EVIDENCE ON THE CARCINOGENICITY OF

METHYL TERTIARY BUTYL ETHER
(MTBE)

DRAFT

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PREFACE

The Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65, California Health and Safety Code 25249.5 *et seq*.) requires the publication and annual updating of a list of those chemicals "known to the state" to cause cancer or reproductive toxicity. The Act specifies that "a chemical is known to the state to cause cancer or reproductive toxicity...if in the opinion of the state's qualified experts the chemical has been clearly shown through scientifically valid testing according to generally accepted principles to cause cancer or reproductive toxicity." The lead agency for implementing Proposition 65 is the Office of Environmental Health Hazard Assessment of the California Environmental Protection Agency. The "state's qualified experts" regarding findings of carcinogenicity are identified as the members of the Carcinogen Identification Committee of the OEHHA Science Advisory Board, and of reproductive toxicity findings, the Developmental and Reproductive Toxicant Identification Committee (22 CCR 12301).

The Local Drinking Water Protection Act of 1997 [Assembly Bill 592 (Kuehl) and Senate Bill 1189 (Hayden)] requires that the OEHHA Science Advisory Board make recommendations on or before January 1, 1999 as to whether methyl tertiary butyl ether (MTBE) should be listed under Proposition 65 as a carcinogen or reproductive toxicant. A public request for information relevant to the carcinogenicity assessment was announced in the *California Regulatory Notice Register* on December 5, 1997.

This draft document *Evidence on the Carcinogenicity of Methyl Tertiary Butyl Ether* was developed to provide the Carcinogen Identification Committee with relevant information for use in its deliberations. The document reviews the available scientific evidence on the carcinogenic potential of methyl tertiary butyl ether. A public meeting of the Committee to discuss this evidence is scheduled for December 1998. The exact meeting date will be published in the *California Regulatory Notice Register*. Written public comment on the document should be submitted to OEHHA by November 24, 1998, in order to be considered by the Committee in advance of the meeting. During the December meeting, the public will have an opportunity to present verbal comments to the Committee.

1. EXECUTIVE SUMMARY

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Methyl tertiary butyl ether (MTBE) (CAS No. 1634-04-4) is a synthetic solvent used almost exclusively as an oxygenate in unleaded gasoline to improve combustion efficiency. Currently, MTBE is added at 11% volume to almost all of the gasoline used in California. It also has had limited use as a therapeutic drug for dissolving cholesterol gallbladder stones. Due to its use as a fuel additive, MTBE is a high volume production chemical. It was the second most-produced chemical in the U.S. in 1997.

Epidemiological studies of the carcinogenic effects of MTBE are not available. Carcinogenicity of MTBE has been observed in animal studies, by oral and inhalation routes. Specifically, tumors have been observed in rats of both sexes in lifetime gavage studies, in male rats in a 24-month inhalation study of a different strain, and in male and female mice in 18-month inhalation studies. Statistically significant increases in Leydig interstitial cell tumors of the testes were observed in both the inhalation and gavage studies involving the two different strains of rats. Statistically significant increases in renal tubular tumors were also observed in male rats exposed via inhalation, and increases in leukemias and lymphomas (combined) in female exposed orally. In the inhalation studies in mice, statistically significant increases in hepatocellular carcinomas were observed in males, and adenomas and combined adenomas and carcinomas in females.

At present, the mechanism by which MTBE induces the various tumors observed in animals remains unknown. MTBE has demonstrated little or no genotoxicity *in vitro* or *in vivo*. Two primary metabolites of MTBE, formaldehyde and tertiary butyl alcohol (TBA), both exhibit tumorigenic activity in animal studies. TBA and MTBE both induce renal tumors in the male Fisher rat; and formaldehyde and MTBE both have produced lymphohematopoietic cancers in Sprague Dawley rats exposed orally.

There is evidence for the carcinogenicity of methyl tertiary butyl ether (MTBE), based on several findings from animal studies. However, critics have questioned the interpretation of each of the individual findings. Some have questioned the use of a combined incidence category for statistical analysis of the leukemias and lymphomas observed in female rats. Some have argued that the cancers observed in male rats -- the renal tubular in the Fisher rat and the Leydig cell tumors in the Fisher and Sprague Dawley rat -- are not relevant to humans because of species specific mechanisms of carcinogenesis. For the renal tubular tumors, some hypothesize the possible involvement of α2u-microglobulin nephropathy, and for the testicular tumors, on possible interspecies differences in the hormonal control of Leydig cells. In mice, the increase in hepatocellular tumors was observed in the high dose male and female groups. An argument has been made that the mechanism of induction of these tumors, although unknown, may be non-genotoxic, and consequently the findings may not be relevant to humans exposed at environmental levels. In this report, the carcinogenicity studies are reviewed and the issues that have been raised are
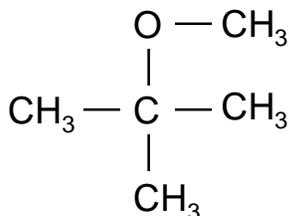
addressed in the context of a detailed discussion of pathology and mechanisms for each tumor site.

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2. INTRODUCTION

2.1. Identity of methyl tertiary butyl ether (MTBE)

$$O-CH_3$$
$$|$$
$$CH_3-C-CH_3$$
$$|$$
$$CH_3$$

Methyl tertiary butyl ether: $C_5H_{12}O$
Molecular Weight = 88.15   CAS Registry No. 1634-04-4

Synonyms: methyl tertiary-butyl ether; methyl tert-butyl ether; methyl t-butyl ether; MTBE; MtBE; tertiary-butyl methyl ether; tert-butyl methyl ether; methyl-1,1-dimethylethyl ether; 2-methoxy-2-methylpropane; 2-methyl-2-methoxypropane;

MTBE is a synthetic alkyl ether with a pungent, terpene-like odor (ATSDR, 1996). It is a clear, colorless liquid at room temperature, with a melting point of -109 °C and a boiling point of 53.6 - 55.2 °C (Merck, 1989). MTBE's density at 20 °C is 0.7404 - 0.7578 g/ml. MTBE is miscible in gasoline, moderately soluble in water (43 - 54.3 g/L), and highly soluble in alcohol and other ethers (Squillace *et al.*, 1997). MTBE is unstable in acidic solutions (Merck, 1989). It is volatile (vapor pressure 245 mm Hg at 25 °C), highly flammable and combustible.

2.2. Occurrence and use[1]

MTBE is a synthetic solvent used almost exclusively as an oxygenate in unleaded gasoline to improve combustion efficiency. It also has had limited use as a therapeutic drug for dissolving cholesterol gallbladder stones (Leuschner *et al.*, 1994). MTBE is not known to occur naturally. Reformulated gasoline with MTBE has been used nationwide to meet the

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[1] Information in this section is adapted from *Public Health Goal for Methyl Tertiary Butyl Ether (MTBE) in Drinking Water* (OEHHA, 1998).

1990 federal Clean Air Act Amendments requirements for reducing carbon monoxide and ozone. About 40% of the U.S. population lives in areas where MTBE is included in oxyfuel or reformulated gasoline. Federal and State law required the exclusive sale of reformulated gasoline in California beginning in 1996. Currently, MTBE is added at 11% volume to almost all of the gasoline used in California (a small percentage of the gasoline used in California contains ethanol as an oxygenate, rather than MTBE).

Due to its use as a fuel additive, MTBE is a high volume production chemical. It was the second most-produced chemical in the U.S. in 1997. California produced 181 million gallons of the 2.9 billion gallons of MTBE estimated to be produced in the U.S. in 1997. In addition, MTBE is imported for use in California. It can be manufactured from isobutene, also known as isobutylene or 2-methylpropene (Merck, 1989), which is a product of petroleum refining. It is produced primarily by combining methanol with isobutene, but can also be produced by combining methanol with TBA (ATSDR, 1996).

MTBE is present in ambient air in California. Potential sources of MTBE in ambient air are the manufacture and distribution of oxyfuel and reformulated gasoline, vehicle refueling, and evaporative and tailpipe emissions from motor vehicles. Monitoring for MTBE was initiated by the California Air Resources Board in 1996. Preliminary data suggest a statewide average of approximately 2 parts per billion volume (ppbv) with higher average concentrations in the South Coast of about 4 ppbv. These values are similar to limited data from other states.

MTBE has become a drinking water contaminant in California because of its high water solubility and persistence in solution. Potential sources of drinking water contamination are leaking underground storage tanks, recreational power-boating and refinery wastewater. MTBE has been detected in groundwater in connection with leaking underground storage tanks by water quality management authorities in Santa Clara, Orange, Solano and San Francisco counties, among others. MTBE can reach concentrations of 10 ppm near the source of the fuel release. MTBE has been detected in lakes and reservoirs, with concentrations higher in reservoirs that allow use of gasoline-powered boats. Affected California lakes include Lake Tahoe, Shasta, Donner, Merced, Havasu, Clear Lake and Canyon Lake. Beginning in 1997, monitoring of drinking water sources for MTBE was instituted by the California Department of Health Services. As of June, 1998, MTBE was detected in 25 of 671 public water systems and 46 of 3173 drinking water sources monitored. Six groundwater wells have reported concentrations above 20 μg/L, the lower limit of the U.S. EPA Hazard Advisory level of 20-40 ppb. Two groundwater well fields in Santa Monica and one in Santa Clara have been shut down due to MTBE contamination.

3. DATA ON MTBE CARCINOGENICITY

Two series of carcinogenicity studies have been reported in which MTBE was administered by inhalation to rats and mice of both sexes. In a third series of carcinogenicity studies, MTBE was administered by gavage to male and female rats. MTBE has also been tested in several *in vitro* and *in vivo* genotoxicity assays in bacterial and mammalian cells.

3.1.   Epidemiological studies of carcinogenicity in humans.

No data on long-term effects of human exposure to MTBE relevant to cancer risk were found in recent literature searches performed by OEHHA.

3.2.   Carcinogenicity studies in animals.

The carcinogenic activity of MTBE has been investigated in male and female Sprague-Dawley rats administered MTBE by gavage (Belpoggi *et al*., 1995; 1997), and in male and female Fischer 344 rats (Chun *et al.*, 1992; Bird *et al.*, 1997) and CD-1 mice (Burleigh-Flayer *et al*., 1992; Bird *et al.*, 1997) exposed to MTBE by inhalation. In rats receiving MTBE by gavage for 24 months, statistically significant increases in Leydig interstitial cell tumors of the testes were observed in males, and statistically significant increases in lymphomas and leukemias (combined) were observed in females. An increase in the incidence of uterine sarcomas was also observed in MTBE-exposed female rats, but was not statistically significant at the $p < 0.05$ level. In rats exposed to MTBE by inhalation for up to 24 months, statistically significant increases in the incidences of renal tubular tumors and Leydig interstitial cell tumors of the testes were observed in males. In mice exposed to MTBE by inhalation for up to 18 months, statistically significant increases in the incidences of liver tumors were observed in females (hepatocellular adenomas; hepatocellular adenomas and carcinomas combined) and males (hepatocellular carcinomas). These studies are described in more detail below. There is additional discussion in Section 3.4 regarding the pathology of the tumors observed, and issues that have been raised regarding their significance.

Rat Oral Exposure: Belpoggi et al. 1995; 1997

Groups of 60 male and 60 female eight-week old Sprague-Dawley rats were administered MTBE in olive oil by gavage at doses of zero (oil only), 250 or 1,000 mg/kg body weight/d, four d per week for 104 weeks. Animals were maintained until natural death; the last animal died at 174 weeks of age. No difference in water or food consumption, or in mean body weights was observed between treated and control animals of either sex. A dose-related decrease in survival was observed in females. At 56 weeks of age, survival was approximately 98%, 85%, and 78% in controls, low- and high-dose females, respectively; at 88 weeks of age, survival in those same groups was approximately 76%, 60%, and 43%. In males, there was no difference in survival between the controls and the

low-dose animals. However, after 88 weeks, survival in high-dose males exceeded that of low-dose and control males. At 104 weeks of age, survival was approximately 30% in low-dose and control males and 43% in high-dose males; at 120 weeks of age, survival in those same groups was approximately 11% and 32%.

A dose-related increase in the combined incidence of lymphomas and leukemia was observed in female rats (Table 1). The authors reported that the increase was highly significant ($p < 0.01$) in the high-dose group and marginally significant in the low-dose group, when analyzed using a log-ranked test as described by Mantel (1966) and Cox (1972). When analyzed using the Fisher Exact test, the combined incidence of lymphomas and leukemia in high-dose females was significantly different from controls at the $p = 0.001$ level. Historical control incidence rates in this laboratory for lymphomas and leukemias (combined) was < 10% in female Sprague-Dawley rats (Belpoggi *et al.*, 1995). The authors also noted an increase in uterine sarcomas in the low-dose females (5/60 versus 1/60 in controls); however, this increase did not reach statistical significance ($p = 0.1$ by Fisher's Exact test). In males, a statistically significant increased incidence of Leydig cell tumors of the testes was observed in the high-dose group (Table 1). The authors reported that this increase was significant at the $p = 0.05$ level using a prevalence analysis for nonlethal tumors (Hoel and Walburg, 1972).

Table 1. Tumors in Sprague-Dawley Rats Receiving MTBE by Gavage, 0, 250, or 1,000 mg/kg/d, Four d/wk for 104 Weeks (Belpoggi *et al.*, 1995; 1997)

Tumor site and type		Dose[a] (mg/kg/d)		
		0	250	1,000
Females				
Hemolympho-reticular tissues (including mesenteric lymph nodes)	Lymphomas and leukemias	2/58[b] (3.4%) $p < 0.01$[d]	6/51[b] (11.8%)	12/47[b,c] (25.5%) $p = 0.001$[e]
Males				
Testes	Leydig interstitial cell tumors	2/26[f] (7.7%)	2/25[f] (8.0%)	11/32[f,g] (34.4%) $p = 0.015$[e]

[a] Administered in olive oil, four d/week, for 104 weeks.

[b] Number of lesion-bearing animals/total alive at 56 weeks of age, when the first leukemia was observed.

[c] Incidence relative to control group was significant ($p < 0.01$) using a log-ranked test (Mantel, 1966, Cox, 1972), as reported by Belpoggi *et al.* (1995). ($p = 0.001$).

[d] P-value for dose-related trend by the Cochran-Armitage trend test.

[e] P-value for incidence relative to control group by the Fisher Exact test

[f] Number of lesion-bearing animals/total alive at 96 weeks of age, when the first Leydig cell tumor was observed.

[g] Incidence relative to control group was significant at the $p = 0.05$ level using prevalence analysis for nonlethal tumors (Hoel and Walburg, 1972), as reported by Belpoggi *et al.* (1995).

Rat Inhalation Exposure: Chun et al., 1992; Bird et al., 1997

Groups of 50 male and 50 female eight-week old Fischer 344 rats were exposed to zero, 400, 3,000, or 8,000 ppm MTBE vapor by inhalation (corresponding to analytical mean concentrations of 403, 3,023, or 7,977 ppm, or 1,453, 10,899, 28,760 mg/m^3). The animals were exposed for six h/d, five d/week for 24 months, except for the mid- and high-dose males, which were terminated at 97 and 82 weeks, respectively, due to a dose-

dependent increased mortality rate from chronic progressive nephropathy. Low-dose males also experienced an increase in nephropathy that was associated with a slight increase in mortality and a decrease in survival. Survival times for females were not significantly different between exposed and control rats. However, there were slightly more deaths due to chronic progressive nephropathy in the mid- and high-dose females than in the low-dose and control females. Body weight gain and absolute body weight were decreased in both sexes of the high-dose group. Exposure-related increases in kidney and liver weights were reported in mid- and high-dose females, but not in males. Chun *et al.* (1992) concluded that the maximum tolerated dose (MTD) was exceeded in both sexes at high- and mid-dose levels, based on increased mortality. Other observed effects of MTBE exposure included anesthetic effects in rats of both sexes in the mid- and high-dose groups.

A detailed histopathology examination was performed on all animals in the control and high-dose groups, and on all animals that died or were sacrificed moribund. Only a limited histopathology examination was performed on nonmoribund animals from the low- and mid-dose groups that survived to terminal sacrifice; for males, only the liver, kidneys, testes and gross lesions were evaluated, while for females, only the liver and gross lesions were examined microscopically (Bird *et al.*, 1997). At the request of the MTBE Task Force, Experimental Pathology Laboratories, Inc. (1993) re-evaluated the histopathologic slides of kidneys from all male and female rats used in the Chun *et al.* (1992) study, and confirmed the study pathologist's conclusion that MTBE increased the severity of chronic progressive nephropathy in rats of both sexes. No histopathologic re-evaluation of the kidney tumors was performed.

In males, a statistically significant increase in renal tubular adenoma and carcinoma (combined) was observed in the mid-dose group (Table 2). In high-dose males renal tubular adenomas were increased; however, this increase did not reach statistical significance (Table 2). The sensitivity of the bioassay to detect a dose-related increase in renal tumors in the high-dose group is likely to have been reduced by the high rate of early mortality, and the early termination of this treatment group at week 82. Despite the reduced sensitivity of the bioassay, a statistically significant increase in Leydig interstitial cell testicular tumors was observed in mid- and high-dose males, with a clear dose-response evident (Table 2). Historical laboratory control values for Leydig testicular tumors in Fischer rats ranged from 64 to 98% (Bird *et al.*, 1997).

In female Fischer 344 rats exposed to MTBE vapor, a single rare renal tubular cell adenoma was observed in one mid-dose animal; no treatment-related increases in tumor incidence were observed (Chun *et al.*, 1992; Bird *et al.*, 1997). MTBE treatment of females was associated with several nonneoplastic kidney lesions, however. Both female and male rats exposed to MTBE experienced a dose-related increase in mortality from chronic progressive nephropathy. Increases in microscopic kidney changes indicative of chronic nephropathy were seen in all treated males and in mid- and high-dose females. All treated males had increases in the severity of mineralization and interstitial fibrosis of the

kidney, while increases in mild to moderate glomerulosclerosis, interstitial fibrosis, and tubular proteinosis were observed in females.

Table 2. Tumors in Male Fischer 344 Rats Receiving MTBE by Inhalation, 0, 400, 3,000, or 8,000 ppm, for up to 24 Months[a] (Chun *et al.*, 1992; Bird *et al.*, 1997)

Tumor site and type		Concentration[b] (ppm)			
		0	400	3,000	8,000
Kidney	renal tubular adenoma	1/35[c]	0/32[c]	5/31[c]	3/20[c]
	renal tubular carcinoma	0/35[c]	0/32[c]	3/31[c]	0/20[c]
	renal tubular adenoma and carcinoma (combined)	1/35[c] (3%)	0/32[c] (0%)	8/31[c] (26%) $p < 0.01$[d]	3/20[c] (15%)
Testes	Leydig interstitial cell tumors	32/50 (64%)	35/50 (70%)	41/50 (82%) $p < 0.05$[d]	47/50 (94%) $p < 0.001$[d]

[a] Mid- and high-dose animals were terminated at 97 and 82 weeks, respectively, due to a dose-dependent increased mortality rate from chronic progressive nephropathy.

[b] Administered as MTBE vapor six h/d, five d/week.

[c] Survival-adjusted tumor incidence rates were used to attempt to control for excess early mortality in the mid- and high-dose groups (US EPA, 1995).

[d] P-value for incidence relative to control group by the Fisher Exact Test.

Mouse Inhalation Exposure: Burleigh-Flayer et al., 1992; Bird et al., 1997

Groups of 50 male and 50 female eight-week old CD-1 mice were exposed to zero, 400, 3,000, or 8,000 ppm MTBE vapor by inhalation (corresponding to analytical mean concentrations of 402, 3,014, or 7,973 ppm or 1,442, 10,816, or 28,843 mg/m^3). The animals were exposed for six h per d, five d per week, for 18 months. Increased mortality and decreased mean survival time were observed only for male mice in the high-dose group. A slightly increased frequency of obstructive uropathy, a condition which occurs

spontaneously in this mouse strain, was observed in high-dose males; however, deaths due to the condition were within the range noted for historical controls. Body weight gain and absolute body weights were decreased in high-dose males and females. Dose-dependent increases in liver weights were observed in both sexes. Kidney weights were increased in high-dose females and in low- and mid-dose males. Burleigh-Flayer *et al.* (1992) concluded that the MTD was exceeded in both sexes at the high-dose level. Other observed effects of MTBE exposure included anesthetic effects in mice of both sexes in the mid- and high-dose groups.

A detailed histopathology examination was performed on all animals in the control and high-dose groups, and on all animals that died or were sacrificed moribund. Only a limited histopathology examination was performed on nonmoribund animals from the low- and mid-dose groups that survived to terminal sacrifice; for males, only the liver, spleen and submandibular lymph nodes were evaluated, while for females, only the liver, uterus and stomach were examined microscopically (Bird *et al.,*1997).

In females, a statistically significant increased incidence of hepatocellular adenomas was observed in the high-dose group (Table 3). The incidence of hepatocellular adenomas and carcinomas (combined) was also increased in high-dose females, however, only two hepatocellular carcinomas were reported, one each in the low- and high-dose groups. In males, a statistically significant increase in hepatocellular carcinomas was observed in the high-dose group (Table 3). Bird *et al.* (1997) noted that the combined incidence of adenomas and carcinomas in high-dose males was similar to the historical incidence for male CD-1 mice of 33%. However, after correcting for the number of animals alive at 49 weeks, when the first hepatocellular adenoma was observed in males, the incidence in the high-dose group was 43% (16/37, see Table 3), representing a clear increase above the cited historical incidence in male CD-1 mice. Burleigh-Flayer *et al.* (1992) concluded that the increased incidence of liver tumors in the high-dose groups (adenomas in females and carcinomas in males) could be attributed to MTBE exposure. The ability of this study to detect increases in tumor incidence was likely decreased by the shortened study length (18 versus 24 months).

**Table 3. Tumors in CD-1 Mice Receiving MTBE by Inhalation,
0, 400, 3,000, or 8,000 ppm, for up to 18 Months[a]
(Burleigh-Flayer *et al.*, 1992; Bird *et al.*, 1997)**

Tumor site and type		Dose[b] (ppm)			
		0	400	3,000	8,000
Females					
Liver	hepatocellular adenoma	2/50	1/50	2/50	10/50 $p < 0.05$
	hepatocellular carcinoma	0/50	1/50	0/50	1/50
	hepatocellular adenoma and carcinoma (combined)	2/50	2/50	2/50	11/50 $p < 0.01^{c}$
Males					
Liver	hepatocellular adenoma	11/47[d]	11/47[d]	9/46[d]	12/37[d]
	hepatocellular carcinoma	2/42[e]	4/45[e]	3/41[e]	8/34[e] $p < 0.05^{c}$
	hepatocellular adenoma and carcinoma (combined)	12/47[d]	12/47[d]	12/46[d]	16/37[d]

[a] Male mice in the high-dose group experienced early mortality.
[b] Administered as MTBE vapor six h/d, five d/week.
[c] P-value for incidence relative to control group by the Fisher Exact test.
[d] Number of lesion-bearing animals/total alive at 49 weeks, when the first hepatocellular adenoma was observed.
[e] Number of lesion-bearing animals/total alive at 63 weeks, when the first hepatocellular carcinoma was observed.

3.3. Other relevant data

In addition to the reported animal bioassays, additional evidence related to the possible carcinogenicity of MTBE is available. This includes studies of genetic toxicity, observations of the pharmacokinetics and metabolism, and structure-activity comparisons.

3.3.1. Genetic Toxicology

MTBE has been tested for genetic damage in 6 species in over 10 different *in vitro* and *in vivo* assays (see Table 4 for summary). To date, the results of most tests have been negative. Positive results have been reported only for the mouse lymphoma assay for forward mutations (ARCO, 1980; Mackerer *et al*., 1996) and the rat lymphocyte comet assay for DNA strand breaks (Lee *et al*., 1998). The high volatility of MTBE presents technical difficulties for testing in bacterial and cultured cell systems. Negative results observed in these assay systems should be viewed with caution if there is no explicit discussion of precautions taken to assure adequate exposure of test cells. The *in vivo* assay systems used to test MTBE were primarily chromosomal damage assays, with two exceptions being the spleen lymphocyte *hprt* mutation assay (Ward *et al.*, 1995) and the *in vivo-in vitro* mouse hepatocyte unscheduled DNA synthesis assay (Vergnes and Chun, 1994). Only one *in vivo* assay system, the *hprt* mutation assay, had the potential to detect gene mutations. However, this assay is relatively insensitive in detecting genotoxic chemicals with known false negatives. No published data have been identified to date that address the genotoxic potential of MTBE in humans.

Assays for induction of point mutations by MTBE *in vitro* have included the *Salmonella* reverse mutation assay, and tests for forward mutation in mouse lymphoma cells and Chinese hamster fibroblastic cells. Three separate groups have reported negative results in the *Salmonella* test, with and without activation by rat liver metabolic enzymes (ARCO, 1980; Kado *et al*., 1998; Cinelli *et al*., 1992). However, only one of these groups (Kado *et al*., 1998) used an experimental design that prevented volatilization of the ether. MTBE tested negative for forward mutations in V79 cells, a hamster fibroblastic cell line (Cinelli *et al*., 1992). Two independent tests of MTBE in the mouse lymphoma cell assay for forward mutation at the TK locus have reported positive results (ARCO, 1980; Mackerer *et al*., 1996). In the ARCO study, positive results were obtained only in the presence of a rodent S9 metabolic system. Mackerer and colleagues used 10% S9 in all experiments and performed the assay in closed vials. They obtained mutant frequencies ranging from 4- to 11-fold above the average control frequency. Mutagenic activity was inhibited by addition of formaldehyde dehydrogenase to the test system, leading the authors to conclude that MTBE mutagenicity was due to S9-mediated formation of formaldehyde from MTBE.

In addition to these *in vitro* tests for gene mutation, an *in vivo* assay for *hprt* mutation was reported in a meeting abstract (Ward *et al*., 1995). CD-1 mice were treated by gavage for three weeks at a range of doses. Following a three week recovery period, spleen lymphocytes were assayed for the presence of mutations at the *hprt* locus. No increases were observed in the MTBE-treated mice.

Several studies have addressed the ability of MTBE to induce gross chromosomal damage. As with the gene mutation assays summarized above, most results were negative. However, a positive result in the comet assay was reported in a recent meeting abstract (Lee *et al*., 1998). The comet assay measures DNA strand breaks; as such it can indicate

DNA damage induced by xenobiotics, as well as the more severe DNA fragmentation associated with apoptosis. Rats were treated with MTBE by gavage, and lymphocytes assessed for alkaline-labile strand breaks. A significant increase in DNA strand breaks was reported for the highest dose group. An increase in apoptotic comets was also observed in lymphocytes from exposed rats, but this result was not statistically significant for any one dose group.

Three groups have reported negative results in the micronucleus assay (Vergnes and Kintigh, 1993; McKee *et al*., 1997; Kado *et al*., 1998). Micronuclei can include fragments of chromosomes due to double strand breaks, or whole chromosomes lost in mitotic errors. In two studies, CD-1 mice were exposed to a range of MTBE doses, with no subsequent elevation in the frequency of micronuclei in erythrocytes. Analysis of micronuclei in bone marrow cells from exposed Swiss-Webster mice also failed to find an effect. No studies of micronucleus formation *in vitro* were identified.

Chromosome aberrations have been evaluated in one mouse and two rat strains in four studies (ARCO, 1980; Vergnes and Morabit, 1989; Ward *et al*., 1994 as cited in ATSDR, 1996; McKee *et al*., 1997). Findings from all four studies, two of which administered MTBE by inhalation and two by the oral route, were negative. Chromosomal aberration frequency was also measured in cultured rodent cells exposed to MTBE, with negative results (ARCO, 1980).

Assays for induction of DNA synthesis, potentially indicating repair of damaged DNA, were carried out by three different groups. Whether exposure was performed *in vivo* or to hepatocytes removed to culture, no induction of DNA repair by MTBE was observed in these studies.

Two groups have reported a lack of activity in the *Drosophila* recessive lethal assay, a multigenerational test in which male flies are exposed to MTBE in the diet, and potential DNA damage is observed in future generations.

In summary, there is limited evidence for genotoxicity of MTBE; most evidence supports a lack of genotoxic activity. However, a potential role for genetic damage can not be ruled out. Metabolism and genotoxic effects of MTBE specific to target tissues such as the kidney and testes require more investigation. While a role for formaldehyde in liver carcinogenicity may be unlikely (see section 3.4), there is little to no knowledge of the production and detoxification rates for this metabolite in the other target tissues. Assays for clastogenicity and chromosomal rearrangements have only assessed damage in bone marrow, which is not thought to be a target tissue for MTBE-induced carcinogenesis (see discussion of the Sprague-Dawley rat leukemias and lymphomas in section 3.3.4).

Table 4. Genotoxicity of MTBE

Assay	Species	Strain/Cells	Exposure[1]	Result	Reference
Bacterial systems					
Reverse mutation	*Salmonella typhimurium*	TA 98 TA 100 TA 1535 TA 1537 TA 1538	0, 625, 1250, 2500, 5000 and 10,000 µg/plate. With and without S9 activation.	Negative	Cinelli *et al.*, 1992
Reverse mutation	*Salmonella typhimurium*	TA98 TA100 TA104 TA1535	0, 30, 90, 300, 925, 1850, 3700, and 7400 µg/tube, in tubes to control evaporation, with and without S9 activation.	Negative	Kado *et al.*, 1998
Reverse mutation	*Salmonella typhimurium*		With and without S9 activation	negative	ARCO, 1980
Yeast systems					
Reverse mutation	*Saccharomyces cerevisiae*	D4		negative	ARCO, 1980

Table 4. Genotoxicity of MTBE (*continued*)

Invertebrate systems					
Recessive lethality	*Drosophila*	OR-R		negative	Sernau, 1989
Recessive lethality	*Drosophila*	OR-R	In food, at 0, 0.01, 0.05, 0.1, 0.2, and 0.3%	negative	McKee *et al.*, 1997
Mammalian systems, in vitro exposure					
Gene mutation	Hamster	V79		negative	Cinelli *et al.*, 1992
Forward mutation	Mouse	L5178Y/TK	0, 1, 2, 3, 4 µl/ml, 3 h, in closed vials, with activation and +/- formaldehyde dehydrogenase	positive	Mackerer *et al.*, 1996
Forward mutation	Mouse	L5178Y/TK	With and without activation	positive	ARCO, 1980
Chromosomal aberrations	Hamster	CHO	With and without activation	negative	ARCO, 1980
Sister chromatid exchange	Hamster	CHO	With and without activation	equivocal	ARCO, 1980
Unscheduled DNA synthesis	Rat	primary hepatocytes	0, 1-10 mg/ml	negative	Cinelli *et al.*, 1992

Table 4. Genotoxicity of MTBE (*continued*)

Mammalian systems, in vivo exposure					
Comet assay	Rat	Sprague-Dawley, males only, lymphocytes	0, 40, 400, 800 mg/kg, gavage, 28 d	positive	Lee *et al.*, 1998
HPRT mutation	Mouse	CD-1, spleen lymphocytes	0, 1, 10, 100, 1,000 mg/kg, gavage, three weeks	negative	Ward *et al.*, 1995
Micronucleus	Mouse	CD-1, erythrocytes	0, 1440-28,800 mg/m^3 6h/d, 2 d	negative	Vergnes and Kintigh, 1993
Micronucleus	Mouse	CD-1, erythrocytes	0, 400, 3000, 8000 ppm, inhalation, 6h/d, 2d	negative	McKee *et al.*, 1997
Micronucleus	Mouse	Swiss-Webster, erythrocytes	0, 250, 500, 1000, 1500, 1750 mg/kg, i.p.	negative	Kado *et al.*, 1998
Chromosomal aberrations	Rat	F344, bone marrow cells	0, 800, 4000, 8000 ppm, inhalation, 6h/d, 5d	negative	Vergnes and Morabit, 1989
Chromosomal aberrations	Rat	Sprague-Dawley, bone marrow cells	0, 30, 90, 296 mg/kg/d, oral. One dose or five doses, 24 h apart.	negative	ARCO, 1980

Table 4. Genotoxicity of MTBE (*continued*)

Chromosomal aberrations	Rat	F-344, bone marrow cells	0, 800, 4000, 8000 ppm, inhalation, 6h/d, 5d	negative	McKee *et al.*, 1997
Chromosomal aberrations	Mouse	CD-1	0, 1 - 1,000 mg/kg, oral, 3 weeks	negative	Ward, 1994 as cited in ATSDR, 1996
Unscheduled DNA synthesis	Mouse	Primary hepatocytes	0, 400, 3000, 8000 ppm, inhalation, 6h/d, 2d	negative	McKee *et al.*, 1997
Unscheduled DNA synthesis	Mouse	Primary hepatocytes	0, 1440-28,800 mg/m^3 6h/d, 2 d	negative	Vergnes and Chun, 1994

[1] Exposure concentrations for inhalation experiments are the reported target concentrations.

3.3.2. Structure-Activity Comparisons

MTBE and similar ethers generally undergo metabolism at the ethereal bond to form the corresponding alcohol and an aldehyde (Savolainen *et al.*, 1985). Other structurally similar ethers include ethyl tertiary butyl ether (ETBE) and tertiary-amyl methyl ether (TAME). No studies have been reported to date on the carcinogenicity of ETBE or TAME. Published data on the genotoxic potential of ETBE and TAME are few in number; ETBE and TAME tested negative in the *Salmonella* reverse mutation assay, and TAME did not induce micronuclei in mouse bone marrow cells following exposure *in vivo* (NSTC, 1997). In a recent review of gasoline toxicity, Caprino and Togna (1998) briefly refer to an unpublished report in which TAME induced "chromosome effects" in Chinese hamster ovary cells. MTBE is manufactured from either isobutene and methanol, or TBA and methanol. NTP has reported some evidence of carcinogenic activity for isobutene in male rats (NTP, 1997), and for TBA in male rats and female mice (NTP, 1995).

3.3.3. Pharmacokinetics and Metabolism

Overview

The pharmacokinetics of MTBE have been recently reviewed (ATSDR, 1996; NSTC, 1997; US EPA, 1997a; OEHHA, 1998). Available information is mainly limited to humans and rats, and indicates that the pharmacokinetics and metabolism of MTBE are reasonable similar in these two species. Very little information was located for mice. MTBE is readily absorbed by inhalation (in humans and rats) and orally (rats), and absorbed to a modest extent dermally (rats). MTBE is probably widely distributed in the body. MTBE is metabolized *in vivo* by cytochromes P450 to TBA, and, probably, formaldehyde. The data suggest that MTBE induces its own metabolism to a limited extent, and that some degree of saturation occurs. TBA is also probably widely distributed. Information on the distribution and fate of formaldehyde produced from MTBE is lacking. TBA is further metabolized to several compounds. The primary metabolites of TBA are 2-methyl-1,2-propanediol and a-hydroxyisobutyric acid. Additional metabolites are the glucuronide and sulfate conjugates of TBA, and acetone. Both MTBE and TBA are eliminated by exhalation in rats and humans, but the unchanged compounds do not appear appreciably in urine. TBA is eliminated more slowly than MTBE, especially in humans. Metabolites of TBA are eliminated in the urine in rats and humans, but elimination in feces is negligible. Physiologically based pharmacokinetic (PBPK) models have been developed for rats and extended to humans.

Absorption

Studies indicate that MTBE is readily absorbed by inhalation in humans. In 10 male volunteers exposed to MTBE at 5, 25, or 50 ppm during 2 h of light exercise, net respiratory uptake ranged from 32% to 42% (Johanson *et al.*, 1995). Blood levels of MTBE increased rapidly at the beginning of the exposure, and leveled off at the end. At

50 ppm MTBE in air, the peak blood level was 13 μmol/L (1.14 mg/L). Average blood levels were proportional to air concentrations (Johanson *et al.*, 1995; Nihlen *et al.*, 1998). In another study, inhalation of MTBE for 1 h at 1.39 ppm led to peak blood concentrations of MTBE of 8.2 μg/L in one male and 14.1 μg/L in one female at the end of the exposure. No leveling off was evident during the 1 h exposure (Buckley *et al.,* 1997; Prah *et al.*, 1994). In two males and two females exposed by inhalation to MTBE for 1 h at 1.7 ppm, blood concentrations of MTBE peaked at the end of exposure at 17.1 ppb (average of individual peak blood concentrations of 14.85, 16.65, 17.35, and 19.70 ppb). Some leveling off of blood concentration was evident between 30 minutes and 60 minutes of exposure (Cain *et al.,* 1996). Environmental studies have also indicated that exposure to MTBE results in increased blood levels (ATSDR, 1996).

MTBE was also rapidly and extensively absorbed by rats in inhalation studies using both single and repeated exposure sessions. In rats exposed to 400 or 8000 ppm MTBE for 6 h, plasma concentrations of MTBE increased rapidly, then leveled off after about 2 h. Peak plasma concentrations of 14 and 493 mg/L were found for the low and high concentration. Little difference between males and females was found. At the end of repeated exposure for 6 h/d for 15 d at 400 ppm, a plasma concentration of 9 mg/L was reported, suggestive of induction by MTBE of its own metabolism (Miller *et al.*, 1997). Similar observations were made when rats were exposed to MTBE for 6 h/d, 5 d/week, for 2-15 weeks at 50-300 ppm. At the end of 2 weeks at 300 ppm, blood concentration was 67 nmol/g (5.9 mg/kg). Blood concentrations were approximately linearly related to MTBE concentrations in the air. MTBE blood concentrations varied somewhat after 6, 10, or 15 weeks exposure, but were roughly similar to those at 2 weeks (Savolainen *et al.*, 1985).

No human data for oral or dermal absorption of MTBE were located. In rats exposed by gavage to 40 or 400 m/kg, absorption was rapid and complete. After a 40 mg/kg gavage, a peak plasma concentration of about 18 mg/L was achieved in about 15 minutes. In rats exposed dermally to 40 or 400 mg/kg for 6 h, absorption was 16 and 34%, respectively. The peak plasma concentration at 40 mg/kg after about 2 h was about 0.3 mg/L (Miller *et al.*, 1997).

Distribution of MTBE

MTBE is a small organic molecule with high lipid solubility and some water solubility. As such, it is expected to easily cross biological membranes and be widely distributed (ATSDR, 1996).

As discussed in the section above, MTBE is readily taken up and distributed via blood in humans exposed by inhalation (Buckley *et al.*, 1997; Johanson *et al.*, 1995; Nihlen *et al.*, 1998; Prah *et al.*, 1994). In humans treated with MTBE by intracystic infusion for dissolution of gallstones, MTBE was found in blood, fatty tissue, and breast milk. At treatment end, the concentration in breast milk was similar to that in blood; the

concentration in fat was 3-4 times higher (Leuschner *et al.*, 1991). No data on distribution of MTBE in humans following oral or dermal exposures were located.

Rats were exposed to MTBE at 50-300 ppm by inhalation for 6 h/d, 5 d/week for 2, 6, 10, or 15 weeks. MTBE was found in blood, brain, and perirenal fat at the end of exposure at all time points. Concentrations in brain were similar to those in blood. Concentrations in fat were on the order of 10-20 times those in blood (Savolainen *et al.*, 1985).

PBPK models incorporate distribution of MTBE to numerous tissues (Borghoff *et al.*, 1996; Rao and Ginsberg, 1997). For rats, the experimentally measured tissue:air partition coefficient for blood was 11.5 (similar to saline). A similar blood:air partition coefficient (17.7) was found for humans (Johanson *et al.*, 1995). Partition coefficients for rat liver, kidney and muscle ranged from 6.5 to 35.8, whereas that for fat was considerably higher at 115.6 (similar to oil) (Borghoff *et al.*, 1996).

Distribution of TBA

Following human inhalation of MTBE, TBA has been found in the blood (Buckley *et al.*, 1997; Johanson *et al.*, 1995; Nihlen *et al.*, 1998; Prah *et al.*, 1994). Following intracystic infusion of MTBE for the dissolution of gallstones, TBA was found in the blood, and, in 1 subject, in breast milk, at a concentration slightly less than that in blood (Leuschner *et al.*, 1991).

In rats exposed to MTBE by inhalation at 50-300 ppm for 6 h/d, 5 d/week for 2-15 weeks, TBA was found in blood, brain, and perirenal fat at all time points. Concentrations in brain were similar to those in blood, but concentrations in fat were considerably lower (Savolainen *et al.*, 1985).

PBPK models for MTBE incorporate distribution of TBA to numerous tissues (Borghoff *et al.*, 1996; Rao and Ginsberg, 1997). For rats, the experimentally measured tissue:air partition coefficient for blood was 481 (similar to saline) and those for liver, kidney and muscle ranged from 400 to 543. The coefficient for fat was lower at 191 (similar to oil) (Borghoff *et al.*; 1996). Thus, TBA has a relatively greater affinity for water compared to lipids than does MTBE.

Metabolism

A proposed metabolic scheme based upon the limited available evidence is shown in Figure 1. It should be noted that the evidence supporting this scheme is highly variable in quality.

The initial step in MTBE metabolism is the conversion of MTBE to TBA and formaldehyde. In humans, elevated TBA has been found in blood following MTBE exposure. The peak concentrations of TBA in blood were roughly comparable to those of MTBE (Buckley *et al.*, 1997; Cain *et al.*, 1996; Johanson *et al.*, 1995; Leuschner *et al.*, 1991; Nihlen *et al.*, 1998; Prah *et al.*, 1994). Similarly elevated TBA levels have been

found in rat blood and plasma following MTBE exposure (Savolainen *et al.*, 1985; Miller *et al.*, 1997).

Microsomes from human liver metabolized MTBE to TBA *in vitro* (Hong *et al.*, 1997b). The specific activity varied about 2-fold for 8 liver samples obtained in the U.S. Of two samples from China, activity for one was at the high end of the U.S. range, and the other slightly exceeded the highest activity U.S. sample. The involvement of cytochromes P450 was indicated by microsomal location, NADPH dependence, and inhibition by carbon monoxide. Human CYP2A6 and 2E1, expressed in a baculovirus system, were able to metabolize MTBE, with 2A6 having about 9-fold higher activity per nmol protein than 2E1 (Hong *et al.*, 1997b). Another study, reported as a meeting abstract (Poet and Borghoff, 1998), also found that microsomes prepared from individual human livers metabolized MTBE *in vitro*. The kinetics of MTBE disappearance indicated a high affinity, low capacity pathway and a low affinity, high capacity pathway. The former correlated with activity markers for cytochrome P4502E1, and the latter correlated with activity markers for 2A6. Considerable individual variability in activity was found. The V_{max} for the low capacity pathway varied by 3.7 fold, and for the high capacity pathway by 26 fold. Metabolic products were not reported (Poet and Borghoff, 1998).

An *in vitro* study using rat liver microsomes incubated with 1 mM MTBE found equimolar production of TBA and formaldehyde (Brady *et al.*, 1990). The involvement of cytochrome P4502E1 was indicated by an approximately 4-fold increase in metabolism following acetone pretreatment, and loss of 35% of activity following incubation with monoclonal antibodies for this protein. The involvement of P4502B1 was suggested by increased MTBE metabolizing activity following pretreatment with phenobarbital (an inducer of 2B1) and an increase in this protein (assessed by immunoblotting) following MTBE pretreatment (Brady *et al.*, 1990). In studies of metabolic activity of purified rat P450s *in vitro*, 2B1 had a 16-fold higher demethylation activity per nmol protein than 2E1, which in turn had a 10-fold or greater demethylation activity than 2C11 and 1A1 (Turini *et al.*, 1998). A series of inhibition and induction studies also supported a substantial role for 2B1 and a lesser role for 2E1 (Turini *et al.*, 1998). Another study, reported as a meeting abstract, also found production of formaldehyde from MTBE by rat liver microsomes, and induction with phenobarbital pretreatment (Poet and Borghoff, 1997b). In a separate study (Hong *et al.*, 1997a), microsomes prepared from various rat tissues were tested for MTBE metabolizing activity. Olfactory mucosa were found to have the highest specific activity: 46-fold higher than liver. No activity was detected in rat lungs, kidneys, or olfactory bulb of the brain (Hong *et al.*, 1997a).

A study of the apparent anti-estrogenic effects of MTBE in female mice found elevated liver P450 levels following MTBE exposure (Moser *et al.*, 1996b). Mice were treated with MTBE by inhalation at 8000 ppm for 6 h/d, 5 d/week, for up to 3 weeks. Total liver P450 protein increased by 40% after 3 d and 200% after 3 weeks. Induction of specific enzyme activities, including activity indicative of the P4502B family, was observed. Treatment by gavage at 1.8 g/kg/d for 3 d resulted in similar increases in P450 protein

levels and enzyme activity. MTBE increased estrogen metabolism, but this study did not examine metabolism of MTBE *per se* (Moser *et al.*, 1996b).

A series of experiments in rats involving either inhalation (400 or 8000 ppm) or oral (gavage: 40 or 400 mg/kg) exposures to MTBE found a shift in elimination from urine to exhalation at the higher exposures (see below). This is suggestive of a saturation of some metabolic pathway (Miller *et al.*, 1997; Bioresearch Laboratories, 1990[b] as cited in ATSDR, 1996). A PBPK model for rats found an improved fit to elimination data by using a 2-pathway model, with a high-affinity, low capacity component and a low affinity, high capacity component (Borghoff *et al.*, 1996).

Further metabolism of TBA in rats and humans is indicated by the pattern of urinary metabolites. In rats exposed to ^{14}C-MTBE, approximately 70% of urinary radioactivity co-migrated with α-hydroxyisobutyric acid on a C-18 HPLC column. Another peak, co-migrating with 2-methyl-1,2-propanediol, accounted for 14% of radioactivity. Two other peaks, accounting for 10% and 5% of radioactivity were identified. No TBA was identified in urine (Miller *et al.*, 1997). In other studies, rats were exposed to [2-^{13}C]MTBE by inhalation, and urinary metabolites analyzed by nuclear magnetic resonance and gas chromatography/mass spectrometry (Bernauer *et al*., 1998). Qualitatively, the major urinary metabolites were α-hydroxyisobutyric acid, 2-methyl-1,2-propanediol, and (putative) TBA-sulfate. Quantitative analysis of MTBE metabolites was not performed. Minor metabolites were TBA, acetone and (putative) TBA-glucuronide. Similar urinary elimination products were found after administration of [2-^{13}C]TBA by gavage at 250 mg/kg. In a single male human volunteer administered [2-^{13}C]TBA as a single oral dose of 5 mg/kg, the major elimination products were α-hydroxyisobutyric acid and 2-methyl-1,2-propanediol. Minor products were TBA, (putative) TBA-glucuronide and (putative) TBA-sulfate (Bernauer *et al*., 1998). Urinary excretion of the MTBE metabolites found in the rat inhalation study above also occurs in humans after MTBE exposure by inhalation (Amberg *et al.* unpublished, cited in Bernauer *et al.,* 1998; Dekant, personal communication).

Metabolism of TBA has also been indicated by experiments where TBA was administered to rats by injection. Following injection of TBA at 1 g/kg, acetone was found in the blood. Similar results were obtained using [β-^{14}C]-TBA or [α-^{13}C]-TBA. Using [β-^{14}C]-TBA, ^{14}C-acetone and [14]CO_2 were recovered as major products. However, the ^{14}C-acetone and [14]CO_2 were not produced in equal amounts, and relative quantities varied greatly from experiment to experiment (Baker *et al.*, 1982). Rat liver microsomes *in vitro* converted TBA to acetone and formaldehyde; the reaction was NADPH-dependent (indicating the involvement of cytochrome P450). The reaction was also inhibited by hydroxyl radical scavengers and catalase, indicating the involvement of H_2O_2 (Cederbaum and Cohen, 1980; Cederbaum *et al.*, 1983).
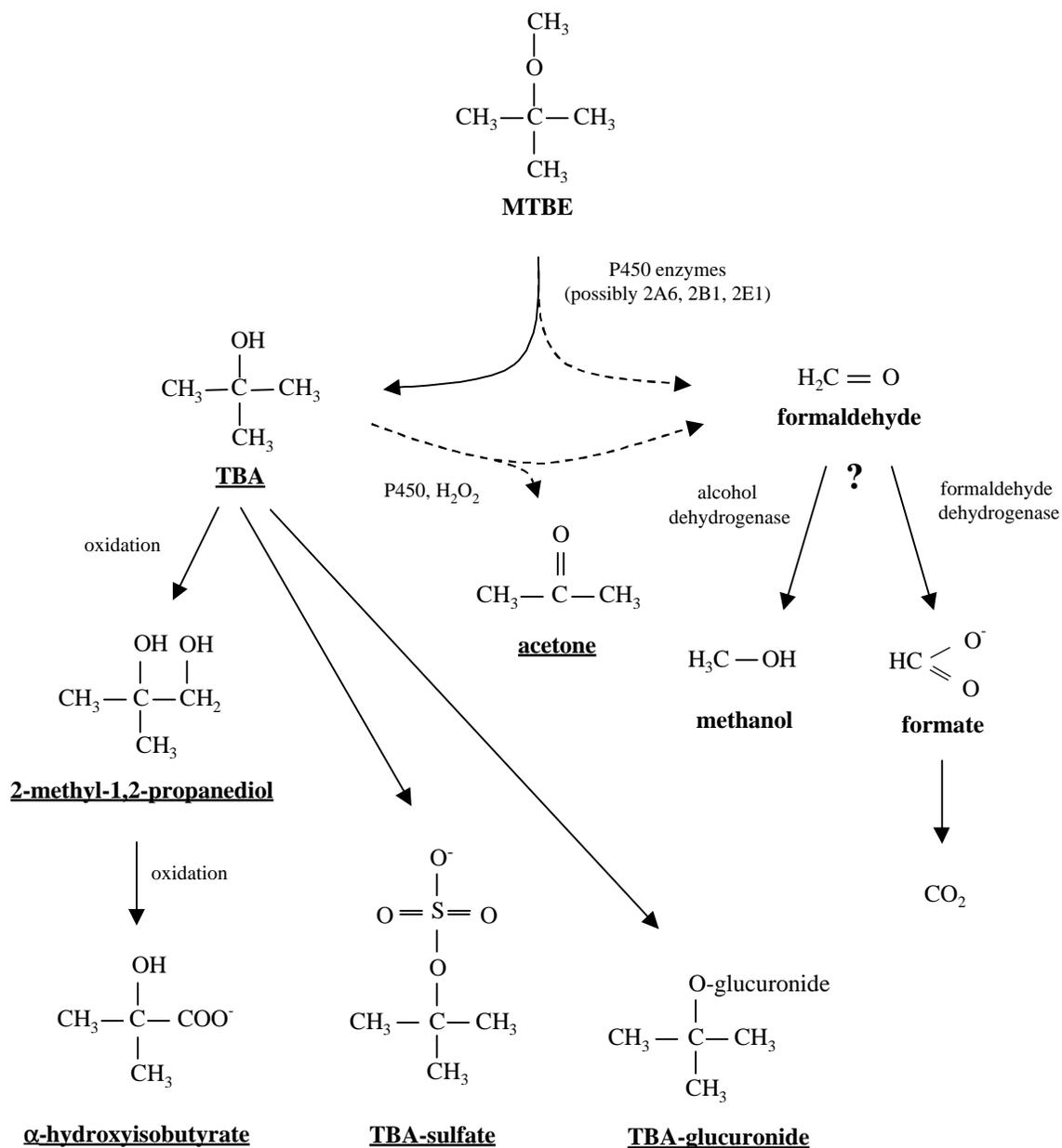
An early paper examined the conjugation of numerous alcohols in the rabbit. When TBA was administered by gavage at 350 mg/kg, additional glucuronide conjugated material was

found in the urine. The additional conjugate amounted to 24.4% of the administered dose of TBA (Kamil *et al.*, 1953).

There is less information concerning the fate of the methyl group of MTBE. As discussed above, the initial product *in vitro* is formaldehyde. However, this has not been clearly demonstrated *in vivo*. Formaldehyde is a normal metabolic intermediate in mammals. In the absence of exogenous exposure, formaldehyde has been found in blood from humans, monkeys, and rats at levels of 2-3 mg/L. It is oxidized to formic acid by two pathways. Formic acid, in turn, can be oxidized to CO_2, excreted in urine, or incorporated into various biological molecules. Formaldehyde can also react directly with biological macromolecules (e.g., DNA and proteins) (IARC, 1995). There is very limited direct information concerning methanol as an MTBE metabolite; trace amounts of methanol were detected when blood and urine of gallstone patients treated with MTBE were analyzed (Leuschner et al. 1991).

Figure 1. Proposed Metabolic Pathways for MTBE

Underlined compounds have been detected in urine after MTBE exposure (Bernauer *et al*., 1998); dashed pathways are based on *in vitro* evidence.

MTBE

P450 enzymes
(possibly 2A6, 2B1, 2E1)

$H_2C == O$
formaldehyde

TBA

P450, H_2O_2

oxidation

alcohol
dehydrogenase

?

formaldehyde
dehydrogenase

acetone

$H_3C — OH$
methanol

HC, O^-, O
formate

2-methyl-1,2-propanediol

CO_2

oxidation

α-hydroxyisobutyrate

TBA-sulfate

O-glucuronide

TBA-glucuronide

Elimination

Data are available on the elimination of MTBE and TBA in humans and rats, and also on elimination of TBA (following TBA exposure) in mice.

As described earlier, 10 male volunteers were exposed by inhalation to MTBE at 5, 25, or 50 ppm during 2 h of light exercise. Elimination in breath and urine was followed for 22 h. After the end of exposure, MTBE concentration in blood dropped rapidly. Decay could be resolved into 4 phases with $t_{1/2}$ = 1 m, 10 m, 1.5 h, and 19 h. Respiratory excretion (including exposure and post-exposure) ranged from 32% to 47% of respiratory uptake. In contrast, blood TBA leveled off following the end of MTBE exposure, began to decline after about 6 h, and declined with a half-life of about 10 h. Less than 0.1% of the inhaled MTBE was eliminated in urine as MTBE within 24 h. Less than 1% of the inhaled MTBE was excreted in the urine as TBA within 24 h, suggesting further metabolism of TBA (Johanson *et al.*, 1995; Nihlen *et al.*, 1998).

In a more limited study, 2 male and 2 female humans were exposed to MTBE by inhalation at 1.7 ppm for 1 h. The blood concentration of MTBE dropped rapidly after exposure, reaching one-half of the peak 40 minutes later. No reduction in blood TBA was evident 90 minutes after exposure (Cain *et al.*, 1996).

In another small study including pharmacokinetic modeling, a man and a woman were exposed to MTBE by inhalation at 1.39 ppm for 1 h. The blood concentration of MTBE dropped rapidly after exposure. A one component model yielded clearances with $t_{1/2}$ = 36 min and 37 min, respectively. A three component model yielded residence times of 5 min, 60 min, and 32 h. In contrast, blood concentrations of TBA continued to increase after the end of MTBE exposure. In the male, TBA concentration in blood peaked about 1-2 h after the end of MTBE exposure, and dropped to about one-half 7 h after the end of exposure. In the female, TBA blood concentration was still increasing 7 h after the end of MTBE exposure. After exposure to MTBE, both MTBE and TBA were found in urine samples in concentrations similar to those in blood. An unexplained observation was that the highest concentrations of TBA in urine were found more than 1 h <u>prior</u> to MTBE exposure. It was thus not clear what the appropriate baseline for elimination of TBA to urine from MTBE exposure was. If it was assumed that all TBA eliminated to urine following MTBE exposure was due to that exposure, the total of MTBE and TBA eliminated to urine was less than 1% of the inhaled MTBE (Buckley *et al.*, 1997; Prah *et al.*, 1994).

Blood and urinary MTBE and TBA were followed in 20-27 humans treated with MTBE by intracystic infusion for gallstone dissolution. MTBE in blood dropped rapidly after treatment and was present in only trace amounts after 12-18 h. TBA was more persistent, and was present at slightly more than one-half the peak concentration 12-18 h after treatment. MTBE and TBA were found in the urine in concentrations similar to those in blood. In one patient, at the end of treatment, MTBE and TBA were found in breast milk at concentrations similar to those in blood (Leuschner *et al.*, 1991).

Rats were exposed to MTBE by inhalation for 6 h at 400 or 8000 ppm (Miller *et al.*, 1997). After exposure, plasma concentrations of MTBE dropped rapidly, with a $t_{1/2}$ of about 0.5 h. Plasma TBA began to drop shortly after the end of exposure to MTBE, with a $t_{1/2}$ of slightly over 3 h. Using ^{14}C-MTBE at 400 ppm, the majority of radioactivity was recovered in urine (64.7% urine, 21.2% expired air), whereas at 8000 ppm, the majority of radioactivity was recovered in expired air (41.6% urine, 53.6 % expired air). This suggests saturation of some metabolic pathway at the higher concentration. Negligible amounts of radioactivity were recovered in feces. When exposed repeatedly (15 d) at 400 ppm, elimination of MTBE was similar to single exposure ($t_{1/2}$ = 0.51 h), but elimination of TBA was somewhat faster ($t_{1/2}$ = 1.8 h). The radioactivity recovered in expired air consisted mainly of MTBE (66% to 79%), with the remainder being TBA. The urinary excretion was mainly (70%) a compound which co-migrated with α-hydroxyisobutyric acid on a C-18 HPLC column. A second peak (14%) co-migrated with 2-methyl-1,2-propanediol. Two other peaks (10% and 5%) were unidentified. No MTBE or TBA was detected in urine (Miller *et al.*, 1997).

In a study where rats were exposed to MTBE by inhalation (described under metabolism, above), the major urinary excretion products identified were 2-methyl-1,2-propanediol, α-hydroxyisobutyric acid, and (putative) TBA-sulfate. The minor products identified were TBA, acetone, and (putative) TBA-glucuronide (Bernauer *et al.*, 1998). Human exposures by inhalation have been reported to produce similar urinary products (Amberg *et al.* unpublished, cited in Bernauer *et al.*, 1998; Dekant, personal communication).

Rats were treated with ^{14}C-MTBE by gavage at 40 or 400 mg/kg. Elimination of radioactivity by exhalation exceeded elimination in urine. Relative elimination by exhalation increased, and by urine decreased, at the higher dose. Elimination in feces was negligible. At the low dose, the $t_{1/2}$ for elimination of MTBE from plasma was 0.52 h and that for TBA was 0.95 h. At the high dose, the $t_{1/2}$ values were 0.79 h and 1.6 h, respectively. (Miller *et al.*, 1997; Bioresearch Laboratories, 1990[b] as cited in ATSDR, 1996).

Rats were treated dermally with MTBE at 40 or 400 mg/kg. The MTBE was injected into a metal chamber that covered an area of the rat's skin. Somewhat slower elimination of MTBE was found than by the inhalation or oral routes. The $t_{1/2}$ for MTBE was 2.3 and 1.8 h, and for TBA was 2.1 and 1.9 h for the low- and high-dose, respectively (Miller *et al.*, 1997).

In addition to data on the elimination of TBA following MTBE exposure, data from rats, mice, and humans on the elimination of TBA following TBA exposure was located. In rats treated with TBA by single injection (1g/kg) or gavage (at a dose chosen to achieve similar blood levels), blood concentrations of TBA dropped to one-half of the peak after about 9-12 h, and to very low levels after about 24 h. In the gavage experiment, pretreatment with TBA appeared to speed elimination slightly (Baker *et al.*, 1982; Thurman *et al.*, 1980). These times for elimination are considerably longer than those reported above for TBA elimination following MTBE treatment. However, the peak TBA

blood levels in the TBA treatment experiments were much higher than in the MTBE treatment experiments.

In female mice receiving 780 mg TBA/kg by gavage, peak blood levels occurred about 1.5 h after administration. Blood levels dropped to one-half the peak after about 6 h and to very low levels after 12 h. Pretreatment with 5 additional doses of TBA did not significantly affect elimination (Faulkner *et al.*, 1989). In mice treated by intraperitoneal injection with TBA at 370, 740, or 1480 mg/kg, peak blood concentrations occurred soon after injection. The time to drop to one-half of the peak increased with dose, from 3 - 4 h at 370 mg/kg to 9 - 10 h at 1480 mg/kg (Faulkner and Hussain, 1989). In mice treated by single injection of TBA at 600 mg/kg, blood concentrations of TBA dropped to one-half of the peak value after about 4 - 5 h, and to very low levels after about 8 h. In contrast, after 3 d of TBA inhalation (concentration approximately 1200 ppm), TBA blood levels dropped to one-half of maximum after about 1 - 1.5 h, and to very low levels after 2.5 h. The blood levels for the injection and inhalation experiments were similar: whether the pronounced difference in rate of elimination was due to differing routes or duration of exposure was not clear (McComb and Goldstein, 1979).

In rats given TBA by gavage (described under metabolism, above), the major urinary metabolites found were 2-methyl-1,2-propanediol, α-hydroxyisobutyric acid, and (putative) TBA-sulfate. Minor urinary metabolites were TBA, acetone, and (putative) TBA-glucuronide (Bernauer *et al*., 1998). In a single human given TBA orally, the major metabolites were 2-methyl-1,2-propanediol and α-hydroxyisobutyric acid. Minor urinary metabolites were TBA, (putative) TBA-glucuronide, and (putative) TBA-sulfate (Bernauer *et al*., 1998).

PBPK models

A PBPK model has been developed for MTBE and TBA in male rats (Borghoff *et al*., 1996), and refined and extended to humans (Rao and Ginsberg, 1997). These models used 7-8 compartments for MTBE and TBA. The best fit to the data for MTBE in rats was obtained by using two saturable metabolic pathways (a high-affinity, low capacity pathway and a low-affinity, high capacity pathway). In the initial model, a reasonably good fit to MTBE absorption and blood data for inhalation, oral (gavage) and injection (intravenous) administration was achieved. However, the fit for TBA in the initial model was poor. The fit of MTBE to humans was reasonable during exposure, but was low after exposure ceased. The authors concluded that additional data were needed on TBA distribution and elimination (Borghoff *et al.*, 1996). The later model improved the fit by fitting MTBE metabolic parameters to both MTBE and TBA empirical data, and by fitting the slowly perfused compartment partition coefficient to the empirical data. Although an improved fit to TBA was obtained, there was still a considerable divergence from experimental values obtained for the rat. The model was extended to humans: the overall time dependence of MTBE and TBA blood concentrations was reproduced, although substantial divergences from experimental data were also found (Rao and Ginsberg, 1997).

Comparison of doses from inhalation and oral routes

Toxicological studies with MTBE have most often used inhalation and oral (gavage) routes. It is therefore helpful in interpreting these studies to compare concentrations used in inhalation studies to doses used in oral studies. Two different approaches have been used to make this comparison. The first used the amount of MTBE inhaled, corrected by an assumed inhalation:oral absorption ratio of 0.5:1 (Dourson and Felter, 1997). The second used a PBPK model to calculate equivalent oral doses based on area under the curve for blood concentrations (OEHHA, 1998) (See Table 5). Neither measure reflects the time-dependence of blood concentration. For inhalation, over the 6 h exposure interval, the MTBE blood concentration rises to a plateau, followed by a rapid drop after the end of exposure. The gavage route produces a sharp peak about 15 minutes after administration, followed by a rapid drop (Miller *et al.*, 1997).

Table 5. Estimated comparison of inhalation to oral (gavage) doses in rats.

MTBE inhalation (6 h/d, 5 d/week) (ppm)	Equivalent oral dose[1] (mg/kg/d)	Equivalent oral dose[2] (mg/kg/d)
0	0	0
400	130	82.9
3000	940	618.8
8000	2700	1848.3

[1] Assumes inhalation:oral absorption ratio 0.5:1 (Dourson and Felter, 1997)
[2] Based upon area under the curve for blood MTBE using a PBPK model (OEHHA, 1998)

3.3.4. Pathology

Lymphomas and leukemias

The tumors observed by Belpoggi *et al.* (1995; 1997) in blood and lymphoreticular tissues, including subcutaneous lymph nodes, in the female Sprague-Dawley rat were diagnosed as leukemias and lymphomas. The authors also reported an increase in dysplastic hyperplasia involving atypical lymphoblasts in lymphoid tissues, which was particularly elevated in low-dose females, and suggested that these hyperplasias may have been precursors to the lymphomas/leukemias.

There has been some debate over whether leukemia and lymphoma incidences were appropriately combined for statistical analysis in the Belpoggi *et al.* (1995; 1997) study. Discussion is somewhat hampered by the fact that the published report does not provide details of the histopathology of these neoplasms. Several reviewers have expressed some reservation about using the leukemia/lymphoma data for risk assessment purposes until further pathological details are available to address the issue of combined incidence. The

study authors have apparently responded to these requests, and have submitted a paper for publication. However, the manuscript is not yet available.

Much of the debate has arisen from a recommendation by the National Toxicology Program (NTP) that, for analysis of Fischer rat data, mononuclear cell leukemias (also known as large granulocytic lymphocyte leukemia or lymphoma) should not be combined with other leukemias/lymphomas (McConnell *et al.*, 1986). NTP also states that histiocytic sarcomas should not be combined with either lymphomas or leukemias. The rationale behind the NTP recommendation for separate analysis rests on both the cell type of origin and background incidence rates of the different neoplasms.

Regarding cell type of origin, the rationale is that neoplasms with distinct cellular origins should be analyzed separately. Histiocytic sarcomas are lymphoma-like tumors of non-lymphocyte origin, typically originating in a phagocytic cell, and therefore could have distinct etiology from neoplasms derived from lymphocytic cells. Analyses of historical data have reported average incidences of histiocytic sarcomas in Sprague-Dawley rats of 1.1% (Frith, 1988), 1.4% (Chandra *et al.*, 1992) and 1.9% (McMartin *et al.*, 1992). It is not clear whether any of the lymphomas reported by Belpoggi *et al.* (1995; 1997) could have been histiocytic sarcomas, but in the absence of firm data to the contrary, it is reasonable to assume that this laboratory's extensive experience in conducting toxicologic pathology evaluations has resulted in accurate diagnoses.

The appropriateness of combining leukemia and lymphoma incidence is somewhat more complex. In rats, the majority of hematopoietic neoplasms originate in cells of the lymphocytic lineage. Myeloid (granulocytic) leukemias are rare (Benirsche *et al.*, 1978; Frith, 1988). This appears to be the case for most strains and for both spontaneous and chemically-induced leukemias and lymphomas. Within the lymphocytic or lymphoblastic leukemias and lymphomas in rats, subclassifications have not been well characterized and the terminology used to refer to subtypes is quite variable (Frith, 1988; IARC, 1990). Primary acute lymphocytic leukemias are quite rare in rats; most cancers of lymphocytic origin observed in rats are lymphomas with eventual dissemination and variable conversion to leukemia (Benirsche *et al.*, 1978).

In a proposed classification of hematopoietic neoplasms in Sprague-Dawley rats, Firth (1988) states "hematopoietic neoplasms derived from lymphoid cells should be referred to as lymphomas. They can be further classified as leukemic or nonleukemic if so desired". This is in agreement with an IARC classification scheme, in which disorders of lymphocytic origin are reported together, with the term "leukemic" as an optional qualifier (IARC, 1993). Thus, subtype analysis of lymphomas/leukemias involving cells of the lymphocytic lineage may not always be useful. Wide adoption and usage of these classification guidelines for hematopoietic neoplasms derived from lymphoid cells in the rat is reflected in the published literature. Due to the common cellular lineage of the majority of lymphomas/leukemias observed in rats, there are abundant instances of reporting combined incidences, under variable classification headings (e.g., Burek and

Hollander, 1977; Rogers *et al*., 1990; Imaida *et al*., 1991; Gross and Dreyfuss, 1993; Komulainen *et al*., 1997).

The rationale for NTP's recommendation to analyze large granulocytic cell leukemia/lymphoma separately from other lymphomas lies in the fact that the Fischer 344 rat has a high background incidence of this disease (also referred to as monocytic leukemia or simply Fischer rat leukemia). Control incidence of this neoplasm reaches 10-50% in this rat strain (IARC, 1990). Studies of the pathology have indicated an origin in a splenic cell of lymphocytic origin, which first generates solid masses in the spleen. The disease then passes through a leukemic phase, and sometimes generates secondary lymphomas as a result of nodular invasion (Moloney *et al*., 1970; Moloney and King, 1973). Because of the unusually high incidence unique to this strain, there appears to be a specific etiology for these leukemias in Fischer rats, which might confound analysis of dose-related effects. Therefore, combining incidence of this tumor type with other leukemia and lymphoma cell types has been deemed inappropriate for this strain.

However, Sprague-Dawley rats do not have high incidences of leukemia/lymphoma in general, and have a much lower incidence of the large granulocytic lymphocyte disease that affects Fischer rats. The reported incidence of the Fischer rat disorder in Sprague-Dawley rats has ranged from 1-7% according to IARC (IARC, 1990). Other reviews have reported lower incidences, e.g., an average incidence of 0.59% in a review of data from 7 bioassays (Frith, 1988). The overall average incidence of malignant lymphomas, a category that includes leukemic dissemination, reported in several reviews of data from multiple bioassays varies from 0.8% (Frith, 1988) to 1.5% (McMartin *et al*., 1992) and 1.9% (Chandra *et al*., 1992). Interestingly, the background incidence of malignant lymphoma is higher in Sprague-Dawley males than in females, while MTBE increased the incidence of lymphomas/leukemias in females only. This further distinguishes the bioassay results from spontaneous cancers.

In summary, in rat strains such as the Sprague-Dawley, which do not have a high incidence of the Fischer rat disease, it is appropriate to combine the incidence of all malignant lymphomas. According to the IARC classification, this would includes large granular lymphocytic leukemia; lymphoblastic, lymphocytic, plasmacytic, and pleomorphic lymphomas; and lymphocytic tumors not otherwise specified (IARC, 1993). A clear presentation of the pathology data from the Belpoggi *et al*. (1995; 1997) study is needed to assure a clear distinction between leukemia/lymphoma and histiocytic sarcoma, which do have different cellular origins. Also, while it is expected based on historical data that very few, if any, of the hematopoietic neoplasms reported were myelocytic in origin, this should be clarified.

Testicular tumors

The testicular tumors observed in both the Sprague-Dawley (Belpoggi *et al*., 1995; 1997) and Fischer 344 (Chun *et al*., 1992; Bird *et al*., 1997) rat strains were diagnosed as Leydig interstitial cell tumors. The majority of Leydig cell tumors observed in rats and humans

are benign, however, up to 10 - 15% of the observed tumors can be malignant (Prentice and Meikle, 1995; Clegg *et al*., 1997). Leydig cell hyperplasia and adenoma are considered to represent a continuous spectrum, and are distinguished based on size (Prentice and Meikle, 1995). Although it is routine practice to pool Leydig cell hyperplasia and adenoma in carcinogenicity studies (Prentice and Meikle, 1995; McConnell *et al*., 1986), neither Belpoggi *et al*. (1995) nor Chun *et al*. (1992) appear to have done so.

The spontaneous incidence of these tumors is typically much lower in the Sprague-Dawley rat, as compared to the Fischer 344 rat (approximately 5% and 88%, respectively at 24-months) (Clegg *et al.,* 1997). The control incidence of these tumors reported by Belpoggi *et al.* (1995) (i.e., 7.7%) is consistent with levels typically observed in the Sprague-Dawley strain. The control incidence at 24 months observed by Chun *et al.* (1992) (i.e., 64%) was lower than that typically observed in the Fischer 344 strain, but within the range (64 - 98%) reported for aged male rats of this strain (Bird *et al.*, 1997; Haseman and Arnold, 1990). The lower spontaneous Leydig cell tumor incidence observed in the Chun *et al.* (1992) study is likely to have facilitated the detection of the dose-dependent increase in Leydig cell tumors in MTBE-treated males, despite the early termination of the mid- and high-dose groups. Interestingly, this same laboratory (i.e., Bushy Run Research Center) reported a similar control incidence of Leydig cell tumors in male Fischer 344 rats (64.9%) in another oncogenicity study (Burleigh-Flayer *et al*., 1997).

The consensus conclusions from a workshop (sponsored by the American Industrial Health Council, the US EPA, and the National Institute of Environmental Health Sciences) on the human relevance of rodent Leydig cell tumors included the statement that "rats are an appropriate model for human Leydig cell growth control under some, but not all, conditions" (Clegg *et al*., 1997). Specifically, the tumor-inducing modes of action of gonadotropin-releasing hormone (GnRH) agonists and dopamine agonists were considered not relevant to humans (Clegg *et al*., 1997). MTBE is not known to be either a GnRH or dopamine agonist.

Kidney tumors

The tumors observed in male Fischer 344 rat kidney tissues (Chun *et al.*, 1992; Bird *et al.*, 1997) were diagnosed as renal tubular adenomas and carcinomas. These two tumor phenotypes are generally considered to be related in origin, with the possibility that adenomas may progress to carcinomas. Therefore, they are normally aggregated for carcinogen identification and risk assessment purposes (US EPA, 1991; IARC, 1998).

As discussed in more detail in section 3.4, the possibility that the male rat-specific α_{2u}-globulin nephropathy plays a significant role in the pathogenesis of MTBE rat kidney tumors has been investigated, and reported to be unlikely (NSTC, 1997; US EPA, 1997a). Briefly, the data indicate that MTBE induces only mild accumulation of α_{2u}-globulin and mild or partial expression of α_{2u}-globulin associated nephropathy in male rats, while clearly exacerbating the expression of non-α_{2u}-globulin rat nephropathy in both males and

females (NSTC, 1997). Support for this conclusion includes the observation that a dose-dependent increase in mortality from chronic progressive nephropathy was observed in male rats at all dose levels, and in females at the mid- and high-dose levels in the rat inhalation bioassay (Bird *et al.*, 1997). Observed microscopic kidney changes included increases in the severity of mineralization and interstitial fibrosis in all treated males, and increases in mild to moderate glomerulosclerosis, interstitial fibrosis, and tubular proteinosis in mid- and high-dose females (Chun *et al.*, 1992). In addition, a rare renal tubular tumor was observed in one MTBE-treated female rat (Chun *et al.*, 1992).

In a separate analysis of a 13-week inhalation exposure study of male rats conducted at the Bushy Run Research Center laboratory, Swenberg and Dietrich (1991) measured the levels of α_{2u}-globulin associated with hyaline droplets in MTBE-treated and control kidneys. Although a slight increase in renal cortex staining for α_{2u}-globulin was observed in MTBE-treated animals, as compared with controls, there was no relationship between the level of α_{2u}-globulin staining and the dose of MTBE received (US EPA, 1997b; Swenberg and Dietrich, 1991). In a study by Lington *et al.* (1997), inhalation of 4,000 and 8,000 ppm MTBE for 13 weeks resulted in a moderate increase in the size of hyaline droplets in male rat kidney, but no MTBE-associated increase in the area or intensity of α_{2u}-globulin immunostaining was observed, as reported by Bird *et al.* (1997). In a four-week inhalation study, exposure to 3,000 and 8,000 ppm MTBE slightly increased the levels of protein accumulated in male rat kidney tubule epithelial cells, but not the levels of α_{2u}-globulin, as compared with controls (Bird *et al.*, 1997). (See section 3.4. for discussion of additional data relevant to the possible role of α_{2u}-globulin in MTBE induced kidney tumorigenicity.)

Liver tumors

The tumors observed by Burleigh-Flayer *et al.* (1992) (and published as Bird *et al.,* 1997) in mouse liver were diagnosed as hepatocellular adenomas and carcinomas. These two tumor phenotypes are generally considered to be related in origin, with the possibility that adenomas may progress to carcinomas. They are normally therefore aggregated for carcinogen identification and risk assessment purposes. The sensitivity of the study to detect treatment-related tumors, especially in the low- and mid-dose groups, may have been compromised by the less-than-lifetime length of the study (18 months).

3.4. Mechanism

The mechanism(s) by which MTBE induces tumors at multiple sites in rats and mice is unknown at this time. It is unclear whether MTBE itself plays a direct role in the observed tumorigenesis, or whether metabolism to one or more active metabolites is required. Two metabolites of MTBE, formaldehyde (Kerns *et al.*, 1983; Sellakumar *et al.*, 1985; Soffritti *et al.*, 1989; Til *et al.*, 1989; Woutersen *et al.*, 1989) and TBA (NTP, 1995), have both been shown to possess tumorigenic activity in animal studies. Interestingly, there is a commonality of tumor sites observed for MTBE and formaldehyde when both are administered by the oral route, and for MTBE and TBA. Leukemias were

observed in male and female Sprague-Dawley rats administered formaldehyde in drinking water (Soffritti *et al.*, 1989), while gavage administration of MTBE induced leukemias and lymphomas in females of the same rat strain. Renal tubular cell adenomas and carcinomas were observed in male Fischer 344 rats administered TBA in drinking water (NTP, 1995; Cirvello *et al.*, 1995), while MTBE administered by inhalation also induced renal tubular cell adenomas and carcinomas in males of the same rat strain. Formaldehyde was classified as a Group 2A carcinogen (i.e., probably carcinogenic to humans) by IARC (1995) based on sufficient evidence in animals and limited evidence in humans. In reviewing the results of the two-year drinking water studies with TBA, NTP (1995) concluded that "there was 'some' evidence of carcinogenic activity of TBA in male Fischer 344/N rats based on increased incidences of renal tubule adenoma or carcinoma (combined)".

It is presently unknown whether the nature or degree of MTBE metabolism is tissue- or sex-specific, or whether there is any relationship between the site of metabolism and target tumor sites. Comparison of the target tumor sites in rats administered MTBE by two different routes of administration is inherently limited by the use of different rat strains in these studies; however, these findings suggest that route-specific distribution and metabolism of MTBE may be of importance in the development of some (e.g., leukemias and lymphomas, renal tumors), but not all treatment-associated tumors (e.g., testicular tumors). It has also been suggested that sex-specific differences in metabolism may underlie the development of leukemias and lymphomas in female, but not male rats (Belpoggi *et al.*, 1995; 1997). This hypothesis remains untested, however.

MTBE was negative in a number of genotoxicity assays, as noted in section 3.3.1., testing positive only in the activated mouse lymphoma forward mutation assay (ARCO, 1980; Mackerer *et al.*, 1996). The MTBE metabolite TBA was not mutagenic in either the *Salmonella* assay (Zeiger *et al.*, 1987) or the mouse lymphoma assay (McGregor *et al.*, 1988). Formaldehyde is genotoxic, testing positive in numerous assay systems (IARC, 1995). Data on formaldehyde-related genotoxicity in MTBE tumorigenesis are too limited to draw any conclusions at this time. Studies conducted in freshly isolated mouse hepatocytes from female CD-1 mice (Casanova and Heck, 1997) did not find any dose-related increase in formaldehyde-associated DNA-protein cross-links or RNA-formaldehyde adducts following MTBE-treatment. Similar results were obtained with freshly isolated hepatocytes from male B6C3F$_1$ mice and male Fischer 344 rats (Casanova and Heck, 1997). These data suggest that formaldehyde is not the active species responsible for MTBE liver tumorigenesis in the mouse. In studies using the *in vitro* mouse lymphoma assay, however, formaldehyde has been implicated as the active species responsible for MTBE's mutagenic activity (Mackerer *et al.*, 1996). DNA-protein cross-link data and RNA-formaldehyde adduct data are not available for the other tumor sites noted after MTBE exposure in laboratory animals. Similarly, MTBE (or MTBE-derived)-DNA adduct data are not available for any of the tumor sites associated with exposure to MTBE.

Several hypotheses have been put forward suggesting that MTBE may act via a variety of primarily nongenotoxic mechanisms, such as the involvement of endocrine modulation in mouse liver and rat testicular tumorigenesis (Bird *et al.*, 1997; Moser *et al.*, 1996b) and α_{2u}-globulin nephropathy in male rat kidney tumorigenesis (Bird *et al.*, 1997; Poet and Borghoff, 1997a; 1997b; Prescott-Mathews *et al.*, 1997a). These hypotheses and the available data relevant to each are discussed below. While no such hypotheses have been put forward regarding the induction of rat leukemia or lymphoma by MTBE, the available mechanistic data are also discussed below.

Mouse liver tumors

Several hypotheses have been proposed (Casanova and Heck, 1997; Bird *et al.*, 1997; Moser *et al.*, 1996b) regarding the mode of action of MTBE in the induction of mouse liver tumors, including formaldehyde-mediated genotoxicity, mitogenic tumor promotion, and estrogen antagonism leading to tumor promotion, perhaps as a result of either an interaction with the estrogen receptor (ER), or decreased estrogen production, or decreased responsiveness to estrogen, or enhanced estrogen metabolism. The data relevant to each of these hypotheses, and the strength of the evidence is discussed below. At the present time, none of these hypotheses are supported by the available mechanistic data.

Formaldehyde-mediated genotoxicity

The findings of Mackerer *et al.* (1996) suggested that MTBE-derived formaldehyde was the active compound responsible for mutagenicity in the *in vitro* mouse lymphoma assay. Casanova and Heck (1997) therefore investigated the effect of MTBE treatment on the formation of formaldehyde-associated DNA-protein cross-links and RNA-formaldehyde adducts in freshly isolated hepatocytes from female CD-1 mice. As described earlier, no dose-related increases in DNA-protein cross-links or RNA-formaldehyde adducts were observed following MTBE treatment, although dose-dependent increases did occur in the formaldehyde-treated positive control cells. The authors concluded that in this isolated hepatocyte system, detoxification of MTBE-derived formaldehyde proceeds more rapidly than metabolism of MTBE to formaldehyde, and suggested that this would also be the case in mouse liver *in vivo*. While these data do not rule out a genotoxic effect of MTBE in the liver, they suggest that if there is one, it is not likely to be mediated by formaldehyde.

No studies investigating whether MTBE induces DNA adduct formation in mouse liver have been reported to date. However, studies in mice indicate that *in vivo* exposure to MTBE does not induce unscheduled DNA synthesis in hepatocytes removed to culture (Vergnes and Chun, 1994; McKee *et al.*, 1997). While the time lag between exposure and labeling may have been too long for active repair to be detected, these studies provide some additional evidence that MTBE is not genotoxic in mouse liver.

Mitogenic tumor promotion

In three separate labeling studies, inhalation exposure to MTBE increased liver cell proliferation in CD-1 mice; at days 3 and 21 (Moser *et al*., 1996a), day 5 but not day 28 (Bird *et al*., 1997), and at day 21 (Chun and Kintigh, 1993 as cited by Moser *et al*., 1996a). Thus, MTBE appears to be a mouse liver mitogen. Bird *et al*. (1997) suggested that MTBE might act as a liver tumor promoter as a result of this mitogenic activity; however, there are no data to support this hypothesis at this time. As described in more detail below, comparative studies were undertaken of unleaded gasoline, which also increases mouse liver cell proliferation, and MTBE in a B6C3F$_1$ mouse liver tumor initiation/promotion model. These indicated that unleaded gasoline was active as a liver tumor promoter, while MTBE was not (Moser *et al*., 1996a).

Estrogen antagonism

Data from several studies indicate that exposure of female mice to MTBE results in a number of effects indicative of hormonal disruption, and in particular, anti-estrogenic activity. Decreases in cystic endometrial cell hyperplasia were observed in CD-1 (Burleigh-Flayer *et al*., 1992) and B6C3F$_1$ mice (Moser *et al*., 1996b). Reductions of relative uterine weights (Moser *et al*., 1996b; 1998) and ovarian weights (Moser *et al*., 1998) were observed in B6C3F$_1$ mice, but not in CD-1 mice (Lee *et al*., 1995; Okahara *et al*., 1998). Increases in hepatic cytochrome P450 content in CD-1 (Lee *et al*., 1995; Moser *et al.,* 1996a) and B6C3F$_1$ mice (Moser *et al.,* 1996a), and increases in activities of the P4502B and 1A families of enzymes in CD-1 and B6C3F$_1$ mice (Moser *et al.,* 1996a) were also observed following MTBE administration.

Estrogens have been shown to suppress liver tumor promotion in female B6C3F$_1$ (Vesselinovitch, 1990) and C57BL/6 X DS-F$_1$ (Yamamoto *et al*., 1993), suggesting that chemicals with anti-estrogenic activity might promote liver tumors in female mice. As mentioned above, unleaded gasoline, which exhibits anti-estrogenic as well as mitogenic activity in female mice, acts as a liver tumor promoter in B6C3F$_1$ mice initiated with *N*-nitrosodiethylamine (DEN) (Moser *et al*., 1996a). However, parallel experiments with MTBE did not find that MTBE had any promoting activity in this DEN-initiated B6C3F$_1$ female mouse liver model (Moser *et al*., 1996a). In fact, MTBE had an apparent inhibitory effect on the growth and development of DEN-initiated liver lesions. The number of macroscopic lesions seen at 32 weeks was reduced from 38/mouse in the animals treated with DEN only to 13.8/mouse in the DEN + MTBE animals. The number of microscopic lesions seen at 32 weeks was reduced from 324 total in the animals treated with DEN only to 159 total in the DEN + MTBE animals. While concluding that MTBE did not promote DEN-initiated liver tumors in B6C3F$_1$ mice, the authors suggested that MTBE may have tumor-promoting activity in other initiation-promotion systems (Moser *et al*., 1996a). To date, no data have been reported on the possible tumor promoting activity of MTBE in other initiation-promotion systems, or in CD-1 mice.

It is generally recognized that strain differences exist with regard to both spontaneous and chemically-induced mouse liver tumor incidences (Dragan *et al.*, 1998). As discussed above, the estrogen and anti-estrogen responsiveness of female liver tumors in the B6C3F$_1$ mouse has been demonstrated; however, no such data are available for the CD-1 mouse. Investigations designed to further characterize the effects of MTBE on other estrogen-related parameters in female mice have continued, using both the CD-1 and B6C3F$_1$ mouse. Additional findings in CD-1 mice include the observations that MTBE had no effect on vaginal cytology, the number of corpora lutea, or uterine peroxidase activity (Okahara *et al.*, 1998). In B6C3F$_1$ mice, MTBE increased the length of the estrous cycle, decreased the number of uterine glands, and decreased the number of epithelial layers in the cervix and vagina. It decreased the rate of DNA synthesis in uterine glands, uterine luminal epithelial cells, and cervical and vaginal epithelium. However, there was no effect on the location or intensity of ER immunoreactivity in the uterus, cervix or vagina (Moser *et al.*, 1998). In addition, this study found that MTBE decreased the zona reticularus (the site of cortisol production) of the adrenal gland.

MTBE increases liver metabolism of estrogen in B6C3F$_1$ mice (Moser *et al.*, 1996b), and increases the activity level of the P4501A family of enzymes, which are capable of estrogen metabolism, in both CD-1 and B6C3F$_1$ mice (Moser *et al.*, 1996a). However, MTBE had no effect on serum estradiol levels in CD-1 (Chun and Kintigh, 1993 as cited by Moser *et al.*, 1996a; Lee *et al.*, 1995) or B6C3F$_1$ mice (Moser *et al.*, 1998). These data suggest that MTBE does not disrupt estrogen homeostasis at the systemic level. Moser *et al.* (1998) demonstrated in B6C3F$_1$ mice that MTBE does not bind to the uterine ER, does not down-regulate the uterine ER, and does not activate the ER or antagonize the effects of estradiol in a mammalian-based transient infection assay. These authors concluded that MTBE's anti-estrogenic effects are not mediated through the ER.

A major limitation of the estrogen antagonism hypothesis is that it only addresses the increase in liver tumors observed in female CD-1 mice, and can not account for the increased incidence of hepatocellular carcinomas seen in the male CD-1 mice. As shown in Table 3, when the tumor incidence data are expressed as the number of lesion-bearing animals per total alive at the time the first hepatocellular carcinoma (or adenoma) was observed (to properly adjust for early mortality experienced in the high-dose males), there is a statistically significant increase in hepatocellular carcinomas in the male mice. The proposed female mouse liver-specific mode of action of estrogen antagonism is therefore predicated on an incorrect conclusion that MTBE induces liver tumors only in the female mouse.

Thus, while MTBE exposure of the mouse is associated with various endocrine-related tissue and cellular responses, the available data (Moser *et al.*, 1996a; 1996b; Moser *et al.*, 1998; Okahara *et al.*, 1998) are insufficient to support an endocrine-mediated mode of action for MTBE-associated liver tumors at this time.

Rat testicular tumors

It has been suggested that MTBE's induction of testicular Leydig cell tumors may be hormonally mediated (Bird *et al.*, 1997). Mechanistic data relevant to the induction of testicular Leydig cell tumors in rats by MTBE are limited to studies reported in an abstract by Day *et al.* (1998). Male Sprague-Dawley rats received MTBE in corn oil by daily gavage at doses of 0, 40, 400, or 800 mg/kg for 28 d. A significant decrease in plasma testosterone levels was observed, however, no changes in "androgen-dependent organ weights", plasma luteinizing hormone (LH) or prolactin levels, or testicular mitochondrial or microsomal P450 levels were noted at 28 d (Day *et al.*, 1998). The available data are insufficient to identify an endocrine-mediated mode of action for MTBE-associated testicular tumors in the rat at this time.

Rat kidney tumors

It has been suggested that an interaction between the male rat specific protein α_{2u}-globulin and MTBE is involved in the induction of the renal tubular tumors observed in male rats (Bird *et al.*, 1997; Poet and Borghoff, 1997a; 1997b; Prescott-Mathews *et al.*, 1997a). Data which suggest that α_{2u}-globulin nephropathy may be involved in MTBE kidney tumorigenesis include the following:

- A mild to moderate increase in the number and size of hyaline droplets in the renal proximal tubule cells of MTBE-treated male rats has been observed.

    ◊ In a 10-day inhalation study, MTBE increased the number of protein droplets within the renal proximal tubules of male rats with a statistically significant concentration-related positive trend (Prescott-Mathews *et al.,* 1997a).

    ◊ In a 14-day gavage study, MTBE increased the formation of hyaline droplets in male rat kidney proximal tubular epithelial cells at the highest dose tested (Robinson *et al.*, 1990).

    ◊ In a 28-day inhalation study, MTBE slightly increased protein accumulation in male rat kidney tubular epithelial cells (Bird *et al.,* 1997).

    ◊ In a 13-week inhalation study, MTBE slightly increased hyaline droplet formation in male rat kidney (Swenberg and Dietrich, 1991).

    ◊ In another 13-week inhalation study, MTBE slightly increased the size of hyaline droplets in male rat kidney (Bird *et al.*, 1997 reporting on findings of Lington *et al.*, 1997).

    ◊ In a 90-day gavage study, MTBE slightly increased the number of hyaline droplets in male rat kidney proximal tubular epithelial cells (Robinson *et al.*, 1990).

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- Protein in the renal proximal tubule cells of MTBE-treated male rats stains weakly for α_{2u}-globulin.

  ◊ In a 13-week inhalation study, MTBE slightly increased hyaline droplet formation and staining for α_{2u}-globulin in male rat kidney but these increases were not dose-dependent (Swenberg and Dietrich, 1991).

  ◊ In a 10-day inhalation study, no increase in α_{2u}-globulin staining could be detected in MTBE-treated male rat kidney by immunohistochemical staining (Prescott-Mathews *et al.*, 1997a).

- Using an ELISA-based method, a mild dose-dependent increase in α_{2u}-globulin-immunoreactivity (approximately 150 μg α_{2u}-globulin/mg total protein in controls versus 200 μg α_{2u}-globulin/mg total protein in the high-dose animals) has been observed in rat kidney cytosol of male rats exposed to MTBE by inhalation for 10 d (Prescott-Mathews *et al.*, 1997a).

- MTBE binds weakly to α_{2u}-globulin *in vitro*. Using a kidney homogenate system, only a very weak interaction between MTBE and male rat renal proteins was detected (Poet and Borghoff, 1997a). This interaction did not survive dialysis or anion exchange chromatography (Poet and Borghoff, 1997a).

- A dose-dependent increase in cell proliferation has been observed in the renal cortex of male rats exposed to MTBE by inhalation for 10 d (Prescott-Mathews *et al.*, 1997a).

- Agents which are thought to induce renal tubular tumors via an α_{2u}-globulin-mediated mechanism are nongenotoxic. MTBE has demonstrated little genotoxicity *in vitro* or *in vivo*.

Data which argue against a significant role for α_{2u}-globulin nephropathy in MTBE kidney tumorigenesis include the following:

- Male rat specificity for nephropathy and renal tumorigenicity has not been observed.

  ◊ In a two-year inhalation study, MTBE exacerbated chronic progressive nephropathy and increased mortality associated with chronic progressive nephropathy in a dose-dependent manner in both in female and male rats (Chun *et al.*, 1992; Bird *et al.*, 1997).

  ◊ A rare kidney tumor was observed in one MTBE-treated female rat in the two-year inhalation study (Chun *et al.*, 1992; Bird *et al.*, 1997).

- A clear exposure-related increase in staining for α_{2u}-globulin, an effect typical of classical α_{2u}-globulin nephropathy-inducing agents, has not been observed in male rats treated with MTBE.

  ◊ In a 13-week inhalation study, MTBE slightly increased hyaline droplet formation and staining for α_{2u}-globulin in male rat kidney but these increases were not dose-dependent (Swenberg and Dietrich, 1991).

  ◊ In another 13-week inhalation study, MTBE slightly increased the size of hyaline droplets in male rat kidney, but no increase in the area or intensity of α_{2u}-globulin staining was observed (Bird *et al.*, 1997 reporting on findings of Lington *et al.*, 1997).

  ◊ In a 28-day inhalation study, MTBE slightly increased protein accumulation in male rat kidney, but did not increase α_{2u}-globulin levels (Bird *et al.*, 1997).

  ◊ In a 10-day inhalation study, no increase in α_{2u}-globulin staining could be detected in MTBE-treated male rat kidney by immunohistochemical staining, but using a more sensitive ELISA-based assay a mild increase in the concentration of α_{2u}-globulin (approximately 150 µg α_{2u}-globulin/mg total protein in controls versus 200 µg α_{2u}-globulin/mg total protein in the high-dose animals) was observed (Prescott-Mathews *et al.*, 1997a). This small increase is in contrast to the marked increase seen with classical α_{2u}-globulin nephropathy-inducing agents, such as 2,2,4-trimethylpentane (approximately 200 µg α_{2u}-globulin/mg total protein in controls versus 550 µg α_{2u}-globulin/mg total protein in treated animals) (Prescott-Mathews *et al.*, 1997a).

- α_{2u}-Globulin-positive proteinaceous casts, another effect typical of classical α_{2u}-globulin nephropathy-inducing agents, were not seen at the junction of the proximal tubules and the thin loop of Henle in several short-term studies, including a 10-day inhalation study (Prescott-Mathews *et al.*, 1997a), a 28-day inhalation study (Bird *et al.*, 1997), or a 13-week inhalation study (Swenberg and Dietrich, 1991; US EPA, 1997b). However, in a 90-day oral study a small number of granular casts were observed (Robinson *et al*., 1990).

- Linear mineralization of papillary tubules, another effect typical of classical α_{2u}-globulin nephropathy-inducing agents, has not been reported in rats exposed to MTBE to date.

- Investigators have been unable to detect the binding of MTBE to α_{2u}-globulin or male rat renal proteins *in vivo* (Prescott-Mathews *et al.*, 1997b), and only a very weak interaction between MTBE and male rat renal proteins has been detected *in vitro*, using a kidney homogenate system (Poet and Borghoff, 1997a). This interaction did

not survive dialysis or anion exchange chromatography (Poet and Borghoff, 1997a), in contrast to observations with classical α_{2u}-globulin nephropathy-inducing agents, where typically 20-40% of bound ligand is retained after dialysis (NSTC, 1997).

The available data on renal tumorigenesis indicate that MTBE induces only mild accumulation of α_{2u}-globulin and mild or partial expression of α_{2u}-globulin associated nephropathy in male rats, while clearly exacerbating the expression of non-α_{2u}-globulin rat nephropathy in both males and females (NSTC, 1997). The US EPA (1991) established three criteria for causation of an α_{2u}-globulin effect:

> (1) increased number and size of hyaline droplets in renal proximal tubule cells of treated male rats;
>
> (2) accumulating protein in the hyaline droplets is α_{2u}-globulin; and
>
> (3) additional aspects of the pathological sequence of lesions associated with α_{2u}-globulin nephropathy are present.
>
> If the response is mild all of the typical lesions may not be observed, however, some elements consistent with the pathological sequence must be demonstrated to be present.

Evaluation of the available data indicate that the first US EPA criterion has been satisfied, but not the second or third (NSTC, 1997; US EPA, 1997a).

In late 1997, IARC held a workshop to examine, among other issues, the scientific basis for possible species differences in mechanisms by which renal tubular cell tumors may be produced in male rats (IARC, 1998). The final draft of the consensus report from this workshop outlines seven criteria which all must be met by agents causing kidney tumors through an α_{2u}-globulin-associated response in male rats. These criteria are the following:

> (1) Lack of genotoxic activity (agent and/or metabolite) based on an overall evaluation of *in vitro* and *in vivo* data
>
> (2) Male rat specificity for nephropathy and renal tumorigenicity
>
> (3) Induction of the characteristic sequence of histopathological changes in shorter-term studies, of which protein droplet accumulation is obligatory
>
> (4) Identification of the protein accumulating in tubular cells as α_{2u}-globulin
>
> (5) Reversible binding of the chemical or metabolite to α_{2u}-globulin
>
> (6) Induction of sustained increased cell proliferation in the renal cortex

(7) Similarities in dose-response relationship of the tumor outcome with the histopathological end-points (protein droplets, α_{2u}-globulin accumulation, cell proliferation)

The data summarized above indicate that the second, fourth and seventh IARC criteria have not been satisfied. With regard to the third criterion, the classical α_{2u}-globulin-associated accumulation of granular casts has not been observed in several shorter-term studies. Similarly, linear mineralization of papillary tubules, which is also part of the characteristic sequence of histopathological changes, has not been observed. With regard to the fifth criterion, MTBE appears to reversibly bind to α_{2u}-globulin only very weakly. As to the sixth criterion, there are no data available to evaluate whether MTBE induces a sustained increase in cell proliferation in the renal cortex.

Thus, based on both the US EPA (1991) and IARC (1998) criteria, α_{2u}-globulin nephropathy does not appear to play a significant role in MTBE kidney tumorigenesis.

Rat lymphomas and leukemias

There are limited mechanistic data concerning the possible mechanisms of action of MTBE in inducing hematopoietic neoplasms. Two studies have investigated potential genetic damage in lymphocytic cells. As described above in section 3.3.1., increased tail moments were observed in the comet assay performed on peripheral lymphocytes isolated from Sprague Dawley rats exposed to 800 mg/kg MTBE by gavage (Lee *et al.*, 1998). Increased comet tails are indicative of DNA strand breaks. Female rats were not tested. In another study, CD-1 mice administered MTBE by gavage did not show mutations at the *hprt* locus in spleen lymphocytes (Ward *et al*., 1995). These two assays are sensitive to different types of genetic damage, thus these findings are not necessarily inconsistent. No conclusions can be made at this time concerning the ability of MTBE to cause genetic damage in lymphocytes. It is not known whether observed leukemias and lymphomas were induced in part by MTBE-associated chromosomal damage, such as that measured in the comet assay.

Formaldehyde, a genotoxic metabolite of MTBE, has been associated with increased hematopoietic cancers in both animals and humans. Consistency across studies has not been observed, however. In a population-based case/control study elevated odds ratios for leukemia and lymphoma were observed but did not reach statistical significance (Linos *et al*., 1990). Significantly increased standard mortality ratios for leukemia and all lymphatic/hematopoietic cancers were seen in one of eleven studies of formaldehyde-exposed cohorts (IARC, 1995). In one drinking water study in Sprague-Dawley rats dose-related increases in lymphoblastic leukemia and lymphosarcoma were observed relative to drinking water controls (Soffritti *et al*., 1989). Two other drinking water studies in Wistar rats failed to identify increased rates of hematopoietic cancers. The extent of metabolism of MTBE to formaldehyde in bone marrow or lymphocytic tissues is not known. In summary, the available data are inadequate to suggest a mechanistic pathway responsible for producing leukemia/lymphoma in response to MTBE exposure.

4. SUMMARY AND CONCLUSIONS

4.1.   Summary of evidence

Epidemiological studies of the carcinogenic effects of MTBE are not available. Carcinogenicity of MTBE has been observed in both sexes of the rat in lifetime gavage studies (Belpoggi *et al.*, 1995; 1997), in male rats of a different strain in a 24-month inhalation study (Chun *et al.*, 1992; Bird *et al.*, 1997), and in male and female mice in 18-month inhalation studies (Burleigh-Flayer *et al.*, 1992; Bird *et al.*, 1997). Statistically significant increases in Leydig interstitial cell tumors of the testes were observed in rats orally exposed, and in a different rat strain exposed via inhalation. While some reviewers have pointed out that Leydig cell tumors in the rat are not always predictive for humans, MTBE has not been shown to act via either of the mechanisms that are thought to be specific to rats. A statistically significant increase in renal tubular tumors was observed in one of the test strains of male rats. While it has been suggested that interaction with α-2u-microglobulin may play a role in MTBE-induced kidney tumors, neither the criteria established by IARC nor those established by the US EPA to define α-2u-microglobulin based mechanism are met by the MTBE data. The mechanism of kidney tumor induction remains uncertain. A statistically significantly increase in incidence of leukemias and lymphomas (combined) occurred in female rats of one strain. While the study report does not provide pathological details that would help to further characterize these cancers, the authors' use of a combined category for statistical analysis in this strain is supported by an IARC tumor classification scheme and historical data. Statistically significant increases in hepatocellular carcinomas were observed in male mice, and statistically significant increases in adenomas and combined adenomas and carcinomas were observed in female mice.

MTBE has demonstrated little or no genotoxicity *in vitro* or *in vivo*. The mechanism by which MTBE induces tumors at multiple sites in animals remains unknown (NSTC, 1997; Mennear, 1997). Additional supporting evidence is provided by the carcinogenic activity of formaldehyde and TBA, two primary metabolites of MTBE, which share target tumor sites in common with MTBE. Both TBA and MTBE cause renal tumors in one strain of rat, and both orally administered formaldehyde and MTBE were associated with lymphohematopoietic cancers in a different strain.

4.2.   Other Reviews

Recent authoritative reviews by the White House National Science and Technology Council and the US Environmental Protection Agency (US EPA) have considered the carcinogenicity evidence summarized in this document, and have concluded, based on the weight of evidence, that MTBE poses a cancer hazard, while a review of the National

Research Council did not state a conclusion about the likelihood of a human cancer hazard.

In 1996, the Health Effects Institute (HEI), with support from US EPA, conducted a review of the literature. HEI concluded that MTBE is a rodent carcinogen (HEI, 1996), but noted that the implications for human health risk were complicated by toxicity at the doses which induced cancers in rodent bioassays. The HEI report stated "Considering that the mechanisms of action of these and other nonmutagenic rodent carcinogens are poorly understood, it would seem imprudent to dismiss these results as irrelevant" (HEI, 1996 p.94).

Based on MTBE carcinogenicity in two species, by two routes of exposure, and at multiple organ sites and on the fact that one metabolite is a probable human carcinogen and another induced tumors at the same site as MTBE, the final report of the White House National Science and Technology Council (NSTC) concluded that there is "sufficient evidence that MTBE is an animal carcinogen" (NSTC, 1997, p. 4-26) and that the "weight-of-evidence supports regarding MTBE as having a carcinogenic hazard potential for humans" (NSTC, 1997, p. 4-26). The National Research Council reviewed the HEI document and an earlier draft of the NSTC report (NRC, 1996). The review did not state a conclusion about the likelihood of a human health hazard, but did express reservations about the relevance of the renal tubular cancers and the leukemias and lymphomas in rats to humans health. These issues have been discussed in Sections 3.3 and 3.4, above. In 1997, the US EPA developed a drinking water advisory for MTBE, which states "the weight of the evidence indicates that MTBE is an animal carcinogen and the chemical poses a carcinogenic potential to humans" (USEPA, 1997a). US EPA expressed uncertainties about quantitative risk estimation for low dose exposures. Therefore, there is agreement from comprehensive reviews of the science by US EPA and NSTC that MTBE poses a carcinogenic hazard to humans. However, uncertainties remain about the nature and extent of risk at very low doses, and about the particular tumor sites that are most relevant to humans.

4.3.   Conclusion

Based on the information reviewed in the preparation of this document, there is evidence for the carcinogenicity of MTBE in animals. MTBE causes leukemias/lymphomas in female rats, renal tubular tumors and Leydig cell tumors in male rats, and hepatocellular tumors in mice. Positive animal carcinogenicity data and some further concordance in tumor sites for formaldehyde and TBA, metabolites of MTBE, provide support for this conclusion. However, uncertainties remain about the nature and extent of risk at very low doses, and about the particular tumor sites that are most relevant to humans.

5. REFERENCES

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