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Full Length Research Paper

Contamination of drinking water by methyl tertiary-butyl ether (MTBE) and its effect on plasma enzymes

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When lead in car fuel was removed in the United States beginning from 1979, it was replaced with methyl tertiary-butyl ether (MTBE). This move came as a result of the discovery of the deleterious effects of lead on health and the environment. As of January 2001, leaded car fuel in Saudi Arabia was also replaced with MTBE, at a concentration of 12 - 15%. MTBE dissolves readily in water and evaporates quickly. This study focused on the possible health toxicity of MTBE in drinking water, as manifested by changes in the activities of certain plasma enzymes, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyltransferase (GGT), lactate dehydrogenase (LDH), and creatine kinase (CK). One hundred and twenty male Wistar rats were exposed to five different MTBE concentrations (0.0, 1,000, 1,500, 2,000, and 2,500 ppm) for 60 days. The results showed increased levels (U/l) of ALT at all MTBE concentrations by 48.8, 19.2, 16.9, and 15.4%, respectively. The AST concentration (U/l) increased significantly (29.2%) only at 2,000 ppm MTBE. This significant increase of blood plasma enzymes, which are related to liver function, may indicate injury or damage to liver cells. However, there were no significant differences between the MTBE concentrations in ALP, GGT, LDH or CK and AST. This may indicate there was no marked potentiating acute liver damage induced by MTBE.

Key words: Methyl tertiary-butyl ether, plasma enzymes, gasoline.

INTRODUCTION

Low levels of MTBE have been used in gasoline in the United States (U.S.) since 1979 (between 0.5 - 3.5% w/v) to replace lead, which is a toxic metal. Since 1992, MTBE has been used at high concentrations in gasoline (15% w/v) to fulfill the oxygenate requirements set in some U.S. states by Congress in the 1990 Clean Air Act Amendments. In 1994, MTBE was the 18th most important chemical in volume produced in the U.S. In 1999, over 200,000 barrels per day were produced in the U.S., which was almost exclusively used as a fuel additive in motor gasoline (WHO, 1998).

In January 2001, leaded car fuel in Saudi Arabia was replaced by unleaded fuel and was consequently distributed by all gas stations across the Kingdom. This move came as a result of the mounting evidence that lead causes deleterious effects to health and the environment. MTBE, which is a synthetic and organic substance, was produced in the Kingdom by SABIC and delivered to Saudi Aramco for distribution. Moreover, MTBE is produced in other Arab Gulf States in very large quantities (EIA, 2002).

MTBE is part of a group of chemicals commonly known as “oxygenates” because they increase the oxygen content of gasoline. They therefore increase gasoline’s octane number and reduce automotive emissions, such as carbon monoxide and hydrocarbons. The use of oxygenated fuels such as MTBE is anticipated to increase over the next few decades (Costantini, 1993).

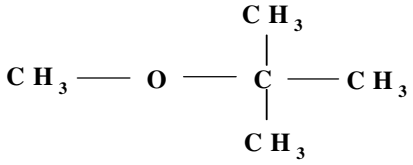
Yet, the issue of MTBE has been controversial, in part because of concerns about potential inhalation health effects and more recently, because of an added concern about MTBE-contaminated drinking water (EPA, 2004).

The U.S. Environmental Protection Agency (EPA) now requires monitoring of MTBE and other oxygenate compounds in ground water at leaking underground storage tank sites nationwide, since environmental officials

classify this additive as a hazardous substance (EPA, 2004). To further complicate the problem, the major metabolites of MTBE exposure in humans are methanol, formaldehyde, and tertiary butyl alcohol (TBA), which are produced as a result of microsomal oxidation by cytochrome P-450 enzymes (CYPs) (Hutcheon et al., 1996). These active metabolites are known to be toxic to humans (Casarett and Doull, 2001).

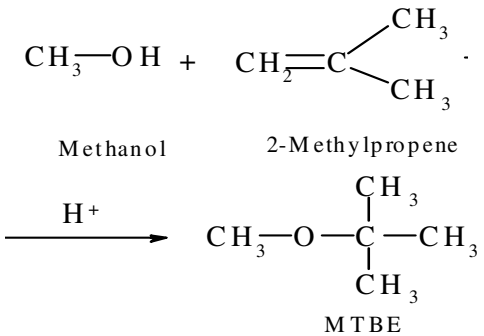
Despite the growing concerns over the use of MTBE, few biochemical data on this chemical have been published. Most studies on health issues concentrated on the neurotoxicological aspects (Daughtrey et al., 1997), genotoxicity (Kado et al., 1998), mutagenecity and carcinogenecity (Zhou et al., 2000), the induction of programmed cell death (apoptosis), and inhibition of cell cycle progression (Vojdani et al., 1997). Recently, the results of Lin et al. (2007) revealed that high concentrations of MTBE significantly increased plasma membrane damage and the ratio of necrotic cells with decreased spermatogenic cell capability. Moreover, MTBE increased the production of reactive oxygen species (ROS) and enhanced lipid peroxidation, which play an important role in the cytotoxicity of spermatogenic cells (Lin et al., 2007).

MTBE's chemical formula is $C_5H_{12}O$, and its molecular weight is 88.15. The structural formula of MTBE is shown as:



MTBE is a clear, colorless, flammable liquid with a distinctive ethereal odor. Its density is 0.741 g/ml, boiling point 55.2°C, melting point -109°C, flash point -28°C, vapor pressure 245 mm Hg, and specific gravity 0.74. It is soluble in alcohol and ether, and its solubility in water is 4.8 g/100 g. No information is available about its pH. Other names for MTBE are 2-Methoxy-2-methylpropane; tert-Butyl methyl ether; or Methyl, 1-dimethyl ethyl ether (WHO, 2000).

MTBE is prepared by the acid-catalyzed addition of methanol to 2-methylpropene (WHO, 2000), as follows:



Humans are exposed to MTBE on a daily basis through, for example, touching the skin or breathing contaminated air while pumping gasoline, breathing exhaust fumes while driving cars, and breathing MTBE-contaminated air near highways or in cities. Exposure can also occur while drinking, swimming, or showering in water that has been contaminated with MTBE. MTBE is used as a therapeutic drug to dissolve gallstones (ATSDR, 2001) and to allow for faster and cleaner lipid recovery (because of MTBE's low density) (Matyash et al., 2008). Therefore, this study focused on the possible health toxicity of MTBE in drinking water, as manifested by changes in the activities of certain plasma enzymes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyltransferase (GGT), lactate dehydrogenase (LDH), and creatine kinase (CK).

MATERIALS AND METHODS

Animals

The source animals in this study were as follows: 120 male Wistar rats that had a mean initial body weight of 139.14 ± 1.76 g at 6 weeks of age. The rats were randomly selected from a rat colony, bred from animals obtained in 1976 from Olac Ltd, U.K., in the experimental animal unit of King Fahad Medical Research Center, Jeddah. They were housed in an air–conditioned room maintained at 24°C and exposed to a 12 h dark/light cycle. Animals were kept in plastic cages, on wood shaving bedding. They were fed on standard food produced by Grain, Silos and Flour Mills Organization, Western Province, Saudi Arabia. The animal house was approved and licensed. Rats were then divided into five groups: 40 rats for the control group, and 20 rats for each of the other four groups. Five rats were kept in each cage. Rats were individually weighed at the beginning of the study and at the end of the experiment period (60 days).

MTBE sample

The MTBE sample was provided by Saudi-Aramco, Jeddah, Saudi-Arabia, and was used without further purification.

Drinking water

Drinking water containing different concentrations of MTBE (0.0, 1,000, 1,500, 2,000, and 2,500 ppm) was supplied daily for 60 days. Tap water mixed with MTBE was available for the rats 24 h a day. The water was changed every 6 days. The concentration of MTBE in drinking water was calculated as shown in (Table 1).

The water under study was prepared by taking the proper amount of water in a beaker and placing it on the balance. The weight was then adjusted to zero grams, and the required MTBE concentration was added. This procedure was used to prevent the evaporation of MTBE. The solution was then placed in a measured conical flask, and filled up with water. Each cage was supplied with a certain concentration of MTBE, ranging from 1,000 - 2,500 ppm.

Blood samples collection

Blood samples were individually obtained from the 120 rats at the

Table 1. Concentration of MTBE in water.

Group no.	No. of rats	Conc. of MTBE in water	
		ppm	g/L
1	40	0.0	0.0
2	20	1,000	1.0
3	20	1,500	1.5
4	20	2,000	2.0
5	20	2,500	2.5

Table 2. Amounts of plasma samples, reagent one (R1) and reagent two (R2), for detection of certain plasma enzymes [alanine aminotransferase (ALT), aspartate amino-transferase (AST), Alkaline phosphatase (ALP), γ-glutamyltransferase (GGT), lactate dehydrogenase (LDH), and creatine kinase(CK)] in Wistar rats.

Enzymes	ALT	AST	ALP	GGT	LDH	CK
Sample	10	10	11	6	7	7
Reagent 1	250	250	250	250	250	250
Reagent 2	50	50	50	-	50	50

end of the experimental period (60 days). Samples were collected in Lithium heparin (L.H.) and EDTA tubes by cardiac puncture under slight diethyl ether anesthesia using a vacationer with (22G×1") needles. Tubes with anticoagulants were gently inverted end over end 7 to 10 times after collection. All test tubes were kept in an ice box containing an ice bag or dry ice.

The L.H. tubes were then centrifuged at 3,000 rpm for 3 min (using cold centrifuge). Clear plasma was carefully separated using pipettes (plasma samples can be stored in an Epindorf tube at 80°C for repeated reading). All experiments were carried out at the King Fahad Medical Research Center.

Plasma enzymes determination

Plasma enzymes studied include: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyltransferase (GGT), lactate dehydrogenase (LDH), and creatine kinase (CK). The output quantities are shown in Table 2; all were measured photometrically at proper wavelength.

For assaying alanine aminotransferase and lactate dehydrogenase activities, Bablok's method was used (1988).

The procedures to determine AST, ALP, GGT and CK were used as mentioned by Tietz (1995).

RESULTS AND DISCUSSION

The ALT concentration (U/l) of blood plasma in treated rats increased significantly (Table 3) at all MTBE concentrations (1,000, 1,500, 2,000 and 2,500 ppm) by 48.8, 19.2, 16.9, and 15.4%, respectively.

The AST concentration (U/l) of blood plasma in treated rats increased significantly (29.2%) only at the MTBE concentration of 2,000 ppm (Table 3). At MTBE concentrations of 1,000, 1,500, and 2,500 ppm, however, there was no significant difference in AST concentration of the blood plasma as measured in treated rats.

There were no significant differences between the MTBE concentrations (1,000, 1,500, 2,000, and 2,500 ppm) in the ALP, GGT, LDH, and CK concentrations (U/l) of blood plasma in treated rats (Table 3).

ALT is a key enzyme indicator of liver function, which is present in the cytosol of hepatocytes and mitochondria. This enzyme is known to be released from these cells into blood circulation upon liver injury or disease (Andreoli et al., 2001). Furthermore, it is known that an increase of ALT activity in plasma is observed in conditions involving necrosis of hepatocytes, myocardial cells, skeletal muscle cells, and erythrocytes (Wallach, 2000). This study showed that MTBE, at all concentrations used, causes a significant increase of plasma ALT and AST (only at 2,000 ppm) activities. This conclusion is in agreement with the findings of Elovaara et al. (2007), who revealed that ALT and AST were elevated in blood plasma after administration of MTBE via exposure to MTBE (10 - 20 mmol/kg) for 3 days in the rats (Elovaara et al., 2007). Furthermore, Elovaara observed that there was a slight or no modifying effect on the NADPH: Quinone oxidoreductase (NQO1), glutathione S-transferase (GST), and UDP-glucuronosyltransferase (UGT) activities. This study showed that there were no significant effects in the ALP, GGT, LDH, and CK activities of blood plasma in rats treated with MTBE.

The rate of MTBE clearance from the blood after the host has been exposed to MTBE depends on the route of exposure. This point was studied in human volunteers exposed to MTBE via the three possible routes (Prah et al., 2004). MTBE disappeared more quickly from human blood if it was taken orally in drinking water as compared to inhalation and dermal routes. Oral exposure to MTBE causes its rapid conversion to tertiary-butyl alcohol (TBA). The biotransformation of MTBE to TBA is mediated by cytochrome P-450 enzymes (CYPs), which constitute the main metabolic pathway of phase 1 metabolism. This means that oral exposure to MTBE results in a greater MTBE metabolism to TBA than other routes. TBA is a very water soluble compound and has a blood: air partition ratio of 462:1(Prah et al., 2004). The slower TBA elimination from the blood may make it a better indicator of MTBE exposure than measuring MTBE itself. However, there could be other TBA sources than MTBE, since TBA is used in various consumer products such as some perfumes, cosmetics, drugs, paint removers, and industrial solvents. Other major metabolites produced from MTBE metabolism, such as 2-Hydroxy isobuteric acid, 2-methyl-1,2-propanediol, were identified and quantified in rat urine (Benson et al., 2003).

MTBE exhibited a clear but differential inducing effect on MTBE-metabolizing CYP forms, with no marked effect on phase II activities. This may reflect the importance of these pathways *in vivo*, which explained the unchanged concentrations of all the enzymes used in this study except

Table 3. Effect of different MTBE concentrations on plasma enzymes of rat (at day 60)*.

		ALT (U/l)	AST (U/l)	ALP (U/l)	GGT(U/l)	LDH (U/l)	CK(U/l)
Control n = 40		80.42 ± 1.99	130.92 ± 8.14	182.0 ± 5.72	36.38 ± 1.44	592.86 ± 54.16	528.41 ± 57.95
1,000 ppm, n=20		119.69 ± 9.63	160.46 ± 17.62	193.2±6.65	34.29 ± 2.0	536.25 ± 85.78	541.0 ± 67.72
Control Vs 1,000 ppm	p <	0.001	NS	NS	NS	NS	NS
1,500 ppm, n = 20		95.9 ± 5.41	148.5 ± 20.26	184.0±6.95	34.4 ± 2.2	498.78 ± 80.09	412.88 ± 69.38
Control Vs 1,500 ppm	p <	0.013	NS	NS	NS	NS	NS
2,000 ppm, n = 20		94.05 ± 5.2	169.11 ± 16.78	197.55±7.48	40.0 ± 1.4	564.14 ± 70.86	449.0 ± 69.22
Control Vs 2,000 ppm	p <	0.011	0.033	NS	NS	NS	NS
2,500 ppm, n = 20		92.84 ± 4.8	133.85 ± 14.33	184.6 ± 9.82	43.9 ± 4.87	498.08 ± 44.43	453.92 ± 47.08
Control Vs 2,500 ppm	p <	0.025	NS	NS	NS	NS	NS

Aveage ± SE; Significance (p) < 0.05
* End of the experimental period
ALP = Alkaline phosphatase
AST = aspartate aminotransferase
GOT = glutamate oxaloacetate transaminase
ALT = alanine aminotransferase
GPT = glutamate pyruvate transaminase
GGT = γ-glutamyltransferase
LDH = lactatedehydrogenase
CK = creatine kinase.

for ALT. In conclusion, there was no marked potentiating, acute liver damage induced by MTBE.

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