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Bioassay of methyl tertiary-butyl ether (MTBE) toxicity on rainbow trout fish

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Abstract

Methyl tertiary-butyl ether (MTBE) has been widely used as a gasoline additive. Water resource contamination due to spillage and accidental leakage of gasoline during fuel transportation may cause an important threat to aquatic life. In this work, the bioassay of MTBE toxicity on rainbow trout was performed. MTBE solutions of 250, 500, 750, 1000, and 1250 mg L^{-1} were prepared in five aquariums and aerated for 96 h. LC_{50} values of each experiment were measured according to probit analysis. A mathematical relationship between time exposure and LC_{50} of MTBE for rainbow trout was developed. The relative toxicity of MTBE to rainbow trout and to other organisms was also reviewed. It was shown that the toxicity of MTBE does not change significantly in the time exposure between 24 and 72 h varying from 878 to 831 mg L^{-1}, respectively. After 72 h of rainbow trout exposure to MTBE, LC_{50} value gradually decreased and reached 773 mg L^{-1} in 96 h of contact time. In conclusion, the result of this work showed that the toxicity of MTBE to rainbow trout was relatively low.

Keywords: Methyl tertiary-butyl ether (MTBE); Rainbow trout; Relative toxicity; Water pollution; Fuel additive

1. Introduction

Methyl tertiary-butyl ether (MTBE) has been widely used as a gasoline additive all over the world. It is commonly added to fuel to reduce the emission of carbon monoxide by converting carbon monoxide to carbon dioxide [1]. MTBE was originally added to gasoline on 1979 as a replacement for lead to help engines burn cleaner and became more widely used in the 1990s with passage of the revised Clean Air Act [2]. Although the use of this additive was banned in some developed countries, it is extensively used in developing countries as a substitute for tetraethyl lead. Transportation of fuel would be considered as a concern of water resource contamination due to spillage and accidental leakage of gasoline.

In fact, properties such as high water solubility, low adsorption to granulated activated carbon, low Henry's constant, and slow biodegradability that make MTBE an ideal fuel oxygenate cause it more difficult to remove in water. Therefore, the toxicity of MTBE to aquatic life could be a matter of concern, especially in countries which are shifted from lead additive to MTBE as a gasoline additive. Toxicity of MTBE to some freshwater organisms has been studied earlier. LC50 (96 h) values of MTBE for invertebrates such as *Daphnia magna* and *Neomysis mercedis* were measured as 542 and 236 mg L^{-1}, respectively [3]. Toxicity of MTBE to *Mysidopsis bahia* was even higher, since the related LC_{50} (96 h) of MTBE was reported as high as 136 mg L^{-1} [4]. The LC_{50} (96 h) values of MTBE for fishes such as *Pimephales promelas* and *Menidia beryllina* were measured as 929 and 574 mg L^{-1}, respectively [4]. Geiger et al. [5] reported the LC_{50} (96 h) of MTBE for *P. promelas* as 672 mg L^{-1}. In another work, LC_{50} (96 h) of MTBE for *P. promelas* was measured as high as 706 mg L^{-1} [6]. Recently, even toxicity of MTBE in trees was investigated [7]. However, further toxicity tests should be performed to indicate the model of MTBE toxicity on aquatic vertebrates.

The rainbow trout (*Oncorhynchus mykiss*) is a species of salmonid native to tributaries of the Pacific Ocean in Asia and North America as well as much of the central, western, eastern, and especially the northern portions of the United States. Rainbow trout survives in water from 3 °C in the winter to 21 °C in the summer, but the optimum temperature is between 10 and 16 °C [8]. As rainbow trout is very sensitive to pollutants, it has been considered as a candidate for bioassay tests in many studies.

The aims of the present work were to (1) investigate the toxicity responses obtained from the bioassay using rainbow trout (*O. mykiss*), (2) present the mathematical relationship between time

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exposure and LC_{50} of MTBE for rainbow trout, and (3) compare the toxicity of MTBE to rainbow trout to its toxicity to other organisms, which have been reported in previous publications.

2. Materials and methods

2.1. MTBE solutions

The bioassay was performed using five concentrations in separate 20-L aquariums. MTBE solutions of 250, 500, 750, 1000, and 1250 mg L^{-1} were prepared by diluting the pure MTBE (>99% pure), which was produced by Merk Company with the Order No. of 8.18109.1000. The density of the mentioned commercial MTBE was 740 g L^{-1}.

2.2. Aquarium setup

Six separate aquariums were made to conduct the bioassay test. The aquariums were made up of glass and the length, width, and height of each module were 35, 30, and 30 cm, respectively. Five aquariums were used for five mentioned concentrations in this work and one aquarium was considered as control in which MTBE was not added. The water depth in each aquarium was adjusted to 20 cm. As the optimum temperature for survival and growth of rainbow trout was about 12–19 °C, all aquariums were manipulated in a container which was filled with water and also connected to the cooling system. The outer sides of the aquariums were kept cool by recirculating the cold water from the refrigerator to the container of aquarium. Each unit was equipped with diffused aeration to supply the oxygen requirement of at least 6 mg L^{-1}. Aquariums were sealed prior to the fish loading and addition of MTBE.

2.3. Fish adaptation stage

Rainbow trout fish, scientifically named *O. mykiss*, were transferred from a fish farming unit to the adaptation plastic container in the laboratory. The water content of adaptation container was 80 L and was kept cool using indirect contact with ice. In the adaptation stage of the work, fish were maintained in the adaptation container a week before loading to the aquariums. Fish were fed every other day during the adaptation stage and the feeding was discontinued 24 h before transferring to the test aquarium. The water quality parameters of dilution water are presented in Table 1. Dilution water was made up of tap water, which was dechlorinated by sodium thiosulfate and passed through a column of activated carbon. In the adaptation stage, fish were accustomed to the water quality parameters and laboratory conditions. Environmental conditions such as temperature, dissolved oxygen, and pH were monitored every day. If the mortality rate of fish in the adaptation stage was more than 5%, the fish were not transferred to the test aquariums.

2.4. Bioassay test procedure

The MTBE solutions of 250, 500, 750, 1000, and 1250 mg L^{-1} were prepared in five aquariums and aerated for 96 h. The concentration of MTBE was determined at 2, 4, 6, 8, 24, 48, 72, and 96 h after aeration. Since MTBE is volatile, the concentrations of MTBE in each aquarium were supposed to be decreased due to air stripping during the test. According to the rates of MTBE loss due to aeration in aquariums, the required MTBE makeup rates were calculated and continuously injected through a makeup solution plastic bag to the aquariums in order to maintain the MTBE levels constant.

After preparing the solutions in aquariums, 10 rainbow trout fish were transferred to each aquarium and the numbers of dead fish and environmental conditions such as temperature, dissolved oxygen, and pH were recorded at 2, 4, 6, 8, 24, 48, 72, and 96 h during the bioassay test. The average weight and length of fish were 2 g and 5 cm, respectively. The water temperature varied in the range of 17 ± 2 °C and the dissolved oxygen level was 7 ± 1 mg L^{-1}. The tests were performed in a dark condition for about 10 h/d.

Table 1
Water parameter data which were used in this work for filling the aquariums

Parameters	Quantity	Method
pH	8	pH meter
Dissolved oxygen (mg L^{-1})	6.8	DO meter
Conductivity (μmho cm^{-1})	450	Conductivity meter
Total hardness as $CaCO_3$ (mg L^{-1})	168.3	EDTA titration
Non-carbonate hardness as $CaCO_3$ (mg L^{-1})	23.76	EDTA titration
Carbonate hardness as $CaCO_3$ (mg L^{-1})	144.54	Calculation
Total alkalinity as $CaCO_3$ (mg L^{-1})	118	Acid titration
P-alkalinity as $CaCO_3$ (mg L^{-1})	8	Acid titration
MO alkalinity as $CaCO_3$ (mg L^{-1})	110	Acid titration
SO_4^{2-} (mg L^{-1})	48	Turbidimetry
Cl^- (mg L^{-1})	23.49	Argentometry
Ca^{2+} (mg L^{-1})	58.4	EDTA titration
Mg^{2+} (mg L^{-1})	5.8	Calculation
Na^+ (mg L^{-1})	44	Flam photometry
K^+ (mg L^{-1})	8	Flam photometry
Total kejeldal nitrogen (mg L^{-1})	0	Kejeldal nitrogen

3. Results and discussion

All responses in bioassay on rainbow trout fish exposed to MTBE are presented in Table 2. These data were used as the essential variables, which were needed to perform the probit analysis. According to the presented data in Table 2, the probit analysis was performed and the results are presented in Table 3, which shows the LC_{50} values of MTBE in different time exposure and related 95% confidence intervals.

As shown in Fig. 1, a polynomial relationship can be considered as the best fit to explain the mathematical relationship between the time exposure and LC_{50} values of MTBE. The multiple regression coefficient of the following best fit model, which was obtained in this work, appeared to be 0.9931:

$$y = -19.399x^3 + 197.74x^2 - 666.73x + 1585.8$$

In this equation, y represents the LC_{50} values of MTBE as mg L^{-1}, and x stands for time exposure of rainbow trout to the pertinent MTBE concentration. As illustrated in Fig. 1, the toxi-

Table 2
Results of bioassay on rainbow trout fish exposed to MTBE

Average MTBE concentrations ($mg\,L^{-1}$)	No. of rainbow trout fish	No. of dead fish during the experiment (h)							
		2	4	6	8	24	48	72	96
1182.4	10	5	7	7	7	10	10	10	10
1011.0	10	0	0	3	3	10	10	10	10
794.4	10	0	0	0	0	1	2	3	4
519.9	10	0	0	0	0	0	0	0	1
242.5	10	0	0	0	0	0	0	0	0

Table 3
LC_{50} values for each time exposure according to the probit analysis

Parameters	Time of exposure (h)				
	6	24	48	72	96
Lower limit 95% confidence level	1007	808	792	719	667
LC_{50}	1100	878	856	831	773
Upper limit 95% confidence level	1237	977	997	927	873

city of MTBE does not change significantly in the time exposure between 24 and 72 h varying from 878 to 831 $mg\,L^{-1}$, respectively. After 72 h of rainbow trout exposure to MTBE, LC_{50} value gradually decreased and reached to 773 $mg\,L^{-1}$ in 96 h of contact time. A three-dimensional model was also prepared from the outputs of probit analysis and is depicted in Fig. 2. This model also confirms the different features of MTBE toxicity beyond the exposure time of 24–72 h. The MTBE toxicity on invertebrates has been studied and reported in many publications. The results of these studies are compared to the findings of the present work. In 1998, the measured LC_{50} (24 h) for *Brachionus calyciflorus* was reported as 960 $mg\,L^{-1}$ by UC Davis Aquatic Toxicology Laboratory [3]. In this work, the LC_{50} (24 h) for rainbow trout measured as 878 $mg\,L^{-1}$. In Fig. 3, toxicity values from the current work are compared to data published by other workers for MTBE in studies with different organisms.

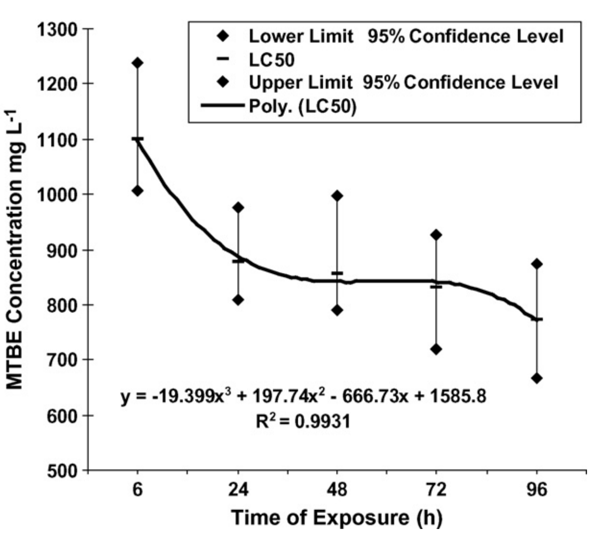

Fig. 1. Variations of LC_{50} values versus time exposure.

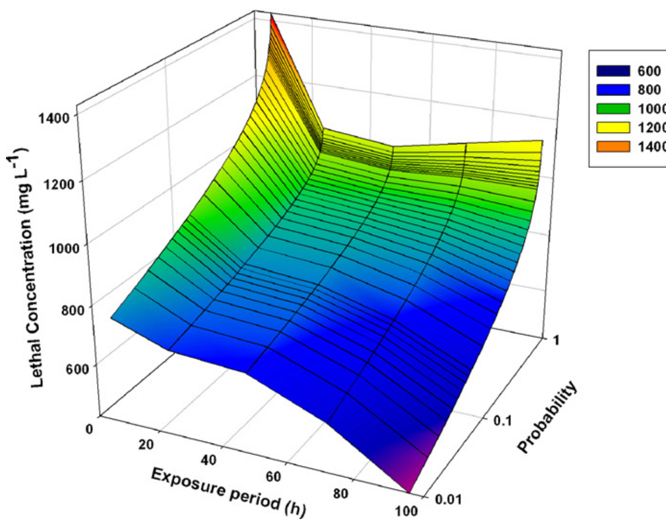

Fig. 2. Three-dimensional model according to the outputs of probit analysis.

As shown in Fig. 3, the LC_{50} (96 h) for rainbow trout is higher than those for all invertebrates. Relative toxicity of MTBE to any organism could be defined as the ratio of LC_{50} of MTBE for rainbow trout to the LC_{50} value for other organism. As shown in Fig. 4, the relative toxicity of MTBE to all vertebrates are higher than one. Even in the fish category, the relative toxicity of MTBE to *P. promelas*, *P. promelas*, and *M. beryllina* appears to be more than one, which means that the toxic effect of MTBE to them are higher than the toxicity to rainbow trout. However, it should be noted that the relative toxicity of MTBE to different fish does not appear to be significant.

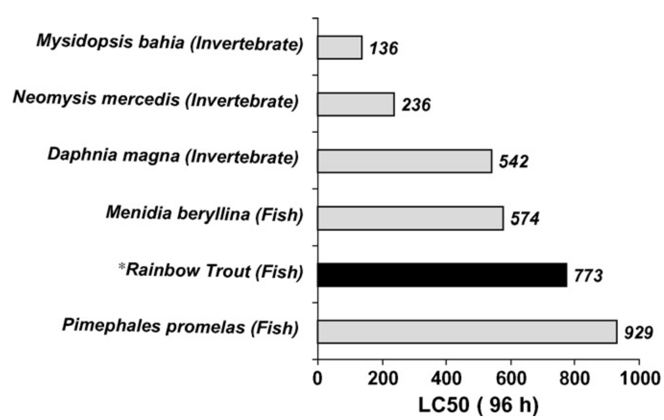

Fig. 3. Comparison the LC_{50} (96 h) values of previous studies to LC_{50} (96 h) for rainbow trout (* rainbow trout in present work).

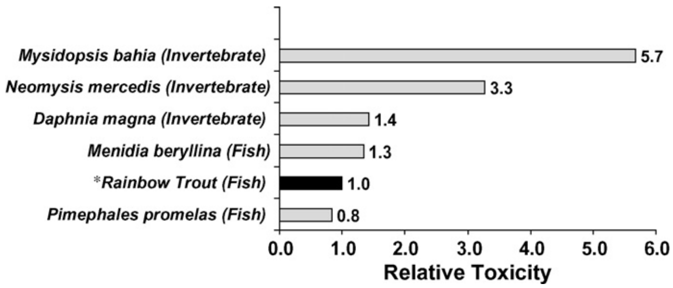

Fig. 4. Relative toxicity of MTBE on different organisms (in comparison to the MTBE toxicity in this work).

4. Conclusions

In conclusion, the result of this work showed that the toxicity of MTBE to rainbow trout was relatively low. The LC_{50} values which were obtained in this research and those were investigated in other toxicity studies could be considered as tools to describe the acute toxicity effects of MTBE in accidental leakage, drainage, or spillage in water bodies. It was also concluded that the toxicity of MTBE does not change significantly in the time exposure between 24 and 72 h varying from 878 to 831 mg L^{-1}, respectively.

Acknowledgment

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