Epidemiology, Toxicokinetics, and Health Effects of Methyl *tert*-Butyl Ether (MTBE)

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ABSTRACT

This paper reviews the published information assessing the kinetics and potential for adverse health effects related to exposure to the fuel oxygenate, methyl *tert*-butyl ether (MTBE). Data were obtained from previously published reports, using human data where possible. If human data were not available, animal studies were cited. The kinetic profile of MTBE in humans is similar for ingestion and inhalation. The concentrations of MTBE to which the general public is expected to be exposed are orders of magnitude below concentrations that have caused adverse health effects in animals. Controlled human studies have not replicated early epidemiology studies that suggested, but did not confirm, a possible association between MTBE exposure and nonspecific health complaints.

INTRODUCTION

In 1990, the US Congress passed the Clean Air Act Amendments mandating the use of oxygenated fuels in geographic areas that did not meet the National Ambient Air Quality Standards during winter months, when fuel combustion is less efficient. Compliance required the use of gasoline additives known as “oxygenates” (chemicals that increase the oxygen content of gasoline). The most commonly used oxygenate is methyl *tert*-butyl ether. Health complaints of residents in several regions where “MTBE” was used prompted epidemiological evaluation as well as experimental studies in both animal and human models.

These investigations sought not only to confirm the existence or absence of associated human health risk following MTBE exposure but also to establish pharmacokinetic parameters of MTBE. This article critically reviews the relevant studies to date and provides a comprehensive picture of the use, exposure, metabolism, toxicity, and regulation of MTBE.

A possible link between MTBE exposure and health effects has been assessed using a variety of study designs, including both epidemiological investigations of large human populations as well as laboratory studies using animal models. Each study method has inherent strengths and limitations. It is imperative to be mindful of the research design as well as the overall quality of the

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study prior to drawing conclusions and making regulatory recommendations based on the study results. Several comprehensive papers have been written that critically evaluate the current literature in great detail [1–3]. The intended audience of the present review is the working clinician in need of a brief broad review of the available literature without extensive depth. Nonetheless, the conclusions reached by the authors of this review are in agreement with those previously published by others.

The purpose of an epidemiological study is to determine whether an association exists between an exposure and an effect; epidemiological studies do not establish causation. In a retrospective epidemiological investigation, researchers, following residents' health complaints, interview exposed subjects as a data source. When feasible, biological markers such as blood or urine chemical levels may also be measured in order to confirm that exposure actually occurred. These data are then compared with the same metrics in a matched nonexposed control group. Confounders may be controlled both through study design and in the statistical analysis of the collected data. Due to varying exposure levels, circumstances, and durations—as well as the often nonspecific nature of symptoms—it is difficult to draw quantitative dose-response conclusions from epidemiological studies. This is certainly the case with MTBE exposure, in which the primary reported health complaints (e.g., headache, coughing, sneezing, and a burning sensation in the throat) are not specific for MTBE exposure and may easily be attributed to hydrocarbon solvents other than MTBE as well as completely unrelated medical conditions.

Animal studies are used to determine if a given effect is caused by a specific exposure, elucidation of toxicological mechanism, and establishment of dose-response relationships. Multigenerational animal studies are also used to evaluate teratogenicity and reproductive effects of test chemical exposure. Typically, exposed animals are compared with nonexposed animals, often over a range of exposure doses. In order to decrease the possibility of confounding factors affecting results, the environment and exposure levels are strictly controlled and after sacrifice or natural death, the animals are thoroughly necropsied. Further, it is possible to detect both acute and chronic (e.g., carcinogenic) effects over the lifespan of the test animals, which is much shorter than that of humans. Though some animal models are reasonably applied to humans, others are fraught with inconsistencies in predicting human physiological responses.

Controlled human studies, which would ideally provide the most exact and applicable data, are limited by ethical concerns. These experiments can provide useful data about how a chemical is absorbed, to what body compartments and organs it is distributed, how and where it is metabolized and by what route(s) it is eliminated from the body.

METHODS

We performed systematic searches of PubMed and Toxline for articles published up to December 2006, using the key terms

MTBE, toxicokinetics, epidemiology, *t*-butanol, metabolism, oxygenate, reformulated gasoline, and oxyfuel (alone or in combinations), with particular attention for human studies. In parallel, we critically analyzed the US Government reports on the environmental and occupational impacts of oxygenated fuels.

BACKGROUND

Oxygenates are gasoline additives used to increase fuel oxygen content. Increased oxygen content enables internal combustion engines to burn fuel more efficiently and quietly with improved per-gallon mileage. Oxygenates consist simply of a hydrocarbon chain bearing an oxygen atom. Currently, the most widely used oxygenates are low-molecular-weight alcohols (e.g., ethanol, methanol, and isopropyl alcohol) and alcohol ethers [e.g., methyl *tert*-butyl ether (MTBE), ethyl *tert*-butyl ether (ETBE), *tert*-amyl methyl ether (TAME) and diisopropyl ether (DIPE)]. When mixed with gasoline, the ether oxygenates are less volatile and less water soluble than the alcohols. Thus, ether oxygenates are less subject to evaporative loss or partitioning into water as a fuel contaminant than are alcohol additives, which improves year-round engine performance and makes for easier handling, transportation, and storage of the fuel [4]. The most widely used ether oxygenate is MTBE. The estimated production capacity of MTBE in the United States in 1995 was 240,100 barrels per day [5]. In 2005, the reported annual US MTBE production exceeded 47 million barrels.

Synthesis of MTBE is accomplished through the reaction of isobutylene with methanol in the presence of ion exchange resin catalysts. Volatile synthetic by-products are then removed by distillation. The increased demand for MTBE has led to investigation of production methods using other starting materials, including isobutene and isobutanol, through a variety of chemical processes. Although most of the MTBE produced in the United States is manufactured in a few dedicated facilities, the compound is stored and processed in large quantities in more than 30 states [5]. The vast majority of MTBE produced is ultimately used as a gasoline oxygenate. As a result, MTBE may be found essentially anywhere gasoline is found, including waste sites used for fuel disposal.

Governmental regulation of fuel composition has been in effect for well over 30 years. The Clean Air Act (CAA), which banned the use of lead in gasoline, was passed by the US Congress in 1970. The CAA was amended in 1990 to require the oxygenation of two types of fuel: reformulated gasoline (RFG) and oxygenated gasoline (oxyfuel). Reformulated gasoline contains a minimum of 2% oxygen by weight (equivalent to roughly 10% MTBE by volume) and a reduced content of aromatic hydrocarbons such as benzene. The year-round use of RFG is required in geographic areas with high levels of ozone. Oxyfuel is conventional gasoline to which a minimum of 2.7% oxygen has been added. Federal regulations mandate the use of oxyfuel during the winter months in areas with high levels of carbon monoxide (CO) [6]. The US EPA predicted that use of oxyfuels would result in a

13–24% decrease in ambient air CO concentrations as well as reductions in airborne concentrations of volatile organic compounds such as benzene [6]. Emissions testing of vehicles burning fuel containing MTBE reported a 2–10% decrease in CO per percent oxygen by weight. This translated into roughly a 10% reduction in some studies, while other investigations found a smaller or absent effect [6].

As an alternative to surgical removal of the gall bladder (cholecystectomy), dissolution of cholesterol stones in the gall bladder and bile duct has been achieved using MTBE administered via percutaneous transhepatic catheter or endoscope. Although no randomized trials have been performed to date, numerous human case series report promising results with low toxicity and complication rates [7–13]. The reported toxicities associated with MTBE treatment were primarily self-limited nausea and drowsiness, which was rarely severe enough to require treatment cessation. The efficacy of the treatment varied from 50% to 98% in the different studies. In the long term, some recurrence of gallstones was noted in patients treated with MTBE; one follow-up study reported nearly 50% recurrence after several years [10]. This finding is not unexpected as the gall bladder was still present and the factors leading to stone formation were not altered as a result of MTBE dissolution therapy. The authors are aware of no current guidelines in which MTBE dissolution is a recommended therapy for gallstones.

RESULTS

Exposure

Most human exposure to MTBE occurs passively through air and drinking water. Several sources of atmospheric MTBE have been identified involving every step of production and use from manufacture, through distribution, to tailpipe emissions [14]. Of the 149–179 million pound yearly atmospheric burden of MTBE, approximately 73% is due to tailpipe emissions [14]. Petroleum refineries and service stations follow distantly, with each contributing roughly 3 million pounds of MTBE to the atmosphere per year [14]. Other sources contribute amounts smaller by several orders of magnitude.

It is essential to interpret the above data within the proper contextual framework. A 1995 estimate of RFG distribution concluded that about 73 million people (i.e., ~30% of the US population) lived in areas where MTBE was used in RFG or oxyfuels [14]. In 1997, 32 areas in 18 US states participated in the RFG program, and RFG accounted for approximately 30% of gasoline nationwide [15]. Within these areas, exposure is not distributed evenly [16]. People in certain geographical circumstances (e.g., residences in proximity to manufacturing plants, storage facilities, or service stations) and those employed as garage and transportation workers are exposed to higher MTBE concentrations than the general population (Table 1). The mean MTBE exposure concentration for the general public is approximately 1 µg/m³ (~0.28 ppb) [14]. Mean exposure concentrations for service station attendants and workers in the MTBE industry may be thousands of times higher,

Table 1: MTBE Atmospheric concentrations in various populations [14]

Study Groups	Geometric Mean (SD) (µg/m ³)	Arithmetic Mean ^a (µg/m ³)
Manufacturing workers	300 (6.0)	1,500
Blending workers	1,000 (6.0)	5,000
Transportation workers	870 (10.6)	14,000
Distribution workers	470 (6.3)	2,600
Gasoline station workers	2,000 (4.0)	5,200
Mechanics	300 (3.5)	660
Professional drivers	40 (2.5)	61
Commuters	40 (2.5)	61
Other drivers	20 (2.5)	30
Gasoline station customers	150 (4.0)	390
Manufacturing and blending neighbors	3 (2.1)	4
Gasoline station and storage neighbors	30 (3.5)	66
General public	1 (4)	2.6

^aCalculated from distributions, not original data

but the duration of these exposures is typically short [14]. Dose is not solely a function of concentration. Exposure duration must also be considered, as a brief high concentration exposure may actually result in a substantially lower dose when compared with a long-term, low-concentration exposure. For example, gas-station customers may be briefly exposed to high concentrations of MTBE for a few minutes per week. In contrast, persons living in proximity to a production facility are likely to sustain continuous low concentration exposures. Other groups potentially incurring MTBE exposures exceeding those of the general public include automobile commuters and mechanics [14]. Estimated cumulative exposure data are shown in Table 2.

Direct contamination of drinking water with MTBE may occur as a result of wastewater released during manufacture or from fuel spills and storage tank leaks. As the vapor pressure of MTBE is relatively high (245 mmHg at 25° C), the compound readily evaporates into the air. Therefore, surface spills are less likely to directly contaminate drinking water supplies than are leaks from poorly maintained underground storage tanks. Areas with a high density of leaking storage tanks also have higher detection rates of MTBE in groundwater [16]. Of the potentially exposed population, 98.5% receive their drinking water from sources with undetectable levels of MTBE (<2 µg/L), with smaller proportions exposed to higher concentrations (Table 3) [14].

A 2005 study examined 3,964 groundwater samples collected between 1993 and 2002 from sites across the United States, including Alaska and Hawaii [16]. The authors report an MTBE

Table 2: Estimated doses of populations exposed to MTBE in air [14]

Study Groups	Number of Persons	Duration of Event (Events per year)	Geometric Mean Years of Exposure (SD)	Geometric Mean Chronic Dose (SD) (mg/kg/day)	Geometric Mean Lifetime Dose (SD) (mg/kg/day)
Manufacturing workers	883	8 hours (240)	4 (3.0)	5.19E-02 (6.0)	2.97E-03 (8.2)
Blending workers	1,800	8 hours (240)	4 (3.0)	1.73E-01 (6.0)	9.89E-03 (8.2)
Transportation workers	1,489	8 hours (240)	4 (3.0)	1.51E-01 (10.6)	8.60E-03 (13.5)
Distribution workers	7,705	8 hours (240)	4 (3.0)	8.13E-02 (6.3)	4.65E-03 (8.5)
Gasoline station workers	150,000	8 hours (240)	2.5 (3.0)	3.46E-01 (4.0)	1.24E-02 (5.9)
Mechanics	300,000	8 hours (240)	4 (3.0)	5.19E-02 (3.5)	2.97E-03 (5.3)
Professional drivers	900,000	8 hours (240)	4 (3.0)	3.74E-03 (2.5)	2.14E-04 (4.2)
Commuters	30,000,000	1 hours (240)	20 (1.5)	2.47E-04 (2.5)	1.41E-04 (2.7)
Other drivers	22,800,000	1 hours (365)	50 (1.2)	3.75E-04 (2.5)	2.68E-04 (2.5)
Gasoline station customers	52,800,000	3 minutes (70)	50 (1.2)	2.70E-04 (2.5)	1.93E-04 (2.5)
Manufacturing and blending neighbors	815,000	18 hours (350)	8 (2.5)	1.30E-03 (2.1)	1.23E-04 (4.7)
Gasoline station and storage neighbors	11,700,000	18 hours (350)	8 (2.5)	1.30E-03 (6.0)	1.48E-04 (7.5)
General public	60,500,000	24 hours (365)	70 (1.0)	4.00E-04 (4.0)	4.00E-04 (4.0)

Table 3: Extrapolated percentage of population using water by concentration range of MTBE [14]

Concentration range (µg/L)	Percentage of the population exposed
Less than detection limit	98.5
Detection limit, 10	1.21
10–20	0.11
20–200	0.13
200 or more	0.04

detection frequency of 7.6% (300 of 3,964) in drinking water source samples (laboratory reporting range, 0.1–1.66 µg/L). The majority of the 300 MTBE-positive samples were collected in the northeastern United States. Notably, only 0.3% of MTBE-positive samples had concentrations above the lower EPA-recommended MTBE drinking water limit of 20 µg/L [16]. Increased rates of MTBE detection were weakly associated with geographic density of leaking storage tanks, high recharge rates (the rate at which a water source is refilled), MTBE use and high population density [16]. A sampling of wells in California found that only 1.9% (206 of 11,132) had detectable levels of MTBE. In nearly 91% of these positive samples, the MTBE concentration was below even the more stringent California State limit of 13 µg/L [17].

In addition to measurements in environmental matrices, it is also possible to measure MTBE in human biological samples.

These measurements are imperative in establishing an association between exposure to MTBE and health complaints, as detection of MTBE and/or its metabolites within the presenting individual is the only way to unequivocally prove exposure occurred. As such, analytical methods for measurement of MTBE and a key metabolite, *tertiary*-butyl alcohol (TBA; *t*-butanol), in human blood have been developed. Blood concentrations of MTBE and TBA correlate well with ambient air concentrations in both experimental and occupational studies [18–21].

In 1998, Saarinen et al. published an occupational monitoring study of 12 male tanker truck drivers in Finland, in which both MTBE and MTAE (methyl *tertiary*-amyl ether) are used as fuel oxygenates [22]. The drivers were responsible for filling the trucks at supply depots, transporting the fuel and dispensing it into holding tanks at one or more service stations. The investigators measured air levels of MTBE and MTAE as well as urine concentrations of MTBE, TBA, MTAE, and the MTAE metabolite, TAA (*tertiary*-amyl alcohol). A positive correlation was found between time-weighted MTBE exposure data and urine MTBE and TBA concentrations [22]. No such correlation was detected with MTAE exposure and urine MTAE or metabolite (TAA) concentrations. The authors attributed the lack of a discernible exposure/excretion correlation between MTAE exposure and urine MTAE and TAA concentrations to the low levels of these compounds in the air and in the drivers' urine.

Patients treated with MTBE for the dissolution of gallstones were found to have detectable urinary concentrations of MTBE for 5 hours after treatment while TBA persisted in the urine of

these patients for up to 18 hours [8]. Due to its lower volatility, longer half-life and lower detection limit compared with MTBE, some authors suggest that TBA may be a more accurate biological marker of MTBE exposure than parent MTBE [19]. However, the presence of measurable background pre-exposure urinary concentrations of TBA, likely due to sources such as food processing and flavoring, make TBA measurement problematic for chronic low-level MTBE exposure assessment [23]. Two other metabolites, 2-hydroxyisobutyrate and 2-methyl-1,2-propanediol, have also been isolated from the urine of subjects exposed to MTBE [19]. Measurements of MTBE have also been reported in exhaled air [24–27].

It is up to the regulatory authorities of each country to establish what they consider acceptable MTBE levels in their air and drinking water. In the US, the Environmental Protection Agency (EPA) provides nationwide guidelines, although some states (e.g., California) have more stringent requirements. The EPA has not established a reference dose for the presence of MTBE in drinking water, but has published an advisory, in which smell and taste thresholds of 20 and 40 ppm, respectively, are proposed as upper limits [15].

Toxicokinetics

Studies of MTBE kinetics have been performed in both humans and animals. Where possible, this paper will focus on the human data referring to the animal studies for circumstances in which human data are lacking. For the ethical reasons discussed above, the doses to which humans are exposed are substantially lower, and the procedures for collecting results are less invasive, than in animal studies. Animals are typically necropsied at the end of an experiment, while in human studies the researchers are usually limited to blood and urine samples.

Absorption

Human uptake of MTBE has been studied following both inhalation and ingestion exposures. In the reviewed studies, subjects were exposed to MTBE at various low air concentrations ranging from 1.7–50 ppm for 1–4 hours [23,26,27]. Blood concentrations

were found to rise rapidly and then begin to decline immediately upon cessation of exposure. MTBE clearance half-lives ranged from 40 to >120 minutes and were related to exposure concentration (Table 4). Observational studies of exposed workers found a positive correlation between serum concentrations and ambient air levels of MTBE [18,20]. However, inhaled MTBE is not completely absorbed. In fact, as a result of the low blood:alveolar-air partition coefficient of MTBE, when measurements of MTBE in exhaled air collected during exposure were performed, respiratory uptake was determined to be only 40% of the exposure dose [26].

The kinetic profile of MTBE in humans following ingestion of contaminated water has been studied [24,27]. The authors observed peak blood MTBE concentrations 10–20 minutes after ingestion with concentrations and elimination half-lives similar to those observed in inhalation studies. More recently, evidence supporting hepatic first-pass metabolism of MTBE was reported [27]. The case for the existence of a first-pass effect was based on lower concentrations of MTBE in exhaled air and higher TBA:MTBE area under the curve (AUC) ratios when oral (ingestion) and inhalation exposures were compared [27].

In rats, MTBE is distributed rapidly to all tissues studied with the largest percentage of initial body burden detected in the liver [28]. Smaller amounts were found in the lungs, kidney, and testes [28]. Though total organ distribution of MTBE in humans has not been studied, the compound was identified in abdominal wall fat of patients treated with MTBE for gallstone dissolution [8].

MTBE is metabolized in the liver to TBA, 2-hydroxyisobutyrate, and 2-methyl-1,2-propanediol (Figure 1). A small amount (<1%) of inhaled MTBE is excreted unchanged in the urine. Biotransformation is qualitatively and quantitatively similar between rats and humans, and no gender differences were found in either species [23]. Metabolism of MTBE is predominantly mediated by two cytochrome P-450 (CYP) isoenzymes, CYP2A6 and CYP2E1, which in human liver microsomes display turnover numbers of 6.1 and 0.7 nmol TBA/minute per nmol P450, respectively (i.e., CYP2A6 is the more active enzyme by almost an order of magnitude) [29]. Several studies using MTBE and other chemicals have found that CYP2A6 is a low-affinity, high-capacity

Table 4: Summary of Studies Investigating MTBE Uptake and Clearance Following Inhalation Exposure

Study	Sample Size (n)	Exposure Concentration (ppm)	Exposure Duration (hour)	Average Post-Exposure Blood Concentration (μmol/L)	Average Elimination Half-Life (minutes)
(Prah et al. 2004) [27]	14	3.0	1 hour	0.28	36 minutes
(Cain et al. 1996) [31]	4	1.7	1 hour	0.19	40 minutes
(Nihlen et al. 1998) [26]	10	5 25 50	2 hours	1.4 6.5 13	0.8, 12, 90, 21 hours ^a 1.1, 9.6, 96, 20 hours ^a 1.0, 8.6, 84, 17 hours ^a
(Amberg et al. 1999) [23]	6	4 40	4 hours	1.9 6.7	156

^aFour-phase elimination described. Fourth phase reported in hours, all others reported in minutes.

Figure 1: Metabolism of MTBE [5]

enzyme and is thus important in high-dose exposure. In contrast, CYP2E1 is a high-affinity, low-capacity enzyme, and therefore has a greater role in MTBE metabolism following low-dose exposures [30]. Although it is known that TBA undergoes further metabolic transformation to form 2-hydroxyisobutyrate and 2-methyl-1,2-propanediol, the enzyme(s) responsible for these conversions have not been identified.

Significant variation in interindividual blood levels of TBA in humans has been observed with equivalent MTBE exposure con-

centrations [20,31]. Differences in reported prevalence and severity of symptoms between persons exposed to equivalent MTBE concentrations have also been suggested in at least 1 epidemiological investigation [32]. As such, CYP2A6 polymorphism has been offered as an explanation of the differences in complaints, despite comparable MTBE exposure [33]. Although correlating polymorphism of CYP2A6 with serum metabolite levels and symptom severity seems plausible, to date no scientific data supporting this theory exist.

Table 5: Cumulative Excretion and Urinary Half-Lives of MTBE and Metabolites Following Inhalational Exposure to 38.7 ± 3.2 ppm MTBE [23]

	Human (n = 6)			Rat (n = 10)		
	Total excretion (μmol)	Background (μmol)	T _{1/2} (hours)	Total excretion (μmol)	Background (μmol)	T _{1/2} (hours)
MTBE	2.0 ± 1.3	ND ^a	4.3 ± 1.4	ND ^a	ND ^a	—
t-Butanol	29.9 ± 9.4 ^b	0.6 ± 0.4	10.4 ± 1.8	1.2 ± 0.7 ^b	0.3 ± 0.1	3.4 ± 1.0
2-Methyl-1,2-propenediol	205 ± 15.6 ^b	0.3 ± 0.3	9.7 ± 1.5	2.8 ± 1.0 ^b	0.1 ± 0.1	3.4 ± 1.0
2-Hydroxyisobutyrate	943.8 ± 332.3 ^b	221.5 ± 63.2	17.0 ± 2.5	11.1 ± 2.8 ^b	2.1 ± 0.9	4.4 ± 0.9

^aND = Not detected

^bStatistically significant difference from background

Following inhalation, MTBE and its metabolites are eliminated in exhaled air and in the urine. Some MTBE is exhaled as unchanged parent compound. However, urinary excretion is mainly as 2-hydroxyisobutyrate, with smaller amounts of 2-methyl-1,2-propanediol and TBA [23]. The 2-hydroxyisobutyrate metabolite predominates in the urine of both rats and humans exposed to MTBE [23]. Elimination half-lives for these compounds in humans range from 4 to 17 hours, while elimination half-lives in rats were 3.4–4.4 hours (*Table 5*) [23]. Following inhalation exposure to 5–50 ppm MTBE, human metabolic clearance rates ranged from 0.34 to 0.52 L/hour/kg with slightly lower exhalation clearance rates of 0.23–0.32 L/hour/kg for the same exposures [26]. Although three exposure concentrations were tested, a strict parallel between exposure concentration and clearance rate was not observed. Clearances rates and elimination half-lives following oral MTBE administration are not significantly different than the values determined following inhalational exposure [24].

At least one attempt to model the complete kinetic profile of MTBE in humans has been published. Licata et al. used human liver microsomes to develop a 6-compartment physiologically-based model for the human kinetics of MTBE [30]. These in vitro results were extrapolated to whole-body in vivo metabolism as the basis for the model. The predictive ability of the model was then tested using previously published blood concentrations from human volunteer MTBE exposures. The model reliably predicted post exposure MTBE blood levels at all time points for up to 20 hours after a 1-hour inhalation exposure to 1.7 ppm MTBE and a 4-hour inhalation exposure to 40 ppm MTBE. With a 4-hour inhalational exposure to 4 ppm MTBE, the model slightly under-predicted MTBE blood concentrations at early time points, though later time points were adequately predicted. Additional analysis by the authors demonstrated that greater variability existed in the measurement of MTBE blood concentrations than in the concentrations predicted by the model.

Epidemiological Studies

The first suggestion of possible health effects associated with the use of MTBE as a fuel oxygenate came from Fairbanks, Alaska in

1992. In their abstract, Beller and Middaugh reported a survey of a population of individuals presumed to have heavy (taxi drivers; n = 12) or moderate (health-care workers; n = 90) exposure to tailpipe emissions and compared them to a presumed less-exposed control group (university students; n = 101) [34]. Within these groups, the authors found 4 taxi drivers, 26 health-care workers and 15 university students who they believed exhibited symptoms (headache, cough, nausea, mucosal irritation, dizziness, and disorientation) possibly related to “oxyfuel-associated illness.” In this investigation, the reported health complaints appeared to be of an acute, mild, and transient nature. Although the sample size was small, the authors reported a statistically significant increase in symptoms associated with greater tailpipe emissions exposure. The authors designated the reported health complaints as the key symptoms of MTBE exposure-related illness, and this metric was used for subsequent studies.

Great care must be taken in interpreting this report, as it contains substantial methodological limitations. First, neither a biological marker for MTBE exposure (i.e., blood or urine testing) nor measurements of airborne MTBE concentrations from tailpipe emissions were incorporated. Additionally, subject interviews were conducted after negative media reports concerning MTBE, leading to a strong possibility of reporting bias. Furthermore, the reported symptoms are vague and nonspecific rather than pathognomonic markers of MTBE exposure. The authors also failed to rule out the possibility that the reported symptoms were caused by a plausible alternative source such as the gasoline itself, carbon monoxide, or other tailpipe emissions and were not caused by the added MTBE. In addition, the high-exposure and low-exposure (control) groups were not matched demographically, and it is likely that the control subjects were younger than those in the high-exposure group. A similarly designed study performed in Anchorage, AK reported almost identical results [35]. The methodological limitations of both of these studies make application of the reported results problematic. Nonetheless, the use of oxyfuel in Alaska was terminated as a result of health complaints reported to a hotline and the contributions of the two studies discussed above. Despite these limitations and the fact

that these studies were published only as abstracts, they are important to consider as they provide the starting point for controlled human trials investigating the possibility of adverse health effects due to MTBE exposure.

In an attempt to better define true MTBE exposure, Moolenaar et al. compared blood levels of MTBE and the incidence of reported complaints among occupationally exposed workers in Fairbanks, Alaska while MTBE was in use, as well as after its removal [18]. These investigators used as toxicological effects a key symptom profile similar to that proposed by Beller and Middaugh (e.g., headache, cough, nausea, mucosal irritation, dizziness and disorientation, etc.) [34]. The incidence of key health complaints was determined via a survey completed by study participants. Although there was an increased incidence of key complaints reported in subjects comprising the upper quartile of MTBE blood levels, this relationship did not achieve statistical significance. Both blood MTBE concentrations and the reported incidence of key health complaints were significantly decreased following suspension of the oxyfuel program. However, as above, subject awareness of the MTBE discussion in the media may have biased reporting.

In a Stamford, Connecticut study, MTBE blood concentrations of 30 workers who worked near automobiles or gasoline were compared with blood MTBE samples from 29 commuters. Sampling times were at the end of the work shift for workers and immediately following the morning commute for drivers in order to best approximate the end point of MTBE exposure. The subjects with the highest blood levels of MTBE ($>2.4 \mu\text{g/L}$) had a significantly increased chance of exhibiting one or more of the key symptoms defined above (OR 8.9; 95% CI: 1.2–75.6) [20]. However, the broad confidence interval indicates that this study lacks precision and casts substantial doubt on the significance of these findings. Furthermore, the key symptoms for MTBE-related illness lack any specificity for the exposure.

Mohr et al. compared health complaints in New Jersey garage workers in the northern portion of the state, where the oxyfuel program was in effect, and in the southern part of the state, where the oxyfuel program had been phased out [36]. No difference was detected between the low- and high-exposure groups in the prevalence of reported key symptoms.

The Wisconsin Department of Health performed a large telephone survey (29,314 calls with 1,513 interviews) comparing the incidence of alleged MTBE-related health complaints in Milwaukee, Wisconsin and Chicago, Illinois, both of which had reformulated gasoline (RFG) programs in effect, with the remainder of Wisconsin, which did not have such a program [37]. The authors detected an increase in reported symptoms in Milwaukee but not in Chicago. However, participants in Milwaukee were also found to be more likely to report symptoms if they had a cold or flu, smoked cigarettes, or were aware of the RFG program. None of the reported symptoms was found to be attributable to the use of MTBE.

Animal Studies

The acute lethality of MTBE is low with a measured LC_{50} for rats of 23,600 ppm following inhalation and an LD_{50} of 4 g/kg with

oral exposure [38]. Rats inhaling 8,000 ppm MTBE for 6 hours showed significant respiratory, sensorimotor and autonomic effects, including labored breathing, ataxia, weakened reflexes, decreased muscle tone, lacrimation, piloerection and decreased body temperatures when assessed 1 hour after exposure [39]. Other rats exposed to 4,000 ppm for 6 hours displayed only a few of these symptoms (e.g., piloerection, lacrimation and ataxia) with only females demonstrating significant decreases in hind-leg grip strength and body temperature. Animals exposed to 0 (control) or 800 ppm for 6 hours developed no symptoms in either gender. The battery of tests was repeated at 6 and 24 hours post-exposure and none of the above symptoms was observed in these later assessments.

This study also assessed neurotoxicity in rats following subchronic exposure to the same MTBE concentrations [0 (control), 800, 4,000 and 8,000 ppm]. Test animals were exposed for 6 hours daily, 5 days per week for 13 weeks [39]. None of the rats showed any persistent or cumulative neurobehavioral effects for any of the 3 MTBE exposure concentrations. In the high-concentration (8,000 ppm) exposure group, absolute brain weight was reduced by approximately 5% in both genders. However, body weight in this group was also reduced significantly compared with control animals and when brain weight was expressed relative to body weight, there were no significant differences detected. This observation led the authors to conclude that the reduced brain weight was a result of general growth retardation and not specific neurotoxicity, a conclusion supported by the lack of histopathological lesions in central or peripheral nervous tissue in any study animals.

In another subchronic inhalation study that used the same schedule and doses, Lington et al. found that at the highest dose (8,000 ppm) both male and female rats were ataxic when compared to controls [40]. Postexposure necropsies revealed statistically significant increases in liver, kidney, and adrenal gland weights relative to controls when normalized to body weight in the 4,000 and 8,000 ppm groups; no gender effect was observed. Slight but significant increases in normalized liver and kidney weights at 800 ppm were observed only in male rats. Consistent with the observations of Daughtrey [39], absolute brain weight decreased significantly with exposure to 8,000 ppm in both genders, but this effect disappeared when brain weight was normalized to body weight. Microscopic lesions consisting of enlarged hyaline droplets in the kidneys, increased splenic hemosiderosis, and mild lymph node hyperplasia were found only in male rats in the 8,000-ppm exposure group. The authors interpreted these findings as a reflection of physiological, rather than pathological, changes, and thus were determined not to be treatment related. No neuropathological lesions were detected in any of the study animals.

Potential oncogenicity of chronic MTBE exposure has been assessed in animal studies. Belpoggi and coworkers studied the carcinogenic effects of daily gavage dosing at 0 (control), 250, and 1,000 mg MTBE/kg body weight dissolved in olive oil over the natural lifetime (maximum, 104 weeks) in male and female Sprague-Dawley rats [41]. In male rats, the high-dose group had a

significantly increased rate of testicular Leydig cell tumors. In female rats, both dosing groups demonstrated a significant increase in the development of lymphomas and leukemias. An increase in the number of uterine sarcomas was observed only in the 250-mg/kg dosing group. Of particular note is the lack of increased mortality among any treatment group. In fact, the high-dose males had longer survival times. Older rats are known to have greater tumor frequencies than younger animals. Therefore, increased survival period—not MTBE exposure—may be responsible for the appearance of tumors. As a result, several US regulatory agencies have criticized this study, and it is not included in official risk assessments pending independent review of the data.

In another large lifetime study, mice (CD-1) and rats (Fischer 344 CDF) of both genders were exposed to inhaled concentrations of 0 (control), 400, 3,000 and 8,000 ppm MTBE for 6 hours per day, 5 days per week for 18 months (mice) and 24 months (rats) [42]. In mice, early mortality was increased due to exacerbation of uropathy in the 8,000-ppm group. In male mice, statistically significant increases in relative kidney weight over controls were observed at all three MTBE exposure concentrations. Although relative liver weight showed a dose-dependent increasing trend, these results were not statistically significant. In female mice, relative kidney weight was significantly increased in the 8,000-ppm exposure group and relative liver weight increased to a significant degree in both the 3,000- and 8,000-ppm groups. Absolute and relative adrenal weights were significantly increased in male mice only in the 8,000-ppm group, while relative and absolute spleen weights were significantly decreased only in female mice in the highest dosing group. Relative brain weight did not change for either gender at any MTBE concentration studied. The only neoplastic lesion showing an increased incidence with MTBE exposure in mice was hepatocellular adenomas, which significantly increased only in the female 8,000-ppm group.

The observations in rats differed slightly from those in mice. Male rats in both the 3,000- and 8,000-ppm exposure groups required early euthanasia due to excessive mortality from a progressive nephrosis that precluded assessment of end-of-study relative organ weights. In female rats, significant increases in relative liver and kidney weights were observed in the 3,000- and 8,000-ppm groups. Chronic progressive nephropathy was also observed in female rats but to a lesser degree than in male rats and only at the highest exposure doses. Male rats showed an increased incidence of renal tubular cell tumors and interstitial adenomas of the testes with exposure to 3,000- and 8,000-ppm MTBE.

The mechanism of MTBE-induced tumorigenesis is unclear. Two bioassays have shown MTBE to not be genotoxic, effectively ruling out this mechanism even if the tumors are treatment-related [43]. Male rat kidney tumors and nephropathy may be caused by the production of alpha-2-globulin, while hepatic tumors in females are possibly related to defects in estrogen metabolism [44]. It must be noted, however, that the positive animal studies were only associated with increased tumor growth following prolonged MTBE exposures exceeding typical human exposures by 2 to 3 orders of magnitude.

In an attempt to explain the increase in Leydig cell (testicular) tumors and gain a greater understanding of the observed endocrine effects of MTBE exposure, Williams et al. performed an oral exposure experiment [45]. In this experiment, male Sprague-Dawley rats were exposed to doses of 0 (control), 250, 500, 1,000 and 1,500 mg MTBE/kg body weight by gavage in a corn-oil vehicle for 15 or 28 consecutive days. The two study periods were selected to allow comparison of hormonal changes at these time points. After 15 days of treatment, serum prolactin as well as serum and testicular interstitial fluid testosterone were decreased in the 1,500-mg/kg group. After 28 days of treatment, triiodothyronine and luteinizing hormone were decreased in both the 1,000- and 1,500-mg/kg dosing groups. These data do not support MTBE-induced hormonal derangement as the cause of testicular neoplasms in exposed rats.

Concern over the possibility that MTBE exposure may induce alterations in testicular function prompted another animal study. Billiti and coworkers evaluated the acute effects of oral MTBE exposure on testicular function in male CD-1 mice [46]. Study animals were gavaged with MTBE in canola oil at doses of 0 (control), 400, 1,000 and 2,000 mg MTBE/kg body weight 3 times over a 5-day period. No changes were observed in fecal testosterone levels (a noninvasive marker of testicular function) with and without stimulation by injection of human chorionic gonadotropin, a hormone that stimulates reproductive function. Histopathological examination showed a slight increase in the number of tubules with gross disruption but only in the 2,000-mg/kg group.

In order to further understand the possible effects of MTBE exposure on reproduction, several multigenerational studies have been performed. For example, rats exposed to inhaled doses of up to 3,400 ppm showed no significant differences in mating behavior, fertility, pregnancy outcomes, or litter survival compared with controls [47]. The results of a two-generation study using even higher inhaled MTBE doses (up to 8,000 ppm) were similar [48].

Although no treatment-related effects were observed in the absolute reproductive parameters measured in the papers cited above, studies performed in rats, mice and rabbits born to MTBE-exposed mothers have looked for more subtle developmental changes. At airborne MTBE exposures of 2,500 ppm, there was no evidence of treatment-related adverse pregnancy outcomes or birth defects in rats [49]. Although there was a slightly greater incidence of fetal resorption observed in both low-dose (250 ppm) and high-dose (2,500 ppm) MTBE-treated mice, the authors attributed this to two female mice in each of the two indicated treatment groups that had an unusually high number of resorptions, rather than to MTBE exposure. A slight increase in fused sternebrae was observed in mouse pups whose mothers were exposed to MTBE at 2,500 ppm. In a study of mice receiving the larger doses, those with exposure to 4,000 and 8,000 ppm displayed a higher incidence of postimplantation loss, fetal weight was reduced, and anatomic malformations were more common [50]. Exposed rabbits showed no adverse developmental effects at doses up to 8,000 ppm [50].

Human Experimental Studies

Experimental studies of acute MTBE inhalational exposure in human volunteers have been reported [3,23,26,28,31,47,51–3]. The study designs were similar and compared the results of exposures to airborne MTBE (1.4–50 ppm) with clean air (control). Nihlen et al. also compared results between the various MTBE exposure concentrations [26]. The investigators used both subjective (e.g., symptom-rating scales, based on the symptoms found in the epidemiological studies and mood profiles) and objective (e.g., measured ocular and nasal indicators of inflammation, and neurobehavioral evaluation) measures of effect. Only Nihlen et al. found a statistically significant correlation between MTBE exposure and nasal blockage index (a measure of nasal airway resistance calculated from peak expiratory air flows [26]. However, this finding was not concentration dependent and, therefore, is not likely causally related to MTBE exposure. None of the other studies reported any significant effects associated with exposure to inhaled MTBE at the concentrations studied.

COMMENTS

This article presents a review of the kinetics and toxicology of the fuel oxygenate, methyl *tert*-butyl ether (MTBE), which is primarily used to increase the oxygen content of gasoline, thus reducing vehicle emissions. Air and water concentrations of MTBE are very low in regions where the use of this oxygenate is mandated. Those who work in specific jobs and who live near facilities involved in the production and distribution of gasoline may be exposed to relatively higher concentrations. Following ingestion and inhalation, MTBE is absorbed rapidly into the bloodstream, undergoes significant metabolism in the liver and is excreted in urine and exhaled air. Some of the metabolites persist in the body somewhat longer than parent MTBE.

The alleged adverse effects of MTBE on human health are a matter of great debate. Early epidemiological studies suggested an increase in nonspecific subjective complaints including headaches, throat and nose irritation, and dizziness. However, there were serious flaws in the methodology of these investigations, and these results have not been replicated. Controlled human studies of MTBE exposure generally found no adverse health effects in the exposed study subjects.

The epidemiological data for MTBE are limited by methodological designs due to the nature of the exposure circumstance necessitating the use of reference populations rather than control groups. Therefore, the plausibility of a true exposure-related health effect, especially in a sensitive population, must be cross-checked using other means. Such a sensitive population might be the result of polymorphism in metabolic enzymes. The kinetic model developed by Licata incorporates the interindividual variability of the CYP2A6 (i.e., low-affinity, high-capacity) metabolic pathway [30]. The observed variations of up to 25-fold in enzyme activity were not found to have a significant effect on the model for the low MTBE concentrations to which the general public is exposed. Therefore, variations in the development of health

complaints from MTBE exposure alleged in epidemiological studies are unlikely to be the result of CYP2A6 polymorphism.

In animal studies using MTBE exposure doses orders of magnitude greater than those in human studies, some exposure-related adverse effects have been observed. Acute inhalation exposure caused effects associated with neurotoxicity, as well as autonomic nervous system and respiratory effects. Subchronic inhalational exposure was associated with some of the previous effects plus gross and histological changes in various body tissues, specifically the liver and kidneys. Notably, these effects were seen with very high concentration exposures. Chronic ingestion of MTBE was associated with tumors in the liver, kidneys, and testicles as well as tumors of the reticulo-erythropoietic system. Minor changes in reproductive function were detected in some studies, but not replicated in others.

For residents of urban areas, MTBE is ubiquitous in the air. As a result of groundwater transport and ingestion, it reaches an even larger population. Understanding of the issues addressed in this paper is crucial to an informed decision-making process concerning the continued use of MTBE as a fuel oxygenate. The MTBE concentrations to which the general public is exposed are an order of magnitude lower than the recommended EPA drinking water advisory value of 20 µg/L, which is based on odor. However, some workers may still face potentially harmful occupational exposures due to higher-concentration exposures. Although the half-life of parent MTBE is short, metabolites persist in the blood for a longer duration. While the toxicities of some of these metabolites (e.g., methanol, formaldehyde, and formic acid) have been studied, less is known about the health effects of other important MTBE metabolites (e.g., 2-hydroxyisobutyrate and 2-methyl-1,2-propanediol).

Based on the data detailed above, and despite the inherent limitations of the cited studies, it may be reasonably concluded that the current guidelines for exposure reflect sound objective scientific rationale and that the vast majority of persons passively exposed to MTBE need not be concerned with serious adverse health effects. The original human observations, which reported several mild nonspecific short-term subjective complaints, have not been replicated in controlled laboratory studies of human MTBE exposure. Exposure-related adverse health effects in animal studies were only detected at concentrations or dose levels far in excess of those to which the average citizen is exposed through air and groundwater.

Advanced kinetic modeling makes it possible to predict MTBE blood concentrations following various exposures. However, this does not translate well into foretelling toxic effects as most of the available data concerning high-concentration exposures are based on animal studies. Application of the high-dose animal data to large exposures in humans, such as might occur in occupational settings, is fraught with difficulty. For example, a lack of universal consistency in animal results, as well as potential species specificity in observed effects, limits applicability of such results to human exposures. As such, additional appropriate investigations are necessary to better address the issues facing

occupational MTBE exposure. This research must include investigation into the basic science of MTBE metabolites such as 2-hydroxyisobutyrate and 2-methyl-1,2-propanediol, as well as methodologically sound field research of exposed populations in order to better describe exposure conditions and doses as well as determine if any specific MTBE-related health effects can be identified. As MTBE has now seen widespread use for over 15 years, should any specific chronic or cumulative MTBE-related health effects be suggested, careful epidemiological evaluation may be able to define an association.

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