

Review

Toxicology and human health effects following exposure to oxygenated or reformulated gasoline

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Abstract

In order to replace antiknock leaded derivatives in gasoline, legislations were enacted in the United States and other countries to find safer additives and to reduce CO, O₃, and volatile organic compounds (VOCs) in non-attainment areas. Oxygenates commonly used include various alcohols and aliphatic ethers. Methyl *tert*-butyl ether (MTBE) is the most widely used and studied ether oxygenate and is added to gasoline at concentrations up to 15% by volume. Inhalation of fumes while fueling automobiles is the main source of human exposure to MTBE. Humans are also exposed when drinking water contaminated with MTBE. Epidemiological, clinical, animal, metabolic and kinetic studies have been carried out to address human health risks resulting from exposure to MTBE. MTBE is an animal carcinogen, but its human carcinogenic potential remains unclear. Because MTBE functions as a non-traditional genotoxicant, several mechanisms were suggested to explain its mode of action, such as, functioning as a cytotoxic as opposed to a mitogenic agent; involvement of hormonal mechanisms; or operating as a promoter instead of being a complete carcinogen. Some studies suggested that carcinogenicity of MTBE might be due to its two main metabolites, formaldehyde or tributanol. A role for DNA repair in MTBE carcinogenesis was recently unveiled, which explains some, but not all effects. The totality of the evidence shows that, for the majority of the non-occupationally exposed human population, MTBE is unlikely to produce lasting adverse health effects, and may in some cases improve health by reducing the composition of emitted harmful VOCs and other substances. A small segment of the population (e.g. asthmatic children, the elderly, and those with immunodeficiency) may be at increased risk for toxicity. However, no studies have been conducted to investigate this hypothesis. Concern over ground and surface water contamination caused by persistent MTBE has lead the Environmental Protection Agency (EPA) to proposed reducing or eliminating its use as a gasoline additive. The major potential alternatives to MTBE are other forms of ethers such as ethyl *tert*-butyl ether (ETBE) or *tert*-amyl methyl ether (TAME), and alcohols such as ethanol. More definitive studies are needed to understand the mechanism(s) by which aliphatic ethers may pose health and environmental impacts. The switch from MTBE to ethanol is not without problems. Ethanol costs more to produce, poses challenges to the gasoline distribution system, extends the spread of hydrocarbons through ground water in gasoline plumes, and

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in the short-term is unlikely to be available in sufficient quantity. Moreover, its metabolite acetaldehyde is a possible carcinogen that undergoes a photochemical reaction in the atmosphere to produce the respiratory irritant peroxyacetyl nitrate (PAN). Congress is addressing whether the Clean Air Act Amendments (CAA) provisions concerning reformulated gasoline (RFG) should be modified to allow refineries to discontinue or lessen the use of oxygenates. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

The Clean Air Act Amendments (CAA) of 1990 require year round emissions reduction of toxic air pollutants from motor vehicles and ozone-forming volatile organic compounds (VOCs) during high ozone season (US Congress, 1990). The amendments stipulate two types of gasoline, (1) reformulated gasoline (RFG) designed to reduce ozone (O_3) levels in O_3 non-attainment areas (i.e. those exceeding a daily maximum O_3 1-h average of 0.12 ppm more than once per year); and (2) oxygenated gasoline (oxyfuel) designed to reduce carbon monoxide (CO) emissions in CO non-attainment areas during winter. O_3 is formed in the troposphere by sunlight-induced photochemical reactions between nitrogen oxides (NO_x) and VOCs. RFG must contain year-round an oxygenating agent, which may also function as an antiknock compound, of at least 2.0% oxygen by weight; this will reduce O_3 by decreasing fuel evaporation and by limiting NO_x and VOCs exhaust emissions (Erdal et al., 1997). Oxyfuel requires that the oxygenate (alcohols or related ethers) be added to conventional gasoline during the winter months so that the final product contain 2.7% oxygen by weight (Balter, 1997). Currently, methyl *tert*-butyl ether (MTBE) in concentrations up to 15% by volume (\sim 2.7% oxygen by weight) is the predominant chemical oxygenate because of favorable qualities such as its manufacturing and distribution capabilities, performance qualities (high octane number, low sulfur content, blending vapor pressure and boiling point) and its low cost compared with other high-octane components. Ethanol is also used as a fuel oxygenate, but to a lesser extent than methanol, because of its high cost. Toxicities of ethanol and methanol have been detailed else-

where (ATSDR, 1993; Ahmed, 1995). Other oxygenates such as ethyl *tert*-butyl ether (ETBE), *tert*-amyl methyl ether (TAME), *tertiary*-amyl ethyl ether (TAEE), diisopropyl ether (DIPE) and *tert*-butyl alcohol (TBA) are also used. The odor threshold for ether-gasoline blends is 15–80% lower than gasoline alone (White et al., 1995a). Fig. 1 shows the structure and metabolism of the most commonly used aliphatic ethers, the subject of this review.

2. MTBE

2.1. Environmental exposure, compound fate and health risks

MTBE is a volatile ether that is derived from the catalytic reaction of methanol and isobutylene. In 1998, its total worldwide production was 6.6 billion gallons, with the US consuming the most, about 4.3 billion gallons annually (Borghoff and Williams, 2000) and is considered as one of the top 50 chemicals in production (WHO, 1998). Thus, the potential exposure for humans to MTBE is considerable. A minor portion of MTBE is used therapeutically for *in vivo* dissolution of cholesterol gallstones in humans (Hellstern et al., 1998).

MTBE enters the environment during all phases of the petroleum fuel cycle (e.g. auto emissions, evaporative losses from gasoline stations and vehicles, storage tank releases, pipeline leaks and accidental spills, and refinery stock releases). When released into air, the greater part will exist in the atmosphere, with small amounts entering soil and water, with chemical degradation being the major removal source from air. When released into water, a significant amount of MTBE re-

mains dissolved in surface water, with some partitioning into air and a much smaller amount into soil; the key removal process being volatilization (WHO, 1998). Because its high solubility, once released, it moves through the soil and into ground water more rapidly than other chemicals present in gasoline. Once in ground water, it is slow to biodegrade and is more persistent than other gasoline-related compounds (EPA, 1999).

The major non-occupational source of MTBE exposure to the general population is environmental emissions from gasoline (e.g. during time spent at service stations, while driving cars, in parking garages, and in homes with attached garages). These exposures generally occur through inhalation. Inhalation from fumes while fueling automobiles was reported as the principal route for human exposure (Dourson and Felter, 1997). In addition, discharges into the soil or groundwater from leaking stationary sources, such as underground storage tanks (USTs), can contaminate water supply leading to exposures when such wa-

ter is drunk. Dermal contact may also occur through accidental spills of MTBE-blended gasoline, or through use of gasoline as a solvent (NRC, 1996). Brown (1997) estimated arithmetic mean occupational dose via air to range from 0.1 to 1.0 mg/kg per day, while doses from residential exposures, commuting and refueling to be in the range of 0.0004–0.006 mg/kg per day. When refueling cars, the concentrations of MTBE range from less than 1–4 ppm (within the breathing zone and from 0.01 to 0.1 ppm inside cars [1 ppm = 3.57 mg/m³ at 25 °C, 1 atm] (Hartle, 1993). The Environmental Protection Agency (EPA) reference dose (RfD) corresponds to 0.42 mg/kg per day (EPA, 1993). Lifetime doses for workers were in the range of 0.01–0.01 mg/kg per day (Brown, 1997).

The cumulative dose distribution for the entire population of the MTBE-using regions of the US was estimated by combining the distributions of doses and the numbers of people in each exposure category. In the MTBE-using areas, arithmetic

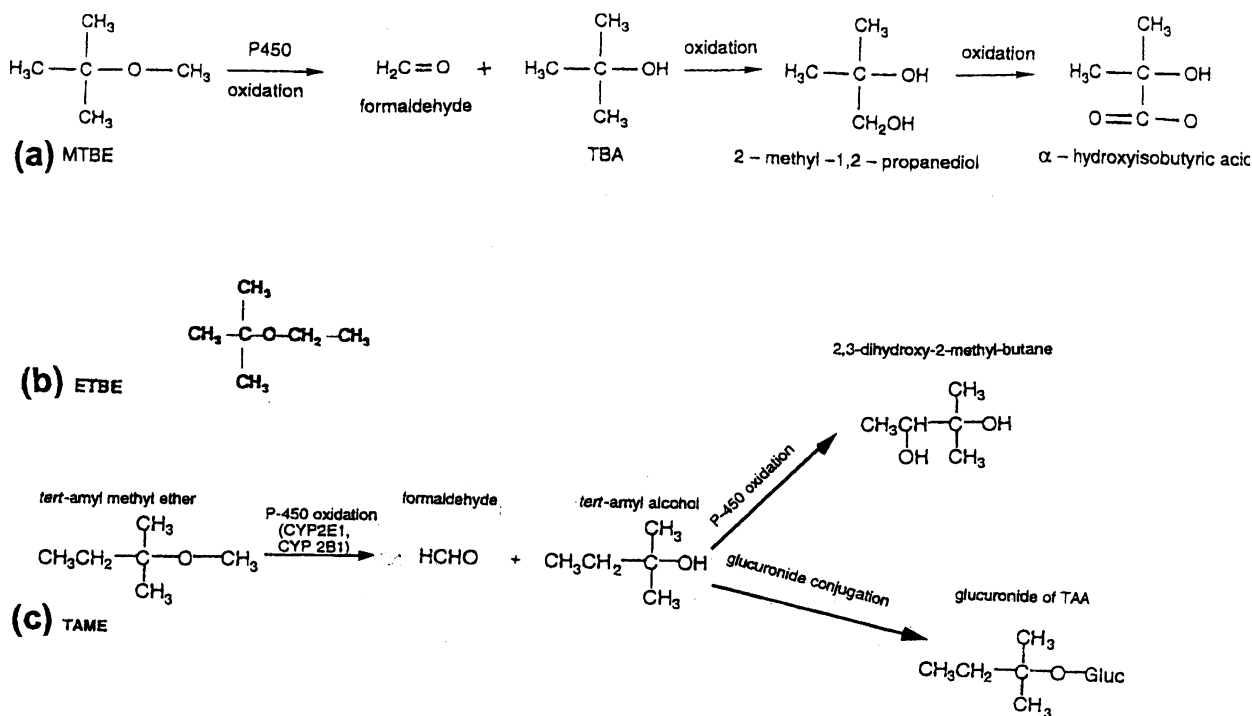


Fig. 1. Structure and metabolic pathways of three commonly used aliphatic ether oxygenates.

mean doses via air were estimated to be 0.0053 and 0.00185 mg/kg per day for the chronic and lifetime cases, respectively (Brown, 1997).

It was further reported that 1.5% of the population use water contaminated with MTBE leakage having an estimated geometric mean concentration of 0.36 µg/l and a 95th percentile concentration of 64 µg/l. Including ingestion, inhalation and dermal absorption of contaminated water, the estimated arithmetic mean dose of the population exposed via water was 1.4×10^{-3} mg/kg per day, which is well below the RfD via either air or water, although chronic doses are higher for occupational exposure (Brown, 1997).

A major issue regarding the use of MTBE concerns its detection at low levels in ground water in numerous locations nationwide and at elevated levels in some municipal drinking water wells and reservoirs (EPA, 1999). In 1998, the state of Maine tested nearly 800 public water supplies and 950 randomly selected private wells and found detectable levels of MTBE in 16% of the public water supplies and 15% of the private wells, although none of the public water supply samples exceeded the state drinking water standard of 35 µg/l, and only 1% of private water samples contained MTBE concentrations above the standard. Roughly 94% of public water supply samples showed MTBE levels that were either not detectable or were below 1; the remaining 6% of samples were between 1 and 35 µg/l (MDHS, 1998).

The most extensive MTBE in drinking water monitoring data is available from California. As of January 2000, 1444 systems had tested 6492 sources of drinking water. MTBE was detected in 52 (0.8%) of these sources, including 31 of 6076 ground water sources (0.5%) and 21 of 416 surface water sources (5%). Overall, 30 (2.1%) of the 1444 public water systems reported detection of MTBE in at least one of their drinking water sources. Although the state database did not include some contaminated wells that have been closed, very few sources had MTBE concentrations exceeding the EPA taste and odor drinking water advisory of 20–40 µg/l. Looking at ground water generally (not only drinking water wells), the California Environmental Protection Agency

has estimated—based on monitoring information of underground storage tank (UST) systems—that MTBE can be expected to be found undegraded in shallow, unused ground water at thousands of UST sites in the state at concentrations in ppm range (CAEPA, 2000).

Nationwide, the data on the presence of MTBE in drinking water are more limited. In July 1999, the EPA-appointed Blue Ribbon Panel on oxygenates in gasoline reported that between 5 and 10% of drinking water supplies in high oxygenate use areas show at least detectable amount of MTBE. The vast majority of these detections were well below the levels of public health concern, and only roughly 1% of detections exceeded 20 µg/l (EPA, 1999). In a 1998 survey of state leaking underground storage tank (LUST) programs undertaken by the University of Massachusetts and EPA, 19 states reported detections of MTBE in public water systems. Among these states, the total number of public wells with MTBE detection was estimated to range from 251 to 422 wells, with the vast majority of detection being below 10 µg/l (Hitzig et al., 1998). Research carried out by the US Geological Survey from 1993 to 1998 for some 2743 monitoring observations and water supply wells in 42 states showed MTBE to be present in 22 of the 42 states at about 5% (145) of the wells, with MTBE levels exceeding 20 µg/l in 0.5% (12) of the wells. Moreover, low concentrations of MTBE were detected in 21% of ambient ground water samples in high MTBE-use RFG or oxyfuel program areas, and 2.3% of samples in low or no MTBE-use areas (USGS, 1999).

The impact of burning cleaner gasoline was conducted to predict changes in the atmospheric concentrations of VOCs resulting from use of RFG and oxyfuels. Modeled ambient air concentrations of VOCs were used to compare baseline gasoline to (1) summer MTBE:RFG; (2) winter MTB:RFG; or (3) MTBE oxyfuel. The model predicted that the addition of MTBE to RFG or oxyfuel would decrease acetaldehyde, benzene, 1,3-butadiene and particulate organic matter (POM), but increases formaldehyde tailpipe emissions (Table 1). The increased formaldehyde

Table 1
Impact of MTBE on vehicle emissions of VOCs in summer and winter

Component	Summer (mg/mile)		Winter (mg/mile)		
	Baseline gasoline	MTBE RFG	Baseline gasoline	MTBE RGF	MTBE oxyfuel
Acetaldehyde	8.1	7.5	14.4	13.4	13.4
Benzene	108.5	71.8	178.4	112.3	113.2
1,3-Butadiene	13.0	12.0	23.0	19.6	17.9
Formaldehyde	15.0	16.5	26.6	30.8	32.6
POM	5.9	5.4	10.5	10.0	10.1
Total	150.5	112.3	252.9	186.1	187.2

Values are estimated based on Mobile 5a modeling of 1995 vehicle fleet emissions. Source, Spitzer (1997); with permission.

emissions were offset by the reduction of formaldehyde formation in the atmosphere from other VOCs. When a range of plausible health estimates was used, the analysis predicted a decline in cancer increase associated with exposure to MTBE:RFG and MTBE oxyfuel. Assuming total US population exposure, the US EPA predicted annual cancer risk estimates associated tail pipe emissions of VOCs for various exposure scenarios (Table 2). An incidence of 437 for 100% use declined 13% (to 379) if baseline gasoline was used exclusively in the summer and MTBE:RFG in winter. If baseline gasoline replaced MTBE in summer and winter, the predicted cancer declined to 362 (17%). The maximum decline (359 or 18%) was achieved when MTBE:RFG was used exclusively in the summer and MTBE oxyfuel in winter. If the current 30% marketplace penetration for MTBE:RFG remained constant, the prediction was that cancer would decline only by 4% (from 437 to 419; Spitzer, 1997). Reductions in 1,3-butadiene exposure accounted for more than 60% of the total predicted cancer incidence, while reduction of benzene exposure, using an alternative risk exposure, accounted for the greatest health benefits. An analysis of microenvironment monitoring data indicated that most exposures to VOCs were significantly below levels of concern, but the health effects associated with short-term exposure to acetaldehyde and benzene warrants more investigation (Spitzer, 1997). Modeling to predict emission changes, ambient O₃ changes and human exposure suggests that even a small MTBE-associated reduction in peak ambient O₃

levels (1–5 ppb) should yield considerable public health benefits (Erdal et al., 1997).

State and local environmental agencies and EPA attributed marked improvements in air quality to the use of fuels containing MTBE and other oxygenates, but the exact role of oxygenates in achieving these improvements is subject to discussion. In Los Angeles, which had the worst air quality in the US, the use of RFG gasoline was credited with reducing ground-level ozone by 18% during the 1996 smog season, compared with weather adjusted data for the same period in 1994 and 1995. Use of RFG also reduced the cancer risk associated with exposure to vehicle emission by 30–40% largely because it uses less benzene, a known human carcinogen (OEHHA, 1999). Whether the oxygenates themselves should be given credit for these improvements has been the subject of debate, with the answer depending to some extent on what one assumes would replace the oxygenates if they were removed. For example, the National Research Council concluded that the addition of commonly available oxygenates to RFG is likely to have little air-quality impact in terms of ozone reduction (NRC, 1999). On the other hand, the EPA's Blue Ribbon Panel concluded that RFG has provided substantial reductions in the emissions of a number of air pollutants from motor vehicles, notably VOCs, CO and mobile-source air toxics (benzene, 1,3-butadiene, and others) in most cases resulting in emissions reductions that exceeded those required by law (EPA, 1999). Less controversy exists regarding oxygenates' role in reducing CO emis-

sions. Both EPA and an interagency group chaired by the White House Office of Science and Technology Policy (OSTP) have reported improvements in CO levels due to oxygenates in some cities with winter oxygenated gasoline programs amounting to 10–14% reductions in ambient CO concentrations (OSTP, 1997). The fuel economy penalty associated with the use of oxy-fuel is ~2–3% and is related to changes in volumetric energy content. However, engine performance is typically not adversely affected by the use of oxygenated fuels (NRC, 1996).

Table 2
Impact of MTBE:RFG and MTBE oxyfuel on predicted cancer incidence in the United States

Scenario	Predicted annual cancer incidence, EPA
Baseline gasoline (summer + winter) × 100% US population exposed (USPE)	437
Baseline gasoline (summer) + MTBE:RFG (winter) × 100% USPE	379
Baseline gasoline (summer) + MTBE oxyfuel (winter) × 100% USPE	369
MTBE:RFG (summer + winter) × 100% USPE	362
MTBE:RFG (summer) + MTBE oxyfuel (winter) × 100% USPE	352
Baseline gasoline (summer × 100% USPE + winter, × 70% USPE) + MTBE:RFG (winter, × 30% USPE)	419
Baseline gasoline (summer × 100% USPE + winter, × 70% USPE) + MTBE oxyfuel (winter, 30% USPE)	416
MTBE:RFG (summer × 100% USPE + winter × 70% USPE) + MTBE oxyfuel (winter, × 30% USPE)	359

Source: modified from Spitzer (1997); with permission.

2.2. Epidemiological and clinical studies

Although several reviews by various state and federal government agencies and national and international organizations in the US and Europe have been conducted to evaluate the potential adverse health effects of MTBE exposure (EPA, 1993, 1999; NCSAB, 1995; HEI, 1996; NRC, 1996; NTP, 1998; ECETC, 1997; NSTC, 1997; OSTP, 1997; WHO, 1998; IARC, 1999; OEHHA, 1999), limited epidemiological and clinical data are actually available on human health effects resulting from exposure to MTBE.

When MTBE-oxygenated gasoline was introduced in the winter of 1992 in Fairbanks and Anchorage, Alaska (4 years later than other states), acute health effects were reported. Non-specific symptoms were characterized at varying levels in the respiratory tract and eyes (burning of nose, throat and eyes, and cough), the gastrointestinal tract (nausea) and the central nervous system (CNS; headache, dizziness and feeling of disorientation). Whereas preliminary studies indicated a positive association between the symptoms and acute exposure as judged by MTBE blood levels (Moolenaar et al., 1994), further studies showed no exposure–response relationship, and no increase in insurance claims from respiratory illness were reported (Gordian et al., 1995; Middaugh, 1995a,b). The Alaskan study had no controls, as it provided no comparative data in either individuals exposed to non-oxygenate gasoline, or in a population not exposed to gasoline.

To resolve some of these issues, the US Center for Disease Control and Prevention (CDC) conducted further studies in Stamford, Connecticut (White et al., 1995a) and in Albany, New York (CDC, 1993). Although these studies were not carried out concurrently, did not use identical methods for identifying study subjects or assessment of health complaints, and were conducted at different times of the year, they provided some insight into causality of the complaints reported in Alaska. The prevalence of key symptoms was similar in Stamford and Albany, both for occupationally exposed individuals and commuters (Table 3). In spite of higher symptom prevalence in occupationally exposed individuals in Fair-

Table 3
Comparison of the CDC studies: Fairbanks, Stamford, Albany

Symptoms	Occupational exposure			Commuters	
	Fairbanks (<i>n</i> = 18; %)	Stamford (<i>n</i> = 105; %)	Albany (<i>n</i> = 105; %)	Stamford (<i>n</i> = 59; %)	Albany (<i>n</i> = 182; %)
Headache	72	27	37	25	24
Eye irritation	67	13	NR ^a	19	NR ^a
Nose/throat burning	50	7	5	7	13
Cough	28	10	21	15	20
Nausea	33	1	6	0	8
Dizziness	44	6	11	2	3
Spaciness	33	6	2	3	7

Source, Balter (1997); with permission.

^a NR, not reported.

banks was higher than in either Stamford or Albany, this difference could not be attributed to MTBE exposure because the post shift MTBE blood concentrations in the Fairbanks and Stamford controls were similar (Balter, 1997). Despite the limitations of the epidemiological studies, if a large segment of a population were affected, they would still be capable of detecting such effects. Table 4 represents results of power calculations for several of the key symptoms. If, for example, the prevalence of headaches were doubled in Stamford compared with Albany, studies of comparable size would be able to detect a statistically significant difference (at $P \geq 0.05\%$) 99% of the time (Balter, 1997).

In a study comparing garage workers in northern New Jersey (high exposure) to those in southern New Jersey (low exposure) no differences were found in the reporting of symptoms attributable to MTBE exposure in this cohort of healthy individuals (Mohr et al., 1994). Although older individuals reported more symptoms, this finding was considered to be due to their preexisting health status rather than to oxyfuel exposure.

A random digit study design, based on response to standardized questions, was undertaken in the Milwaukee area in Wisconsin to investigate the relationship between health complaints and exposure to RFG gasoline. Three geographical areas were chosen, (1) Milwaukee, where there was extensive public resistance to RFG and adverse media coverage; (2) Chicago, IL, where there was

no adverse public reaction to RFG; and (3) a non-Milwaukee, WI, where conventional gasoline was used. Symptoms were significantly higher in Milwaukee than in the other two areas, but symptom prevalence did not increase with increasing exposure when average commuting time was used as a surrogate for exposure. No increase was found in reported symptoms in the oxygenated fuel areas where public resistance and adverse media coverage were absent (Anderson et al., 1995).

A study measuring occupational exposure to MTBE among service station attendants from the Cincinnati, OH area revealed levels below 1 ppm (3.57 mg/m³), even in areas with requirement for use of at least 12% MTBE in motor fuels (Hartle, 1993).

A controlled clinical human chamber study of young, healthy male and female subjects exposed to 1.39 ppm (5 mg/m³) MTBE for 1 h did not report increase in headache, nasal irritation, ocular inflammation, odor intensity, or cognitive or mood changes (Prah et al., 1994).

In a Finnish study investigating changes in neuropsychological symptoms and moods among tanker drivers from three oil companies in various parts of Finland exposed to MTBE as compared with milk delivery drivers from two milk companies from the same locations, 20% of tanker drivers reported acute symptoms of headache, dizziness, nausea, dyspnoea, irritation of saliva excretion at the end of workweek (Hakkola et al., 1997).

In Sweden, a study of acute MTBE vapor on the health of male volunteers, exposure on three occasions—with at least 2 weeks between successive exposures—to 5, 25 and 50 ppm for 2 h during light physical work showed no or minimal effects such as tendency to slight nasal swelling. This effect, however, did not show a clear dose–response relationship (Nihlén et al., 1998a).

Controlled human exposure studies to MTBE added to gasoline in individuals with self-reported sensitivities (SRSs) to MTBE showed no significant differences in symptoms (eye irritation, burning sensation in nose or throat, headache, nausea or vomiting, cough, daytime sleepiness, difficulty concentrating, disorientation, dizziness), neurobehavioral or psychophysiological responses associated with anxiety and/or hyperventilation, between controls and those exposed to gasoline with 11% MTBE. Although these SRSs exhibited increased total symptoms in response to gasoline with 15% MTBE, they did not show a dose–response relationship for MTBE exposure, nor the specific symptoms associated with MTBE that were suggested in the epidemiological studies reported above (Fiedler and Kipen, 2001; Fiedler et al., 2000).

Some hypothesis were suggested to explain these conflicting results, (1) certain individuals abnormally sensitive to chemicals may be particularly sensitive to low levels of MTBE; (2) public and media hysteria cause some individuals to attribute non-specific symptoms to MTBE exposure; and (3) the distinctive and unpleasant odor

of MTBE may trigger stress-related symptoms and increase existing symptom awareness (Balter, 1997; Caprino and Tonga, 1998).

It is also possible that the epidemiological studies may be unable to detect a small subpopulation of sensitive individuals who experience adverse symptoms. Studies of acute effects of MTBE exposure have not examined potentially sensitive populations with underlying respiratory diseases such as children with asthma (McConnell and Tuber, 1998). Nor are there studies on evaporative and exhaust emissions of oxyfuel mixtures, as there is a possibility of synergistic interactions within the emission mixtures that are unpredictable. In addition, these mixtures may contain unidentifiable chemicals that are toxicologically significant (Balter, 1997). The interagency task force concluded that the limited epidemiological and controlled exposure studies conducted to date do not support the conclusion that MTBE as used in the winter oxygenated fuel programs is causing significant increases over background in acute symptoms or illness (OSTP, 1997).

Single day exposure to oxyfuel and its combustion products did not show an immediate effect on the immune system as judged by the level of serum interleukin 6 (Duffy, 1994). However, only one study reported immune reactions to MTBE through hapten carrier reactions, which, in some individuals, end with specific IgG and IgM production (Vojdani et al., 1997b).

Table 4
Probability that epidemiologic studies could detect a two-fold increase in symptoms

Study	Comparison ^a	Symptoms		
		Headache	Eye irritation	Cough
CDC	Stamford/Albany	0.99	ND ^b	0.70
New Jersey	North/south	0.99	0.99	0.92
Wisconsin	Chicago/Wisconsin ^c	0.69	0.48	ND

Oxyfuel exposed compared with unexposed population, $\alpha = 0.05$. Source, Balter (1997); with permission.

^a Exposed cohort/comparison cohort (no oxyfuels program).

^b Symptom data not reported.

^c Wisconsin refers to non-Milwaukee, WI (no RFG program).

2.3. Animal studies

Several toxicity studies in animals were conducted to examine the acute, subchronic, chronic toxicity, carcinogenicity, reproductive effects, developmental abnormalities and teratogenicity employing various routes of exposure to MTBE. In rats, effects were similar in both the inhalation and oral routes; the primary target being the kidney and testis in the males. No oral studies were carried out in mice; the primary target organ of inhalation toxicity was the liver in the females. Table 5 shows the lowest observed adverse effect level (LOAEL) in these studies.

2.3.1. CNS effects

Transient signs of CNS effects normally seen during exposure, or immediately after exposure to ≥ 3000 ppm (ataxia, hypoactivity, blepharospasm and lack of startle reflex) were reported in several inhalation studies in rats and mice, ranging in length from 24 h to 2 years (Dodd and Kintigh, 1989; Robinson et al., 1990; Burleigh-Flayer et al., 1992; Chun et al., 1992; Johnson and Boyne, 1992; Chun and Kintigh, 1993; Bird et al., 1997; Daughtrey et al., 1997; Lington et al., 1997; Bevan et al., 1997a).

2.3.2. Effects on kidney in male rats

Although in only one inhalation study employing CD-1 mice an increase in relative and/or absolute kidney weight was noted after 18-month of exposure, no treatment-related microscopic kidney lesions were observed (Chun and Kintigh, 1993).

Inhalation exposure in rats resulted in treatment-related increases in absolute and relative kidney weight in several studies ranging from 4 weeks to 2 years in both sexes (Dodd and Kintigh, 1989; Chun and Kintigh, 1993; Chun et al., 1992; Bird et al., 1997). The primary neoplastic lesion in MTBE-exposed rats was the increase in the incidence of renal tubular adenomas and carcinomas at the 3000 and 8000 ppm doses in males, but not in females (Bird et al., 1997).

In Sprague–Dawley rats, oral exposure re-

sulted in a significant absolute and relative kidney weight increases in male rats in a 2 and 13 weeks study (Robinson et al., 1990). However, in an oral lifetime study in the same rat strain, no changes in kidney weight, $\alpha_2\mu$ -globulin accumulation, or histopathology were reported (Belpoggi et al., 1995).

A significant increase in hyaline droplet accumulation only in male rats (believed to be due to $\alpha_2\mu$ -globulin) was observed in several studies (Table 5). The accumulation of $\alpha_2\mu$ -globulin in the lysosomes of the proximal tubule cells results in a protein overload causing toxicity, which leads to cell division. The repeated cycles of cell death and division are believed to be associated with development of kidney tumors in rats mediated through a $\alpha_2\mu$ -globulin mechanism (Swenberg and Lehman-McKeeman, 1999). $\alpha_2\mu$ -Globulin was shown to be the only protein involved in the accumulation of protein droplets induced by MTBE (Borghoff and Williams, 2000), and MTBE interacted with $\alpha_2\mu$ -globulin in vitro (Prescott-Mathews et al., 1997) and in vivo (Prescott-Mathews et al., 1999). Following MTBE exposure, male rats had a higher concentration of MTBE in their kidneys compared with female rats, with detectable MTBE levels after exposure to MTBE had ceased (Prescott-Mathews et al., 1997). $\alpha_2\mu$ -Globulin is a specific male rat protein, absent in humans, which has been the cause of kidney tumors seen with several other chemicals (Olson et al., 1990; Baetxke et al., 1991). This species/sex specificity had many investigators question the relevance of male rat nephropathy following human exposure to MTBE (Bird et al., 1997; Mennear, 1997; Stern and Tardiff, 1997; Borghoff and Williams, 2000).

TBA, a metabolite of MTBE, was also shown to cause a low incidence of kidney tumors in male rats (Cirvello et al., 1995), and produced a mild increase in concentration of $\alpha_2\mu$ -globulin in kidney, along with protein droplet accumulation and renal cells proliferation especially in male rats (Borghoff and Williams, 2000). In rodents, the urinary tract is the target organ for TBA toxicity, and males showed more sensitivity than females (Lindamood et al., 1992).

Table 5
The MTBE LOAEL for kidney, liver and CNS effects in different inoculation and oral studies

Species	Route	Exposure levels	Duration	Hyaline droplets ^a	Kidney weight increase	Liver weight increase	Hypertrophy	CNS depression		References
								Ataxia	Hypo-activity	
Rats	Inhalation	0, 400, 3000, 8000 ppm ^{b,c}	4 Weeks	M, 3000 ppm	3000 ppm	3000 ppm	None	8000 ppm	3000 ppm	Chun and Kintigh (1993)
Rats	Inhalation	0, 250, 500, 1000 ppm	13 Weeks	NA	None	None	None	None	None	Greenough et al. (1980)
Rats	Inhalation	0, 800, 4000, 8000 ppm	13 Weeks	M, 8000 ppm	M, 4000 ppm; F, 4000 ppm	4000 ppm	None	8000 ppm	4000 ppm	Dodd and Kintigh (1989)
Rats	Inhalation	0, 400, 3000, 8000 ppm	13 Weeks	NA	NA	3000 ppm	None	8000 ppm	3000 ppm	Bevan et al. (1997a)
Rats	Inhalation	0, 400, 3000, 8000 ppm ^d	24 Months	?	M, ?; F, 3000 ppm	3000 ppm	None	3000 ppm	3000 ppm	Chun et al. (1992)
Rats	Inhalation	0, 800, 4000, 8000 ppm	13 Weeks	M, 8000 ppm	M, 4000 ppm	M, 4000	None	8000 ppm		Lington et al. (1997)
Mice	Inhalation	0, 400, 3000, 8000 ppm	4 Weeks	NA	None	M, 3000; F, 3000 ppm	8000 ppm	4000 ppm	4000 ppm	Chun and Kintigh (1993)
Mice	Inhalation	0, 400, 3000, 8000 ppm ^e	18 Months	None	M, 400; F, 8000 ppm	3000 ppm	M, 3000; F, 8000 ppm	3000 ppm	3000 ppm	Burleigh-Flayer et al. (1992)
Rabbit	Inhalation	0, 1000, 4000, 8000 ppm	2 Weeks	NA	NA	F, 8000 ppm	None	None	None	Bevan et al. (1997b)
Rats	Oral	0, 357, 714, 1071, 1428 mg/kg per day	2 Weeks	M, 1071 mg/kg; F, none	M, 1428 mg/kg per day; F, none	None	None	1428 mg/kg	1428 mg/kg	Robinson et al. (1990)
Rats	Oral	0, 100, 300, 900, 1200 mg/kg per day	13 Weeks	M, 100 mg/kg per day; F, none	M, 900 mg/kg per day; F, none	1750 mg/kg per day	None	1200 mg/kg	1200 mg/kg	Robinson et al. (1990)
Rats	Oral	0, 90, 440, 1750 mg/kg per day	4 Weeks	M, 400 mg/kg per day; F, none	M, none; F, none	None	None	440 mg/kg	440 mg/kg	Johnson and Boyne (1992)

Table 5 (Continued)

Species	Route	Exposure levels	Duration	Hyaline droplets ^a	Kidney weight increase	Liver weight increase	Hypertrophy	CNS depression		References
								Ataxia	Hypo-activity	
Rats	Oral	0, 250, 1000 mg/kg per day ^f	Life time	NA	NA	NA	None	None	None	Belpoggi et al. (1995)
Mice	Inhalation	0, 400, 3000, 8000 ppm ^g	18 Months	NA	M, 3000 ppm	3000 ppm	3000 ppm	3000 ppm	3000 ppm	Bird et al. (1997)
Rats	Inhalation	0, 400, 3000, 8000 ppm ^h	104 Weeks	M, 3000 ppm	M, 3000 ppm	NA	NA	3000 ppm	3000 ppm	Bird et al. (1997)
Rats	Inhalation	0, 800, 4000, 8000 ppm	24 h	NA	NA	NA	NA	4000 ppm	4000 ppm	Daughtrey et al. (1997)

Absolute and relative kidney weight. Modified from Clary (1997); with permission.

^a Accumulation of protein droplets containing $\alpha 2\mu$ in the lysosomes of the proximal kidney tubule cells in male (but not female) rats exposed to MTBE causes cytotoxicity (nephropathy) and stimulates cell division, eventually leading to kidney tumors.

^b 1 ppm = 3.57 mg/m³ at 25 °C (1 atm pressure).

^c 400, 3000 and 8000 ppm in rate equal 223, 1670 and 4450 mg/kg per day; 400, 3000 and 8000 ppm in mice equal 340, 2250 and 6810 mg/kg per day (Mennear, 1997).

^d A statistically significant increase in interstitial cell testicular tumors occurred in male F344 rats at 3000 and 8000 ppm.

^e MTBE caused a statistically significant increase in hepatic adenomas in female CD-1 mice and hepatic carcinomas in male mice.

^f Increased incidence of Leydig cell tumors of the testis and hemolymphoreticular tumors in male Sprague–Dawley rats exposed to 1000 mg/kg, and combined lymphomas and leukemias in females at 250 and 1000 mg/kg.

^g Increased incidence of interstitial cell testicular adenoma in male CD-1 mice exposed to 3000 and 8000 ppm levels, and increased incidence of hepatocellular adenoma in females at 8000 ppm exposure level.

^h Increased incidence and severity of chronic nephropathy in male F344 rats at all exposure levels, and in females exposed to 3000 and 8000 ppm.

2.3.3. Effects on liver in female rodents

A significant increase in liver weight was observed in several inhalation rat studies at high doses in both sexes (Table 5). In CD-1 mice, an increase in hepatocellular hypertrophy in both sexes was also noted in several studies. Although the incidence of hepatocellular carcinoma in the males showed an increase in the high dose males at 8000 ppm, which exceeded the maximum tolerated dose (MTD), combined adenomas and carcinomas were not increased following exposure to MTBE (Bird et al., 1997). After MTBE exposures exceeding the MTD (8000 ppm) female mice showed increased incidence of hepatocellular adenoma (Burleigh-Flayer et al., 1992; Bird et al., 1997). Although this species/sex specific response may suggest involvement of antiestrogenic-like effects of MTBE through interaction with estrogen receptors, which leads to decrease in estrogen circulation, eventually causing the development of hepatic tumors, as reported for unleaded gasoline and other chemicals (Moser et al., 1996b), endocrine alterations were not mediated through the estrogen receptor (Moser et al., 1998). Moreover, unlike gasoline, MTBE did not promote mouse liver tumors (Moser et al., 1996a).

The fact that that MTBE seems to induce its adverse effects only at doses exceeding the MTD indicates that it does not fit traditional genotoxicant mitogens. Cytotoxicants, on the other hand, share a common property of producing cell death followed by increasing cell proliferation in target organs at very high doses (Cohen and Ellwein, 1990; Ahmed and Thomas, 1992; Butterworth et al., 1992). It should be noted that this mouse-specific tumor is of doubtful value in predicting human health effects (Menear, 1997; Stern and Tardiff, 1997; NTP, 1998).

2.3.4. Leydig testicular cell tumors in male rats

Both inhalation studies in Fischer 344 rats and gavage dosages in Sprague–Dawley rats (a strain with low spontaneous incidence of these tumors) suggest a probable association between MTBE exposure and interstitial cell tumors of the testes (Belpoggi et al., 1995; Bird et al., 1997). Occurrence of this tumor coupled with a decrease in

uterine weight, increased estrogen metabolism and hepatic microsomal P450 in female mice suggest that MTBE might disrupt normal hormonal signaling (Borghoff and Williams, 2000).

Studies conducted in Sprague–Dawley rats given high MTBE doses orally for 15 or 28 days resulted in mild changes in hormonal levels. Serum testosterone was decreased in rats treated for 15 but not 28 days, but increased luteinizing hormone levels commonly observed following exposure to chemicals that produce Leydig adenoma were not observed. However, what was observed is decreased leutenizing hormone levels with MTBE dose, a pattern not consistent with Leydig cell tumorigens (Williams et al., 2000).

More studies on the mechanism by which MTBE induces Leydig tumors and their relevance to human are needed. Occurrence of Leydig cell adenoma in test species is of potential concern, as both carcinogenic and reproductive effects, if the mode of induction and potential exposures cannot be ruled out as relevant for humans (Rudo, 1995; Clegg et al., 1997).

Based on the above studies, the EPA estimated that an adult commuter who lives next to a gas station could be maximally exposed for 4 months to oxyfuel concentrations between 0.03 and 0.05 mg/m³ of MTBE (EPA, 1993). The North Carolina Scientific Advisory Board on Toxic Air Pollutants estimated a human cancer risk of 10⁻⁵ from continuous exposure to MTBE for 70 years to be 0.04–0.64 mg/m³ (NCSAB, 1995).

In California, a public health goal of 13 µg/l was adopted for MTBE in drinking water. This figure was based on cancer potency estimates using a polynomial model which fitted the animal data to establish the lower 95% confidence bound on the dose associated with a 10% increased risk of cancer. The cancer potency estimate determined from the geometric mean of the cancer slope factors of the combined male rat kidney adenomas and carcinomas, the male rat Leydig cell tumors and the leukemia and lymphoma in female rats was 1.8 × 10⁻³ (mg/kg per day)⁻¹, assuming a de minimis theoretical excess individual cancer risk level of 10⁻⁶ (OE-HHA, 1999).

2.3.5. Other effects

Many other effects were inconsistently observed in some, but not all studies. For example, an increase in adrenal weight was reported in four rat studies (Johnson and Boyne, 1992; Chun and Kintigh, 1993; Chun et al., 1992; Bevan et al., 1997a). Decreased spleen weight was reported in two mouse studies (Chun and Kintigh, 1993; Bird et al., 1997), and increased spleen weight was seen in two rat studies (Dodd and Kintigh, 1989; Chun et al., 1992). Secondary lesions of hyperplasia of the parathyroid and mineralization of tissue were associated with nephropathy in rats exposed to high doses of MTBE (Bird et al., 1997). A statistically significant increase in the combined incidence of lymphoma and leukemia was reported in female Sprague–Dawley rats given MTBE by gavage in olive oil at 1000 mg/kg (Belpoggi et al., 1995). However, the significance of this finding is questionable because neither cancer caused an increase by itself, nor combining tumors of different histogenic origin is an acceptable evaluative practice (Mennear, 1997; NTP, 1998). Other organ weight changes, and variations in clinical chemistry, hematology and urinalysis parameters were reported (Clary, 1997; Stern and Tardiff, 1997), but their inconsistency make them irrelevant to human health evaluation.

MTBE did not induce reproductive, developmental or teratogenic responses in mice, rats or rabbits (Conway et al., 1985; Biles et al., 1987; Bevan et al., 1997a,b).

The contribution of formaldehyde to MTBE carcinogenesis was investigated in rats and mice. MTBE did not cause nasal tumors following chronic inhalation in rats even though the olfactory mucosa was shown to possess a much greater capacity to metabolize MTBE compared with the liver (Hong et al., 1997a). Thus, formaldehyde was believed not to contribute to MTBE carcinogenicity in this scenario.

2.3.6. Metabolic and kinetic studies

Metabolism of MTBE in rat liver microsomes, P450 showed that equimolar amounts of TBA and formaldehyde were formed by oxidative demethylation (Brady et al., 1990; Hong et al., 1997b) (Fig. 1). Formaldehyde is highly reactive

and is most likely completely metabolized in the liver. TBA is further oxidized into 2-methyl-1,2-propanediol and α -hydroxyisobutyric acid (Hutcheon et al., 1996; Bernauer et al., 1998). In human liver, CYP2A6 is the major P450 isoform responsible for the metabolism of MTBE, ETBE and TAME (Hong et al., 1999).

Mass balance studies in rats using radiolabeled MTBE (intravenous, iv, oral or dermal) showed the majority of MTBE to be rapidly exhaled (20–70% depending on the dose), the remainder being eliminated through the urine (Miller et al., 1997). About 1% of the radioactivity was found in the feces and 2% in the carcass in all groups by 48 h after dosing in all routes of exposure (BioResearch Laboratories, 1990a,b; Table 6). The excretion pattern following inhalation is similar to the iv and oral dosing. Very little, if any, TBA or its metabolites is excreted in the first 6 h, and the amount of TBA excreted in the expired air is \sim 4–6% of the total absorbed dose in the expired air by 6 h exposure, giving the same ratio of MTBE/TBA exhaled by both inhalation and oral routes (Amberg et al., 1999).

The pharmacokinetics of MTBE in humans show that MTBE is cleared very rapidly from blood, making a precise quantifiable link with exposure difficult (Prah et al., 1994), whereas blood levels of TBA remain elevated for a significant duration following cessation of MTBE exposure, similar to what has been observed in rats. This finding makes TBA in blood or urine a better biomarker of cumulative MTBE exposure than the parent compound (Poet et al., 1997; Nihlén et al., 1998b). The partition coefficient of TBA indicates that it is not excreted via lungs to any great extent, and that it is preferentially distributed in body water (Johanson et al., 1995).

Pharmacokinetic studies indicate that MTBE is handled similarly in humans and rats. In the rat, MTBE is rapidly absorbed and rapidly excreted as MTBE and TBA in the expired air and urine, and that MTBA is metabolized to TBA and formaldehyde. However, urinary excretion products in humans (MTBE and TBA) are different from the urinary products of rats (metabolites of TBA) indicating a more complete and/or rapid metabolism of MTBE in rats (elimination half-

Table 6
MTBE balance and distribution data on rodents

Species	Route	Level (~mg/kg)	Duration	Expired air –48 h % of ¹⁴ C ^{a,b}				References
				MTBE and TBA (~6 h; %)	Urine percent of ¹⁴ C (%)	Contents percent of ¹⁴ C (%)	Feces percent of ¹⁴ C (%)	
Rat	Inhalation	400 ppm	1 Day	21.2–22.0 (~72) ^c MTBE-10.7 (~68) TBA-5'-9' (~4)	64.7–65.4 (~24.4) (~24.4)	1–4	~1	BioResearch Laboratories (1990a)
Rat	Inhalation	400 ppm	15 Day	16.9–21.4 (~72) MTBE-11.2 (~68) TBA-5.1 (~4)	67.4–71.6 (~26)	I-4	~1	BioResearch Laboratories (1990a)
Rat	Inhalation	8000 ppm	1 Day	53.6–59.0 (~86) MTBE-22.8 (~80) TBA-4.7 (~6)	35.0–41.6 (~14)	1–4		BioResearch Laboratories (1990a)
Rat	Oral	40 mg/kg	1 Day	45.8–54.4 MTBE-93.3 TBA-6.7	29.0–36.2	~2	~1	BioResearch Laboratories (1990b)
Rat	Oral	400 mg/kg	1 Day	65.2–68.7 MTBE-97.6 TBA-2.3	10.8–16.9	–0.5	~0.3	BioResearch Laboratories (1990b)
Rat	IV	40 mg/kg	1 Day	41.8–46.1 MTBE-93.8 TBA-6.2	25.6–26.5	~2	–1	BioResearch Laboratories (1990b)
Rat	IP	232 mg/kg		95+ MTBE-90 CO ₂ -7.5	3 (Formic acid)	~1	~1	BioDynamics Inc. (1987)
Rat	Dermal	40 mg/kg	1 Day	6.05–9.67	6.12–6.49	–0.8	~0.2	BioResearch Laboratories (1990b)
Rat	Dermal	400 mg/kg	1 Day	19.6–23.2	10.7–12.4			

^a Percent radioactivity recovered, MTBE and TBA values are based on the levels in the air.

^b Is calculated risk value.

^c Figure in bracket is an estimate based on potential excretion during 6 h exposure. From Clary (1997); with permission.

lives for the different urinary metabolites of MTBE were between 7.8 and 17 h in humans compared with 2.9–5 h in rats). Between 35 and 69% of the MTBE, which remained after the end of exposure, was recovered as metabolites in urine of both humans and rats (Amberg et al., 1999).

A physiologically based pharmacokinetic (PBPK) model in rats was developed to describe the dosimetry of MTBE and TBA following inhalation and oral exposures (Borghoff et al., 1996). The model is being tested to verify its ability to predict the dosimetry of MTBE in humans (Borghoff and Williams, 2000).

2.3.7. Short-term tests

The mutagenicity and clastrogenicity of MTBE and its metabolites was evaluated in several *in vitro* and *in vivo* short-term tests. MTBE has tested negative for mutagenicity in, the standard Ames *Salmonella* assay with TA98, TA100, TA104, TA1535, TA1537, TA1538, with and without metabolic activation via the S9 microsomal fraction; *Drosophila*'s-linked-recessive-lethal test; rat bone marrow cytogenetic test; the mouse bone marrow micronucleus test; the N3H/3T3 cell micronucleus test; hprt mutant frequency increase in mouse spleen lymphocytes, and *in vivo*–*in vitro* mouse hepatocyte unscheduled DNA synthesis (UDS) test (Vergnes and Chun, 1994; Ward et al., 1995; McKee et al., 1997; Caprino and Tonga, 1998; Kado et al., 1998; Zhou et al., 2000). On the other hand, in a more recent UDS assay employing rat primary hepatocytes to measure DNA excision repair, a dose–response relationship was observed, and a statistically significant effect at high doses (1000 µg/ml) was observed, indicating that MTBA damages DNA at high doses, and that this damage is repairable by an excision repair system (Zhou et al., 2000).

McGregor et al. (1988) obtained positive results for MTBE, but not TBA, in the L5178Y tk⁺/tk⁻ mouse lymphoma forward mutation assay in the presence of rat S9 activation system. In a modified mouse lymphoma test, the enzyme formaldehyde dehydrogenase (FDH) and its cofactor NAD⁺ were added in large excess during the exposure period such that any formaldehyde produced in this system is converted to formic acid, which is

not genotoxic. An MTBE dose–response increase in the frequency of forward mutations and in cytotoxicity occurred in male rats without FDH present, but not in the presence of FDH and NAD⁺, leading the authors to conclude that formaldehyde derived from MTBE in this system is responsible for MTBE mutagenicity (Mackerer et al., 1996). On the other hand, DNA cross links following formaldehyde exposure in mouse hepatocytes showed only very low levels of cross links due to metabolized formaldehyde, indicating that the rate of formaldehyde production is slow relative to the rate of formaldehyde metabolism; thus metabolized formaldehyde was not considered responsible for MTBE carcinogenesis in this system (Casanova and Heck, 1997).

Tang et al. (1997) studied MTBE and TBA abilities to induce DNA damage of human leukemia cells with the single cell gel electrophoresis technique. Results indicate that at 1–30 mmol/l concentrations, both chemicals cause DNA damage in a dose-dependent pattern.

An *in vivo* test for DNA strand breaks in the rat lymphocyte comet assay after MTBE exposure was positive. 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 dose level (Lee et al., 1998).

Vojdani et al. (1997a), using peripheral human lymphocytes from volunteers with certain genetic makeup and health complaints exposed to 76 ppb MTBE from MTBE-contaminated water for a period of 5–8 years, showed that ~80% of lymphocytes of exposed subjects had a statistically significant increase in the rate of apoptosis, attributable to a discrete block within the cell cycle progression machinery. Programmed cell death reverted almost to the control level by an inhibitor of the nuclear activation factor NF_κβ, which is modulated by reactive oxygen species, illustrating the role this factor plays in oxidative damage (Ahmed, 1999) and the importance of oxidative DNA repair as a mechanism for correcting MTBE induced injury.

A recent study employed Ames *Salmonella* tester strain TA102, which is genetically similar to TA104 in carrying an ochre mutation on *hisG248* gene sensitive to DNA damage detection. However, TA102 strain is proficient in excision repair (*uvrB*⁺), whereas TA104 carries a deletion in the *uvrB* gene, which makes it defective in repairing this damage (Levin et al., 1984). MTBE was weakly mutagenic when tested directly with TA102 strain, and moderately mutagenic with S9 activation. Mutagenicity was inhibited 25–30% by FDH. TA102 revertants were also induced by TBA and by MTBE when human S9 was substituted for rat S9. These results indicate that MTBE and its metabolites induce a mutagenic pathway involving oxidative DNA base and an intact repair system (Williams-Hill et al., 1999).

MTBE is volatile and water-soluble. Given the technical difficulties associated with testing volatile chemicals in bacteria and culture systems, it is possible that careful delivery of MTBE to the genetic material may not have been attained. Additionally, the *in vivo* 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 hepatocytes 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, it is relatively insensitive for detecting genotoxic chemicals with known false negative. Moreover, *in vivo* genotoxicity and metabolism data are not available for a number of organ systems such as rat kidneys and rat testes, which developed tumors in carcinogenicity tests (OE-HHA, 1999). Thus, the overall evidence from short-term tests should be reexamined. It is possible that the lack of mutagenicity/clastrogenicity of MTBE in earlier assays might have been due to inadequacies in either the employed test systems or procedures, or choice of appropriate test systems.

2.3.8. Regulatory classification/response

Much discussion has centered on MTBE carcinogenesis. In spite of some investigators' and representatives of groups attempts to classify

MTBE as a human carcinogen (Belpoggi et al., 1995, 1997; Mehlman, 1996, 1998, 2000), EPA—based on animal studies which looked primarily at inhalation effects, concluded that MTBE poses a potential for carcinogenicity to humans at high doses. However, because of uncertainties and limitations in the data, EPA has been unable to make a confident estimation of risk at low exposure levels, and tentatively classified MTBE as a possible human carcinogen (group C; EPA, 1997).

California's Carcinogen Identification Committee determined not to list MTBE as a human carcinogen. Regarding non-cancer effects, another California advisory committee determined that there was not clear scientific evidence to support listing MTBE as a toxic substance affecting human development or reproduction. These groups generally noted that research gap exists regarding the potential health effects of MTBE, and that the data were particularly limited on health effects associated with MTBE ingestion (OEHHA, 1999).

The International agency for Research on Cancer considered the evidence inadequate for its human carcinogenicity (Group 3), but indicated that there exists limited evidence in experimental animals to warrant concern (IARC, 1999). The National Toxicology Program stated that MTBE is clearly an animal carcinogen, but did not list it as being reasonably anticipated to be a human carcinogen (NTP, 1998). For practical purposes, the interpretation of any health risks associated with the addition of MTBE to gasoline requires a comparison to the health risks associated with conventional gasoline. The Interagency Task Force, EPA, IARC, WHO and some environmental groups have all argued that current knowledge suggests that MTBE is a less serious pollutant than the gasoline components it replaces (OSTP, 1997; WHO, 1998; EPA, 1999; IARC, 1999).

In 1999, the EPA's Blue Ribbon Panel made a number of recommendations—that the winter oxygenated fuels program be continued; that use of MTBE should be reduced substantially and that Congress should act to provide clear federal and state authority to regulate and/or eliminate the use of MTBE and other gasoline additives that threaten drinking water supplies; that Congress act to abolish the current CAA requirement

that 2% of RFG by weight consist of oxygen; that EPA seek mechanisms to ensure that there is no loss of current air quality benefits (i.e. no back-sliding); accelerate laboratory and field research and pilot projects for the development and implementation of cost effective water supply treatment and remediation technology, and harmonize these efforts with other public and private efforts underway; and that a comprehensive set of improvements to the nation's water protection programs be implemented. The panel also suggested that EPA and others should accelerate ongoing health effects and environmental behavior research of other oxygenates and gasoline components that would likely increase in use in the absence of MTBE (EPA, 1998, 1999).

To address concerns raised by the detection of MTBE in ground water and drinking water supplies, EPA issued a drinking water advisory for MTBE in the range of 20–40 µg/l or lower for consumer acceptability reason that will also provide a large margin of safety from adverse health effects. In addition, EPA included MTBE on the monitoring list of unregulated contaminants according to provisions in the Safety Drinking Water Act, and enhanced contamination prevention efforts to implement the UST program. As of January 2001, public water systems are required to monitor MTBE in drinking waters (CRS, 2000). On March 24, 2000, EPA published a proposed rulemaking to limit or entirely ban the use of MTBE in gasoline, limiting the use of MTBE in particular geographical areas or during particular times of the year, limiting the type of facilities in which MTBE can be stored, or limiting the manner in which MTBE can be transported (EPA, 2000). It is expected that the final rule will be published in the fall or winter of 2001.

The principal issue for Congress is whether CAA provisions concerning reformulated gasoline should be modified to allow refiners to discontinue or lessen their use of oxygenates, legislation to permit California refiners to do so has substantial support among the state's congressional delegation. Bills allowing additional flexibility in all states have also been introduced, as have bills that would phase out the use of MTBE (CRS, 2000).

3. Other aliphatic ethers

The use of MTBE and other ethers as gasoline oxygenates is expected to increase in the coming decades (Costantini, 1993); however, their possible adverse effect on human health remains a public health concern. Two other aliphatic ethers structurally similar to MTBE, such as ETBE and TAME (Fig. 1), have been used in reformulated gasoline because ETBE can be produced from renewable sources, and TAME has lower pressure than MTBE resulting in lower separation emissions. Thus, these two ethers have been proposed as alternate oxygenates for MTBE since the 1980s (HEI, 1996; NRC, 1996). Only few studies have been conducted on these ethers; they are listed below.

3.1. ETBE

Like MTBE, ETBE is metabolized in rat liver microsomes and by rat lung tissue and blood into TBA. Rat P450 2B1 was found to have the highest activity towards ETBE and MTBE in a purified reconstituted system (Turini et al., 1998). TBA is further metabolized into 2-methyl-1,2-propanediol and α -hydroxyisobutyric acid, both of which are subsequently excreted in urine (Johanson et al., 1995). The kinetic profile of ETBE in humans could be described by four phases in blood (average half-lives of 2, 18 min, 1.7 and 2.8 h) and two phases in urine (8 min and 8.6 h). Post exposure half-lives of TBA in blood and urine were on average 2 and 8 h, respectively. On an equimolar basis, the 48 h pulmonary excretion of TBA accounted for 1.4–3.8% of the absorbed ETBE. Urinary excretion of ETBE and TBA was low, below 1% of ETBE uptake, indicating further metabolism of TBA, or other routes of metabolism and elimination. The kinetics of ETBE and TBA were linear up to 50 ppm, and TBA is a more appropriate biomarker for ETBE than the parent itself. Acetone levels in blood were higher after ETBE than control human volunteers, indicating that acetone is a byproduct of ETBE metabolism (Nihlén et al., 1998a).

Transient ataxia was observed in male rats following exposure to 5000 ppm of ETBE (Dorman

et al., 1997). In a 4 weeks inhalation study in rats exposed to both ETBE and TAME at doses of 500, 2000 or 4000 ppm, 6 h/day, 5 days/week, ETBE animals did not show treatment-related histopathological changes, although significantly increased liver weights were observed in the mid- and high-dose groups. The 500 ppm was considered a NOAEL for inhalation exposure (White et al., 1995b).

In a subchronic inhalation exposure study in F-344 rats and CD-1 mice exposed to 500, 1750 and 5000 ppm of ETBE for 6 h/day, 5 days/week over a 13 weeks period, degenerative changes in testicular seminiferous tubules, not seen previously for aliphatic ethers, were observed in male rats, but not mice, exposed to 1750 and 5000 ppm ETBE. Moreover, like MTBE, increases in the labeling index of proximal kidney tubule cells in male rats indicating renal cell proliferation, occurrence of regenerative foci, and accumulation of $\alpha_2\mu$ -globulin containing protein droplets associated with syndrome of renal nephropathy were seen in the kidneys of all treated rats. As in MTBE and gasoline exposures, increases in the incidence of centrilobular hepatocyte hypertrophy and rates of hepatocyte cell proliferation were also observed in the liver of both male and female mice in the 5000 ppm group, consistent with a mitogenic response to ETBE (Medinsky et al., 1999).

Oxidative metabolism of MTBE and ETBE was carried out in rat, monkey and human to evaluate the role of involved P450 isozymes. Purified P450 isoforms, differently treated rat liver microsomes, monkey and human hepatic microsomes, and human recombinant P450s expressed in *Escherichia coli* were used. Results showed a linear Michaelis and Menten kinetics with all types of microsomes. Microsomes from phenobarbital and pyrazole treated rats, compared with control microsomes, showed a V_{\max} increase of four and two times, respectively, both in MTBE and ETBE dealkylation, suggesting a catalytic role for 2B1 and 2E1. Inhibition experiments with 4-methylpyrazole, metyrapone, anti 2B1 and anti 2E1, and catalytic experiments with purified rat P450 (1A1, 2B1, 2C11, 2E1) confirmed a primary role of 2B1 and a secondary one of 2E1 in the two ether dealkyla-

tions. With monkey microsomes, in which the orthologous 2B is absent, it was shown that MTBE and ETBE metabolism depends at least partially on 2E1. Finally, with human microsomes, MTBE and ETBE dealkylations were inhibited by methoxypsoralen and correlated with coumarin hydroxylation, but not with enzymatic rates of 2E1 substrates, suggesting a key role of 2A6. Using human recombinant P450s (1A2, 2C10, 2E1, 3A4), the secondary role of 2E1 in humans was confirmed (Amato et al., 1999).

In a computer-automated structure evaluation (CASE) study, where ETBE structure was compared with the structure of known determinants of carcinogenicity in rodents, ETBE was predicted to be neither a genotoxicant nor a carcinogen (Rosenkranz and Klopman, 1991).

3.2. TAME

TAME received serious consideration as an oxygenate in the early 1990s, in spite of its slightly lower octane content than other ethers, as it compares favorably for vapor pressure, boiling point, energy density and water mixability (Caprino and Tonga, 1998; WHO, 1998).

In rats, TAME is oxidized in the liver by cytochrome P450 (CYP) 2E1 and 2B1 into formaldehyde and TAA. TAA is further metabolized by two different mechanisms P450 oxidation leading to 2,3-dihydroxy-2-methyl-butane, and glucuronide conjugation leading to TAA glucuronide (Fig. 1).

A PBPK model that describes the disposition of TAME and TAA in male F-344 rats has been developed in which the compartments for TAME and TAA were flow limited. The TAME model had six compartments, lung; liver; rapidly perfused tissue; slowly perfused tissue; fat and kidney; whereas the TA model had only three compartments, lung; liver; and total body water. The two models were linked through metabolism of TAME to TAA in the liver (Collins et al., 1999).

In the same 4-week rat inhalation study described above for ETBE, TAME resulted in mortality and caused significantly more CNS depression than ETBE, transient changes in either

neuromuscular functions or sensory perception, increased relative liver weight without histopathology or functional changes, and a NOAEL of 500 ppm. Moreover, like MTBE and ETBE, inhalation of TAME showed increased relative liver weight in rats exposed to 4000 ppm (White et al., 1995b). On the other hand, subchronic oral studies with TAME (or MTBE) showed no increase in relative liver weight at doses up to 1 g/kg; the reason for this difference is presently not known (White et al., 1995b).

A 28-day study in Sprague–Dawley rats dosed orally with TAME in corn oil at concentrations of 0.125, 0.5 or 1.0 g/kg per day for 7 days a week resulted in two compound-related deaths out of ten rats in the high dose group, but no other toxicity signs were reported (Daughtrey and Bird, 1995).

A 90-day inhalation study with TAA in CD-1 mice and F-344 rats and beagle dogs was carried out in the 1980s to investigate its toxicity (Dow Chemical USA, 1981). At 1000 ppm, 6 h/day for 5 days/week, male rats and dogs showed increases in absolute and relative liver weights, but no signs of CNS depression were reported at that dose. Blood measurements showed that the excretion of TAA was saturated at the 1000 ppm exposure. Mice showed no signs of CNS depression or organ weight changes at the 1000 ppm.

Short-term tests did not demonstrate evidence of genotoxicity in either the Ames *Salmonella* test, or in mouse micronucleus assay (Daughtrey and Bird, 1995), but a dose-related effect was reported in an in vitro chromosome aberration study in Chinese hamster ovary cells after S9 activation. Moreover, an increased incidence of cleft palate was reported in few pregnant mice, but not rats after exposure to high concentrations of TAME (Caprino and Tonga, 1998).

4. Ethanol

The use of ethanol as a gasoline additive is increasing worldwide, both as a substitute fuel for imported oil and as an oxygenate to minimize air pollution from combustion. In Brazil, for example, approximately one-half of all automobiles run

on gasoline containing 22% ethanol, with the remainder operating on hydrated ethanol (Alvarez and Hunt, 1999). In the US, gasoline containing 10% ethanol is available in many locations, particularly in the Midwest (EPA, 1999). However, the switch from MTBE to ethanol is not without problems. Ethanol costs substantially more to produce than MTBE. It poses a challenge to the gasoline distribution system, as it would separate from gasoline if transported long distances by pipelines, so it must be mixed with non-oxygenated gasoline blendstock close to the market in which it is to be sold. Moreover, in the short-term, it is unlikely that ethanol will be available in sufficient quantity to replace MTBE nationwide (CRS, 2000). Research suggests that because ethanol will likely preferentially biodegrade in groundwater compared with other gasoline components, its presence in a gasoline plume extend the spread of benzene, toluene, ethyl benzene and xylene (BTEX) through ground water (Alvarez and Hunt, 1999). In California where MTBE has been phased-out since March 1999, three state agencies (Air Resources Board, Water Resources Control Board and Office of Environmental Health Hazard Assessment) were required to conduct additional research on the health and environmental impact of ethanol, the most likely substitute. The agencies concluded that if ethanol were substituted for MTBE, there would be some benefits in term of water contamination, but no substantial effect on public health impacts of air pollution (CARB, 1999).

The health effects of ingested ethanol have been extensively investigated (Ahmed, 1995). Given that ethanol is formed naturally in the body at low levels, inhalation exposure to ethanol at the low levels that humans are likely to be exposed are generally not expected to result in adverse health effects in the general population. However, some questions have been raised about potentially sensitive subpopulations (HEI, 1996).

Vehicle exhaust emission data have shown that acetaldehyde (the principal metabolite of ethanol) emissions can increase by as much as 100% with the use of 2.0% weight ethanol–oxygenated gasoline, part of which undergoes photochemical reactions in the atmosphere to make peroxyacetate

nitrate (PAN; Altshuller, 1993). Acetaldehyde is a respiratory irritant at high levels of human exposure, and is currently classified by EPA as a probable human carcinogen (EPA, 1999). PAN is a respiratory irritant to humans. The acute toxicity of PAN is similar to NO₂. Following acute exposure, severe lung lesions and damage to the epithelium of the upper part of the respiratory tract was reported in animals, but data were insufficient to derive a RfC for acute or chronic inhalation exposure to PAN. PAN is a weak point mutagen or clastrogen, and is a known toxin to plant life. The data, however, are insufficient to evaluate its carcinogenicity (Vyskocil et al., 1998).

5. Other alternatives

In addition to ethanol, the most likely alternatives to replace the current volume of MTBE and other ethers in RFG are increased use of refinery streams such as alkylates, reformates, aromatics and other streams resulting from fluid catalytic cracking (FCC) processes. Alkylates are a mix of high octane, low vapor pressure branched chain paraffinic hydrocarbons that can be made from crude oil through well established refinery processes, and are highly favored as streams for blending into gasoline. In general, an increase in the amount of alkylates used in fuel will have no adverse effects on overall vehicle performance. However, the human and aquatic toxicity risk data associated with exposure to alkylates are limited. Aromatic hydrocarbons characterized by unsaturated ring structures of carbon atoms (e.g. benzene, toluene and xylene) and increased use of aromatics would be linked to increased toxic emissions when used in high quantities. At a minimum, testing for non-oxygenated fuel alternatives should include sufficient data to develop an adequate risk assessment. These tests should include inhalation and ingestion data through animal toxicity and human microenvironmental exposure studies using both the additives themselves and the gasoline mixtures of which they are a part. Refiners in California have produced non-oxygenated fuels using low sulfur alkylates and aromatics that met or exceeded all California RFG air quality requirements (EPA, 1999).

6. Conclusions

Compared with MTBE, ETBE is less economical to produce, and TAME is more toxic. Methanol is more economical to manufacture than ethanol, but more studies on the relationship between chronic low level exposure and subtle changes in CNS function need to be carried out (Caprino and Tonga, 1998), and no studies were performed on the magnitude of methanol to reduce CO, O₃ and VOCs in non-attainment areas. Ethanol poses challenges to the gasoline distribution system, and in the short-term are unlikely to be available in sufficient quantities to replace MTBE nationwide. More studies are needed to understand the mechanisms by which aliphatic ethers induce their toxic effect, and their groundwater characteristics ought to be further explored before they are allowed to be placed in widespread use. Gasoline that meets the performance requirement for RFG gasoline without using oxygenates can be made, but the cost of doing so is uncertain, and current laws require the use oxygenates in RFG (CRS, 2000).

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