Indian Scholar
ISSN 2350-109X
www.indianscholar.co.in
An International Multidisciplinary Research e-Journal

THE TOXIC EFFECT OF METHYL TERTIARY BUTYL ETHER (MTBE) ON THE MICE WEIGHT AND KIDNEY

Mohammed Ali Mohammed AL Fakih
&
Hamid mohammed Al-Gabr
Biology Department,
College of Education,
Al bayda'a University,
Yemen.

Abstract

MTBE is a synthetic organic compound added to gasoline. MTBE dissolves readily in water and evaporates quickly. Blood is considered to be a good bio-indicator for many toxic-induced ailments by chemicals that are injected into the body as MTBE. This study focuses on the possible health hazards of MTBE in drinking water as manifested by changes in blood composition of 60 male mice Mf1 (*Mus musculus*) exposed to five different MTBE concentrations (0.0, 1000, 2000, 4000 ppm) for 60 days. The results of the mice' weight, organ kidney weight and hematology profiles were statistically analyzed. At higher MTBE concentration, animals' weight was lost. This injury decreased plasma Urea, Creatinine (CRE) activity.

Introduction

Methyl Tertiary-Butyl Ether (MTBE) is a synthetic organic chemical mainly used to blend with gasoline instead of antiknock leaded. It is blended into gasoline at about 15% by volume to increase octane ratings and produce cleaner burning fuel and reduce automotive emissions such as leaded and carbon monoxide. MTBE is one ether compound which results from reaction of methanol and isobutylene, that used vast amounts in USA. As of January 2001, leaded car fuel in Saudi Arabia was replaced by unleaded fuel and was consequently distributed by all gas stations across the kingdom. It was replaced by the synthetic organic substance MTBE. Because high solubility of MTBE in water, contamination of drinking water occurs from leaking underground fuel-tanks, above ground fuel tanks, pipelines, refueling spills and also from industries, fumes clouds could contain MTBE so when it rains, MTBE passes into the soil, farms and gases, to cause ground water contamination.

MTBE has been used in the United States (U.S.) gasoline since 1979, in low levels (between 0.5 to 3.5% w/v) to replace lead. 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 produced in the U.S.A. In 1999, over 200,000 barrels per day was produced in the U.S., which is almost exclusively used as a fuel additive in motor gasoline (**Gillner, 1998**). In human liver, by cytochrome 2A6 (CYP2A6) is the major P450 isoform responsible for the metabolism of MTBE (**Hong et al., 1999**). Metabolism of MTBE in rat liver microsomes, P450 showed that equimolar amounts of tertiary butyl alcohol (TBA) and formaldehyde were formed by oxidative demethylation (**Brady et al., 1990; Hong et al., 1997;** and **Ahmed F. E., 2001**). Formaldehyde is highly reactive and is most likely completely metabolized in the liver. TBA is further oxidized into 2-methyl- 1,2,propanediol and a-hydroxyisobutyric acid (**Hutcheon et al., 1996;** and **Bernauer et al., 1998**). MTBE is metabolized in human liver microsomal fraction by cytochrom 2A6 (CYP2A6) enzyme to tert-butyl alcohol (TBA) and formaldehyde.

$$MTBE \xrightarrow{CYP2A6} TBA + Formaldehyde$$

TBA is a major circulating metabolite and an exposure marker of MTBE. Formation of TBA in human liver microsomes is NADPH-dependent and is significantly inhibited by carbon monoxide, which inhibits cytochrome P450 (CYP) enzymes. These results provide strong evidence that CYP enzymes play a critical role in the metabolism of MTBE in human livers *(***Hong et al., 2001***)*.

Pharmacokinetic studies indicate that MTBE is handled similarly in humans and rats but in the rat, MTBE is rapidly absorbed and rapidly excreted as MTBE and TBA in the expired air and urine, and that MTBA is metabolized to TBA and formaldehyde. However, urinary excretion products in humans (MTBE and TBA) are different from the urinary products of rats (metabolites of TBA) indicating a more complete and/or rapid metabolism of MTBE in rats (elimination half-lives for the different urinary metabolites of MTBE were between 7.8 and 17 h in humans compared with 2.9– 5 h in rats). Between 35 and 69% of the MTBE, which remained after the end of exposure, was recovered as metabolites in urine of both humans and rats (**Amberg et al., 1999**).

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) produced as a result of microsomal oxidation by cytochrome P-450 enzymes (CYP's) *(***Hutcheon et al., 1996***)*. These active metabolites are known to be toxic to humans **(Casarett & Doull, 2001**).

The results indicate relative organ weight showed change in heart, liver, spleen, lung, kidney, testes, thymus, and prostate after exposure to MTBE at dose levels of 0, 400, 800, and 1600 mg/kg/day, respectively after 2- or 4-weeks treatment period (**Dong-mei, et al., 2009**). The body weights were reduced only in males following 13 weeks of exposure of MTBE and reduced water consumption and urine output were observed in males and females exposed to MTBE and too in kidney globulin levels in males were increased at 1 and 4 weeks of MTBE exposure and tubular cell regeneration was increased in male kidneys exposed to MTBE concentrations of 7.5 mg ml or greater for 13 weeks (**Bermudez., et al., 2012**).

The kidney is a major organ in urinary system. Because high solubility of MTBE in water the contamination of drinking water occurs from leaking underground fuel-tanks , so the kidney was more affected with Toxic MTBE, in inhalation study employing CD-1 mice an increase in relative absolute kidney weight was after exposure to MTBE (**Robinson et al., 1990; Chun and Kintigh, 1993**). Just as found by (**Dodd and Kintigh, 1989; Chun and Kintigh, 1993; Bird et al., 1997**) that inhalation exposure to MTBE in rats resulted in treatment- related increases in absolute and relative kidney weight in several studies ranging from 4 weeks to 2 years in both sexes. (**Bird et al., 1997**) also found that primary neoplastic lesion in MTBE-exposed rats was the increase in the incidence of renal tubular adenomas and carcinomas at the 3000 and 8000 ppm of MTBE doses in males, but not females.

From dangerously matters, histopathology showed hyaline droplet formation in the proximal convoluted tubules in rats kidneys because an accumulation of 2m-globulin, during MTBE exposure. Therefore, in the case of MTBE it was suggested that the extra stress due to the a2m-globulin nephropathy may be a possible reason for the development of kidney tumours in the male rats. In females no kidney tumours were observed although MTBE exerted a chronic progressive nephropathy in females as well as in males (**Borghoff et al. 1996, Prescott-Mathews et al. 1997**). Another study, reported the accumulation of 2m-globulin mechanism in the lysosomes of the proximal tubule cells results in a protein overload causing toxicity which leads to cell division, which are responsible to development of kidney tumors in rats (**Swenberg and Lehman-McKeeman, 1999**). For this reason 2m-Globulin was shown to be the only protein involved in the accumulation of protein droplets induced by MTBE (**Williams and Borghoff, 2000**), and following MTBE exposure, male rats had a higher concentration of MTBE in their kidneys compared with female rats, where MTBE interacted with 2m-globulin in vitro (**Prescott-Mathews et al., 1997**) and in vivo (**Prescott-Mathews et al., 1999**). So The main goal of this study is to assess the health effects of MTBE in drinking water using blood chemistry and hematology as bio-indicators to monitor its toxicity.

Materials and method

To minimize the dangers of the exposure to chemical materials in laboratory during experiment, number of safety procedures were followed. A laboratory coat , safety mask and gloves were worn at all times during experiment. Dealing carefully with the sharp instruments , such as syringe needles, knife, fume hoods when handling MTBE was considered. In this

study, three different concentrations of MTBE in drinking water were used (1000, 2000 and 4000 ppm). Treated waters were offered to 120 male mice, on a daily basis for 60 days.

Equipment and Chemicals

All equipment used in this experiment was located at a governmental hospital in Jeddah. All experimental work was conducted in accordance with King Fahad Medical Research Center regulations, Jeddah, Saudi Arabia**.** Methyl tertiary-butyl ether (CAS No. 1634-04-4) was obtained from Aramco Chemical Company (Milwaukee, WI), Saudi Arabia. The purity of MTBE was 99.9% , and that chemicals we had obtained from Simat (Honeywell international Inc. Germany) for Medical Solutions & Laboratory Medical Supplies Co. in Jeddah

Animals

The animals used in this study were as follows:

60 male mice that had a mean initial body weight of 28.4±0.5g. They were randomly selected from a rat colony bred from animals obtained from Olac Ltd, U.K. in the experimental animal unit of King Fahad Medical Research Center. They were fed on standard food produced by Grain, Silos and Flour Mills Organization, Western Province, Saudi Arabia. The sixty male mice were then divided into four groups, forty mice for control group, and each of three other groups had fifteen mice, every five mice were kept in one cage.

Animals weight

Mice were individually weighed at the beginning of the study before exposure to MTBE, then divided into 4 groups and weighed after 6 weeks.

Drinking Water and Chemical study

Drinking water containing different concentrations of MTBE was supplied daily (0.0, 1,000, 2,000, and 4000 ppm) for 60-days. Tap water and MTBE-treated water were available for the mice 24hr a day. Water was changed every 6 days. The concentration of MTBE in drinking water was calculated as in (Table 1). The water under study prepared by taking the proper amount of water in a beaker and placed on the balance, then adjusted to zero gram, then 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, then filled up with water. According to equations was calculation of urea and Creatinine: hydrolyzed by urease to form ammonia and carbon dioxide.

$$urea + H_2O \xrightarrow{urease} 2NH_3 + CO_2$$

$$ammonia + \alpha - ketoglutarate + NADH \xrightarrow{GLDH} glutamate + NAD + H_2O$$

Urea = Blood urea nitrogen (BUN) X 2.14

In alkaline solution, creatinine forms a yellow-orange complex with picrate

$$creatinine + picricacid \xrightarrow[solution]{alkaline} creatinine - picricacid\,complex$$

Statistical analyses of the obtained data were performed using a Statgraphics computer program package, SIGMA STAT for Windows, and Excel. P<0.05 was the probability level used to determine statistical significance.

Table 1 : concentrations of MTBE in water

Groups	Numbers of mice	concentrations MTBE in water
Tap water control	15	0
1000 ppm MTBE	15	1.0
2000 ppm MTBE	15	2.0
4000 ppm MTBE	15	4.0

Results

60-Day drinking water experiment in adult mice

Animals treated with MTBE for 60 days showed a significant weight in all treated groups and it was loss only at high MTBE concentrations (4000 ppm) (Fig 1). Thus, as compared to control, the average weights were decreased by MTBE concentrations 2000 and 4000 ppm respectively. At lower MTBE concentrations however (2000 ppm), no significant difference in animal weights was noticed.

MTBE concentrations at 4000 and 2000 ppm, kidney weight (g) only decreased significantly (Table 2) as compared to control by 7.5 %. The statistical significance value was less than 0.05. However, no significant difference in kidney weights (g) was observed at MTBE concentrations 1000 ppm (Fig 2).

Table 2: Body and organ weights after MTBE exposure: drinking water study in adult male mice.

Groups	weight of body after 0 days(g)	weight of body after		Absolute weight	
		60 days(g)	P<0.05	of kidney(g)	P<0.05
Tap water control	28.4± 0.037	32.44 ±0.25		0.180 ± 0.011	
1000 ppm MTBE	28.7 ± 0.044	29 ± 0.54	0.001	0.167 ± 0.023	0.019
2000 ppm MTBE	28.6 ± 0.54	27.2 ± 0.22	0.001	0.162 ± 0.019	0.004
4000 ppm MTBE	28.86 ± 0.02	26.9 ± 0.18	0.001	0.158 ± 0.038	0.001

In blood plasma in the treated mice, urea levels (mg/dl) decreased significantly (Table 3) only at MTBE concentration 4000ppm (Fig 3). But at MTBE concentrations 1,000, and 2000 ppm there were no significant differences in urea content (mg/dl) of blood plasma in treated mice.

Table 3: Urea levels and creatinine content of blood plasma after MTBE exposure

Group	Urea		CREA	
	(mg/dl)	**P**<0.05	(mg/dl)	**P**<0.05
Tap water control	45.7±1.38		0.46±0.01	
1000 ppm MTBE	44.4±1.26	NS	0.49±0.01	NS
2000 ppm MTBE	44.1±2.07	NS	0.51±0.05	NS
4000 ppm MTBE	43.8±3.11	0.013	0.518±0.03	NS

All MTBE concentrations in groups (1000, 2000, and 4000 ppm) showed no significant differences in the creatinine content of blood plasma in treated mice (Table 3)(Fig 4).

Figure 1: Effect of different concentrations of the fuel additive methyl-tert-butyl ether (MTBE) on weight of mice after 60

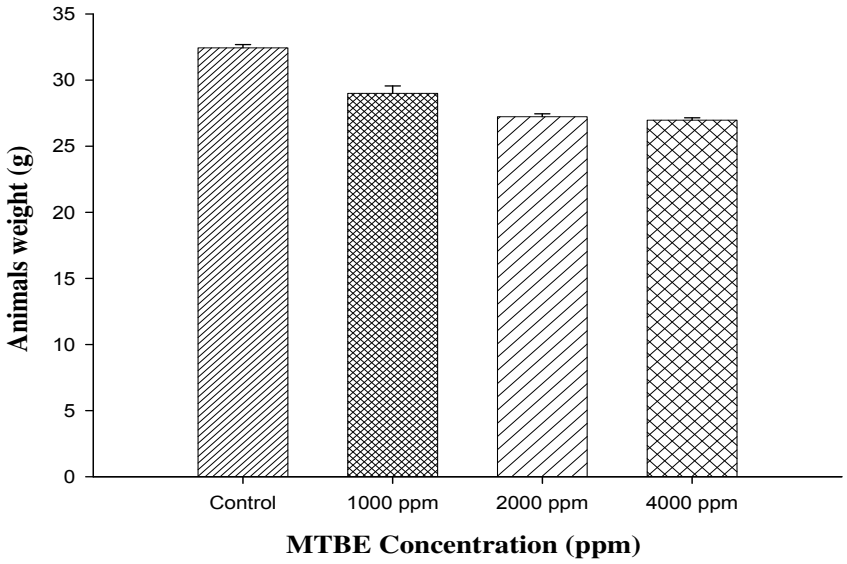


Figure 2: Effect of different concentrations of the fuel additive methyl-tert-butyl ether (MTBE) on kidney of mice after 60 days

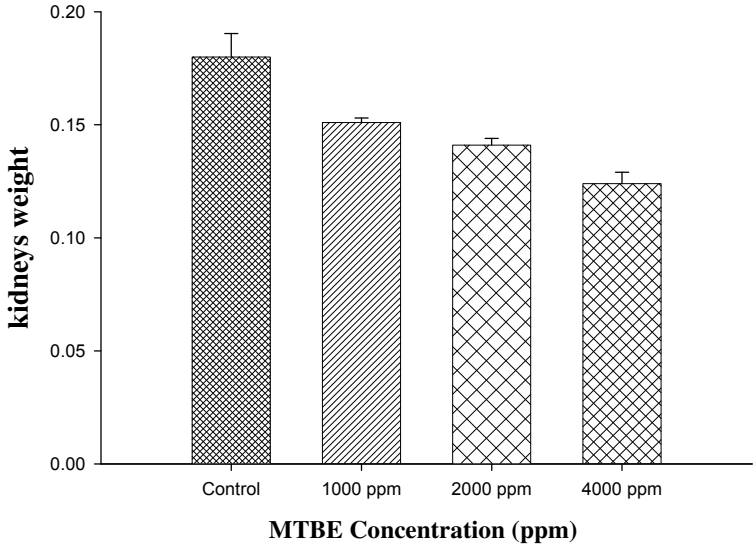
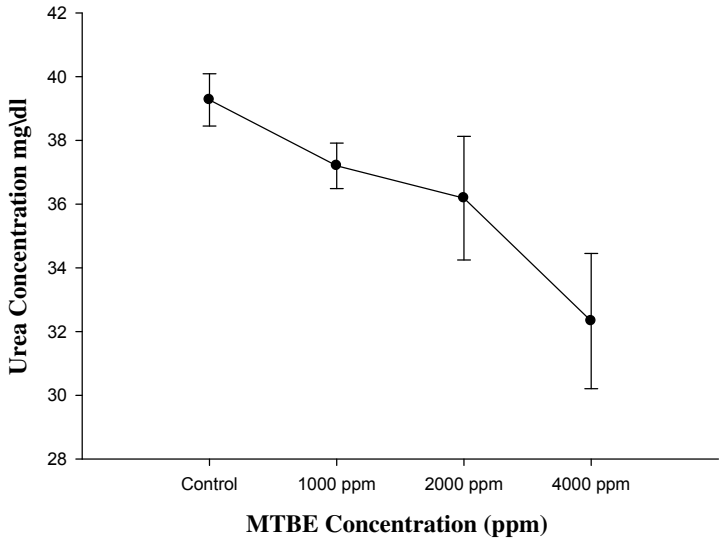


Figure 3: Effect of different concentrations of the fuel additive methyl-tert-butyl ether (MTBE) on plasma urea level (mg/dl) of mice after 60 days



Discussion

Today, after many years of using MTBE Worldwide, the U.S. Geological Survey has announced that MTBE is found in more than a quarter of the national shallow urban wells, in streams, lakes, rain and even in tap water (**Life Streams International Mfg. Co., 2004**). Water contamination with MTBE can lead to human exposure by oral, inhalation and dermal routes (**Prah et al., 1994**). Moreover, the U.S. Geological Survey has also reported that MTBE does not biodegrade

easily in the environment and therefore can affect water supplies for years (**Life Streams International Mfg. Co., 2004**). In fact, MTBE became the second most frequent detected chemical pollutant in drinking water in the U.S. causing Americans to be concerned about the quality of their drinking water (**Prah et al., 2004**).

In this study, the amounts different concentration of MTBE given to the experimented animals (adult mice aged 6 weeks and weighing 28,4 g on average) in drinking water. But according to the precautionary principal, which states that when any substance raises threats of harm to human health, as being suspected to be carcinogenic for example, a precautionary measure should be taken even if unrealistic concentrations were given to experimented animals (i.e. zero risk or no risk). In other words, according to this toxicological principal, if any chemical found to induce cancer in animals or humans at any level, it may therefore pose a danger to humans. This principal may justify the use of high concentrations of the suspected chemical to assess the required zero risk. MTBE is known to produce cancer in rodents, such as lymphoma, leukemia, testicular tumors, thyroid tumers, liver tumors and kidney tumors (**Belpoggi et al., 1995 and Williams & Borghoff, 2001**). Therefore, MTBE is considered to be a suspected carcinogen to humans (**Tang et al., 1997**).

Animal body weights were recorded on the first day of the study, once every 10 day period in all groups control and three groups which were treated of different concentration from MTBE, and before experiment final before collecting samples to analysis. Most of MTBE-treated animals experienced a significant weight loss. Maximum weight loss (nearly 20 %) was achieved at the highest concentration of MTBE (4000 ppm) after 60 days of treatment when compared to the weight of Tap water control, it agrees with results of *(***Dong-mei, et al 2009***) and (***Bermudez., et al. 2012***).* while another observation of the effects of MTBE in drinking water was the increase of the weights of some internal organs (e.g. liver, kidneys and heart) in the treated animals, as well as effects on the behavior of these animals with an obvious fatigue was observed on treated individuals (**Alkazmi et al., 2017***)* this disagree with us whereby showed decrease of body's weight and kidney weight. Several studies have shown that MTBE given to rodents, by various routes, caused significant weight loss *(***Jarvis, 2000***).* Loss of weight of animals treated with MTBE was also reported in studies using MTBE to dissolve stones of gallbladder (**Yoshikawa et al., 1994***).*

Plasma biochemical constituents were investigated. At low MTBE concentration (2000 ppm), a significant decrease in urea was recorded, which may be due to liver damage or diuresis or starvation (**Kaplan and Szabo, 1983**) or renal failure (**Andreoli et al., 2001**). MTBE may also cause some inhibition in the enzymes that are required for synthesis of urea (**Andreoli et al., 2001 and Backer W. S., 2013***).*

Conclusion

MTBE is widely used throughout the world, whereby used improve octane ratings and in clinical medicine to dissolve cholesterol gall stones, and reduce emissions of some pollutants in industry to improve miscibility of solvents **(Yoshikawa et al., 1994).** In recent years its toxicity has been studied systemically by different institutes, but the studies were different to the safety MTBE of gasoline additive **(Zhou W. & Ye S., 1999)**. More studies are needed to understand the mechanisms by which aliphatic ethers induce their toxic effect, and their groundwater characteristics ought to be further explored before they are allowed to be placed in widespread use.

The main objective of this study is to assess the possible health hazards of MTBE in drinking water of male mice as manifested by changes in the blood chemistry as urea and creatinine, and in the weight of body and kidneys. 60 adult male mice, aged 6 weeks and weighing 28.4 g on average exposed to four different MTBE concentrations (0.0, 1,000, 2,000, 4,000 ppm) for 60 days, 15 mice for control, and 15 rats for each concentration.

The results of the mice' weight, weight kidneys , blood chemistry, profiles were statistically analyzed. In the present study, animals lost weight at high MTBE concentrations (2,000, and 4,000 ppm) due to stomach and intestine irritations in addition to nausea, vomiting and diarrhea. Also, the offensive taste and odor of MTBE made rats lose their appetite. Kidneys weight were reduced at a higher MTBE concentration (2,000, and 4,000 ppm) inducing kidneys injury, this is in accordance with study of **Chun and Kintigh (1993)**. As evident from the results, MTBE decreased urea which may be due to liver damage or diuresis or starvation or renal failure.

ACKNOWLEDGEMENTS

First and foremost, all praise is to Allah, and we are indebted to all the staff members of the Biology Department, Faculty of Science, king Abdualziz University for the great effort they provided and granted us to complete this study. We also want to thank Prof. Mohammed Al-anisi, Department of English language, University of Albayda, Yemen for rectifying the language.

REFFRENCES

Ahmed F. E., 2001. Toxicology and Human Health Effects Following Exposure to Oxygenated or Reformulated Gasoline. *Toxicol Lett*, 123. (2-3): 89-113.

Alkazmi, M.L., Sahhaf, Y. Z., Malak, A. H., Abulreesh, H. H., (2017). Effects of Methyl Tert-butyl Ether (MTBE) on the Mucosal Immunity in the Small Intestine of the White Albino Mice. *Annual Research & Review in Biology* . 15(4): 1-11.

Amberg, A., Rosner, E., and Dekant, W. 1999. Biotransformation and kinetics of excretion of methyl *tert*-butyl ether in rats and humans. *Toxicol. Sci*. 51 (1): 1– 8.

Andreoli T., Carpenter C., Griggs R., and Loscalzo J. 2001. Cecil Essentials of Medicine. 5th edition.

Backer W. S. 2013.The impact of Methyl tertiary-butyl ether (MTBE) on contaminated drinking water in organic blood chemistry . Journal of Saudi Chem Soci (Available online 7 May 2013) from :http://dx.doi.org/10.1016/j.jscs.2013.04.008 **.**

Belpoggi, F., Soffritti, M., and Maltoni, C. 1995. Methyl-*tertiary*butyl ether (MTBE)—a gasoline additive—causes testicular and lymphohaematopoietic cancers in rats. Tox. Ind. Health 11, 119– 149.

Bernauer, U., Amberg, A., Schartzow, D., and Delcant, W. 1998. Biotransformation of 12C- and 2– 13C-labeled methyl *tert*butyl ether, ethyl *tert*-butyl ether and *tert*-butyl alcohol in rats: identification of metabolites in urine by 13C nuclear magnetic resonance and gas chromatography/mass spectrometry. Chem. Res. Toxi. 11, 651– 658.

Bird, M.G., Burleigh-Flayer, H., Chun, J.S., Douglas, J.F., Kneiss, J.J., and Andrews, L.S., 1997. Oncogenicity study of inhaled methyl *tertiary*-butyl ether (MTBE) in CD-1 mice and F-344 rats. Appl. Toxicol. 17, 45– 55.

Blue Ribbon Panel on Oxygenates in Gasoline. 1999. Achieving Clean Air and Clean Water: The Report of the Blue Ribbon Panel on Oxygenates in Gasoline. EPA 420-R99021.www.epa.gov/oms/consumer/fuels/oxypanel/blueribb.ht

Borghoff SJ, Prescott-Mathews JS and Poet TS 1996. The mechanism of male rat kidney tumours induced by methyl *tert*-butyl ether and its relevance in assessing human risk. CIIT Activities, Chemical Industry Institute of Toxicol 16, 1-8.

Brady, J.F., Ning, X.F., and Yang, C.S., 1990. Metabolism of methyl *tertiary* butyl ether by hepatic microsomes. Toxicol. 64, 157– 160.

Bermudez, E., Willson, G., Parkinson, H., Dodd, D., (2012). Toxicity of methyl tertiary-butyl ether (MTBE) following exposure of Wistar Rats for 13 weeks or one year via drinking water. *J Appl Toxicol*. 2012 32(9):687-706.

Casarett L., and Doull J. (2001). Toxicology: The Basic Science of Poisons Collier Macmillan Canada, Ltd, Toronto, London.

Chun, J.S., and Kintigh, W.J. 1993. Methyl *Tertiary* Butyl Ether: 28 Day Vapor Inhalation in Rats and MICE. Union Carbide Corporation, Bushy Run Research Center Draft 93N1241, October 29, 1993. Export, PA.

Chun, J.S., Burleigh-Flayer, H.D., and Kintigh, W.J., 1992. Methyl *Tertiary* Butyl Ether: Vapor Inhalation Oncogenicity Study in Fischer 344 Rats. US EPA Docket Number OPTS 42098. Washington, DC.

Dodd, D.E., and Kintigh, W.J., 1989. Methyl *Tertiary* Butyl Ether (MTBE): Repeated (13-Week) Vapor Inhalation Study in Rats with Neurotoxicity Evaluation and MTBE: Single Exposure Vapor Inhalation Neurotoxicity Study in Rats. US EPA/OTS Document Number 0889-0689. Washington, DC.

Dong-mei, L., Yi, G., Chun-Tao, Y., Yu-feng, H., Xiao-dong, H,. (2009). Effects of subchronic methyl tert-butyl ether ether exposure on male Sprague-Dawley rats. *Toxicol Ind Health.* 25 (1):15-23.

EPA (Environmental Protection Agency) (2004). MTBE (Methyl tertiary butyl ether) and under ground storage tanks.

Jarvis, O., 2000. Methyl *Tertiary* Butyl Ether (MTBE).

Hong, J.-Y., Yang, C.S., Lee, M., Wang, Y.-Y., Huang, W.-Q., Tan, Y., Patten, C.J., and Bondocc, F.Y., (1997). Role of cytochromes P450 in the metabolism of methyl *tert*-butyl ether in human livers. Toxicol. 71, 266– 269.

Hong, J.-Y., Wang, Y.-Y., Bondoc, Y., Lee, M., Yang, C.S., Hu, W.-Y., and Pan, J., 1999. Metabolism of methyl *tert*-butyl ether and other gasoline ethers by human liver microsomes and heterologously expressed human cytochromes P450: identification of CYP2A6 as a major catalyst. Toxicol. Appl. Pharmacol. 160, 43– 48.

Hong J., Wang Y., Mohr S., Bondoc F., and Deng C. (2001). Human cytochrome P450 isozymes in metabolism and health effects of gasoline ethers. Res Rep Health Eff Inst, 7(27):95-109.

Hutcheon, D.E., Arnold, J.D., ten Hove, W., and Boyle, J. III., 1996. Disposition, metabolism and toxicity of methyl *tertiary* butyl ether, an oxygenatefor reformulated gasoline. J. Toxicol. Environ. Health 47, 453– 464.

Kaplan A., and Szabo L. 1983 Clinical Chemistry Interpretation and Techniques. 2nd.ed Lea & Febiger, Philadelphia,. Life Streams International Mfg. Co. 2004. Wholly water ® the

ultimate drinking water purifier. From:http:// webcache. googleusercontent.com /search? q=cache: ytUt7Pqswp0J : www. wholly water.Com / MTBE.htm + &cd =1&hl=en&ct=clnk.

Prah, J.D., Goldstein, G.M., Devlin, R., Otto, D., Ashley, D., House, D., Cohen, K.L., and Gerrity, T., 1994. Sensort, symptomatic, inflammatory, and ocular response to and the metabolism of methyl *tertiary* butyl ether in a controlled human exposure experiment. Inhal. Toxicol. 6, 521– 538.

Prah, J., EPA, D. Ashley, B. Blount, M. Case, T. Leavens, J. Pleil, and F. and Cardinali. 2004. " Dermal, Oral, and Inhalation Pharmacokinetics of Methyl Tertiary Butyl Ether (MTBE) in Human Volunteers" . Toxicol Sci. 77 (2): 195-205.

Prescott-Mathews, J.S., Wolf, D.C., Wong, B.A., and Borghoff, S.J., 1997. Methyl *tert*-butyl ether causes _2_-globulin nephropathy and enhanced renal cell proliferation in male Fischer-344 rats. Toxicol. Appl. Pharmacol. 143(2): 301– 314.

Prescott-Mathews, J.S., Poet, T.S., and Borghoff, S.J. 1999. Evaluation of the in vivo interaction of methyl *tert*-butyl ether with a2m-globulin in male F-344 rats. Toxicol. Appl. Pharmacol. 157(1): 60–67.

Robinson, M., Bruner, R.H., and Olson, G.R., 1990. Fourteenand 90-day oral toxicity studies of methyl *tertiary*-butyl ether in Sprague–Dawley rats. J. Am. Coll. Toxicol. 9(2): 525–540.

Swenberg, J., and Lehman-McKeeman, L.D., 1999. α 2-Urinary globulin-associated nephropathy as a mechanism of renal tubule cell carcinogenesis in male rats. In: Capen, C.C., Dybing, E., Rice, J.M., Wilbourn, J.D. (Eds.), Apecies Differences: Thyroid, Kidney and Urinary Bladder Carcinogenesis. IARC Scientific Publication No. 147(2): 95– 118.

Tang G., Wang J and Zhuang Z., 1997. Cytotoxicity and genotoxicity of methyl tert-butyl ether and its metabolite to human leukemia cells. ZhonghuaYu FangYi Xue Za Zhi, 31 (6) 334-7.

Tietz N., 1995. Clinical Guide to Laboratory Tests. 3rd edition, W. B. Saunders, Philadelphia, PA

.**US Congress, 1990**. Clean Air Act Amendments of 1990, Public Law 101-549 (November 15, 1990). US Congress, Washington, DC.

Zhou W., and Ye S. 1999. Subchronic oral methyl tertiary butyl ether (MTBE) exposure in male sprague-dawley rats and effects on health of MTBE exposed workers. Journal of occupational health, 41: 33-38.

Williams T. M., and Borghoff S.J., 2001. **Characterization of tert-butyl alcohol binding to alpha2u-globulin in F-344 rats.** Toxicol. Sci., 62 (2):228-235.

Yoshikawa M., Arashidani K., Katoh T., Kawamoto T. and Kodama Y., 1994. Pulmonary elimination of methyl tertiary butyl ether after intraperitoneal administration in mice. Arch. Toxicol., 68: 517- 519.