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Health Effects of Oxygenated Fuels

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The use of oxygenated fuels is anticipated to increase over the next decades. This paper reviews the toxicological and exposure information for methyl tertiary-butyl ether (MTBE), a fuel additive, and methanol, a replacement fuel, and discusses the possible health consequences of exposure of the general public to these compounds. For MTBE, the health effects information available is derived almost exclusively from rodent studies, and the exposure data are limited to a few measurements at some service stations. Based on these data, it appears unlikely that the normal population is at high risk of exposure to MTBE vapor. However, in the absence of health and pharmacokinetic data in humans or in nonhuman primates, this conclusion is not strongly supported. Similarly, there are a number of uncertainties to take into consideration in estimating human risk from the use of methanol as a fuel. Although methanol may be toxic to humans at concentrations that overwhelm certain enzymes involved in methanol metabolism, the data available provide little evidence to indicate that exposure to methanol vapors from the use of methanol as a motor vehicle fuel will result in adverse health effects. The uncertainties in this conclusion are based on the lack of information on dose–response relationship at reasonable, projected exposure levels and of studies examining end points of concern in sensitive species. In developing a quantitative risk assessment, more needs to be known about health effects in primates or humans and the range of exposure expected for the general public for both compounds.

Introduction

Oxygenated fuels include replacement fuels such as methanol and ethanol and fuel additives such as methyl and ethyl tertiary-butyl ethers (MTBE and ETBE) and tertiary-amyl methyl ether (TAME). The interest in these and other alternative fuels in the last decade stems not only out of a concern for the country's dependence on petroleum but also out of a desire to improve air quality. It is anticipated that fuels with a higher oxygen content than conventional gasoline and diesel fuel will produce less CO and hydrocarbons and may lead to lower ozone levels in some regions. However, the introduction of oxygenated fuels would increase exposure of the general public to methanol or ethanol or the oxygenated additives and also to their combustion byproducts. Exposure would be by inhalation of unburned fuel in the exhaust or fuel vapors either during refueling or normal heating of the fuel tank or the engine. The issue of health effects will include both combustion and evaporative emissions of complex mixtures that will further undergo atmospheric transformation. However, because such studies have not been reported, it is only possible to discuss components of the mixtures, namely, MTBE and methanol. This paper presents an overview of the information on exposure, metabolic transformation, and health effects of the two oxygenated fuel components, MTBE and methanol, which are now being used and are projected to be used more widely in the United States in the future and whose effects following inhalation exposure have been better characterized.

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MTBE

MTBE was originally introduced in the United States in the late 1970s as an octane enhancer at the time of lead phase-out. Its use has increased recently in areas of the country with severe air pollution problems. Currently, approximately 20% of the gasoline sold in the United States contains MTBE at levels ranging from 2 to 15% (R. Wilson, personal communication). Ambient concentrations of MTBE at service stations are expected to be below 1 ppm based on some actual measurements ranging from 0.1 to 1 ppm (*1*).

The research conducted on the health effects of MTBE has been sponsored primarily by the MTBE Health Effects Evaluation Task Force on behalf of several manufacturers who have agreed to perform certain health effects studies in accordance with Section 4 of the Toxic Substances Control Act. Some of the information available is derived from acute human clinical studies of parenteral exposure to MTBE as a potential therapeutic agent to dissolve gallstones.

Metabolism and Pharmacokinetics

MTBE is a colorless liquid with limited water solubility (4 g/100 g water). In rats, the first step in the metabolism of MTBE is demethylation, with formation of tertiary-butyl alcohol (TBA). This compound is further oxidized to various products including 2-methyl-1,2-propanediol,

α-hydroxyisobutyric acid (*2,3*), acetone and formaldehyde (*4*), and formic acid (*5*). Interestingly, TBA is not thought to be a substrate of alcohol dehydrogenase like methanol and other alcohols. The transformation of TBA to formaldehyde appears to proceed *in vitro* via a mechanism involving the interaction of TBA with hydroxyl radicals generated from hydrogen peroxide produced by microsomes (*4*).

The disposition of MTBE in rats has been examined after inhalation, oral, intravenous, and intraperitoneal exposure using radioactive MTBE (Table 1). After exposure by inhalation to 400 ppm for 6 hr (average total dose 285 mg/kg*), MTBE blood levels (a parameter often used to characterize body burden) reached a peak value at the end of the exposure, whereas TBA peaked 30 min after exposure (*7*). At a higher exposure concentration (8000 ppm for 6 hr or 5700 mg/kg), the peak MTBE concentration was higher than expected from that at 400 ppm (35 times instead of 20 times greater), suggesting a possible saturation of the enzyme catalyzing the demethylation of MTBE. At the lower concentration, 400 ppm, the major route of MTBE elimination appeared to be via the kidneys into the urine (65% of the radioactivity recovered) with some MTBE eliminated via the lungs (17–22%) (*2*). At the higher exposure, 8000 ppm, however, a larger fraction of the recovered radioactivity was eliminated by the lungs (54-59%) than through the urine (17–22%) (*2*).

After oral and intravenous administration of MTBE at a dose of 40 mg/kg, the peak blood MTBE concentration was achieved within 5–15 min (*8,9*). The peak level of TBA was achieved approximately 2 hr after dosing. About half of the MTBE dose was eliminated through the lungs (46–54% of oral dose and 42–46% of intravenous dose) and one-third through the urine (26–35%) (*3*). These results are similar to those reported by Exxon (*8*) showing that 50–55% of MTBE oral dose was exhaled 3 hr after exposure to 40 mg/kg. The pulmonary elimination of MTBE was slightly more rapid after intravenous dosing compared to oral dosing, resulting in a lower peak plasma MTBE level. After oral exposure to 400 mg/kg MTBE, the fraction of the dose eliminated via the lungs increased to 65–69%, similar to the pattern observed after inhalation (*3*). However, unlike what was observed after inhalation, the MTBE plasma peak levels were more or less proportional to the dose. Thus, the increase in the proportion of MTBE exhaled is probably due to the fact that, at higher blood concentrations, more MTBE is partitioned into the air. Plasma MTBE was cleared with a half-life ranging from 0.5 to 0.6 hr after inhalation and from 0.5 to 0.9 hr after intravenous and oral administration (*7,9*).

The values reported above for the disposition of MTBE represent the total radioactivity exhaled. When the composition of the exhaled material was characterized, it was found that 1–4% of the exhaled material (after oral and intravenous exposure) and 7–30% (after inhalation) was TBA and the remainder was MTBE (Table 1). In general, the fraction of the exhaled material that was TBA decreased with increasing MTBE doses. Less than 1% was CO_2, indicating that little MTBE is completely oxidized (*2,3*). Of the total exhaled MTBE, 80–94% was eliminated during the first 3 hr after dosing, and virtually complete elimination had occurred 6 hr after dosing. In parallel, the percentage of TBA in exhaled air increased after 3 hr (*2,3*).

In summary, in rats MTBE is quickly absorbed and distributed in the circulation. Depending on the dose and route of exposure, 6–50% of the MTBE dose does not appear to undergo metabolic transformation but is exhaled as such via the lung. The proportion of MTBE exhaled increases with the dose. In terms of the MTBE peak blood levels, exposure to 400 ppm for 6 hr (total dose 285 mg/kg)

*For the purpose of comparison to other routes of exposure, the MTBE dose after inhalation exposure can be calculated as the product of the MTBE concentration in mg/m^3, the ventilation rate in L/hr, and the duration of exposure. The value obtained would also need to be corrected for the percentage of MTBE that is not absorbed by the lung, which is not known. For the rat, the following constants have been used:

$$\text{mg/kg} = \frac{(\text{ppm} \times 3.61) \times (8.3\ \text{L/hr} \times 1/1000) \times \text{hr exposure}}{0.25\ \text{kg body weight}}$$

The ventilation rates for the same species differ in various texts by as much as 4-fold. The values used here have been taken from Hallenbeck (*6*). For humans the ventilation rate value from the same reference is 833 L/hr.

Table 1. Methyl tertiary-butyl ether disposition in rats.[a]

Route of exposure	Sex	Peak blood levels, μg/mL	% Radioactivity exhaled, 0–48 hr	Tertiary-butyl alcohol, % of exhaled radioactivity	
				0–3 hr	0–48 hr
Inhalation					
400 ppm, 6 hr	M	15	17	28	65
	F	14	22	31	65
8000 ppm, 6 hr	M	556	54	10	42
	F	513	59	7	35
Intravenous, 40 mg/kg	M	12	42	4	26
	F	8	46	4	26
Oral					
40 mg/kg	M	17	46	4	35
	F	11	54	4	27
400 mg/kg	M	123	65	1	16
	F	115	69	1	11

[a]References: Inhalation exposure: peak blood levels (*7*), percent exhaled (*2*). Intravenous and oral exposure: peak levels (*9*), percent exhaled (*3*).

is approximately equivalent to an oral or intravenous exposure of 40 mg/kg (Table 1). However, at these comparable exposures, the percentage of MTBE exhaled differs by approximately a factor of two.

Acute Studies

The acute inhalation concentration of MTBE needed to produce death in 50% of rats (LC_{50}) has been calculated in different studies to be 18,000 ppm (*10*), 23,600 ppm (*11*), and 39,500 ppm (*12*). The acute oral dose needed to produce death in 50% of rats (LD_{50}) has been calculated to be about 3.9 g/kg (*12*). Death is preceded by signs of ocular and mucous membrane irritation, ataxia (inability to coordinate voluntary movements), and central nervous system (CNS) depression (*12*). In nonhuman primates, no toxic signs were observed after two exposures to 3400 and 4800 ppm for 6 hr. The animals became ataxic on the third day during exposure to 8500 ppm (*13*).

In humans, MTBE has been administered for the purpose of gallstone dissolution through a catheter inserted either in the bile duct or the gallbladder at doses ranging from approximately 0.01 g/kg to 0.2 g/kg. The duration of the infusion varied from 1–3 min to 30 min. The infused material was usually aspirated, and the treatment repeated several times (*14–18*). The investigators did not report what fraction of the infused MTBE reached the systemic circulation. The side effects observed were perspiration, transient hypotension, bradycardia, sedation, and a transient elevation of liver enzymes, with the distinctive smell of ether on the patient's breath. The severity of the symptoms varied with the regimen administered and from patient to patient. All symptoms usually disappeared after the termination of the treatment.

In a single exposure study to evaluate acute neurotoxic effects, rats were exposed by inhalation to 0, 800, 4000, and 8000 ppm MTBE for 6 hr (*19*). Some of the animals were evaluated using a battery of functional observations (FOB) including ataxia, rectal temperature, hind limb grip strength, labored respiration, leg splay, and treadmill duration at various times after exposure. The remaining animals were tested for motor activity immediately after the end of the exposure. In the 8000-ppm exposure group, several FOB and motor activity changes were observed. Occasional changes were also detected in the 4000-ppm group, but none were seen in the 800 ppm group. The authors interpreted these transient changes as indicative of CNS sedation.

In conclusion, the results of these studies suggest that inhalation of MTBE vapors causes some toxicity in both rats and nonhuman primates. Concentrations that caused ataxia or sedation were greater than 4000 ppm for 6 hr in both species, suggesting comparable sensitivity to MTBE if pharmacokinetics are similar. The effects observed were transient and disappeared after the exposure was terminated. It remains to be established whether subtle behavioral changes are induced at lower concentrations.

Subchronic Studies

These studies were primarily designed to evaluate whether protracted exposures either by inhalation or gavage in different species caused any toxicological changes. In one study (*20*), rats were exposed for 6 hr/day for 9 days to concentrations of 100, 300, 1000, and 3000 ppm MTBE. Microscopic examination of the nasal mucosa and trachea revealed chronic inflammation in the two high-dose groups. Liver weights were significantly increased in both sexes exposed to 3000 ppm. In another study (*21*), male and female rats were exposed to 250, 500, or 1000 ppm MTBE for 6 hr/day for 13 weeks. The few treatment-related effects were a dose-dependent anesthetic effect and a lower weight gain in the high-concentration group. In addition, male rats displayed a significant increase in red blood cell hemoglobin, an increase in kidney weight, and elevated blood-urea nitrogen (BUN) and lactate dehydrogenase (LDH) values at 1000 ppm. A slight decrease in absolute and relative lung weight and in blood LDH values was observed in females exposed to 1000 ppm.

The Bushy Run Research Center (*22*) exposed four groups of male and female rats for 6 hr/day for 13 weeks to MTBE vapors of 0, 800, 4000, and 8000 ppm primarily to evaluate neurotoxic effects. Body weight gains were depressed in rats of the high-dose group for the first 3 weeks of exposure, and corticosterone levels were elevated at the end of the exposure. Liver, kidneys, and adrenal gland weights increased in a dose-dependent fashion in both sexes in the 4000- and 8000-ppm groups. Ataxia was seen in the 8000-ppm group during the first 4 weeks, and minor changes were found in some functional observational tests (elevated body temperature in female rats at 4000 and 8000 ppm, decreased hind limb grip in male rats at 4000 ppm). Motor activity was decreased in male rats of the 8000-ppm group and increased in female rats of the 4000-ppm group. No histopathological changes were detected in any of the organs except for an accumulation of hyaline drops within the proximal tubules of the kidney of male rats at 8000 ppm.

Robinson et al. (*23*) conducted a 14- and 90-day oral toxicity study in rats. The daily doses, administered by gavage, were 0, 357, 714, 1071, and 1428 mg/kg for the 14 day study and 0, 100, 300, 900, and 1200 mg/kg for the 90-day exposure. At or above doses of 1200 mg/kg, MTBE induced temporary anesthesia and diarrhea. Total weight gain was less for both males and females at doses above 1000 mg/kg. Relative liver weights were increased in females with doses above 1200 mg/kg, and absolute kidney weights were increased in males with doses above 700 mg/kg after either the 14-day or the 90-day exposure. Relative kidney weights were also increased with the dose after the 90-day exposure, and absolute lung and spleen weights were significantly reduced in females exposed for 14 days to the highest dose. Male rats in the two high-dose groups displayed elevated hemoglobin values after 14 days but not after 90 days of exposure. Both males and females showed increased cholesterol levels, and females also had lower BUN and creatinine levels. Males in the high-dose groups from both studies displayed a higher incidence of renal

Table 2. Methyl tertiary-butyl ether subchronic studies.

Species	Exposure[a]	Results	Reference
Rats	100, 300, 1000, 3000 ppm, 6 hr/day for 9 days	Increased liver weight and nasal mucosa inflammation	(*20*)
Rats	250, 500, 1000 ppm, 6 hr/day for 13 weeks	Anesthetic effects, decreased weight gain	(*21*)
Rats	800, 4000, 8000 ppm, 6 hr/day for 13 weeks	Decreased weight gain, increased liver, kidney, and adrenal weights, increased kidney hyaline droplets in females, decreased hind limb grip and motor activity in males	(*22*)
Rats	100, 300, 900, 1200 mg/kg, for 90 days	Increased liver and kidney weight, increased kidney hyaline droplets in males	(*23*)

[a]Numbers in bold indicate concentrations at which effects were seen.

tube disease, which was characterized by increased hyaline droplets in the cytoplasm of proximal tubular epithelial cells.

In general, these studies (summarized in Table 2) suggest that MTBE is not highly toxic to rodents. In all cases, MTBE caused a dose-dependent decrease in weight gain. Other common effects that can be identified after repeated oral doses greater than 1000 mg/kg or repeated inhalation of MTBE concentrations greater than 1000 ppm for 6 hr/day are decreased lung and increased liver weight in females and increased kidney weights in association with higher incidence of renal tube disease in males. The renal pathology observed in male rats appears to be consistent with a sex- and species-specific damage to α_2-globulin, which can be induced also by other hydrocarbons and whole gasoline (*24*). Some hematological parameters such as erythrocyte counts and BUN appeared to also be affected at the high doses. The concentrations of MTBE shown to cause these effects are about three orders of magnitude greater than those measured at service stations.

Reproductive Studies

A few studies have been conducted in rodents to evaluate the effects of MTBE on reproductive outcome (see Table 3 for a summary of the studies). In a single-generation study, male and female rats were exposed to 300, 1300, and 3000 ppm for 6 hr/day before mating (12 weeks and 3 weeks, respectively) and then throughout mating, gestation, lactation, and a second gestation period (*25*). The few effects observed were a dilated renal pelvis in adult females and in some pups and a slight decrease in pup viability and weight in the high dose group. Male and female fertility indices were not affected by the treatments.

In a two-generation reproductive study, rats of both sexes were exposed to 400, 3000, and 8000 ppm 5 hr/day for 5 days/week for 10 weeks before breeding and for 7 days/week during mating, gestation, and the postnatal period (*26*). No effects on reproductive parameters such as fertility indices, live birth, and pup survival were observed. The effects reported included a reduction in body weight gain in F_0 males at 8000 ppm, a reduction in body weight of F_0 adult animals of both sexes, and a reduction in body weight gain in F_1 and F_2 litters. In addition, liver weights were increased in the F_1 adults at 3000 and 8000 ppm, and perinatal deaths were increased in the F_2 at 8000 ppm. In both F_0 and F_1 pre-breed groups, hypoactivity and lack of startle reflex were observed at 3000 and 8000 ppm and ataxia at 8000 ppm. The results obtained in this study are consistent with those reported in the subchronic toxicity studies and the fetal developmental studies described below.

Fetal Toxicity Studies

In a study to evaluate the teratological effects of MTBE, pregnant rats were exposed to 250, 1100, and 3300 ppm for 6 hr/day during gestational days 6–15 (*27*). No adverse effects on maternal body and organ weights, uterine implantation, and fetal size were observed. Two teratogenicity studies were conducted in mice. In one study, mice were exposed to concentrations of 0, 1000, 4000, and 8000 ppm for 6 hr/day from gestational days 6–15 (*28*). At the two highest concentrations, the mothers exhibited a signif-

Table 3. Methyl tertiary-butyl ether reproductive studies.

Species	Exposure[a]	Results	Reference
Rat	300, 1300, 3000 ppm, 6 hr/day, premating, gestation, lactation, second gestation	Dilated renal pelves in adults/pups, decreased pup survival and weight in second generation pups, no effect on fertility indices	(*25*)
Rat	400, 3000, 8000 ppm, 5 hr/day; F_0 premating, gestation, pup growth ($=F_1$); F_1 premating, gestation, pup growth ($=F_2$)	Decreased weight gain in F_0 rats and F_1 litters, increased liver weights in F_1 adults, decreased perinatal deaths in F_2 litters, ataxia and hypoactivity in F_0 and F_1 adults, no effects on reproductive indices	(*26*)

[a]Numbers in bold indicate concentrations at which effects were seen.

icant reduction in body weight and clinical signs of toxicity, including hypoactivity, ataxia, lacrimation, and labored respiration. At these concentrations, the number of viable implants per litter was reduced. Fetal body weights were significantly reduced at 4000 and 8000 ppm. An increased incidence of cleft palate was observed at 8000 ppm and of skeletal malformations at both 4000 and 8000 ppm. In a similar study performed by a different group (*29*), pregnant mice were exposed to 0, 250, 1000, and 2500 ppm 6 hr/day during gestational days 6–15. No adverse effects were observed in the dams. A slight increase in the number of fetal resorptions and in the incidence of fused sternebrae in the offspring were detected, but these effects were not considered to be treatment related.

In pregnant rabbits exposed to concentrations of 0, 1000, 4000, and 8000 ppm MTBE 6 hr/day from gestational days 6–18, a significant reduction of weight gain and food consumption was observed at the two highest exposures, and an increase in relative maternal liver weight was noted at 8000 ppm (*30*). Gestational parameters, including number of corpora lutea, number of implantation, fetal body weight, and number of fetal malformations, exhibited no significant changes.

In conclusion, maternal toxicity was observed in both rats and rabbits at 4000 and 8000 ppm and in mice at 8000 ppm (Table 4). Increased frequency of fetal malformation and decreased number of viable implantations were found only in mice at 4000 and 8000 ppm, indicating the mouse fetus may be more sensitive to MTBE than the rat fetus.

Conclusions

In summary, the studies presented indicate a low toxicity of MTBE in rodents. However, limited information is available regarding health effects in humans or nonhuman primates. Because of the considerable differences in species susceptibility to methanol (see below) and the lack of data in primates, one cannot rule out the possibility that MTBE may be toxic to humans at high doses. In fact, formate is a metabolite of MTBE and may accumulate above a certain dose of MTBE. The contribution of the pathway leading from MTBE to formate is unknown. Based on the rodent data and the current exposures associated with refueling at less than 1 ppm, there seems little likelihood of a severe hazard to healthy individuals under normal use conditions. However, there may be subpopulations that are more susceptible to MTBE. Moreover, with increased market penetration, MTBE exposure levels and the number of people exposed will increase. Pharmacokinetic data on the disposition and metabolism of MTBE in primates and exposure information for other scenarios such as garages, street canyons, and inside the car are necessary to better assess the risk of MTBE exposure to humans. In addition, because of its lipid solubility, MTBE may be a neurotoxicant; neurotoxicity studies would be prudent.

Methanol

Methanol has been used since the beginning of the century as a solvent, and, more recently, as a fuel for racing cars. In the last few years efforts have been made by government officials, state regulators, and others to promote the use of methanol as a replacement to gasoline and diesel fuels for passenger cars, buses, and other fleet vehicles in order to improve air quality. It is predicted that use of methanol-fueled vehicles would decrease the levels of some pollutants; however, this practice would increase exposure of the general public to methanol and, in certain microenvironments, to formaldehyde, a product of methanol combustion.

Some methanol exposure concentrations have been calculated for various scenarios from emission data from a few cars running on methanol using dispersion models that take into account, for the various scenarios, traffic conditions, meteorological conditions, and wind patterns (*31*). The highest concentration, projected to occur in a personal garage, is 375 ppm during the cold start. In public garages, assuming 100% of the vehicles were fueled with methanol, concentrations are projected to be as high as 150 ppm. In other scenarios, the concentrations are expected to be lower than 50 ppm. In the majority of the cases, exposure of the general public would be brief but repeated in time.

Metabolism and Pharmacokinetics

Regardless of the species and route of exposure, methanol is rapidly absorbed and distributed to the tissues in proportion to water content (*32*). Unlike MTBE, most methanol (more than 90%) is metabolized in the liver and

Table 4. Methyl tertiary-butyl ether fetal toxicity studies.

Species	Exposure[a]	Results	Reference
Rat	250, 1000, 3300 ppm, 6 hr/day, gestation days 6–15	No effects in dams/offspring	(*27*)
Mouse	1000, 4000, 8000 ppm, 6 hr/day, gestation days 6–16	Decreased weight gain in dams/fetuses, increased fetal resorption and incidence of skeletal malformations	(*28*)
Mouse	250, 1000, 2500 ppm, 6 hr/day, gestation days 6–15	No effects in dams, increased fused sternebrae in offspring	(*29*)
Rabbit	1000, 4000, 8000 ppm, 6 hr/day, gestation days 6–18	Decreased weight gain in dams, increased liver weight in dams, no fetal toxicity or malformations	(*30*)

[a]Numbers in bold indicate concentrations at which effects were seen.

exhaled as CO_2, and only a small fraction is excreted directly through the lung (2.5%) or in the urine (1%) at methanol doses smaller than 1 g/kg. At 1 g/kg, about 78% of the dose is still recovered as exhaled CO_2 (*32*). In the case of inhalation, on the order of 60–85% of inhaled methanol is absorbed by the lung (*33,34*).

In all mammalian species studied, the sequence of metabolic intermediates leading from methanol to its end products in the liver is the same. The first step is the oxidation of methanol to formaldehyde, mediated primarily by alcohol dehydrogenase in nonhuman primates and humans and by a catalase–peroxidase system in rats (*35*). Despite these differences, Tephly and McMartin (*35*) determined that the rate of formaldehyde production was approximately the same in the different species. The disappearance of methanol in the blood of rats appears to follow first-order kinetics with a half-life of 2–3 hr at body burdens as high as 80 mg/kg and zero-order kinetics at higher doses (*32*). A similar half-life was determined in nonhuman primates exposed up to 2000 ppm for 6 hr (*36*). Formaldehyde is quickly metabolized to formate in all species studied (*37*). Formate is then converted to CO_2 and water by a pathway dependent on tetrahydrofolate, which is derived from folic acid in the diet and is the major determinant of the rate of formate disposition (*38,39*). A small proportion of formate is also excreted in the urine. The folate-mediated metabolism of formate has been shown to proceed about 2–2.5 times faster in rats than in nonhuman primates and humans (*35*) and to lead to toxic accumulation of formate in blood in the latter species at sufficiently high doses of methanol (greater than 200 mg/kg) (*32*). The accumulation of formate normally reaches a plateau between 12 and 24 hr after exposure to methanol.

Detailed pharmacokinetic data on methanol disposition after various types of exposure and in different species are being gathered but are not yet available. In general, blood methanol levels peaked at the end of the exposure period (*40*). In rats, methanol blood levels did not increase in proportion to the vapor concentration. With a 6-hr exposure, there was a greater increase between 1200 and 2000 ppm for 6 hr than between 200 and 1200 ppm (*41*). In nonhuman primates, however, the blood levels appeared to be directly proportional to the exposure up to 2000 ppm (*36*). The blood levels were comparable in rats and monkeys up to exposures of 1200 ppm. Blood formate levels were not increased in either species, indicating that these two species do not substantially differ in the metabolism of methanol at low levels of exposure.

Acute Studies

Methanol has been recognized as a human toxic agent since the end of the nineteenth century. Ingestion has been the predominant route of poisoning, but percutaneous absorption of methanol liquids has also been shown to produce toxic effects.

A review of reports of methanol poisoning present a characteristic sequence of symptoms including metabolic acidosis, loss of motor coordination, and blindness. These symptoms, which occur after an asymptomatic period of 12–24 hr, can lead to coma and death or permanent blindness (*42*) and have been attributed to the accumulation of toxic levels of formate (*43*). Although the visual system, and particularly the optic nerve, appears to be the primary target of methanol damage, autopsies indicate that certain brain areas, such as the basal ganglia, also present pathological abnormalities (*32*). Among the various species tested, only nonhuman primates display the acute effects observed in humans (*44*). As discussed earlier, the differences in species susceptibility have been attributed to the different rates of disposition of formate. In fact, toxic symptoms can be induced by methanol in rats by altering their folate status and, as a consequence, formate accumulation (*38,45*). Therefore, it is possible that people with dietary folate deficiency may also exhibit greater susceptibility to methanol toxicity, though there is no experimental evidence as yet to support this view. This potential is of concern because folate deficiency is observed in some pregnant women and may be associated with an increased rate of neural tube defects (*46,47*).

The LD_{50} for rats has been determined to vary between 7 and 13 mL/kg (*48*); the minimal lethal dose of methanol in humans ranges between 300 and 1000 mg/kg body weight (or 0.375–1.25 mL/kg) (*32*). The estimated methanol dose to increase formate levels in humans is 200 mg/kg (*32*). The exposure in the worst-case scenario for methanol-fueled cars (personal garage) would produce a methanol body burden of less than 1 mg/kg (*32*) . Based on blood formate levels resulting from laboratory and occupational exposure studies, it is considered unlikely that measurable increases in blood formate would occur in human adults even under the worst-case exposure scenario for a personal garage. Thus, acute toxic effects would not be expected from the normal use of methanol fuel. However, methanol poisoning as a result of accidents or abuse may represent a potential health threat if methanol fuel were introduced on a large scale.

A few studies have been conducted to evaluate the effects on behavior of acute, nontoxic methanol exposures. In one pilot study, 12 healthy human subjects were exposed to 192 ppm methanol vapor for 75 min (*49*). Neurobehavioral tests and neurophysiological measurements were made before, during, and immediately after exposure. Most behavioral end points showed no association with methanol exposure. Small but statistically significant effects and trends were found only for the subjective rating of fatigue, measurement of interference by a stimulus on the subject's ability to focus attention, and the latency of the early components of the electrophysiological response evoked by an acoustic and visual stimulus. The functional significance of this component is a matter of controversy. However, none of these effects exceeded the normal range of values. Other tasks measuring reaction time, information processing, and vigilance were not affected by the exposure. Because this pilot study included only one concentration of methanol and a limited number of subjects, it is difficult to determine whether the results obtained are indicative of subtle effects on some human neurobehavioral functions. Additional studies are needed to confirm or disprove this study.

In a study by Mullenix et al. (*50*), adult rats were orally administered the artificial sweetener aspartame (which decomposes in the gut to 40% aspartate, 50% phenylalanine, and 10% methanol) at a dose of 500 or 1000 mg/kg (corresponding to a methanol dose of 50 and 100 mg/kg). One hour after dosing, the animals' spontaneous behavior was recorded by a computer pattern recognition system. No significant changes were observed.

In summary, the information available indicates that methanol is more toxic to humans than rodents, but it is inconclusive about whether less severe acute effects could occur at doses that do not cause overt toxicity, particularly in sensitive subpopulations.

Repeated Exposure Studies

The literature on the effects of repeated exposure to methanol concentrations similar to those that would result from the introduction of methanol fuels is very limited. As a point of reference, the American Congress of Governmental Industrial Hygienists set the threshold limit value (TLV) for occupational exposure at 200 ppm time-weighted average for an 8-hr period (*51*). Such an exposure would result in a methanol body burden of approximately 25 mg/kg (calculated as the product of methanol concentration in mg/m^3, duration of exposure, and ventilation rate divided by body weight. For methanol, the conversion factor from ppm to mg/m^3 is 1.3).

There is some evidence from epidemiological studies of workers exposed to methanol from duplicating machines that repeated sublethal doses (less than 0.3 g/kg body weight and above the TLV of 200 ppm) may cause headache, dizziness, nausea, and blurred vision, indicating that methanol exposure can result in CNS depression (*52,53*). The exposure duration in the latter study was reported to range from 1 hr/day for 1 day/week to 8 hr/day for 5 days/week and had presumably been occurring for about 3 years. The conclusions, however, are based on reporting of symptoms. The effects in these studies are similar in nature to those from acute intoxication, but appear less severe.

In Japan, the New Energy Development Organization (NEDO) (*54*) sponsored a study to evaluate the effects of protracted exposure to methanol vapor (10, 100, 1000 ppm, 21 hr/day for 7–25 months) in nonhuman primates. In a few animals some degeneration of astrocytes or increase in their number in some areas of the brain was observed at the 100 and 1000 ppm exposures, even after the shorter exposure duration. The authors considered these changes to be reversible and did not believe them to be of biological significance. Monkeys exposed to 1000 ppm for more than 2 years also showed degeneration of the inside nucleus of the thalamus, cerebral white cortex, liver, and kidney. In a few animals, from each exposure group, a slight degeneration of the optic nerve was suspected, but the authors concluded that overall no significant degeneration of the optic nerve was observed. However, in monkeys exposed to 3000 ppm or 5000 ppm, but not to 2000 ppm, for 21 hr/day for 20 days and then allowed to recover for 10 months, a slight atrophy of the optic nerve and a reduction in myelinated optic nerve cells was observed. The results of these studies are interesting but are presented with insufficient detail to permit critical evaluation and have not been published as yet in the peer-reviewed literature.

NEDO also conducted a 24-month carcinogenicity study in rats exposed to methanol vapors of 0, 10, 100, and 1000 ppm for 19 hr/day and a 18-month carcinogenicity study in mice exposed to 0, 10, 100, 1000 ppm for 19 hr/day (*54*). The exposures did not affect body weight or survival rates in either species. Pathological examination revealed a slightly higher incidence of tumors in the lung (papillary adenoma) in male rats in the 10 and 1000 ppm groups and of tumors of the adrenal gland (chromaffinoma) in female rats in the 1000 ppm group. No significant histopathological changes were observed in mice. These results suggest that methanol is not carcinogenic in mice and raise the possibility that methanol may be a weak carcinogen in rats.

In summary, these studies provide weak evidence that protracted exposure to methanol vapors may damage the central nervous and visual systems, but additional work would be helpful to define clear dose–response relationships.

Developmental Toxicity Studies

A few studies in rodents have indicated that fetal methanol exposure at high doses can be teratogenic and have developmental effects. In one study conducted by Nelson et al. (*55*), the teratogenic effects of methanol were evaluated in pregnant rats exposed to concentrations of methanol ranging from 5,000 to 20,000 ppm (25–100 times the TLV) or of ethanol from 10,000 to 20,000 ppm for 7 hr/day for the entire duration of gestation. Although methanol was not toxic to the dams, ethanol caused complete narcosis at 20,000 ppm and hyperactivity at the lower dose. The highest concentration of methanol (20,000 ppm) produced a high incidence of congenital malformations and urinary tract and cardiovascular defects. No definite increase in malformations was observed at any ethanol dose.

Other studies compared the teratogenic effects of methanol in rats (*56*) and mice (*57*). Pregnant rats were exposed to 15,000 ppm 7 hr/day on gestational days 7–19, and pregnant mice were exposed to 0, 2000, 5000, and 15,000 ppm methanol vapors 7 hr/day on gestational days 6–15. The rat study confirmed the results of Nelson et al. (*55*) in that exposure to 15,000 ppm was not teratogenic. Mice, however, appeared to be more sensitive. All the exposed mouse dams gained less weight than the controls. Most of the litters of dams exposed to 15,000 ppm were completely resorbed, and 38% of the fetuses surviving to day 17 had exencephaly. Exposure to 5000 ppm resulted in exencephaly in about one-third of the litters and 5–10% of all fetuses. No effects were observed at the 2000-ppm exposure.

In the study by Infurna and Weiss (*58*), early postnatal behavior was evaluated in rats after maternal ingestion of an average daily dose of methanol of 2.5 g/kg during gestational days 15–17 or 17–19. The results indicated that both groups of methanol-exposed pups required longer

than controls to begin suckling (at postnatal day 1) and more time to locate nesting material from their home cage (at postnatal day 10). No signs of overt toxicity were apparent in either the mothers or the offspring. A similar study was conducted by Stanton et al. (*56*) in rats exposed to 15,000 ppm for 7 hr/day on gestational days 7–19. A number of tests were performed during pup development (from postnatal day 13 to day 73), including tests of motor activity, learning, and reaction time, but none was affected by the prenatal methanol exposure.

A study conducted by NEDO (*54*) in rats exposed to methanol for an average of 20 hr/day at 200, 1000, and 5000 ppm during gestation and lactation showed a decreased brain weight in the high-dose group, but no brain histopathological changes. Exposure of pregnant rats to 5000 ppm methanol, but not to 1000 ppm, during gestation days 7–17 caused lower survival and birth rate, visceral and skeletal malformation, and decreased weight of the thyroid and the brain.

In summary, methanol appears to be teratogenic in rats at exposure levels greater than 15,000 ppm for 6 hr or 5000 ppm for 20 hr, and in mice at levels of 5,000 ppm for 6 hr (Table 5). However, these mouse studies are ongoing, and a definite no-adverse-effect level (NOAEL) has not been established. Early postnatal behavior of rats may be affected by *in utero* exposure to 2.5 g/kg, but behavioral tests performed in developing rat pups exposed *in utero* to 15,000 ppm 7 hr/day were not affected.

Conclusions

Ingestion of or dermal exposure to high doses of methanol can cause toxic and life-threatening effects in humans but not in rodents. However, these effects have been attributed to the accumulation of the metabolite formate and should not occur in normal individuals at the predicted exposure levels from normal use of methanol as a fuel. The analysis of the data currently available regarding effects of low-level exposures points out many uncertainties and assumptions that need to be verified to estimate human risk for realistic exposure levels. For example, there is limited information about the dose–response relationship on neurotoxic and visual end points and on fetal development across species at both single and repeated low-level exposure. These end points may be sensitive to methanol exposure. There are also no data about the potential susceptibility to methanol of individuals with dietary folate deficiency. The exposure information that would aid in the design of new studies is limited and is derived from testing a small number of cars whose engines were not optimized to run on methanol. This information would also enable extrapolation of the available data to human exposure levels.

Discussion

In general, MTBE appears to have relatively low toxicity in rodents. No specific target organs have been identified except for the male rat kidney and possibly the female rat lung and liver. The effects observed, changes in organ weight and renal hyaline droplet accumulation, were induced by MTBE concentrations much greater and of much longer duration than those to which humans would be exposed from use of MTBE in gasoline. There is limited information about health effects in other species. One study of nonhuman primates was exclusively aimed at evaluating gross psychomotor effects at high concentrations. The only human studies are clinical studies in which MTBE was infused to the gall bladder for gallstone treatment. These studies indicate that, with the exception of sedation, MTBE did not cause any overt toxicity. Taken together, the results of all these studies suggest that exposure to MTBE derived from its use as a component of gasoline is unlikely to pose a health risk to the general public. This conclusion is limited by the fact that pharmacokinetic data in nonhuman primates and humans, which would be useful for extrapolation of the rodent findings to humans, are not available, and some possible relevant end points have not been studied.

Methanol is known to be toxic in humans and nonhuman primates, but it is considerably less toxic in rodents. The acute toxicity studies raise concern about the introduction

Table 5. Methanol fetal toxicity studies.

Species	Exposure[a]	Results	Reference
Rat (teratology)	5000, 10,000, 20,000 ppm, 7 hr/day, gestation days 7–15	Congenital malformations, urinary tract, and cardiovascular defects	(*55*)
Rat (teratology)	15,000 ppm, 7 hr/day, gestation days 7–19	No teratogenic effects	(*56*)
Rat (teratology)	200, 1000, 5000 ppm, 22 hr/day, gestation days 7–17	Decreased weight gain in dams, increased fetal resorptions, decreased pup survival, visceral and skeletal malformations	(*54*)
Mouse (teratology)	2000, 5000, 15,000 ppm, 7 hr/day, gestation days 6–15	Decreased weight gain in dams. Increased execenphaly, increased fetal resorptions	(*57*)
Rat (fetal development)	2.5 g/kg/day, gestation days 15–17 or 17–19	Delayed suckling and nesting behaviors	(*58*)
Rat (fetal development)	15,000 ppm, 7 hr/day, gestation days 7–19	No effect on motor activity, learning, or reaction time	(*56*)

[a]Numbers in bold indicate concentrations at which effects were seen.

of methanol as a fuel for two reasons. First, there are safety issues that need to be taken into consideration to prevent accidents and abuse and reduce occupational exposure. Second, there is concern that even the low levels of exposure projected from use of methanol as a fuel, if protracted, may cause subtle effects, particularly to the visual and nervous system. In 1987 a special report issued by the Health Effects Institute (HEI) (*59*) concluded that exposure to methanol vapor from the use of methanol as a motor vehicle fuel was not likely to result in adverse health effects. However, the report also identified research needs to reduce the level of uncertainty about this conclusion. Numerous studies are currently funded, not only by HEI, but also by other agencies, to address some issues of concern. Similarly, work is being done on MTBE that will clarify possible oncogenic effects. One active area of methanol research is that of developmental effects of methanol in various species. The results so far available indicate that the mouse fetus is more sensitive to maternal methanol exposure than the rat fetus. This was also observed with MTBE exposure. Additional studies would be helpful to determine which species is the best model for humans.

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