Survey of Methyl Tertiary Butyl Ether (MTBE) toxicity using bioassay on *Daphnia magna*

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In this study, the toxicity of MTBE on the crustacean *Daphnia magna* (Cladocera) has been studied. At the beginning, for finding the range of sensitivity of *D. magna* to MTBE, a test had been conducted at 24h. The initial and final tests with synthetic MTBE has been done with containing solutions of water accommodated fractions (WAFs) in periods of 24 and 48h and then static bioassay was calculated in two phases of tests and the data were obtained and analyzed by SPSS 13 by using probit analysis. In 24h, lc_{10}, lc_{50} and lc_{90} for MTBE on *D. magna* were 345, 646 and 941mg/l and for 48h, they were 361, 476 and 893mg/l, respectively. Also, the maximum allowable concentration (MAC) of MTBE (WAFs) on *D. magna* was determined in 24 and 48h, 64.6 and 47.6mg/l, respectively. Results indicated that *D. magna* could be used for toxicity tracing MTBE in surface and ground water, because these Crustaceans have perfect resistance against pollution of MTBE. It is also concluded that after 24 and 48h exposure time, there was no significant difference observed in the activity and mortality of *D. magna*.

Since Methyl Tertiary Butyl Ether (MTBE) was used widespread as a fuel oxygenated in gasoline, it commonly contaminates ground water, drinking water, coastal waters, and other aquatic bodies (Werner *et al*., 2001). Ecotoxicologic studies of MTBE were mainly performed in freshwater ecosystem and reported the concentration of acute toxicity, ranging from 136 to 1000mg/L which varied according to organism species. Therefore, the toxicological studies mixture of chemicals is growing (Hernondo *et al*., 2002). The main goal of this study was to evaluate toxic effects of MTBE using Crustacean *(D. magna*). The second goal of this research included assigning of (MAC) of MTBE and 10% concentration of this material in water.

Static bioassay was calculated in two phases of examination and the data obtained were analyzed by SPSS 13 using probit analysis.

In this stage, the toxicity test has been done on *D. magna* using water accommodated fractions prepared in the laboratory in 24 and 48h. The results showed that in 24h, mortality of *D. magna* begun with concentration of 310mg/L and in 48h mortality ended with concentration of 30mg/L and 497mg/L. The results indicated in which concentrations the mortality was zero and in which concentrations mortality was 100 percent. At the end, by entering these concentrations into SPSS software, data were analyses in probit model. The analyzation of data was preformed by using ANOVA and effect values of MTBE on *D. magna* with use of variance ratio (F), chi-square (x^2) and values of regression coefficient (Tables 1 to 4).

Data on toxicity of MTBE suggest that this compound has a low toxicity towards aquatic organisms. Acceptable data on the toxicity of MTBE to freshwater organisms were available for three species of fish, six species of aquatic invertebrates, and three species of plants. For example at concentrations of 388-2600mg/l, MTBE is toxic to vertebrates (Rousch & Sommerfeld, 1998). Acute toxicity values (96h LC_{50}) for the rainbow trout (*Oncorhynchus mykiss*) (Hocket, 1997) and bluegill sunfish (*Lepomis macrochirus*) ranged from 887mg/l to 1054mg/l (Hocket, 1999). The 96h LC_{50} estimate for juvenile fathead minnows (*Pimephales promelas*) was 980mg/l. Also the toxicity value of MTBE on trout fish had been studied and the obtained results showed that 96h Lc_{50} value for this fish was 752mg/l (Beigy, 2006). On the other hand, MTBE in water and soil of gasoil which pumps around Tehran had also been studied by Ardalani (2003).

Acute toxicity values for invertebrates, ranged from 340mg/l (48h LC_{50}) for *Ceriodaphnia dabia* (Hocket, 1997) to 1742mg/l (48h E50 survival) for *Chironomus tentans* (Hocket, 1999). Depending on the time of exposure, MTBE is a toxic to invertebrates at a concentration of 57 to >1000mg/l (Werner *et al*., 2001). EC_{50} values of 7.4-41.8mg/l have been reported for acute toxicity of MTBE towards bacteria such as *Salmonella tyhimurium* and *Photobacterium phosphoreum* (Kado *et al*., 1998). In microalgae, MTBE is toxic at the concentration of 184-4800mg/l, when tested with species such as *Selenastrum capricornutum* and *Synechococus leopoliensis* (Werner *et al*., 2001). MTBE's toxicity on *D. magna* (EC_{50}=720mg/l) is content with a reported 96h EC30 measurement of 681mg/l (Hernando *et al*., 2002). Other researchers have claimed that MTBE is not toxic to *D. magna* at the concentration of 1-1000mg/l in exposures up to 48h (Gupta & Lin, 1995). These results, obtained with the static mode of the *D. magna* assay are explicable by volatilization of MTBE. Volatilization can greatly reduce the concentration of MTBE seen by the test organisms over duration of the assay (Hernando *et al*., 2002). In conclusion, MTBE alone is toxic to the tested species at concentrations that are significantly greater than the levels of pollutants

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commonly detected in the environment (Hernondo *et al*., 2002).

In this research, we used standard Checkousluaky (No. 46 6807) (Asobeda & Ukuosa, 1995) (Table 9) in limitation between 100 to 1000 which showed MTBE had low toxicity on *D. magna* and also MTBE did not have significant effects on the organism's mortality. These crustaceans have perfect resistance against pollution of MTBE and the results indicated that *D. magna* could be used for toxicity tracing MTBE in surface and ground water. Therefore, MTBE that was determined after 48h exposure (according to standard of Checkousluaky) had a low toxicity.

On the whole, the animals are sensitive to pollution in all seasons and this depends on metabolic activities and physiological manners of them. So, different species have different sensitivities regarding water accommodated fractions (WAFs) (Shahlapour, 1992). Therefore, MTBE and concentration of pollution in certain times of the year may cause mortality for a species of animals and does not have any effect on the others. Generally circular forms forms Shows more toxicity to chain forms (Piri and Falahi, 1998). The other case is toxicity of some refined since we can't generalize the results yield of finding with the MTBE to other oil products at all.

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Table 1: ANOVA for data of logarithmic (11) in 24h

Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	14,826	1	14,826	1,793	,189
Residual	281,174	34	8,270	-	-
Total	296,000	35	-	-	-

Table 2: ANOVA for data five treat in 48h

Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	1,342	1	1,342	,351	,562
Residual	61,158	16	3,822	-	-
Total	62,500	17	-	-	-

Table 3: Coefficient for data of logarithmic (11) in 24h

Model	Unstandardized Coefficients		Standardized Coefficients	T	sig
	-		-		
-	B	Std. Error	Beta	-	-
Case Sequence	,062	,046	,224	1,339	,189
(Constant)	,190	,979	-	,195	,847

Table 4: Coefficients for data of five treat in 48h

Model	Unstandardized Coefficients		Standardized Coefficients	T	Sig.
-	B	Std. Error	Beta	-	-
Case Sequence	,053	,089	,147	,593	,562
(Constant)	,667	,961	-	,693	,498

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