Subchronic Oral Methyl Tertiary Butyl Ether (MTBE) Exposure in Male Sprague-Dawley Rats and Effects on Health of MTBE Exposed Workers*

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Abstract: Subchronic Oral Methyl Tertiary Butyl Ether (MTBE) Exposure in Male Sprague-Dawley Rats and Effects on Health of MTBE Exposed Workers: Wei ZHOU, et al. Department of Environmental Health, Shanghai Medical University—Methyl tertiary-butyl ether (MTBE) is used to reduce carbon monoxide and ozone in urban air and to boost fuel octane. There are yet no data on the toxicity and health effects of MTBE in China. To evaluate the possible toxicity of domestic MTBE, 40 male, 8-week-old Sprague-Dawley rats, weighing 180–200 g, were gavaged with different concentrations of MTBE. MTBE, dissolved in soybean oil, was administered at doses of 1000, 600 or 200 mg/kg b.w., and a control group with soybean oil, once daily, five days per week, for 90 days. There were no marked differences in body weight growth and food intake among the 4 groups. The results showed that MTBE significantly increased the weights of liver and kidneys, but the levels of serum enzymes and proteins were not greatly changed. No apparent pathological changes in the main organs (liver, kidneys, testes, and lungs) were found by light microscopy, but electron microscopy analysis showed significant changes in liver cells of all treatment groups, including cell nuclear condensation, fat drops and lysosomes in cells, and smooth endoplasmic reticulum (SER) dispersion. The effects on the health of 96 MTBE occupationally exposed workers and 102 controls were also investigated by a questionnaire, and we found that workers who reported health complaints in MTBE exposed group (62 cases, 64.6%) were significantly more numerous (P<.001) than those in the control group (16 cases, 16.7%), and the OR value was 9.80 (95% C.I is 4.74–20.53). The most frequently reported symptoms in the exposed group were eye irritation (19.8%), dizziness (18.8%), burning sensation in the nose or throat (17.7%), insomnia (13.5%), nausea or vomiting (13.5%), headache (12.5%), fatigue (12.5%), poor memory (12.5%), irritability (6.3%) and skin irritation or redness (5.2%). The data suggested that MTBE may be toxic to male SD rats at high doses, and MTBE may have harmful effects on exposed workers.

Key words: Subchronic, Methyl Tertiary Butyl Ether, SD rats, Health effects, Workers

Methyl tertiary-butyl ether (MTBE), the main component of oxygenated fuel to enable more complete combustion, is used to reduce carbon monoxide and ozone in urban air, and to boost fuel octane. The concentration of MTBE added to gasoline is up to 15%, and the percent of MTBE-containing gasoline has increased from 8 to 22% in some places1, 2. Environmental release of MTBE may occur at industrial sites involved in the manufacture of MTBE or in the blending of MTBE with gasoline; during the storage, distribution and transference of MTBE-blended gasoline; and from spills or leaks or fugitive emissions at automotive service stations3. So human inhalation and ingestion exposures to MTBE may occur at petroleum terminals and garage stations, and near gasoline pumps the well water also can be contaminated with MTBE through underground storage tank leakage, which enhances the exposure risk in individuals residing in the area4. MTBE is rapidly absorbed after oral or inhalation exposure, and is rapidly distributed in the blood to all parts of the body including the brain5, 6. Animal lethality data indicate that MTBE is low in acute toxicity, and acutely toxic oral doses can result in nervous system effects as well as muscular weakness and inflammation of the stomach and small intestines7, 8. MTBE also causes
mild skin irritation and moderate eye irritation. Subchronic oral and inhalation tests showed that MTBE increased kidney and liver weights, and some changes in blood chemistry in high dose groups. But no indications of significant immunological, cardiovascular, hematological, reproductive, hepatic damage or pulmonary function effects were noted in relative low exposure doses. MTBE did not appear to show mutagenic potential in the Ames bacterial assay or any clastogenicity in cytogenetic tests, and the potential for in vivo mutagenic activity was low; but some investigators showed that MTBE could induce neoplasm in animals, and advised the classification of MTBE as a B2 probable human carcinogen or C carcinogen.

In some areas where MTBE-oxygenated gasoline has been used, there have been widespread complaints of non-specific health effects including neurotic symptoms such as headache, nausea, eye irritation, and respiratory and allergic abnormalities attributed to the gasoline, and in some places the addition of MTBE to gasoline has been suspended. But epidemiological studies conducted in Alaska to assess the health effects of MTBE in gasoline could not be interpreted relative to MTBE-oxygenated gasoline, and studies carried out in some other places including New Jersey, Stamford, Connecticut, and New York did not indicate any significant adverse health effects attributed to MTBE. Human clinical studies also show that a two-hour exposure to pure MTBE (5 to 50 ppm) does not appear to be associated with any negative health effects. Some people think that the adverse symptoms may be due to the combination of MTBE with gasoline.

In China, the use of oxygenated additives in gasoline is very limited. Now, because the government has come to realize the importance of reducing automobile emissions, MTBE will appear on the market in the near future. In order to evaluate the possible toxicity of domestic MTBE, studies were carried out to determine the effect of MTBE on rats, and the health effects of MTBE occupationally exposed workers.

Materials and Methods

Animal experiments

The MTBE sample was supplied by Shanghai Oil Refinery, and the purity is 98.8%. Male Sprague-Dawley rats, 8 weeks old, weighing 180–200 g, were provided by the laboratory animal center of Shanghai Medical University. The 40 rats were randomly divided into 4 treatment groups, treated with doses of 1,000, 600 or 200 mg/kg b.w MTBE, and the control group was gavaged with soybean oil. Every single dose of MTBE was administered by gavage in 1 ml of soybean oil, once daily for 5 days each week for 90 days. During the experiment, the solutions were prepared weekly and maintained at 4°C, and individual animal weights were measured once weekly.

Before sacrificing, the blood was collected from the tail vein and some serum enzymes were measured with an automatic biochemical analyzer (HITACHI 7170A, JAPAN), including LDH (lactic dehydrogenase), AST (aspartate amino transaminase), ALT (alanine amino transaminase), TP (total protein), ALB (albumin), GLO (globulin), A/G (albumin/globulin); BUN (blood urea nitrogen) and Cr (creatinine). The weights of the liver, kidneys, testes, and lungs were measured, the tissues underwent histopathological examination by light microscopy, and the livers were also examined with an electron microscope.

Morphologic Studies

The liver, kidneys, testes, and lungs were excised and processed for morphologic analysis by light microscopy. Fragments of the tissues were fixed in 10% formalin, dehydrated in graded alcohol, passed through xylen and embedded in paraffin. Sections (4 μm) were stained with hematoxylin-eosin.

As MTBE is metabolized in liver, this tissue was processed for electron microscopy. Hepatic tissue slices were fixed by immersion in cacodylate-buffered 2.5% glutaraldehyde at 4°C for 4 h. The tissue samples were washed for 1 h in cacodylate buffer and then postfixed for 1 h in buffered 1% osmium tetroxide, dehydrated in graded ethanol and embedded in Epon 812. Semithin sections (1 μm thick) stained with toluidine blue were used for conventional light microscopy evaluation. Thin sections (500 A thick) were stained with uranyl acetate and lead citrate and examined with a Zeiss-900 electron microscope at 50 KV.

Statistical analysis was performed by Dunnett’s t-test.

Health effects of MTBE in occupationally exposed workers

The epidemiology study took place in several petroleum factories of China. 96 workers (aged from 20 to 49, mean age 29.3 ± 6.1) who were occupationally exposed to MTBE and with no current diseases or drug use, including operators, lab analysts, blenders, and maintenance foremen were investigated. Among these workers, 65 were male and 31 were female; 40 were smokers and 56 were nonsmokers, and among the 56 nonsmokers 22 were negative smokers; 9 workers drank and 87 did not drink. The duration of exposure was 1 to 10 years. The TWA (time weighted average) concentrations of MTBE in workplaces ranged from 10 ppm to 56 ppm (36 mg/m³ to 202 mg/m³). The 102 controls (aged from 20 to 49, mean age 28.1 ± 6.9) were from the same factory and had not been exposed to any harmful chemicals besides MTBE, and included managers of factories, doctors in hospitals and logistic workers. Of these controls, 69 were male and 33 were female; 45 were smokers and 57 were nonsmokers, and among the 57 nonsmokers 20 were negative smokers; 6 workers drank and 96 did not drink.
There was no significant difference between the two groups in age, gender, smoking or drinking habit.

Each worker was asked to answer a questionnaire on confounding factors. And the questionnaires were administered personally by one of the study investigators. The contents of the investigation were as follows: (1) name, gender, birth date, address of family, marriage, original hometown and education. (2) occupational exposure: type of work, length of service, exposure time per working day. (3) smoking and drinking habits: time of starting and giving up, amount of smoking and drinking, and negative smoking. (4) history of diseases and drugs. (5) health situation: whether they had experienced either new onset or increased frequency of health complaints since they began to do their current work. The list of symptoms including dizziness, headache, eye irritation, burning sensation in the nose or throat, anxiety, spaciness or disorientation, insomnia, fever, sweats or chills, inability to concentrate, irritability, fatigue, poor memory, skin irritation or redness, muscle aches, nausea or vomiting, fatigue, fever, diarrhea, cough, difficulty in breathing, sneezing, bronchitis, rashes and others. The questionnaires were collected and the data were analyzed with an Epi Info 6 and SAS statistical package (SAS Institute, Cary, NC). Logistic regression was used to identify confounding factors.

Results

Animal Studies

During the 13 weeks of treatment, there were no significant differences between the treated and control groups in water and food consumption and mean body weight gain. Except that the highest group showed signs of ataxia in the first two weeks, no obvious behavioral changes were observed in the MTBE-treated animals. The liver, kidney, testes, and lung weights are shown in Table 1. Both the absolute and relative weights of livers and kidneys were significantly increased in the 600 and 1,000 mg/kg groups, but the weights of testes and lungs were scarcely changed.

Except for LDH and AST, the serum chemistries did not change greatly in any of the treated groups, compared with the control (Table 2). Although there were significant differences between the treated and control groups in LDH and AST activities, these values were in the normal range.

No apparent pathological changes were observed by light microscopy in any of the treated groups. In electron microscopy, changes were observed in the liver cells of all treated groups, including cell nuclear condensation, fat droplet and lysosome appearance in cells and smooth endoplasmic reticulum (SER) disintegration. At higher doses, more severe changes were observed (see Fig. 1 to Fig. 4).

Epidemiological survey

Workers in the MTBE occupationally exposed group who reported health complaints (62 cases, 64.6%) were significantly more numerous (P<.001) than in the control group (16 cases, 16.7%), and the OR value was 9.80 (95% C.I. is 4.74–20.53). The most frequently reported symptoms in the exposed group were eye irritation or redness, muscle aches, nausea or vomiting, fatigue, fever, diarrhea, cough, difficulty in breathing, sneezing, bronchitis, rashes and others. The questionnaires were collected and the data were analyzed with an Epi Info 6 and SAS statistical package (SAS Institute, Cary, NC). Logistic regression was used to identify confounding factors.

Table 1. Organ weights of male SD rats subchronically treated with MTBE (mean ± S.D.)*

<table>
<thead>
<tr>
<th>MTBE (mg/kg)</th>
<th>Absolute Weight (g)</th>
<th>Relative Weight (g/100 body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>liver</td>
<td>kidney</td>
</tr>
<tr>
<td>0</td>
<td>10.59 ± 1.07</td>
<td>2.38 ± 0.20</td>
</tr>
<tr>
<td>200</td>
<td>12.07 ± 1.36*</td>
<td>2.43 ± 0.23</td>
</tr>
<tr>
<td>600</td>
<td>12.87 ± 1.11*</td>
<td>2.61 ± 0.22</td>
</tr>
<tr>
<td>1,000</td>
<td>13.44 ± 1.50*</td>
<td>2.70 ± 0.23*</td>
</tr>
</tbody>
</table>

*Significantly different from control by Dunnett’s t-test (P<0.05).

Table 2. Effect of various MTBE doses on serum chemistries in rats (mean ± S.D.)*

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>LDH (IU/L)</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
<th>TP (g/L)</th>
<th>ALB/GLO Ratio</th>
<th>BUN (mmol/L)</th>
<th>Creatinine (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>873.1 ± 288.5</td>
<td>102.9 ± 34.0</td>
<td>35.6 ± 10.1</td>
<td>67.4 ± 9.2</td>
<td>1.2 ± 0.2</td>
<td>7.0 ± 1.1</td>
<td>59.9 ± 6.4</td>
</tr>
<tr>
<td>200</td>
<td>589.7 ± 167.2*</td>
<td>71.1 ± 13.5*</td>
<td>31.4 ± 6.5</td>
<td>62.7 ± 8.8</td>
<td>1.2 ± 0.2</td>
<td>6.2 ± 0.9</td>
<td>53.4 ± 3.8</td>
</tr>
<tr>
<td>600</td>
<td>687.4 ± 132.2</td>
<td>72.6 ± 8.1*</td>
<td>33.2 ± 6.3</td>
<td>66.6 ± 6.7</td>
<td>1.1 ± 0.1</td>
<td>6.6 ± 1.1</td>
<td>56.0 ± 4.2</td>
</tr>
<tr>
<td>1,000</td>
<td>630.6 ± 130.4</td>
<td>75.4 ± 9.9*</td>
<td>44.6 ± 5.6</td>
<td>72.3 ± 4.9</td>
<td>1.1 ± 0.1</td>
<td>6.2 ± 0.8</td>
<td>55.5 ± 4.6</td>
</tr>
</tbody>
</table>

*Significantly different from control by Dunnett’s t-test (P<0.05).
(19.8%), dizziness (18.8%), burning sensation in the nose or throat (17.7%), insomnia (13.5%), nausea or vomiting (13.5%), headache (12.5%), fatigue (12.5%), poor memory (12.5%), irritability (6.3%) and skin irritation or redness (5.2%, Table 3). Logistic regression analysis showed that gender, age, MTBE exposure time per working day, length of service, smoking and drinking habits did not significantly affect the symptoms.

Discussion

MTBE is widely used throughout the world, and in recent years its toxicity has been studied systematically by different institutes, but there is conflicting evidence as to the safety of MTBE. One group of scientists indicated that based on the results of current studies, there is not enough evidence to show that MTBE in gasoline is hazardous to humans, particularly at the low concentrations (0.3–0.5 ppm) likely to be encountered in fuel use. And from the standpoint of CO reduction, it has been successful, so its use should be encouraged. Others suggest that the use of MTBE failed to lower ambient CO to levels protective of public health, that MTBE showed signs of carcinogenicity in some animal experiments, and also that oxygenated MTBE-containing gasoline causes a variety of diseases and illnesses in humans, so the use of MTBE in gasoline should be banned.

The results of animal experiments showed that because the absolute and relative weights of liver and kidneys...
were significantly high in the 600 and 1,000 mg/kg groups, probably livers and kidneys are the target organs of MTBE in rats. Though there is no evidence that MTBE induces liver tumors in rats, we still put emphasis on the liver in this study, as MTBE is mainly metabolized in the liver. The results showed that MTBE could damage the cell organelles of the liver even though liver function remained normal. It is known that when chemicals enter the body, they usually affect the sensitive cells firstly, and cell function can be kept normal temporarily when this kind of damage is not serious. Electron microscopy is helpful in identifying this kind of gentle or early damage caused by chemicals. We did not find any positive changes in any of the main organs including the liver, kidneys, lungs and testicles by light microscopy.

In China MTBE has not been added to gasoline, so there are no exposed people except workers in petroleum factories. The investigation of MTBE occupationally exposed workers is helpful in making clear the health effects of MTBE on exposed people. According to this investigation, the frequency of health complaints in MTBE exposed people is higher than that in the controls, which suggested that MTBE may be harmful to peoples’ health. But the frequency of health complaints was not consistent with the exposure time per working day or the length of service in the exposure group, which means that no exposure-response relationship existed. And in this study the frequency of single symptoms is lower than in data reported earlier18, 20, which may be due to the combination of MTBE with gasoline. It is known that exposure to MTBE at gasoline stations when self-service refueling or commuting is 2–10 ppm, and the presence of MTBE in ambient air, public buildings and residences is in the range of 0.001–0.10 ppm21, which concentrations are much lower than the concentration in the workshops of refineries. Further studies should be carried out to explore the toxicity of gasoline containing MTBE, and the health effects on those who are exposed to MTBE should be explored when MTBE is added to gasoline.

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