	Marine barge, pipeline, and rail car personnel; trucking personnel included only for transport of neat MtBE.	< 30 min 30 min-6 h 6-9 h TWA >9 h TWA	62/66 23/27 9/10 1/1	0.3-1,050.0 0.04-700.0 0.03-711.9 0.3	13.8 2.2 0.2 0.3	0.08-291.2 0.01-194.2 0.008-197.5 0.08	3.8 0.61 0.01 0.08
Fuel Mixtures		< 30 min 30 min-6 h 6-9 h TWA >9 h TWA	60/64 64/92 28/42 8/8	0.001-507.9 0.02-59.4 0.01-26.2 0.2-4.5	2.4 0.4 0.1 1.5	0.0003-140.9 0.006-16.5 0.003-7.3 0.06-1.2	0.7 0.1 0.03 0.4
MtBE Distribution in Fuel Markers							
	Marketing terminal and trucking personnel.	< 30 min 30 min-6 h 6-9 h TWA >9 h TWA	93/129 9/10 62/87 46/47	0.0-14.0 0.3-4.1 0.01-2.2 0.1-6.2	0.8 1.0 0.1 0.7	0-3.88 0.08-1.14 0.003-0.61 0.03-1.72	0.22 0.280 0.03 0.19

[a] Adapted from API (1995; cited by HEI 1996) TWA = Time Weighted Average

2.6 Environmental Fate

2.6.1 Air

MtBE is inevitably released into the atmosphere during its production, transportation, and use. The atmospheric half-life of MtBE in a regional airshed is about three days. The determining factor of half-life is the concentration of atmospheric hydroxyl radical (OH) because it reacts

with and degrades MtBE. (Wallington *et al.* 1988) reported the reaction of MtBE [$(CH_3)_3C(OCH_3)$] with (OH) is the main atmospheric reaction. Tertiary-butyl formate, $(CH_3)_3COCHO$, is the major degradation product. $2(CH_3)_3C(OCH_3) + 2OH$ -----> $2(CH_3)_3COCHO + 3H_2$. Other products include methyl acetate $CH_3CO(OCH_3)$, acetone (CH_3COCH_3), tertiary-butyl alcohol $(CH_3)_3COH$, and formaldehyde (HCHO).

Urban areas, however, have lower levels of OH than do rural areas. As a result, MtBE remains longer in city air which would result in a longer half-life (Squillace *et al.* 1997).

2.6.2 Surface Water and Storm Runoff

MtBE enters streams mainly via storm water runoff and groundwater discharge. Direct spills of gasoline can also add to MtBE concentrations. Fuel-use patterns, weather, and unpredicted spills are some of the factors affecting MtBE concentrations in streams and rivers. Volatilization is the main way MtBE is depleted from flowing water. Table 2-8 summarizes estimated half-lives.

Table 2-8. Estimated half-life of MtBE in a stream or river[a]

Water Depth (m)	Water temp. (°C)	Half-life (in days) for each water velocity (m/s)				
		0.032	0.1	0.32	1.0	3.2
0.1	5	0.17	0.14	0.12	0.11	0.10
0.32	5	0.72	0.54	0.43	0.37	0.34
1.0	5	3.3	2.3	1.7	1.4	1.2
3.2	5	16	11	7.2	5.4	4.3
10	5	85	52	35	23	17
0.1	25	0.063	0.042	0.031	0.024	0.021
.32	25	0.32	0.20	0.13	0.10	0.077
1.0	25	1.7	1.0	0.63	0.42	0.31
3.2	25	8.9	5.2	3.2	2.0	1.3
10	25	49	28	16	10	6.3

[a] River or stream under calm air conditions

Squillace *et al*. (1997)

Volatilization of MtBE is affected by the rate of transport from water to air/water interface and the subsequent rate of transport from air/water interface into air. Factors affecting these rates include wind speed, air temperature, water velocity, depth of river, and water temperature. MtBE half-lives are longest in deep, slow moving, cold streams and rivers. Warm, shallow, and fast moving streams dissipate MtBE more rapidly. Calculations for MtBE half-lives in windy conditions are very similar to those for calm air conditions (Squillace *et al*. 1997).

2.6.3 Ground Water

MtBE enters groundwater via point and non-point sources.. Point sources are discharges from a particular location such as spills, both industrial and accidental, or releases from storage tanks and UTSs. Because MtBE is weakly absorbed into the soil and aquifer maters, it will move with the groundwater flow, away from point sources of contamination. Non-point sources are associated more with land uses and are more extensive. Examples include agricultural runoff, urban runoff, automotive emissions, and atmospheric deposition (NSTC 1997).

Water percolation is the primary mechanism of MtBE shallow groundwater infiltration. Air concentrations are consistently higher than groundwater concentrations, even shallow groundwater, because MtBE evaporates rapidly when exposed to the atmosphere (Brown 1997). Squillace *et al.* (1997) estimated that it would take less than 10-15 years for shallow groundwater levels to reach surface air concentrations (assuming a water table depth of 5 m, and an infiltration rate of 10 s/cm). Groundwater concentrations are, however, elevated around certain sources where the air concentrations of MtBE are high, such as parking garages, gas stations, and roadways. Precipitation around these areas would have higher concentrations of MtBE, which would then percolate into the groundwater.

MtBE in groundwater biodegrades very slowly. MtBE is also resistant to microbial attack. Aerobic microcosms do not degrade MtBE after 100 days of incubation and degradation ceases when MtBE levels reach 1 mg/L. Some success has been attained using anaerobic and methanogenic bacterial agents for bioremediation. Point sources are usually treated with pumping followed by above-ground treatment in the form of soil vapor extraction (SVE). SVE is used to increase air flow and vacuum volatile hydrocarbons. MtBE removal is complicated by MtBE's high water solubility. Heating water may be an effective way of removing MtBE. Hydrogen peroxide may also be used (with iron as a catalyst) to remove MtBE, but this approach is ineffective in aerobic or near-neutral (pH > 6.5) to alkaline environments (Squillace *et al.* 1997).

2.7 Regulations

The U.S. EPA has not established a standard for MtBE contamination of drinking water. EPA's Human Health and Criteria Division (HECD) of the Office of Water has recently updated an advisory for MtBE in drinking water. To protect consumers from toxic effects, the advisory recommended keeping levels of contamination below the range of 20-40 μg/L for MtBE (U.S. EPA 1997; http://www.epa.gov/ostwater/drinking/mtbe.html).

Table 2-9. Regulations

EPA Regulations	
PART 63 SUBPART R–National Emission Standards for Gasoline Distribution Facilities (Bulk Gasoline Terminals and Pipeline Breakout Stations). Promulgated: 62 FR	For bulk gasoline terminals and pipeline breakout stations that handle reformulated or oxygenated gasoline containing 7.6 percent by volume or greater MtBE.

EPA Regulations	
9092, 02/28/97.	
PART 80 SUBPART D–Reformulated Gasoline. Promulgated: 59 FR 7813, 02/16/94.	Gasoline that complies with one of the standards specified in 80.41 (a) through (f) that is relevant for the gasoline, and that meets all other relevant requirements prescribed under 80.41, shall be deemed certified. MtBE levels are strictly enforced.
PART 86 SUBPART R–General Provisions for the Voluntary National Low Emission Vehicle Program for Light-Duty Vehicles and Light-Duty Trucks. Promulgated: 62 FR 31242, 06/06/97.	Fuel specifications for light-duty vehicles and light-duty trucks. MtBE concentration will fall between 10.8-11.2 percent volume.
PART 372–TOXIC CHEMICAL RELEASE REPORTING: COMMUNITY RIGHT-TO-KNOW. Promulgated: 53 FR 4525, 02/16/88. U.S. Codes: 42 U.S.C. 11023 and 11048.	The information collected under this part is intended to inform the general public and the communities surrounding covered facilities about releases of MtBE; to assist research, and to aid in the development of regulations, guidelines, and standards.
PART 716–HEALTH AND SAFETY DATA REPORTING. Promulgated: 51 FR 32726, 08/15/86.	This subpart sets forth requirements for the submission of lists and copies of health and safety studies on MtBE since it was selected for priority consideration for testing rules under section 4(a) of the Toxic Substances Control Act (TSCA).
PART 799–IDENTIFICATION OF SPECIFIC CHEMICAL SUBSTANCE AND MIXTURE TESTING REQUIREMENTS. Promulgated: 49 FR 39817, 10/10/84. U.S. Codes: 15 U.S.C. 2603, 2611, 2625.	This part identifies MtBE as a substance for which data are to be developed, specifies the persons required to test (manufacturers, including importers, and/ or processors), prescribes the tests that are required including the test standards, and provides deadlines for the submission of reports and data to EPA.

3 Human Studies

Methyl tertiary-butyl ether (MtBE) studies in humans have primarily addressed blood concentrations and kinetics of MtBE and its metabolite, tertiary butyl alcohol. Tests were performed with the cooperation of healthy volunteers, commuters, and workers exposed to motor vehicle exhaust or gasoline fumes (Hutcheon *et al*. 1996).

Acute toxicity findings have been reported after incidental/accidental exposures to gasoline and additives, including MtBE (Reese and Kimbrough 1993). Further, the toxicokinetics and acute affects of MtBE have been studied in male volunteers (Johanson *et al*. 1995). MtBE has been used, as mentioned in Section 2.1, to dissolve cholesterol gallstones (Hutcheon *et al*. 1996; Pauletzki *et al*. 1995). None of these studies reported data on chronic toxicity or any particular cancer. Therefore, it is concluded that, to date, there are no known human studies that examine any potential associations between MtBE exposure and cancer.

4 Studies of Cancer in Experimental Animals

The data-base of toxicology and carcinogenesis studies of methyl tertiary-butyl ether (MtBE) includes inhalation studies in male and female rats and mice (Bird *et al*. 1997) and a study in which MtBE was administered in olive oil by gavage (Belpoggi *et al*. 1995). Relevant carcinogenesis studies on the metabolite, tertiary-butyl alcohol (TBA), were also conducted in rats and mice by administering MtBE in drinking water (Cirvello *et al*. 1995). These studies are discussed further in Section 6.

4.1 MtBE Administered by Inhalation

4.1.1 MtBE Inhalation Studies in Rats (Bird et al*. 1997)*

Groups of 50 male and 50 female Fischer 344 rats, aged six to seven weeks, were exposed by inhalation, to 0, 400, 3000 or 8000 ppm of MtBE vapor (>99% pure) in air for up to 24 months (6 h/d, 5 d/wk). Food and water were available *ad libitum* except during the six-hour exposure periods. Chamber concentrations were monitored periodically (specific times not given), and mean chamber concentrations of MtBE (±Std. Dev.) during the study were stated to have been 0, 403±8.4, 3023±56.9, and 7977±116.7 ppm.

Animals were housed individually in stainless steel cages. Experimental parameters measured included body weight (weekly through week 13 then biweekly); hematology and serum chemistry, on both high-dose and control animals (at 12 months and at study termination); and urinalysis for weeks 79 and 80. Because of reduced survival, male rats from the 3000 and 8000 ppm groups were sacrificed at weeks 97 and 82, respectively. Surviving control and low-dose males, as well as all surviving females, were sacrificed after 24 months.

Each animal was subjected to complete necropsy. Upon necropsy, organ weights were determined for the brain, liver, kidneys, lungs, spleen, adrenal glands, and testes. Organs, tissues, and lesions were examined microscopically. Tissues examined from high-dose and control animals included all observed gross lesions, tumors, and numerous organs. The liver, kidneys, testes, and gross lesions in low- and mid-dose males, and the liver and gross lesions of low- and mid-dose females were also examined microscopically. Tumor incidence data were analyzed using the Fisher's Exact Test, a method that does not adjust for differences in survival between the control and exposure groups.

Exposure to 8000 ppm MtBE caused reductions in absolute body weight and body weight gains by both males and females. By week 82, when the high-dose male group was terminated because of toxicity and excessive premature deaths, the mean absolute body weight of high-dose males was decreased by 19% relative to controls. Absolute body weights and body weight gains by males of the 3000 ppm group were not significantly different from controls despite the fact that MtBE produced sufficient toxicity and mortality to warrant terminating that group at week 97. In high-dose females that survived to the scheduled 105 week study termination, mean absolute body weight was decreased by 26% relative to controls. Neither the 400 nor the 3000 ppm exposure regimens affected body weights or body weight gains in females.

Exposure to both 3000 and 8000 ppm MtBE decreased survival of males and resulted in early termination of those groups. The rats of the 3000 ppm group were terminated at week 97 when 88% of the animals had died, and the rats of the 8000 ppm group were terminated at week 82 when 82% had died. Chronic, progressive nephropathy was determined to be the primary cause of the early deaths among dosed males. The mortality rates for dosed female rats were not significantly affected by MtBE. The incidence of advanced, chronic, progressive nephrosis was somewhat greater in dosed females that died during the study than in control females that also died before the scheduled termination of the study. The authors suggested that the maximum tolerated dose was exceeded in male rats exposed to 8000 ppm MtBE (Bird *et al*. 1997).

Organ weight analysis was not conducted for mid- and high-dose males since these groups were terminated early. The mean relative (to body weight) kidney weights of mid- and high-dose females were increased (18% and 29%, respectively) and absolute and relative liver weights were also increased in these groups.

Microscopic examination of kidneys confirmed an exposure-related increase in incidence and severity of chronic progressive nephropathy in males from all dosed groups, and to a lesser degree, in females from the 3000 and 8000 ppm groups.

Exposure of males to 3000 and 8000 ppm of MtBE was associated with increased incidences of renal tubular cell adenomas and carcinomas in the mid-dose group (Table 4-1). The incidence of combined tumors in the mid-dose group was significantly elevated relative to controls. Among female rats, one renal tubular cell adenoma was observed in the 3000 ppm exposure group (data not shown).

Table 4-1. Incidence of renal tumors in male F344 rats exposed to MtBE for up to 104 weeks

	MtBE Target Concentration (ppm)			
	Control	400	3000	8000
No. animals examined	50	50	50	50
Renal tubular Adenoma	1	0	5	3
Renal tubular Carcinoma	0	0	3	0
Adenoma/ Carcinoma combined	1	0	8[a]	3

[a]P<0.05 by Fisher's Exact Test

Bird *et al*. (1997)

An exposure-related increase in incidence of interstitial cell adenoma of the testes was observed, This incidence was significantly increased ($p<0.05$) in the 3000 and 8000 ppm groups (see Table 4-2). The incidence of interstitial cell adenomas of the testes in control rats was similar to the historical control rate for National Toxicology Program (NTP) inhalation studies in F344 rats (Nyska *et al.* 1998).

Table 4-2. Incidence of Leydig cell tumors of the testes in F344 rats exposed to MtBE for up to 104 weeks

	MtBE Target Concentration (ppm)			
	Control	400	3000	8000
No. animals examined	50	50	50	50
Interstitial cell Adenoma	32 (64%)	35 (70%)	41[a] (82%)	47[a] (94%)

[a]P<0.05 by Fisher's Exact Test

Bird *et al*. (1997)

4.1.2 Inhalation Studies in Mice (Bird et al*. 1997)*

Groups of 50 male and 50 female CD-1 mice (age not specified) were exposed by inhalation, to 0, 400, 3000, or 8000 ppm of MtBE vapor (>99% pure) in air for up to 18 months (6 h/d, 5 d/wk). Food and water were available *ad libitum* except during the six-hour exposure periods. Chamber concentrations were monitored periodically (specific times not given) and mean chamber concentrations of MtBE (±Std. Dev.) during the study were stated to have been 0, 403 ± 8.4, 3023 ± 56.9, and 7977 ± 116.7 ppm.

Animals were individually housed in stainless steel cages. Experimental parameters measured included body weight, water consumption (control and high-dose for 15 hours during weeks 50 and 51), hematology, serum chemistry, and urinalysis on high-dose and controls (at weeks 50, 51, and study termination). At study termination (18 months), all surviving animals were sacrificed.

At death, each animal was subjected to complete necropsy. At terminal sacrifice, bone marrow smears were prepared from ten randomly selected animals per group, and organ weights were determined for brain, liver, kidneys, lungs, spleen, adrenal glands, and testes. Organs, tissues, and observed lesions were preserved and examined microscopically. For high-dose and control mice all lesions, observed tumors, and 47 tissues and organs were collected for histopathological evaluation. In addition the liver, spleen, and submandibular lymph nodes of males and the liver, uterus, and stomach of females of the 400 and 3000 ppm groups were examined.

Absolute mean body weights of female mice exposed to 8000 ppm were significantly lower (7%) than those of the controls. Male mice, exposed to 8000 ppm of MtBE, had reduced survival relative to concurrent controls. The study was terminated at 18 months (as originally planned) when 49% of the high-dose males had died as compared to 33% in the control group. Reduced survival of high-dose males was attributed to increased incidence of obstructive uropathy in the high-dose group. The survival of dosed females was unaffected by MtBE.

Mean relative liver weights were increased in a dose-related fashion in male mice, but these differences were not statistically significant. Increases in relative liver weights in females dosed with 3000 or 8000 ppm (9% and 39%, respectively) were statistically significant ($p < 0.01$). Mean relative kidney weights were significantly increased in all exposed male groups and in the 8000 ppm females.

Non-neoplastic lesions were observed in sections of the livers and kidneys of exposed mice of both sexes. Kidneys had high incidences of tubular proteinosis, amyloidosis, and interstitial nephritis in both sexes and lymphoid infiltrates in males. These lesions were distributed among mice of dosed and control groups and were considered incidental. Although the method of grading was not described, the lesions were considered more severe in exposed than in control animals. No treatment-related tumors of the kidney were observed in mice of either sex. Hepatocellular hypertrophy was increased in both sexes of mice. There were no increases in hepatocellular degeneration or necrosis in exposed mice. The incidences of cystic endometrial cell hyperplasia was decreased in the 3000 ppm and 8000 ppm female mice.

Incidences of significantly increased hepatocellular neoplasms and related non-neoplastic changes associated with MtBE administration are summarized in Table 4-3. The only neoplasm with a statistically significant increased incidence was hepatocellular adenoma in high-dose females. The incidence of hepatocellular carcinoma in female mice was unaffected by MtBE. In male mice, the incidence of hepatocellular carcinoma was higher in the high-dose group; this increase was not significant by the Fisher's Exact Test. However, this method of analysis does not adjust for differences in survival between control and exposure groups. The combined incidence of adenomas and carcinomas was not significantly increased in the high exposure group of males. Because the duration of this study was only 18 months, it is not possible to determine whether MtBE induces late developing tumors in the low- or mid-exposure groups in mice, or whether more hepatocellular adenomas would have progressed to carcinomas (NSTC 1997).

Table 4-3. Liver tumors in CD-1 mice dosed for up to 18 months with MtBE

Lesions	MtBE Target Concentration (ppm)			
	Control	400	3000	8000
Females				
No. animals examined	50	50	50	50
Hepatocellular Adenoma	2	1	2	10[a]
Hepatocellular Carcinoma	0	1	0	1
Adenoma/Carcinoma combined	2	2	2	11[a]
Males				
No. animals examined	49	50	50	49
Hepatocellular Adenoma	11	11	9	12
Hepatocellular Carcinoma	2	4	3	8
Adenoma/Carcinoma combined	12	12	12	16

[a]P<0.01 by Fisher's Exact Test

Bird *et al.* (1997)

4.2 Orally Administered MtBE

4.2.1 Rats (Belpoggi et al. 1995)

Groups of 60 male and 60 female Sprague-Dawley rats, eight weeks of age, were administered MtBE (>99% pure) in olive oil by gavage. Doses of 0, 250, or 1000 mg/kg, were administered four days per week for 104 weeks. Upon cessation of dosing, when rats were 112 weeks of age, animals were kept under observation until natural death.

Experimental parameters included food and water consumption and individual animal body weights. At death, each animal was subjected to complete necropsy with organs and tissues being preserved for microscopic examination. Histopathological evaluation was performed on samples from 35 tissues and organs and on any gross lesions.

Orally administered MtBE had no effect on food or water consumption and there were no variations in body weight gains in dosed animals of either sex. Dysplastic proliferation of lymphoreticular tissue was increased in treated female rats (1 control, 15 low-dose, 9 high-dose).

Survival of all male groups was similar through experimental week 80. Thereafter, survival of the high-dose males was greater than that of controls or low-dose animals until week 136. At week 104, survival of male rats was 30% in the control and 250 mg/kg groups and 45% in the 1000 mg/kg group. In females MtBE caused a dose-related decrease in survival with differences between dosed and control animals obvious after 56 weeks when approximately 98% of the controls were alive and about 85 and 78% of the low- and high-dose animals, respectively, were alive. At week 104, survival of female rats was 50%, 38%, and 30% in the control, low-dose, and high-dose groups, respectively. The causes of early deaths in dosed females were not determined. Statistical methods that adjust for intercurrent mortality were used for the analysis of tumor incidence: Incidental Tumor Test for non-lethal tumors (Leydig cell testicular tumors) and the Life Table Test for lethal tumors (lymphomas and leukemias).

The oral administration of 1.0 g/kg of MtBE to male rats was associated with an increased incidence of Leydig cell tumors of the testes (Table 4-4).

Table 4-4. Incidence of Leydig cell tumors of the testes in Sprague-Dawley rats dosed for up to two years with MtBE by olive oil gavage

Oral Gavage Dose of MtBE (mg/kg)			
	Control	250	1000
No. animals examined	60	60	60
Leydig cell tumors	2 (3.3%)	2 (3.3%)	11[a] (18.3)

[a] P<0.05 by Incidental Tumor Test

Belpoggi *et al.* (1995)

In female Sprague-Dawley rats the administration of MtBE caused a dose-related and statistically significant increase in the incidence of combined lymphomas and leukemias (Table 4-5).

Table 4-5. Incidence of Lymphomas and Leukemias (combined) in female Sprague-Dawley rats dosed for up to two years with MtBE by olive oil gavage

Oral Gavage Dose of MtBE (mg/kg)			
	Control	250	1000
No. animals examined	60	60	60
Lymphoma/ Leukemia combined	2 (3.3%)	6 (10%)	12[a] (20%)
Dyspastic proliferation of lymphoreticular tissue	1	15	9

[a]P<0.01 by Life Table Test

Belpoggi *et al.* (1995)

4.3 Summary of Carcinogenicity Studies

Chronic inhalation of 3000 ppm of MtBE caused an increased incidence of renal tubular cell neoplasms in male Fischer 344 rats. The absence of a significant increased incidence of renal tubular cell tumors in male rats exposed to 8000 ppm was possibly due to decreased survival in that group. Chronic inhalation exposure of rats to MtBE was also associated with increases in the incidence of testicular interstitial cell neoplasms. There were no increases in neoplasms in any organ of similarly exposed female rats.

Chronic inhalation exposure of CD-1 mice to 8000 ppm of MtBE caused a statistically significant increase in the incidence of hepatocellular adenomas in female mice. There was not a concomitant increase in the incidence of hepatocellular carcinomas. There were no statistically significant increases in the incidences of neoplasms in male mice; however, the incidence of hepatacellular carcinomas was greater in high dose males than in controls.

Chronic, oral administration of 1.0 g/kg of MtBE as a corn oil gavage, four days per week, was associated with an increased incidence of Leydig (interstitial) cell tumors of the testes in Sprague-Dawley rats. Chronic, oral administration of 250 mg/kg or 1.0 g/kg doses of MtBE caused increased incidences of lymphomas and leukemias (combined) in female Sprague Dawley rats. A follow-up characterization of the lymphomas and leukemias induced by MtBE in female rats has shown that this reponse was due mostly to increases in the incidence of immunoblastic lymphomas (Maltoni *et al.* 1998).

5 Genotoxicity

5.1 Prokaryotic Systems

5.1.1 Induction of Reverse-Mutation in Salmonella typhimurium

Methyl tertiary-butyl ether (MtBE) was tested at seven dose levels of 30 to 7400 μg/tube in *Salmonella typhimurium* tester strains TA98, TA100, TA104, and TA1535, with and without the addition of metabolic enzymes (rat liver S9) at four concentrations (0, 300, 600, and 1200 μg S9/mL final concentration). A closed system was used to minimize the loss of MtBE. No convincing evidence of genotoxic potential was found; the maximum tested concentration was clearly bactericidal (Kado *et al*. 1998).

5.2 Lower Eukaryotic Systems

5.2.1 Induction of Sex-linked Recessive Lethal Mutations in Drosophila melanogaster

The *Drosophila* sex-linked recessive lethal (SLRL) test is an assay for lethal mutations which uses male flies that were briefly treated with MtBE and were mated to Basc strain females which carry multiple-inverted X-chromosomes. MtBE was administered in the food at levels from 0.01 to 0.3%. MtBE did not induce sex-linked recessive mutations in *Drosophila* at any treatment level, but showed toxicity at the highest concentrations (McKee *et al*. 1997).

5.3 Mammalian Systems *In Vitro*

5.3.1 DNA Adduct Formation in Isolated Hepatocytes of Rodents

MtBE is metabolized into formaldehyde (HCHO), a potentially mutagenic intermediate capable of forming DNA-protein cross-links (DPX) and RNA-formaldehyde adducts (RFA). Freshly isolated hepatocytes from CD-1 mice incubated with MtBE-(O-methyl-^{14}C) showed that DPX and RFA were detected, but the adduct yields were very small (~ 1 picomole/mg nucleic acid) and independent of MtBE in the hepatocyte suspension over a wide concentration range (0.33-6.75 mM). Similar results were obtained using hepatocytes from male B6C3F1 mice and male F-344 rats. Induction of cytochrome P450 by pretreatment of mice with MtBE prior to isolation of hepatocytes did not result in a measurable increase in the yields of either DPX or RFA. In contrast, there was a marked, concentration-dependent increase in the yields of both DPX and RFA when [^{14}C]formaldehyde was added directly to the medium (Casanova and Heck 1997).

5.3.2 Induction of Gene Mutations in Mouse Lymphoma Cells in Culture

MtBE was shown to be mutagenic in an *in vitro* gene mutation assay using mouse lymphoma cells when tested in the presence, but not in the absence, of rat liver S9. In this study, MtBE was tested to determine if its metabolite, formaldehyde, in the presence of S9, was responsible for the observed mutagenicity. Mouse lymphoma strain L5178Y tk$^{+/-}$ cells were dosed over a range of 1-4 μl/mL. MtBE induced a dose-related increase of mutant frequency, while reducing total growth to near zero, at the highest concentration, as shown in Table 5-1. To determine the effect of formaldehyde dehydrogenase (FDH) on the mutagenicity and cytotoxicity of MtBE, a second lymphoma assay was run with the same concentrations of MtBE in the presence of S9 mix, with and without excess FDH and its co-factor (NAD^+), as shown in Table 5-2. These results confirm the previous findings that formaldehyde, derived from MtBE, is responsible for the mutagenicity of MtBE in the presence of rat liver S9 (Mackerer *et al*. 1996).

Table 5-1. Mutagenicity of MtBE in the activated mouse lymphoma L5178Y tk $^{+/-}$ assay

Test article	Conc.[a]	% Total mutant growth[b]	Frequency[c]
MtBE	4.0	0.3	4.00
	3.0	8.4	2.49
	2.0	25.5	2.01
	1.0	47.8	1.57
Untreated	-	102.5	0.18
	-	97.8	0.26
Formalin[d]	0.065	39.7	1.23
DMBA[e]	5.0	2.6	4.45
	2.5	49.3	1.98

[a] Concentrations are µl/mL culture medium for MTBE and formalin and µg/mL culture medium for DMBA.
[b] % Total Growth = (Relative Suspension Growth) x (Relative Cloning Efficiency) - 100.
[c] Mutant Frequency = (Mean No. of TFT Colonies per Dish)/(Mean Number of VC Colonies per Dish) x (2×10^{-4}).
[d] Formalin is a 37% solution of formaldehyde in water, stabilized with 10% methanol.
[e] DMBA is 7,12-dimethylbenzanthracene, a compound which is converted to a mutagenic form by the S9 activation system. It is used as positive control.
(Mackerer *et al.* 1996)

Table 5-2. Mutagenicity of MtBE in the activated mouse lymphoma L5178Y $tk^{+/-}$ assay with and without FDH

Test article	Conc. of medium (µl/mL)	FDH[a]	% Total growth[b]	Mutant frequency[c]
MtBE	4.0	-	9.2	1.97
	3.0	-	24.7	1.74
	2.0	-	48.0	1.45
	1.0	-	61.1	0.96
	4.0	+	73.9	0.19
	3.0	+	98.9	0.16
	2.0	+	103.3	0.17
	1.0	+	117.7	0.11
HMPA [d]	25.0	-	18.8	0.36
	22.5	-	31.9	0.32
	25.0	+	22.0	0.20
	22.5	+	26.5	0.20
Formalin [e]	0.065	-	55.2	0.98
	0.065	+	94.2	0.27
Control	-	-	100.0	0.16
	-	+	100.0	0.15

[a]FDH and NAD^+ were present (+) or absent (-) in the treatment medium.

[b] % Total Growth = (Relative Suspension Growth) x (Relative Cloning Efficiency) 100.

[c] Mutant Frequency = (Mean Number of TFT Colonies per Dish)/(Mean Number VC Colonies per Dish) x (2 x 10^{-4}).

[d] HMPA used as a positive control. It requires metabolic activation to release formaldehyde and is mutagenic to lymphoma cells in the activated assay.

[e] Formalin is a 37% solution of formaldehyde in water, stabilized with 10% methanol.

5.4 Mammalian Systems *In vivo*

5.4.1 Induction of DNA Repair (Unscheduled DNA Synthesis) in Hepatocytes of Rodents

Groups of ten male and ten female CD-1 mice were exposed to MtBE by inhalation six hours a day for two consecutive days. The target concentrations were 400, 3000, and 8000 ppm. The animals were then sacrificed 16 hours after the second exposure and the hepatocytes were then prepared. The data showed that MtBE exposure did not result in any increase in unscheduled DNA repair in hepatocytes of male or female CD-1 mice (McKee *et al*. 1997).

5.4.2 Induction of Micronuclei in the Bone Marrow of Rodents

MtBE was tested in Swiss-Webster mice (five males, five females per group). The mice were administered single interperitoneal injections of MtBE in olive oil at five doses ranging from 0.25 to 1.75 g/kg and were sacrificed 24 hours later. There were no significant treatment-related increases in micronucleus frequency (Kado *et al*. 1998).

Male and female CD-1 mice were exposed to MtBE by inhalation for six hours a day for five consecutive days. The target concentrations were 800, 3000, and 8000 ppm (McKee *et al*. 1997). The animals were then sacrificed 24 to 48 hours after the final treatment. The results indicated that the treatment did not result in the production of significant increases in micronucleus frequency; MtBE was not considered to be clastogenic in this assay system.

5.4.3 Induction of Chromosome Aberrations in the Bone Marrow of Rodents

Groups of five male and five female F-344 rats were exposed to MtBE by inhalation for six hours a day for five consecutive days. The target concentrations were 800, 4000, and 8000 ppm. The animals were then sacrificed either six or 24 hours after the final treatment. There were no significant treatment-related increases in chromosome aberrations (McKee *et al*. 1997).

6 Other Data Relevant to Assessment of the Carcinogenic Potential of MtBE for Humans

6.1 Summaries by Reviewing Body

Several critical reviews of methyl tertiary-butyl ether (MtBE) carcinogenesis studies have been reported and are summarized by reviewing body:

6.1.1 Office of Science and Technology Policy

The United States Office of Science and Technology Policy sponsored an Interagency Assessment of Oxygenated Fuels which included review and interpretation of MtBE carcinogenicity studies (NSTC 1997).

1. This review group noted there were no epidemiological studies on the carcinogenic potential of MtBE in humans.

2. Studies in rats and mice show that MtBE is carcinogenic at multiple organ sites (male rat testes and kidney, female rat hematopoietic system, and mouse liver) after administration by inhalation or oral gavage.

3. The group concluded that the data constituted sufficient evidence indicating that MtBE is an animal carcinogen and it should be regarded as having a human hazard potential.

6.1.2 National Research Council (NRC)

The NRC convened a Committee on the Toxicological and Performance Aspects of Oxygenated Motor Vehicle Fuels (NRC 1996). They reviewed the conduct and interpretation of the three carcinogenesis studies described in Section 4. A brief synopsis of their conclusions and recommendations follows:

1. The committee decided that the male rat kidney tumor data should not be used to estimate cancer potency because its probable causation, α_{2u}-globulin nephropathy, is not thought to be relevant to humans. When this opinion was rendered, the α_{2u}-globulin mechanism had been proposed, but without supportive evidence.

2. The use of lymphoma and leukemia data (Belpoggi *et al*. 1995) should be questioned until a thorough third party review of the study is done .

3. The most reliable data available for risk assessment relates to the induction of benign liver tumors in female mice exposed to 8000 ppm of MtBE by inhalation. The committee cautioned that, although this amounts to weak evidence of carcinogenicity, it should not be discounted.

6.2 Toxicokinetics and Metabolism of MtBE

6.2.1 Studies in Rats (Miller et al*. 1997)*

6.2.1.1 Absorption

MtBE is rapidly and completely absorbed from the gastrointestinal and respiratory tracts. It is slowly absorbed via the dermal route.

Miller *et al*. (1997) reported that areas under the curve for plasma MtBE were nearly identical after intravenous and oral administration of 40 mg/kg to Fischer 344 rats. Maximum plasma concentration was achieved 15 minutes after the oral (gavage) dose. During inhalational administration (400 or 8000 ppm, 6h) peak plasma concentrations of MtBE were achieved at four to six hours. Peak plasma concentrations, after exposure to 8000 ppm, deviated somewhat from linearity. Observed levels were about 75% higher than would be predicted based on peak concentration after the 400 ppm exposure.

MtBE is cleared from the plasma by exhalation and by oxidative metabolism to equimolar amounts of tertiary butyl alcohol (TBA) and formaldehyde in the liver. The lack of linearity in achieved peak plasma concentrations after MtBE inhalation may reflect saturation of the implicated metabolic pathway at the higher concentrations. In humans, the area under the plasma concentration curve (AUC) (from $t=t\rightarrow\infty$) for MtBE and TBA increased linearly with two-hour exposures to up to 50 ppm (Johanson *et al*. 1995; Nihlen *et al*. 1998a). MtBE applied to the skin, relative to inhalation, was absorbed more slowly and less completely with peak concentrations achieved after two to four hours. Dermal absorption was only 16% after application of 40 mg/kg and it was 34% after the 400 mg/kg dose.

6.2.1.2 Plasma Pharmacokinetics

In Fischer 344 rats, MtBE was cleared from plasma by expiration of the parent molecule and by its metabolism to TBA. TBA was rapidly formed with peak concentrations achieved within one to four hours after oral, intravenous, or dermal administration and within six hours of inhalational dosing. Plasma concentrations of MtBE in rats declined rapidly with a half-life of about 0.5 hours. Plasma concentrations of TBA in rats declined more slowly exhibiting plasma half-life values of about one to three hours. In humans, the kinetic profile of MtBE in blood was characterized by four phases with average half-lives of 1 min., 10 min., 1.5 h, and 19 h; the half life of TBA in human blood was 10 h (Johanson *et al*. 1995; Nihlen *et al*. 1998a).

At higher MtBE exposures (*i.e.*, 8000 ppm vs. 400 ppm by inhalation or 400 mg/kg vs. 40 mg/kg orally), the C_{max} for plasma TBA in male rats was less than proportional to the concentration of MtBE. This was considered as evidence that metabolic conversion of MtBE to TBA was saturated by administration of the higher MtBE doses.

Selected pharmacokinetic parameters for MtBE and TBA, in rats, are summarized in Table 6-1.

Table 6-1. Pharmacokinetic parameters for MtBE and TBA computed for male Fischer 344 rats dosed with MtBE by intravenous, oral, or inhalation routes

	MtBE		TBA	
Exposure	$AUC_{(0-\infty)}$ (ug h mL^{-1})	Plasma $t_{1/2}$ (h)	$AUC_{(0-\infty)}$ (ug h mL^{-1})	Plasma $t_{1/2}$ (h)
Intravenous				
40 mg/kg	10.7	0.45	26.7	0.92
Oral				
40 mg/kg	17	0.52	39	0.95
400 mg/kg	230	0.79	304	1.6
Inhalation				
400 ppm	84.3	0.52	404	3.3
8000 ppm	2960	0.57	6010	3.4

Miller *et al*. (1997)

6.2.1.3 Disposition of Administered MtBE

After intravenous administration of ^{14}C-MtBE to rats, radioactivity was recovered in exhaled air (60%) and urine (35%) with minor amounts being recovered from feces (2%) and tissues/carcass (0.4%). Most of the dose recovered in exhaled breath (~91%) was eliminated during the first three hours after dosing and was identified as MtBE (97.4%). TBA and CO_2 accounted for only 1% and 1.6% of the ^{14}C in breath after dosing with MtBE. Urinary and fecal elimination of radioactivity (35% and 2% of the dose, respectively) contained neither MtBE nor TBA.

Six hours after inhalation exposure to 8000 ppm MtBE (for six hours), roughly half the administered dose was excreted in the breath. ^{14}C measured was associated with MtBE at a level of 79% and TBA at 21%. About 41% of the inhaled dose appeared in the urine (as metabolites other than TBA). After inhalation exposure to 400 ppm (six-hour exposure), approximately 21% was excreted in the breath (66% as MtBE and 34% as TBA). Renal elimination of metabolites (other than TBA) accounted for 65% of the dose. Seven days after exposure, an additional 13% of the dose was recovered from tissues and carcass.

Data were not presented regarding disposition of MtBE and metabolites after oral administrations.

6.2.1.4 Metabolites of MtBE Excreted in the Urine

Miller *et al*. (1997) reported the detection of four metabolites in the urine of rats administered MtBE by the inhalation route. The major metabolite, comigrated with synthetic α-hydroxyisobutyric acid, and accounted for 70% of the total MtBE associated metabolites excreted in the urine. A second metabolite, identified as 2-methyl-propane-1,2 diol accounted for 14% of the urinary metabolites. Two additional, unidentified metabolites accounted for 15% of those in the urine. Neither MtBE, nor TBA, were detectable in the urine.

The experimental observations are consistent with the following bio-transformation steps for MtBE in rats:

1. MtBE undergoes oxidative demethylation to form TBA (the major circulating MtBE metabolite) and formaldehyde (which has never been detected *in vivo* but was demonstrated in *in vitro* studies). This metabolic step is likely carried out by cytochrome P-450-mediated oxidative pathways. MtBE is known to induce cytochrome P-450 enzymes *in vitro* (Brady *et al*. 1990), hence, repeated administrations of MtBE would be expected to induce MtBE metabolism.

2. Formaldehyde is thought to be metabolized to methanol and acetone (Brady *et al*. 1990) which explains why it has not been identified in *in vivo* metabolism experiments with MtBE.

3. TBA undergoes further oxidative metabolism to 2-methyl-1,2-propanediol and α-hydroxyisobutyric acid; the major urinary metabolites of MtBE (and TBA).

6.2.1.5 Metabolism of MtBE at Extrahepatic Sites

MtBE metabolism by rat liver microsomes has been demonstrated (Brady *et al*. 1990). Hong *et al*. (1997) reported that both mouse and human livers are active in metabolizing MtBE to TBA. Recently, Hong *et al*. (1997) reported that the olfactory mucosa from Sprague-Dawley rats is highly proficient at metabolizing MtBE. The metabolic activity of the olfactory mucosa was 46-fold higher than that of the liver. No metabolic activity was detected in microsomes isolated from lung, kidney, or olfactory bulbs of the brain. The metabolic activity of rat olfactory mucosa was shown to be nicotinamide adenine dinucleotive phosphate (NADPH) dependent and inhibitable by carbon monoxide; observations consistent with a role for cytochrome P-450 enzymes.

Given that the metabolism of MtBE to TBA is accompanied by the generation of equimolar amounts of formaldehyde, the potential of the olfactory mucosa to metabolize this chemical is significant. Formaldehyde gas is a demonstrated nasal carcinogen in rats and epidemiological studies suggest a causal relationship between exposure to formaldehyde and nasopharyngeal cancer (IARC 1995). No MtBE-related lesions in the nasal turbinates of inhalationally exposed rats were reported (Bird *et al*. 1997).

6.2.2 Studies Conducted in Humans

Human volunteers were exposed to MtBE (5, 25, or 50 ppm) by inhalation for two hours during light physical exercise (Johanson *et al*. 1995; Nihlen *et al*. 1998b). Respiratory uptake under these conditions was relatively slow (42-49%). Unlike experimental results with rats, the kinetics of MtBE and TBA in humans, as reflected by plasma concentrations, was proportional to exposure concentration. This difference probably reflects the fact that at the high concentrations inhaled by rats the metabolic capacity (MtBE to TBA) was saturated.

Nihlen *et al*. (1998a) reported that MtBE was metabolized to TBA via linear kinetics in humans exposed to up to the 50 ppm concentration, and the plasma half-life of TBA was considerably longer than that of MtBE. The half-life for TBA in blood of humans (ten hours) (Nilhen *et al*. 1998a) is longer than in rats (one to three hours) (Miller *et al*. 1997). The recovery of MtBE and TBA during the 24 hours after exposure was only 33-48% of total dose. The major fraction was

expired as MtBE with less than one percent of the dose being recovered from the urine as MtBE or TBA. These low recoveries of urinary metabolites are consistent with observations from other reports (Prah *et al*. 1994; Buckley *et al*. 1997).

6.3 Carcinogenicity and Genotoxicity of MtBE metabolites

6.3.1 Carcinogenicity Studies of t-butyl alcohol in Male and Female Fischer 344/N Rats (NTP 1995; Cirvello et al. 1995)

Groups of 60 male F-344 rats were given drinking water containing 1.25, 2.5 or 5 mg/mL TBA. Female F-344 rats were given drinking water containing 0, 2.5, 5, or 10 mg/mL TBA. These drinking water concentrations were stated to deliver average daily TBA doses of 85, 195, or 420 mg/kg in males and 175, 330 or 650 mg/kg in females. Animals were maintained on study for up to two years.

The survival rates of high-dose animals of both genders were significantly lower than those of controls. The final mean body weights for dosed males were from 15 to 24% lower than those of controls, and final mean body weight of the high-dose female group exhibited a 21% decrement relative to controls. At an interim sacrifice (15 months), relative kidney weights in the mid- and high-dose male groups were elevated. Relative and absolute kidney weights of all dosed female groups were elevated.

Microscopic assessment of tissues revealed both neoplastic and non-neoplastic lesions in the kidneys of male rats. Non-neoplastic changes included nephropathy, transitional epithelial cell hyperplasia, mineralization, and renal tubular cell hyperplasia. Neoplastic lesions included renal tubular cell adenomas and carcinomas. Both National Toxicology Program (NTP) standard pathological evaluation and extended evaluation (step sections) were used to assess neoplastic changes. The combined data are summarized in Table 6-2.

Table 6-2. Incidences of kidney lesions in male and female F-344/N rats dosed for up to two years with TBA in their drinking water

	Average dose of tertiary-butyl alcohol (mg/kg/d)			
	0	**90**	**200**	**420**
Kidneys Examined	50	50	50	50
Non-neoplastic Lesions				
Nephropathy	(M) 49 (3.0)[a]	(M) 49 (3.1)	(M) 50 (3.1)	(M) 49 (3.3)

	Average dose of tertiary-butyl alcohol (mg/kg/d)			
	0	90	200	420
	(F) 48 (1.6)	(F) 47 (1.9)*	(F) 48 (2.3)**	(F) 50 (2.9)**
Mineralization	(M) 26 (1.0) (F) 49 (2.6)	(M) 28 (1.1) (F) 50 (2.6)	(M) 35 (1.3) (F) 50 (2.7)	(M) 48**(2.2) (F) 50 (2.9)
Linear Mineralization	(M) 0	(M) 5	(M) 24	(M) 46
Transitional epithelium, hyperplasia	(M) 25 (1.7) (F) 0	(M) 32 (1.7) (F) 0	(M) 36**(2.0) (F) 3 (1.0)	(M) 40**(2.1) (F) 17**(1.4)
Tubular cell hyperplasia	(M) 14 (2.1) (F) 0	(M) 20 (2.3) (F) 0	(M) 17 (2.4) (F) 0	(M) 25** (2.8) (F) 1 (1.0)
Neoplastic Lesions				
Tubular cell adenoma	7	7	9	9
Tubular cell adenoma (multiple)	1	4	10**	4
Tubular cell carcinoma	0	2	1	1
Tubular cell adenoma or carcinoma (combined)	8	13	19**	13

Unless otherwise stated values refer to males. (M) - male, (F) - female

[a] Average severity: 1=minimal, 2=mild, 3=moderate, and 4=marked

* $p < 0.05$; ** $p < 0.01$

Cirvello *et al*. (1995)

The severity of nephropathy and the incidence/severity of transitional cell hyperplasia of the kidney were increased in both genders of rats. There was no evidence of a neoplastic effect in females. The NTP noted that the increased incidences of transitional cell hyperplasia might have been related to the presence of renal calculi. The NTP concluded that there was "some evidence of carcinogenicity in male rats but no evidence in females"(1995). The carcinogenic effect of TBA in Fischer 344 rats, administered orally, is nearly identical to that of inhaled MtBE in the same species and strain. Since MtBE is rapidly metabolized to TBA, when administered to F-344 rats by inhalation, the results may indicate that MtBE's renal effects are mediated through TBA. However, unlike TBA, MtBE did not cause transitional cell hyperplasia in male or female F-344 rats. It is possible this difference reflects a dose-related effect associated with the observed production of renal calculi.

6.3.2 Carcinogenicity Studies in Male and Female B6C3F$_1$ Mice

Male and female B6C3F$_1$ mice, in groups of 60, were given drinking water containing 5, 10, or 20 mg/mL of TBA. Average daily doses of TBA delivered to animals were 0, 535, 1035, or 2065 mg/kg for males and 0, 510, 1015, and 2105 mg/kg for females. Survival of the high-dose, male group was significantly lower than that of controls. The mean body weight of the high-dose, female group was 10-15% lower than that of controls from week 13 to the end of the study.

Increased incidences of thyroid gland follicular cell hyperplasia were observed in dosed males and in dosed females. The highest dose of TBA administered to female mice was associated with

a significant increase (p=0.039) in the incidence of thyroid gland follicular cell adenomas (control 2/58, low-dose 3/60, mid-dose 2/59, and high-dose 9/59).

The NTP (1995) concluded that there was "equivocal evidence of carcinogenicity" in male mice, based on a marginally increased incidence of follicular cell adenomas of the thyroid gland in mid-dose animals (controls 1/60 vs. mid-dose 4/59). The NTP (1995) also concluded that there was "some evidence of carcinogenicity" in female mice.

6.3.3 Genotoxicity Studies of TBA (NTP 1995)

TBA was tested for induction of genetic damage, *in vitro* and *in vivo*, and all results were negative (NTP 1995). In *in vitro* experiments (with or without metabolic activation), TBA was negative in *S. typhimurium* and mouse lymphoma cell mutation tests. It caused neither sister chromatid exchanges, nor chromosomal aberrations in cultured Chinese hamster ovary cells. TBA also failed to cause increases in the incidence of micronucleated erythrocytes in mice exposed via drinking water exposure for up to 13 weeks.

6.4 Carcinogenicity and Genotoxicity Studies of Formaldehyde

6.4.1 Carcinogenicity of Formaldehyde (IARC 1995)

The carcinogenicity of the second metabolite of MtBE, formaldehyde, has been reviewed by International Agency for Research on Cancer (IARC) (1995). Formaldehyde causes tumors in the nasal turbinates of rodents when the gas is administered by inhalation. The carcinogenic potential associated with other routes of administration (*i.e*. oral, dermal, and subcutaneous injection) remains questionable. The cancer epidemiology of formaldehyde in humans is suggestive of a causal relationship between exposure and nasopharyngeal cancer.

6.4.2 Genotoxicity of Formaldehyde

The genotoxic potential of formaldehyde has been studied widely and was reviewed by IARC (1995). The National Science and Toxicology Council (NSTC) (1997) stated that formaldehyde produced positive results when tested for genetic toxicity. It produced DNA strand breaks and mutations in bacteria, yeast, fungi, and human and rodent cells, chromosomal aberrations (ABS) and sister chromatid exchange (SCE) in cultured human and rodent cells, and sex-linked recessive lethal mutations and reciprocal translocations in *Drosophila.*

Casanova and Heck (1997) have assessed the ability of MtBE to produce RNA-formaldehyde adducts (RFA) and DNA-protein cross links (DPX). These studies were performed using freshly isolated hepatocytes from female CD-1 mice, male $B6C3F_1$ mice, and Fischer 344 rats. MtBE is metabolized to equimolar concentrations of TBA and formaldehyde by hepatic microsomes (Brady *et al*. 1990). When formaldehyde, *per se*, was added to the incubation mixture as a positive control agent, it produced clear and dose-related increases in both DPX and RFA. When MtBE was added, low levels of DPX and RFA were detected, but the levels were independent of concentrations of MtBE. The low yields of DPX and RFA measured in this study were suggested to be the result of a high rate of formaldehyde oxidation to formate relative to the intracellular rate of formaldehyde production.

6.5 MtBE, α_{2u}-globulin Nephropathy, and Tubular Cell Neoplasms in Rats

Risk assessment criteria have been established by EPA for examining male rat kidney α_{2u}-globulin evidence. The three criteria are: (1) increased number and size of hyaline droplets in renal proximal tubule cells of treated rats; (2) accumulating protein in the hyaline droplets is α_{2u}-globulin; and (3) additional aspects of the pathological sequence of lesions associated with the α_{2u}-globulin nephropathy are present. EPA specifies that all three criteria must be met in order for the α_{2u}-globulin process to be considered (NSTC 1997).

Based on studies conducted at the Chemical Industry Institute of technology (CIIT) (Prescott-Mathews *et al*. 1997; Poet and Borghoff 1997), the National Research Council (NRC) panel stated that the criteria for causation, with respect to MtBE induced kidney tumors in male rats and α_{2u}-globulin, had been fulfilled and that MtBE-induced kidney tumors in male rats should not be used for human risk assessment (NRC 1996). The NRC report did not relate this conclusion to the EPA criteria.

The review by NSTC (1997) concluded that current data provide a basis for satisfaction of EPA's first criterion. Evaluation of the MtBE inhalation kidney tumor response is difficult because the evidence shows mild α_{2u}-globulin accumulation and symptomatic nephropathy, with some of the nephropathy being intermingled with a background of non α_{2u}-globulin rat nephropathy in both males and females. The other two EPA criteria were not fulfilled.

Studies using immunohistochemical staining for α_{2u}-globulin showed a slight increase in male rats exposed to MtBE as compared to controls, although an exposure-related increase in staining was not observed (Swenberg and Dietrich 1991; Fowler and Chun 1993; cited by Prescott-Mathews *et al*. 1997). Furthermore, proteinaceous casts, localized at the junction of the proximal tubules and the thin loop of Henle, did not stain positively for α_{2u}-globulin (Swenberg and Dietrich 1991). Swenberg and Dietrich (1991) suggest that other factors are involved in MtBE-induced nephropathy and renal carcinogenicity in male rats, because classical effects of the α_{2u}-globulin nephropathy-inducing agents are not evident in rats exposed to MtBE.

Studies from CIIT indicate that ten-day exposures to MtBE (at 0, 400, 1500, or 3000 ppm) produce protein droplet accumulation and renal epithelial cell proliferation in proximal tubules of male F344 rats but not in female rats (Prescott-Mathews *et al*. 1997; NSTC 1997). The authors concluded that the mild α_{2u}-globulin increase in male rats exposed to MtBE was not totally responsible for the increase in protein droplet accumulation. In a separate study, Borghoff *et al*. (1992; cited in NSTC 1997) reported that treating male F344 rats with unleaded gasoline for ten days produced dose-related increases in protein droplets, α_{2u}-globulin accumulation, and renal epithelial cell proliferation. NSTC (1997) suggest that there are other factors contributing to the protein droplet and cell proliferation responses in rats exposed to MtBE based on comparable increases in protein droplets and cell proliferation after exposure of male rats to MtBE or unleaded gasoline, but large differences in levels of α_{2u}-globulin accumulation by these two exposures.

Poet and Borghoff (1997) found that the interaction between kidney cytosolic proteins and MtBE did not withstand dialysis or an ion exchange chromatography. This has been attributed to the

weak binding affinity (~$2x10^{-4}$M) of MtBE with α_{2u}-globulin. NSTC (1997) notes that, for other chemicals that induce α_{2u}-globulin accumulation in the male rat kidney, approximately 20-40% of the ligand remains bound after dialysis in buffer. Examples of these include 1,4-dichlorobenzene and its metabolite 2,5-dichlorophenol (Charbonneau *et al*. 1989; cited by NSTC 1997), which also have weak binding affinities for α_{2u}-globulin. This leads to the assumption that interactions between MtBE and α_{2u}-globulin appear to be different from those of other chemicals that induce the accumulation of this protein (NSTC 1997). Renal tubular necrosis and accumulation of hyaline droplets containing small amounts of α_{2u}-globulin can occur by mechanisms that do not necessarily involve binding to this protein (Melnick *et al*. 1996; cited by NSTC 1997).

U.S. EPA (1993b) explains that a nephrotoxic response in the female rat suggests the possibility of other processes leading to, or influencing, the kidney tumor response. Nephropathy was increased in female rats exposed to MtBE or TBA. TBA, a metabolite of MtBE, is also carcinogenic to the male rat kidney (NSTC 1997). Renal tubular cell replication was not increased in male rats at the doses used in the TBA bioassay. NTP (1995) concluded that the mechanism for renal cytotoxicity by TBA was not limited to an increased accumulation of α_{2u}-globulin.

Several key issues require further investigation to increase an understanding of the possible involvement of α-globulin in the male rat kidney response. According to NSTC (1997), these are:

"(1) the basis for accumulation of moderate to large protein droplets in proximal tubular epithelial cells of male rats exposed to MtBE when there is only a mild increase in kidney α_{2u}-globulin concentration.

(2) a determination of whether the weak interaction between MtBE and α_{2u}-globulin is sufficient to account for the hyaline droplet nephropathy observed in male rats exposed to MtBE.

(3) characterization of the role of TBA in MtBE-induced kidney nephropathy.

(4) an evaluation of the impact of non α_{2u}-globulin nephropathy in the kidney of male and female rats exposed to MtBE or TBA."

NSTC (1997) states that, based on available experimental data relating MtBE exposure with α_{2u}-globulin nephropathy, it is reasonable to believe that other modes of action are operating in the male rat kidney tumor response.

6.6 Effects of Short-term Exposure to MtBE on Hepatocellular Proliferation and Tumor Promotion in Mice

Male and female CD-1 mice were exposed to 0, 400, 3000 or 8000 ppm of MtBE, by inhalation, 6 h/d, 5 d/wk/4wk. Twenty-four hours prior to sacrifice mice were infused with 5-bromo-2-deoxyuridine (BrdU). Livers were removed, fixed, and immunochemically stained for BrdU (Bird *et al*. 1997). Increased hepatocellular proliferation was observed in the livers of female mice exposed to 8000 ppm of MtBE for five days. BrdU incorporation was not significantly

increased after four weeks of MtBE exposure. There was no evidence of hepatocellular proliferation in male mice exposed to MtBE for either time period.

The results, presented by Bird *et al*. (1997), were similar to those presented by (Moser *et al*. 1996b), who conducted similar experiments with female $B6C3F_1$ mice. They also reported that exposure to 8000 ppm MtBE, for either 16 or 32 weeks, failed to promote the development of hepatocellular neoplasms in female mice initiated with N-nitrosodiethylamine.

The hypothesis that the female mouse tumor response, after chronic exposure to MtBE, was a result of endocrine alterations (an anti-estrogenic effect) was based on the finding of a decreased incidence of uterine cystic hyperplasia in exposed mice. Follow-up studies showed that exposure of female mice to 8000 ppm MtBE caused decreased uterine, ovarian, and pituitary weights, increased the length of the estrus cycle, decreased the number of epithelial layers of the cervix and vagina, and decreased the zona reticularis in the adrenal cortex. However, MtBE did not bind competitively to estrogen receptors, did not alter serum estrogen levels, and did not alter estrogen receptor immunoreactivity in the uterus, cervix, or vagina. Hence, effects of MtBE in endocrine-related tissues in female mice are not mediated through the estrogen receptor (Moser *et al.* 1998).

6.7 Comparison of Results in Various Species

NSTC (1997) summarized its conclusions on the overall carcinogenicity of MTBE as follows:

Inhalation exposure to MtBE produced increased incidences of kidney and testicular tumors in male rats and liver tumors in mice. Oral administration of MtBE produced an increased incidence of leukemia and lymphomas in female rats and testicular tumors in male rats. Two metabolites of MtBE, TBA and formaldehyde, show carcinogenic activity in animals, with some responses paralleling those seen with MtBE (rat kidney and leukemia) and some responses being at different sites (thyroid gland and nasal cavity). The mechanisms and role of MtBE and its metabolites are not fully understood, therefore, a low-dose hazard should not be excluded.

Based on the amount of evidence on MtBE carcinogenicity (positive in two species, by two routes of exposure, and at multiple organ sites), other supporting factors (e.g., one metabolite is a 'probable' human carcinogen and the other also induces male rat kidney tumors), and considering various uncertainties, it is reasonable to regard this alkyl ether oxygenate as posing a potential carcinogenic hazard and risk to humans.

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