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Toxicity of Methyl Tertiary Butyl Ether to *Daphnia magna* and *Photobacterium phosphoreum*

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Methyl tertiary butyl ether (MTBE) is a liquid organic compound added to gasoline to increase its oxygen content and to reduce the emission of carbon monoxide during combustion in many urban areas (Anderson 1993). In order to meet the 1990 Clean Air Act amendments, gasoline must contain 2.7% oxygen (by weight) or 15% (by volume) of MTBE in gasoline to meet the regulations for the control of carbon monoxide emissions. Health effects caused by inhalation of MTBE include headaches, dizziness, irritated eyes and nausea; Mehlman (1990) reported that MTBE is one of cancer-causing chemicals. Intracaval injection of MTBE (0.2 mg/kg) caused the highest mortality (100%) in rats (Akimoto et al 1992). General anesthetic effect induced by MTBE was found at or above 1200 mg/kg body weight (Robinson et al 1990); Rosenkranz and Klopman (1991) predicted that MTBE is neither a genotoxicant nor a carcinogen. Nevertheless, the safety of using MTBE in oxygenated fuels is now being questioned from its potential as groundwater pollutant.

Gasoline is normally stored in underground tanks. An estimated 1.5 to 10 million underground tanks were used to store gasoline mixed with MTBE; 85% of these underground tanks were buried over 20 yr ago with no corrosion protection (Page 1988). Ten to 35% of these tanks may be leaking (gasoline mixed with MTBE) currently resulting in groundwater pollution (Eiceman et al 1987). Little information is available on the toxicity of MTBE in water. The objective of this study was to measure the toxicity of of MTBE to two organisms (*Daphnia magna* and *Photobacterium phosphoreum*).

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MATERIALS AND METHODS

MTBE (99.89 %) (EM Science, Gibbstown, New Jersey) was dissolved in methanol (1:1) before serial dilutions with distilled water to yield 1, 10, 50, 100, 200 or 1000 mL/L of MTBE. *Daphnia magna* from Ward's Natural Sciences Establishment Inc. (Rochester, New York) were grown in a 4-gal aquarium at 20 °C and fed 1 mL of daphnia growth medium (8g/180mL) thrice a week. Five *Daphnia magna* neonates (less than 24 hr old) were collected and placed in MTBE solutions. The experiment was replicated twice. The activity and mortality of *Daphnia magna* were recorded after 5 min, 4, 24, 36 and 48 hr (USEPA 1987).

A toxicity analyzer (Microtox Model 2055; Beckman Instr. 1982, Carlsbad, California) was used to measure changes in the light output of the bioluminescent bacteria (*Photobacterium phosphoreum*) at 15 °C after exposure to MTBE. The effective concentration (EC_{50}) causing a 50 % decrease in the light output was determined at 5 and 15 min (USEPA 1987; Beckman Instr. 1982). EC_{50} was calculated using the built-in computerized program, through least square analyses of percent luminescence inhibition versus percent concentration of the sample. Two replicates were tested. A reduction in the EC_{50} values signifies an increase in toxicity. This "Microtox" test has been shown to model quite well against more traditional aquatic assay organisms including fathead minnows and rainbow trout (Dutka and Bitton 1986).

RESULTS AND DISCUSSION

MTBE showed no effect on the activity or mortality of *Daphnia magna* even at 1000 mL/L.

"Microtox" appears to be a more sensitive test than *Daphnia magna*. The EC_{50} (mean $\pm$ S.D.) of MTBE was 54.3 $\pm$ 14.9 and 41.8 $\pm$ 16.7 mL/L at 5 and 15 min, respectively. EC_{50} of benzene was reported to be 2 mL/L by Bulich et al 1981; Gupta and Li (1993) reported an EC_{50} of 7.0 mL/L for a mixture of 0.8 mL gasoline (which contains benzene, ethylbenzene, toluene, xylenes and many other hydrocarbons) in 100 mL H_2O. Phenol and $NiCl_2.6\ H_2O$ have an EC_{50} of 26 and 251 mg/L, respectively at 15 min (Liu and Dutka 1984). The toxicity of many other gasoline hydrocarbons (benzene, toluene, xylenes) is higher (Gupta and Li 1993) than the toxicity of MTBE.

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