

# Toxicity of MTBE to Freshwater Organisms

Inge Werner and David E. Hinton  
School of Veterinary Medicine  
Dept. of Anatomy, Physiology and Cell Biology  
UC Systemwide Toxic Substances Research & Teaching Program  
- Lead Campus Program in Ecotoxicology -  
University of California  
Davis, CA 95616.

## Background

Increased input of MTBE into aquatic systems has led to concerns about its effect(s) on aquatic life. Depending on time of exposure and endpoint measured, the compound is acutely toxic to various aquatic organisms at concentrations of 44 mg/L to >1000 mg/L in invertebrates (Table 1; Bengtsson and Tarkpea, 1983; Gupta and Lin, 1995; BenKinney et al., 1994; Hockett and Russell, 1997; Boeri et al., 1994a), and 388 mg/L to >3000 mg/L in vertebrates (BenKinney et al., 1997; Geiger et al., 1981; Veith et al., 1983; Bengtsson and Tarkpea, 1983; Hockett and Russell, 1997; Paulov, 1987). Bacterial assays were most sensitive with toxicity to *Salmonella typhimurium* measured at 7.4 mg/L within 48 hours (Kado et al., 1997). In microalgae, decrease in growth was observed at 2,400 mg/L and 4,800 mg/L within 5 days (Rausch and Sommerfeld, 1998). MTBE does not appear to bioconcentrate in fish and is rapidly excreted or metabolized (Fujiwara et al., 1984).

The US EPA 3 species test (US EPA, 1994) employs standardized protocols to measure toxicity to selected species representing three trophic levels: a primary producer, the green algae *Selenastrum capricornutum*, a primary consumer, the water flea *Ceriodaphnia dubia*, and a secondary consumer, the fathead minnow *Pimephales promelas*. When tests result in a significant reduction in growth, survival, or reproduction of the test organisms, the potential for negative ecological impacts can be inferred.

In order to gain further insight into potentially deleterious effects of MTBE in California's surface waters, toxicity tests were conducted at the UC Davis Aquatic Toxicology Laboratory with two organisms indigenous to the Sacramento/San Joaquin Delta and the Sacramento River watershed, the mysid shrimp *Neomysis mercedis* and the rotifer *Brachionus calyciflorus*. Both these species are considered members of the plankton (free-floating) community indigenous to the Sacramento/San Joaquin Delta and the Sacramento River watershed. Additionally, to detect potential developmental effects of MTBE in fish, the UC Davis Aquatic Toxicology Laboratory performed a series of tests using embryos of the teleost fish medaka, *Oryzias latipes*. In addition, water samples from a related project (Dr. T. Young) were tested by the EPA 3 species test.

This study was done to evaluate toxicity of MTBE breakdown products potentially accumulating during common water treatment procedures.

## **METHODS**

### **US EPA 3 Species Test**

*Ceriodaphnia dubia* were from an in-house culture maintained at the Aquatic Toxicology Laboratory, UC Davis. *Selenastrum capricornutum* were obtained from the University of Texas Starr collection (#1648), and *Pimephales promelas* were purchased from Aquatox in Hot Springs, Arkansas, USA. Upon arrival, the fish were acclimated to laboratory control water for 6 hours before use in a test.

Prior to the bioassays, water samples were mixed rigorously in the original container, filtered through a 60  $\mu\text{m}$  screen (*C. dubia*, *P. promelas* only), warmed to 25°C and aerated at a rate of 100 bubbles/minute until the dissolved oxygen concentration was approx. 8.5 mg/l (103% saturation). For *S. capricornutum* tests, water was filtered through a Gelman-type A/E glass fiber filter and allowed to warm to 20°C.

Tests were initiated within 24 hrs of water collection. All organisms were maintained at 20/25 $\pm$ 1°C under a 16:8 light to dark regime (*C. dubia*, *P. promelas*) or continuous light (80  $\pm$  8  $\mu\text{E}/\text{m}^2/\text{s}$ ; *S. capricornutum*).

***C. dubia*:** Seven day screening bioassays followed guidelines given by US EPA (1994). Briefly, tests were set up using ten replicate containers of one 6-18 hr old neonate each. Toxicity endpoints were altered reproduction (number of offspring per female) and mortality. *C. dubia* were pipetted into fresh test water every 24 hours until termination of exposure (7 d). Animals were fed daily. When 100% mortality occurred within 24 hours, dilutions of the respective water sample were tested to estimate the toxic units present. A toxic unit is defined as the actual concentration of a specific chemical divided by the 96-hour LC50 for the species of interest.

***S. capricornutum*:** Four day screening bioassays followed guidelines given by US EPA (1994). To ensure that cells were in exponential growth, algae were transferred to the US EPA algal media 6 days prior to initiation of the test. Upon termination, cell counts were measured using a Coulter Counter (model ZM).

***P. promelas*:** Seven day screening bioassays followed guidelines given by USEPA (1994). Tests were initiated using 48 hr old larvae with toxicological endpoints being tissue growth, measured as average dry weight per surviving fish, and mortality.

**Controls:** Well water from UC Davis Ecology Institute, diluted with glass distilled water (total hardness:  $78.8 \pm 18.5$  mg/l (n=38)), served as laboratory control water for *C. dubia* and *P. promelas* bioassays. Glass distilled water was the control water for algal tests. Both control waters were made fresh for each series of samples tested.

### **Bioassays Using *Neomysis mercedis***

Animals were obtained from J. Brezina Co. (Dillon Beach, CA). For acclimation, organisms were placed in control water consisting of de-ionized EPA moderately hard (DIEPAMH) water amended to an electric conductivity of 2500  $\mu$ mhos with filtered seawater, at least eight hours prior to test initiation.

*Neomysis* bioassays consisted of 7-8 treatments of ten replicates with one animal per replicate. Test duration was 96 hours with daily renewal of 50% of the treatment water. Organisms were fed approximately 20 *Artemia* nauplii (24 hours old) each day. All tests were conducted at a temperature of 19°C.

Dilutions of MTBE or ethanol in control water were made before the start of the test and each day prior to test renewal in a 500 ml volumetric flask. All solutions were thoroughly mixed, placed into separate 600 ml beakers, covered with plastic wrap and placed in the exposure chamber to maintain them at 19°C. Then, 40 ml of each solution was poured into each of ten Pyrex beakers (vol.=50 ml). One animal was loaded into each test beaker using a 25 cm long glass pipette. Approximately 5 neomysids were drawn into the glass rod at a time and then dispersed into the test beakers one at a time. Finally, mysids were fed, and each beaker was covered with plastic wrap secured with a rubberband (closed system only) to limit the amount of volatilization of the chemical. Beakers were then placed into a 19°C waterbath. At test termination, mortality and any unusual physical or behavioral characteristics were recorded. Each mysid was placed in glycerol on a depression slide and its length measured from the telson to the caudal-most portion of the tail.

Chemical analysis was performed on the initial renewal water for the control and 3 of the MTBE treatments, as well as on the water for the control and the same three MTBE treatments at test termination (for methods see report of Dr. T. Young). All waters were submitted for chemical analysis to the laboratory of Dr. T. Young, Dept. of Environmental Engineering, UC Davis.

### **Bioassays Using *Brachionus Calyciflorus***

## *Toxicity of MTBE to Freshwater Organisms*

Live rotifer cultures were obtained from Aquatic Research Organisms, Hampton, NH. Organisms were maintained in soft spring water on a diet of equal parts of concentrated *Selenastrum capricornutum* and Rotirich™ (Aquatic Research Organisms, Hampton, NH). Rotifer bioassays were 24 hours in duration and consisted of 7 treatments of five replicates with five animals per replicate. Solutions of MTBE and ethanol were prepared using control water (Sierra Spring water).

Tests were conducted at 25°C in 3.7 ml clear plastic vials with caps to minimize volatilization. MTBE or ethanol solutions were prepared immediately prior to test initiation. Rotifers were placed into each container under a dissecting microscope using a red backlight. Water quality parameters were measured in controls only. Mortality was recorded after 24 hours.

### **Developmental Effects of MTBE in Medaka (*Oryzias latipes*)**

Groups of 20 male and 20 female, actively breeding 8 month old medaka were placed in 4 liter beakers containing 3.5 liters of experimental solutions, which included a control and solutions of 10 µg/l, 100 µg/l, 1 mg/l, 100 mg/l and 700 mg/l MTBE (nominal). Measured concentrations were: 11.4 µg/l, 286.4 µg/l, 3.4 mg/l, 95.4 mg/l and 480.0 mg/l. The adult fish were exposed to MTBE over night. Eggs were oviposited the next morning and fertilization allowed to take place in the presence of MTBE. Later, resultant embryos were collected, and individual embryos were placed into glass vials containing MTBE solutions at the above concentrations (20 replicates per treatment). They were observed daily for mortality and developmental defects until hatching. After hatching, larvae were transferred to clean reconstituted water in 1 liter beakers, fed and maintained for 1 week to determine potential delayed effects.

## **RESULTS**

### **Toxicity of MTBE to US EPA 3 Species Test Organisms**

Presented here are results from a literature search on toxicity test results for MTBE with the three species included in the EPA 3 Species Test.

*Selenastrum capricornutum*: In 5 day tests, an increase in growth was measured at a nominal concentration of 600 mg/L. Algal growth decreased at 4,800 mg/L (Rousch and Sommerfeld, 1998). BenKinney et al. (1994) found a 96 hour effect concentration (not further specified) of 184 mg/L (measured concentration).

*Ceriodaphnia dubia*: The 5 day 'lowest observed effect concentration' (LOEC) for mortality was measured at 580 mg/L, with a 'no observed effect concentration' (NOEC)

## *Toxicity of MTBE to Freshwater Organisms*

at 342 mg/L. The LOEC for reproductive effects was 342 mg/L, with a NOEC of 202 mg/L (ENSR, 1997).

*Pimephales promelas*: The 96 hour LC50 (concentration at which 50% of test organisms died) was determined to be 672 mg/L (Geiger et al., 1981) and 706 mg/L (Veith et al., 1983). BenKinney et al. (1994) reported a 96 hour LC50 of 929 mg/L (measured concentration). Growth was affected after 7 days at 388 mg/L (LOEC), with a NOEC at 234 mg/L (ENSR, 1997).

### **Toxicity of MTBE to California Resident Organisms**

*Neomysis mercedis*: Tests using *N. mercedis* involved 'open' and 'closed' system test designs. The 96 hour LC50 in the 'open system' was determined to be 663-767 mg/l (measured concentration; Table 2 a, b). The measured 96 hour LC50 in the 'closed system' was 236 mg/l (Table 2d).

*Brachionus calyciflorus*: The measured 24 hour LC50 for MTBE was 960 mg/l (nominal: 1,410 mg/l; Table 3a).

### **Toxicity of Ethanol to US EPA 3 Species Test Organisms**

A literature search was conducted on toxicity test results for ethanol in *C. dubia* and *P. promelas* (AQUIRE data base, 1998). Standard bioassays were conducted at the UC Davis Aquatic Toxicology Laboratory with *S. capricornutum* using algal cell growth as an endpoint. Although LC50 values were more than an order of magnitude higher for ethanol, ethanol affected *C. dubia* reproduction at lower concentrations than did MTBE.

*Selenastrum capricornutum*: Ethanol concentrations of up to 16,000 mg/l did not cause a measurable effect on algal growth. At concentrations above 16,000 mg/l increased bacterial growth led to depletion of micronutrients, and did not allow conclusions about ethanol toxicity.

*Ceriodaphnia dubia*: The 2 day and 10 day LC50s are 8,808 mg/L and 1,806 mg/L, respectively. Reproductive success measured as the number of neonates produced within 7 days is reduced at 26 to 33 mg/L.

*Pimephales promelas*: The 4 day LC50 is 13,480-14,200 mg/L, whereas the 1 day LC50 is >18,000 mg/L.

### **Toxicity of Ethanol to California Resident Organisms**

## *Toxicity of MTBE to Freshwater Organisms*

*Neomysis mercedis*: The nominal 96 hour LC50 was determined to be 11,397 mg/l in the 'open system' (Table 2a). For the 'closed system', the 96 hour LC50 was 7,465 mg/l (Table 2c).

*Brachionus calyciflorus*: The 24 hour LC50 for ethanol was 7,090 mg/l (Table 3b).

### **Toxicity of MTBE Breakdown Byproducts to US EPA 3 Species Test Organisms**

**(for experimental design, please refer to report of Dr. Tom Young)**

Toxicity was assayed in MTBE containing water samples treated with UV/peroxide. With the US EPA 3 species test (US EPA, 1994), growth of *S. capricornutum* was significantly reduced (Table 4a), and 100% mortality occurred within 24 hours in *C. dubia* (Table 4b). A dilution series of the toxic water sample was tested using *C. dubia*, indicating that between 8 and 16 toxic units were present, i.e. the concentration of the compound causing the toxicity was 8 to 16 times higher than its 96-hour LC50 concentration (Table 4c). Growth and survival of *P. promelas* were not affected (Table 4d).

Principal residue compounds detected in the treated water were hydrogen peroxide and tert butyl formate. No information is presently available on the toxicity of hydrogen peroxide or tert butyl formate for the bioassay organisms.

### **Developmental Effects in Japanese Medaka (*Oryzias latipes*)**

No statistically significant developmental defects were observed at any MTBE concentration. There was no delay in hatching due to MTBE exposure, nor was there an effect on fry viability.

## **CONCLUSIONS**

Collectively, the available bioassay data suggests that at the commonly observed environmental MTBE exposure levels found in surface waters (no detect to a high of 100 parts per billion) MTBE should not be toxic to aquatic life (Table 1). In addition, negative findings in the developmental test of medaka, *Oryzias latipes*, over a range of concentrations and including exposures during water hardening of fertilized eggs, indicate that developmental toxicity is not likely to occur at environmental MTBE exposure levels.

Although LC50 values were more than an order of magnitude higher for ethanol, ethanol affects *C. dubia* reproduction at lower concentrations than MTBE. However, as

## *Toxicity of MTBE to Freshwater Organisms*

concluded for MTBE, these levels are far in excess of any concentrations that might be anticipated to occur environmentally.

Results from tests on 'treated' water containing MTBE indicate that the UV/peroxide treatment of MTBE containing water may be associated with greater toxicity to aquatic organisms (algae, *S. capricornutum*, and water flea, *C. dubia*) than that seen by MTBE itself.

## References

AQUIRE. 1998. Aquatic Information Retrieval. Office of Toxic Substances of the US EPA, Washinton, D.C.

Bengtsson B. E. and M. Tarkpea. 1983. The Acute Aquatic Toxicity of Some Substances Carried by Ships, *Marine Pollution Bulletin*, Vol. 14, No. 6, pp. 213-214.

BenKinney M.T., J.F. Barbieri, J.S. Gross, and P.A. Naro. 1994. Acute Toxicity of Methyl-Tertiary-Butyl Ether to Aquatic Organisms, Stonybrook Laboratories Inc., Princeton, NJ; abstract, 15<sup>th</sup> Annual SETAC Meeting, 30 October-3 November 1994.

ENSR Fort Collins Environmental Toxicology Laboratory. 1997. Short-Term Sub-Chronic Toxicity of MTBE to the Fathead Minnow (*Pimephales promelas*) Under Static-Renewal Test Conditions. Final Report, for Atlantic Richfield Corporation, Study Number: 0480-378-005-001, Study Director: J. Russell Hockett.

ENSR Fort Collins Environmental Toxicology Laboratory. 1997. Short-Term Sub-Chronic Toxicity of MTBE to *Ceriodaphnia dubia* Under Static-Renewal Test Conditions. Final Report, for Atlantic Richfield Corporation (ARCO), Study Number: 0480-378-004-001, Study Director: J. Russell Hockett.

Fujiwara, Y. et al. 1984. Biodegradation And Bioconcentration Of Aldyl Ethers. *Yukayaken*, Vol.33:111-114, translated for EPA by Scitran.

Geiger D.L., D.J. Call and L.T. Brooke. 1981. Acute Toxicities of Organic Chemicals to Fathead Minnows (*Pimephales promelas*), Volume IV, , University of Wisconsin—Superior.

Gupta G. and Y.J. Lin. 1995. Toxicity of Methyl Tertiary Butyl Ether to *Daphnia magna* and *Photobacterium phosphoreum*, *Bulletin of Environmental Contamination and Toxicology*, Vol. 55, pp.618-620.

Kado N.Y., P.A. Kuzmicky, G. Loarca-Pina and M.M. Mumtaz. 1998. Genotoxicity Testing of MTBE in the Salmonella Microsuspension Assay and Mouse Bone Marrow Micronucleus Test. *Mutation Research*, Vol. 412:131-138.

Mancini, ARCO Chemical. 1997. Physicochemical and Ecotoxicological Properties of Gasoline Oxygenates. Abstract, SETAC 18th Annual Meeting, November 1997

Paulov S. 1987. Action of the Anti-Detonation Preparation Methyl tert-Butyl Ether on the Model Species *Rana temporaria*, , *Biologia*, Vol 42, pp.185-189.

*Toxicity of MTBE to Freshwater Organisms*

Rousch J.M. and M.R. Sommerfeld. 1998. Liquid-Gas Partitioning of the Gasoline Oxygenate MTBE under Laboratory Conditions and its Effect on the Growth of Selected Algae. *Archives of Environmental Contamination and Toxicology*, Vol. 34:6-11.

US EPA. 1994. US Environmental Protection Agency. 1994. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Report No. 600/4-91/002, 3rd edition, US EPA, Washington DC, USA.

Veith G.D., D.J. Call, and L.T. Brooke. 1983. Estimating the Acute Toxicity of Narcotic Industrial Chemicals to Fathead Minnows. *Aquatic Toxicology and Hazard Assessment: Sixth Symposium*. ASTM STP 802, American Society for Testing and Materials, Philadelphia, pp. 90-97.

*Toxicity of MTBE to Freshwater Organisms*

Table 1: Toxicity of MTBE in Aquatic Organisms

Species	Effect	Effect Concentration	Reference
BACTERIA:			
<i>Salmonella typhimurium</i>	reduction in number of cells (48 hours)	7.4 mg/l	Kado <i>et al.</i> , 1997
<i>Photobacterium phosphoreum</i>	reduced light emission (15 min.)	41.8 mg/l	Gupta and Lin, 1995
MICROALGAE:			
<i>Selenastrum capricornutum</i>	decrease in growth (5 days)	4,800 mg/l (nominal)	Rousch and Sommerfeld, 1998
<i>S. capricornutum</i>	EC50	184 mg/l (measured)	BenKinney <i>et al.</i> , 1994
<i>Navicula pelliculosa</i>	decrease in growth (5 days)	2,400 mg/L (nominal)	Rousch and Sommerfeld, 1998
<i>Synechococcus leopoliensis</i>	decrease in growth (3 days)	2,400 mg/l (nominal)	Rousch and Sommerfeld, 1998
INVERTEBRATES:			
<i>Brachionus calyciflorus</i>	LC50 (24 hrs)	960 mg/l (measured)	UC Davis Aquatic Toxicology Laboratory, 1998
<i>Nitocra spinipes</i>	LC50 (96 hrs)	>1000 mg/l	Bengtsson and Tarkpea, 1983
<i>Daphnia magna</i>	LC50 (96 hrs)	681 mg/l (measured)	BenKinney <i>et al.</i> , 1997
<i>D. magna</i>	LC50 (96 hrs)	542 mg/l	Hockett and Russell, 1997
<i>Ceriodaphnia dubia</i>	survival (5 days)	LOEC : 580 mg/l NOEC: 342 mg/l	ENSR, 1997
<i>C. dubia</i>	reproduction (5 days)	LOEC: 342 mg/l NOEC: 202 mg/l	ENSR, 1997
<i>Neomysis mercedis</i>	LC50 (96 hrs)	236 mg/l (measured)	UC Davis Aquatic Toxicology Laboratory, 1998
<i>Mysidopsis bahia</i>	LC50 (96 hrs)	136 mg/l (measured)	BenKinney <i>et al.</i> , 1997

*Toxicity of MTBE to Freshwater Organisms*

Table 1, continued

<i>M. bahia</i>	LC50	44 mg/l	Boeri <i>et al.</i> , 1994
AMPHIBIA:			
<i>Rana temporaria</i> (tadpoles)	LC50	2,500 mg/l	Paulov, 1987
FISH:			
<i>Pimephales promelas</i>	LC50 (96 hrs)	672 mg/l	Geiger <i>et al.</i> , 1981
“	LC50 (96 hrs)	706 mg/l	Veith <i>et al.</i> , 1983
“	reduced growth (7 days)	LOEC: 388 mg/l NOEC: 234 mg/l	ENSR, 1997
“	LC50 (96 hrs)	929 mg/l (measured)	BenKinney <i>et al.</i> , 1997
<i>Menidia beryllina</i>	LC50 (96 hrs)	574 mg/l (measured)	BenKinney <i>et al.</i> , 1997
<i>Alburnus alburnus</i>	LC50 (96 hrs)	>1000 mg/l	Bengtsson and Tarkpea, 1983
<i>Onchorhynchus mykiss</i>	LC50	887 mg/L	Hockett and Russell, 1997

*Toxicity of MTBE to Freshwater Organisms*

Table 2a. Toxicity of MTBE and ethanol to *Neomysis mercedis*  
(open system, 2 July, 1998)

MTBE		96 hr LC50	
Treatment	Mortality (%) x	nominal	1,260 mg/l
		measured	767 mg/l
Laboratory control	0		
0.67 g/l MTBE	0		
0.89 g/l MTBE	14		
1.11 g/l MTBE	0		
1.33 g/l MTBE	70(3)		
1.55 g/l MTBE	80(4)		
1.77 g/l MTBE	100(3)		
1.99 g/l MTBE	100(2)		

Ethanol		96 hr LC50	
Treatment	Mortality (%) x	nominal	11,397 mg/l
Laboratory control	0		
1.24 g/l ETOH	10		
2.48 g/l ETOH	0		
4.98 g/l ETOH	0		
9.95 g/l ETOH	60(4)		
19.9 g/l ETOH	100(1)		
39.8 g/l ETOH	100(1)		
79.6 g/l ETOH	100(1)		

Water Quality:

Parameter	Initial	Final
EC	2194	2196
pH	8.08	7.66
DO	9.1	8.3
Temp	19.6	21.2

*Toxicity of MTBE to Freshwater Organisms*

Table 2b. Toxicity of MTBE to *Neomysis mercedis*  
(open system, 25 June, 1998)

MTBE		96-hr LC50	
Treatment	Mortality (%) x	Nominal	1,385 mg/l
		Measured	663 mg/l
Laboratory control	0		
0.12 g/l MTBE	0		
0.23 g/l MTBE	0		
0.46 g/l MTBE	0		
0.92 g/l MTBE	0		
1.85 g/l MTBE	100(1)		
3.69 g/l MTBE	100(1)		

Water Quality:

Parameter	Initial	Final
EC	2518	2660
pH	7.98	7.87
DO	8.9	8.7
Temp	19.6	20.1

*Toxicity of MTBE to Freshwater Organisms*

Table 2c. Toxicity of MTBE and ethanol to *Neomysis mercedis*  
(closed system, 21 July, 1998)

MTBE		96-hr LC50	
Treatment	Mortality (%) x	Nominal	635 mg/l
Laboratory control	0		
0.05 g/l MTBE	0		
0.12 g/l MTBE	0		
0.23 g/l MTBE	0		
0.46 g/l MTBE	20		
0.92 g/l MTBE	100(1)		
1.85 g/l MTBE	100(1)		

ETOH		96-hr LC50	
Treatment	Mortality (%) x	Nominal	7,465 mg/l
Laboratory control	0		
0.16 g/l ETOH	0		
0.31 g/l ETOH	0		
0.62 g/l ETOH	0		
1.24 g/l ETOH	0		
2.49 g/l ETOH	0		
4.98 g/l ETOH	0		
9.95 g/l ETOH	100(2)		

Water Quality:

Parameter	Initial	Final
EC	2250	2770
pH	7.93	7.96
DO	8.6	9.4
Temp	20.1	18.9

*Toxicity of MTBE to Freshwater Organisms*

Table 2d. Toxicity of MTBE to *Neomysis mercedis*  
(closed system, 15 September, 1998)

MTBE	Mortality (%) x	96-hr LC50	
		Nominal	Measured
		375 mg/l	236 mg/l
Laboratory control	0		
0.05 g/l MTBE	10		
0.12 g/l MTBE	0		
0.23 g/l MTBE	0		
0.46 g/l MTBE	70		
0.92 g/l MTBE	100(1)		
1.85 g/l MTBE	100(1)		

Water Quality:

Parameter	Initial	Final
EC	2590	2650
pH	8.33	7.84
DO	8.7	8.6
Temp	20.7	18.4

*Toxicity of MTBE to Freshwater Organisms*

Table 3a. Toxicity of MTBE to *Brachionus calyciflorus*

Treatment	Mortality	
	x	se
Laboratory Control	4.0 <sup>P</sup>	4.0
0.46 g/l MTBE	4.0	4.0
0.92 g/l MTBE	28.0	8.0
1.85 g/l MTBE	80.0*	8.9
3.69 g/l MTBE	84.0*	4.0
7.39 g/l MTBE	100.0*	0.0
14.77 g/l MTBE	100.0*	0.0

P. The laboratory control met the criteria for

test acceptability.

\* indicate a significant increase in mortality

when compared to the laboratory control.

Mortality endpoints were analyzed with Dunnett's Test ( $p < .05$ ).

se = standard error

24-hr LC50:	1,410 mg/l (nominal) 960 mg/l (measured)
-------------	---

*Toxicity of MTBE to Freshwater Organisms*

Table 3b. Toxicity of ethanol to *Brachionus calyciflorus*

Treatment	Mortality (%)	
	x	se
Laboratory Control	0.0 <sup>P</sup>	0.0
1.99 g/l ETOH	8.0	4.9
3.98 g/l ETOH	24.0	14.7
7.96 g/l ETOH	52.0*	8.0
15.92 g/l ETOH	80.0*	11.0
31.84 g/l ETOH	100.0*	0.0
63.68 g/l ETOH	100.0*	0.0

P. The laboratory control met the criteria for test acceptability.

\* indicate a significant increase in mortality when compared to the laboratory control.

Mortality endpoints were analyzed with Dunnett's Test (p<.05).

24-hr LC50	7,090 mg/l (nominal)
------------	----------------------

Analyzed with TOXIS statistical program.

*Toxicity of MTBE to Freshwater Organisms*

Table 4a. Toxicity of UV/peroxide treated MTBE containing water to *S. capricornutum* (96 hour growth)

Treatment	Cell Count		% CV	Final pH at 96 hours
	x	se		
Laboratory Control	185.4 <sup>P</sup>	16.7	18.0	8.55
Treated sample	5.2*	0.7	24.8	8.37

P. The laboratory control met all EPA criteria for test acceptability. The coefficient of variation was 18.0% in this treatment.

\* indicate a significant reduction in growth compared to the laboratory control. Cell counts were analyzed using Dunnett's Test (p<.05).

Table 4b. Toxicity of UV/peroxide treated MTBE containing water to *C. dubia* (reproduction and mortality after 7 days)

Treatment	Reproduction <sup>1</sup> (neonates/adult)		Mortality <sup>1</sup> (%)	Final pH at 24 hours
	x	se		
Laboratory Control	24.4 <sup>P</sup>	1.6	0 <sup>P</sup>	8.39
Treated sample	*	*	100(1)*	8.32

P.The laboratory control met all EPA criteria for test acceptability. 100% of the daphnids had a third brood.

\* indicate a significant reduction in reproduction or increase in mortality relative to the laboratory control water.

The mortality endpoint was analyzed using Fisher's Exact Test.

*Toxicity of MTBE to Freshwater Organisms*

Table 4c: Toxicity of dilutions of UV/peroxide treated MTBE containing water to *C. dubia* (7 day test)

Treatment	Mortality <sup>1</sup> (%)	Final pH at 24 hours
Laboratory Control	5 <sup>P</sup>	8.34
12.5% Sample	100(2)*	8.34
6.25% Sample	0	8.35
3.13% Sample	5	8.34
1.57% Sample	5	8.35
0.78% Sample	0	8.34

P. The laboratory control met all EPA criteria for test acceptability.  
 \* indicate a significant reduction in reproduction or increase in mortality relative to the laboratory control water.  
 The mortality endpoint was analyzed using Fisher's Exact Test.

Table 4d: Toxicity of UV/peroxide treated MTBE containing water to *P. promelas* (7 day growth)

Treatment	Growth <sup>1</sup> (mg/indiv.)		Mortality		Final pH at 24 hours
	x	se	x	se	
Laboratory Control	0.415 <sup>P</sup>	0.008	0 <sup>P</sup>	0.0	8.35
Treated sample	0.414	0.019	0.0	0.0	7.42

P. The laboratory control met the criteria for test acceptability.  
 Mortality endpoints were analyzed with Dunnett's Test (p<.05).