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ScienceDirect
Journal of Environmental Sciences 21(2009) 514–519
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Sensitivity of green and blue-green algae to methyl *tert*-butyl ether

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Received 25 August 2008; revised 07 November 2008; accepted 10 December 2008

Abstract

The toxicity of methyl *tert*-butyl ether (MTBE) to *Chlorella ellipsoidea* and *Aphanizomenon flos-aquae* was tested and assessed for a 15-d incubation with concentrations of MTBE from high (2.00×10^4 mg/L) to low (2 mg/L). The results showed that the toxicity was low when the concentration of MTBE was in the range 1.00×10^4–2.00×10^4 mg/L (the greatest inhibition of growth-rate was 70%–71%, occurred during the day 1–5). Low concentrations (2–500 mg/L) stimulated algal growth up to the greatest effect of 85%–200% when the concentration of MTBE was 50–100 mg/L during day 3–5. The toxicity of MTBE (72–120 h EC_{50}) was 6.65×10^3–9.58×10^3 mg/L for *C. ellipsoidea* and that is 1.14×10^4–2.00×10^4 mg/L for *A. floc-aquae*. We found that the toxicity and ecological risk of MTBE for the algal community structure were low and the toxicity was influenced by the duration time of the test.

Key words: methyl *tert*-butyl ether; toxicity; green algae; ecological risk; cyanobacteria

DOI: 10.1016/S1001-0742(08)62301-3

Introduction

Methyl *tert*-butyl ether (MTBE), which is emitted into the atmosphere as an unburned hydrocarbon, has been added to gasoline since 1979. MTBE consumption has increased substantially and its concentration reached 0.088 mg/L in lakes in California, USA (Werner *et al*., 2001). Algae, essential components of the aquatic ecosystem, produce oxygen and organic substances on which most other life forms depend and provide food for other organisms, including fish and invertebrates (Ma and Chen, 2005; Wong, 2000). Chemical effects on algae can directly affect the structure and function of an ecosystem, resulting in oxygen depletion and primary productivity decrease (Ma *et al*., 2004). However, little is known about the toxicity of MTBE to algae (BenKinney *et al*., 1994; Borden *et al*., 2002; Hernando *et al*., 2003; Rousch and Sommerfeld, 1998). Rousch and Sommerfeld (1998) conducted a study on the effects of MTBE on three unicellular algal species, *Selenastrum capricornutum*, *Navicula pelliculosa* and *Synechococcus leopoliensis*. Their growth was measured as an increase in cell numbers after 3–5 d, and algae were exposed to 600–9600 mg/L MTBE solutions. They found that the growth of the three algae decreased significantly at a nominal concentration range 2400–9600 mg/L MTBE. Contrary to these findings, Benkinney *et al*. (1994) determined a 96-h EC_{50} of 184 mg/L for *S. capricornutum*. The large discrepancy between these two studies cannot be explained easily on the basis of the information given (Werner *et al*., 2001). In this study, we conducted a 15-d toxicity test to determine the toxicity of MTBE to algae, and we studied the toxicity of MTBE at concentrations from high to low to *Chlorella ellipsoidea* and *Aphanizomenon flos-aquae* during 15 d.

1 Materials and methods

1.1 Chemicals and tested organisms

MTBE (purity ⩾ 99.0%) was purchased from Merck Schuchardt OHG., Germany. *C. ellipsoidea* and *A. flos-aquae* were obtained from the Institute of Wuhan Hydrobiology, the Chinese Academy of Sciences.

1.2 Nutrient medium

The medium used for the algal growth inhibition test was prepared in accordance with the China State Environmental Protection Bureau Guidelines 201, using SE medium, which is composed of distilled water and the following chemical ingredients (mg/L): $NaNO_3$ 250, $K_2HPO_4{\cdot}3H_2O$ 75, $MgSO_4{\cdot}7H_2O$ 75, $CaCl_2{\cdot}2H_2O$ 25, KH_2PO_4 175, NaCl 25, Na_2CO_3 20, $Na_2SiO_3{\cdot}5H_2O$ 100, $FeCl_3{\cdot}6H_2O$ 5, and an A_5 solution (1 mL, composition of A_5 solution is H_3BO_3 2.86 g/L, $MnCl_2{\cdot}4H_2O$ 1.81 g/L, $ZnSO_4{\cdot}7H_2O$ 0.22 g/L, $CuSO_4{\cdot}5H_2O$ 0.079 g/L, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 0.39 g/L) and Fe-EDTA (1 mL, composition of Fe-EDTA is Na_2EDTA 10 g/L, $FeCl_3{\cdot}6H_2O$ 0.81 g/L). The culture medium was sterilized at 121°C, 1.05 kg/cm^2 for 30 min.

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1.3 Test methods

Algal cells were propagated in a 250-mL Erlenmeyer flask containing 100 mL of liquid SE medium and kept on a rotary shaker (100 r/min) at 22°C, and illuminated with cool-white fluorescent lights at a continuous light intensity of 450 μmol/(m^2·s). Portions (30 mL) of the medium containing algal cells (initial cell concentration OD_{680} = 0.008) were placed into sterile 50 mL Erlenmeyer flasks. A wide range of concentrations was examined earlier to find a suitable toxicity range for the substance tested, and similar concentrations were used in this study. Algae were grown in medium containing different concentrations of MTBE, incubated for 15 d on an orbital shaker (100 r/min) at a temperature of 22°C and a continuous light intensity of 450 μmol/(m^2·s). Cell counts were correlated with absorbance over time for every 24 h on a UV-2401PC spectrophotometer (Shimadzu, Japan). In this test, we choose the most suitable wavelength 680 nm to monitor culture growth. Tests with each concentration of MTBE were replicated three times. Appropriate control systems containing no MTBE were included in each experiment. Control and treated cultures grew under the same temperature, photoperiod and shaking conditions as the stock culture. In each experiment, percentage inhibition values relative to growth in control system were calculated using optical density. Chlorophyll-*a* (Chl-*a*) analysis was done after the filtration of 20 mL samples of medium 0.45 μm-pore size Whatman GF/C membranes (Whatman, England) and extraction with 90% acetone. The chl-*a* content was estimated by a trichromatic method. The dry weight of algae was determined with a digital balance after filtration through a 0.45-μm pore size membrane at 105°C for 8 h. Statistical analysis was conducted with Microsoft Excel 2003.

1.4 Statistical evaluation

The EC_{50} values were calculated using linear regression analysis of transformed MTBE concentrations as the natural logarithm data versus percentage inhibition. No observed effect concentration (NOEC) is the test concentration immediately below the lowest significant concentration. Weighted analysis of variance (ANOVA) was used, followed by a one-sided Dunnett's test using a 5% significance level to obtain the lowest observed effect concentration (LOEC). The NOEC was taken to be the test concentration immediately below the LOEC (Saker and Neilan, 2001). Statistical analysis was by linear regression analysis using SPSS version 11.0.

2 Results and discussion

2.1 UV-B induced DNA damage in *Arabidopsis thaliana*

2.1.1 Correlation between chl-*a* content or dry weight and the absorbance

The optical density at 680 nm (OD_{680}) of the growth medium of *C. ellipsoidea* and *A. flos-aquae* was measured with a spectrophotometer. There was a significant relationship between both dry weight and chl-*a* content with OD_{680} for the two algae. The linear regression equations are shown in Fig. 1 (all coefficients of correlation $R^2 > 0.98$, $p < 0.001$). The growth of algal and biomass were calculated by measurement of OD_{680}.

2.2 Toxicity of MTBE to two algae at various concentrations and time

The growth inhibition of MTBE to *C. ellipsoidea* and *A. flos-aquae* is shown in Figs. 2 and 3. When the concentration of MTBE was 1.00×10^4–2.00×10^4 mg/L (Figs. 2a and 3a), the maximum growth inhibition of 70% occurred on day 5 for the green alga, and there was no inhibitory effect during the day 10–15. For the blue-green alga, however, a maximum growth inhibition of 71% occurred at day 1, and then decreased gradually during day 2–14, until there was no inhibitory effect at day 15.

When the concentration of MTBE was 2.00×10^3–5.00×10^3 mg/L (Figs. 2b and 3b), maximum inhibition of 48% and 46% occurred on day 5 and day 2 for the green alga and the blue-green alga, respectively, and there was no effect on day 8–15 and day 14–15, respectively. However, there were slight negative inhibitory effects (i.e., stimulating algal growth) on day 10–15, and on day 12 (10%) for the green alga and on day 11 (19%) for the blue-

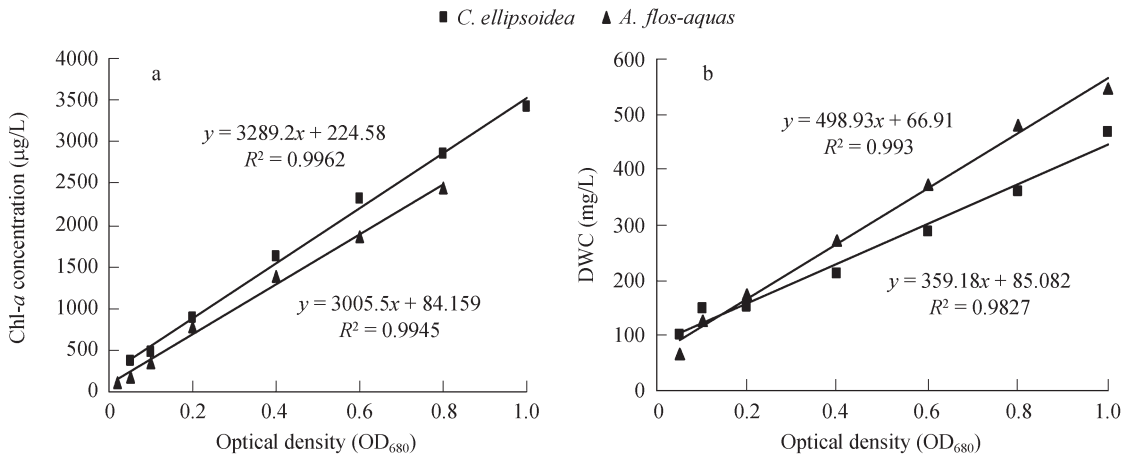

Fig. 1 Relationship between OD_{680} and chl-*a* (a), dry weight (b).

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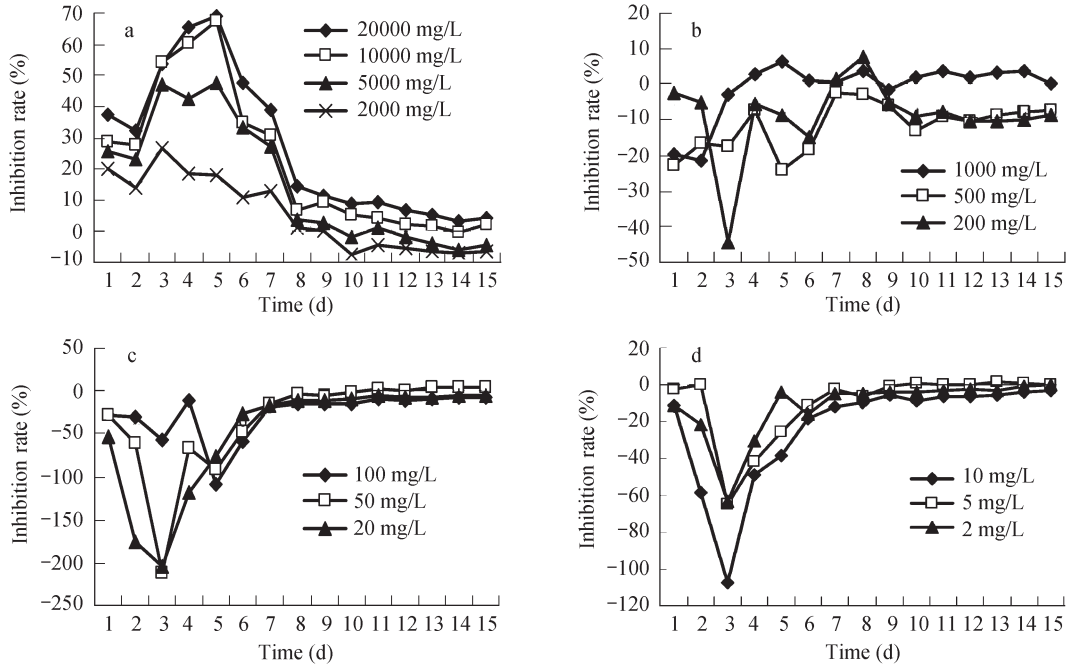

Fig. 2 Inhibition of *C. ellipsoidea* by different MTBE concentrations.

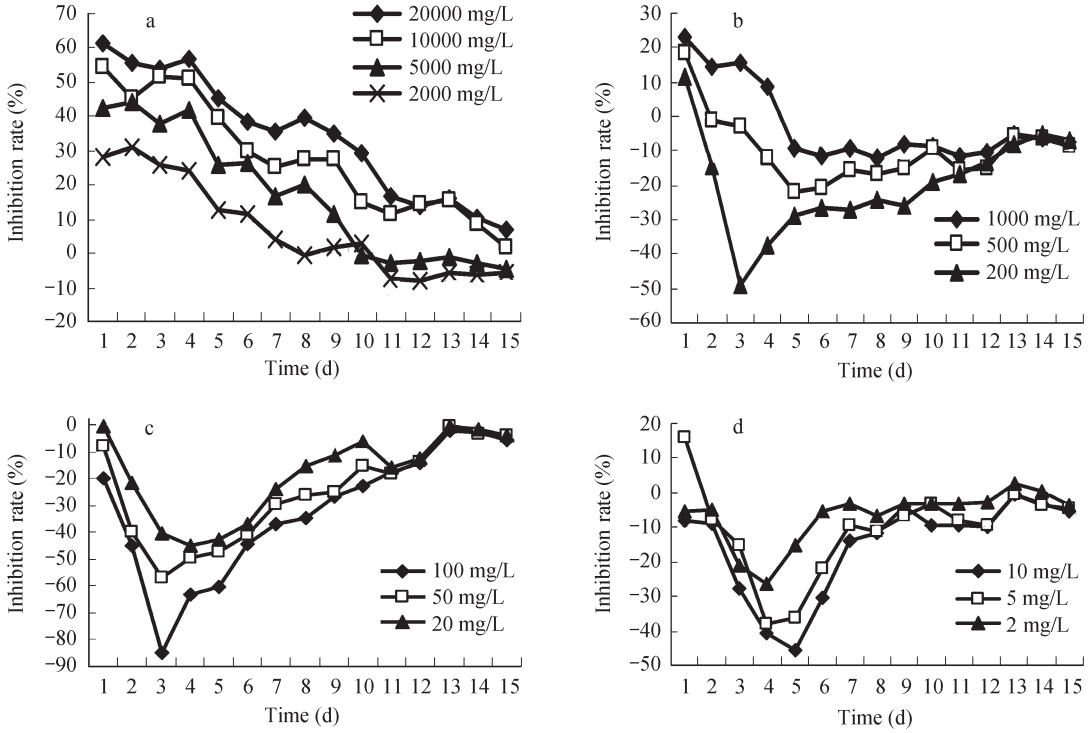

Fig. 3 Inhibition of *A. flos-aquae* by different MTBE concentrations.

green alga.

Figures 2b and 3b display the toxicity of MTBE at 5.00×10^2–1.00×10^3 mg/L during 15 continuous days. There was a slight inhibitory effect on day 1 (17%) for the green alga and on day 3 (21%) for the blue-green alga. There was a moderate stimulatory effect up to 43% on day 2 for the green alga and to 32% on day 6 for the blue-green alga. The stimulatory effect occurred before day 2 at this concentration range for the two algae, and decreased gradually with time.

When the concentration of MTBE was 200 mg/L (Figs. 2b and 3b), there was a slight increase in inhibition of growth (17% and 14%) for the two algae at day 1, but a stimulatory effect occurred from day 2. However, the stimulatory effect disappeared from day 7 for the green alga. The maximum stimulatory effect of 13%–60% during day 2–12 disappeared during day 13–15 for the blue-green alga. For 100 mg/L MTBE, the stimulatory effect occurred from the day 1 and vanished gradually over day 13–15 for the two algae. The maximum stimulatory effect was on day

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5 for the green alga (109%) and on day 3 for the blue-green alga (85%).

The growth inhibition at a concentration of MTBE of 20–50 mg/L is shown in Figs. 2c and 3c. There were strong stimulatory effects on algal growth during day 1–12, with a maximum of 200% and 56% for the green alga and the blue-green alga on day 3, respectively, and vanished gradually over day 13–15 for the two algae.

When the concentration of MTBE was 10 mg/L, a strong stimulatory effect occurred during day 2–7 for green alga (maximum 100% on day 5) and the blue-green alga (maximum 53% on day 6) (Figs. 2d and 3d). This effect disappeared over day 10–15 for the green alga and over day 13–15 for the blue-green alga. When the concentration of MTBE was 5 mg/L (Figs. 2d and 3d), there was a slight inhibitory effect on day 1 and 2, a stimulatory effect on day 3 and 4, and no effect on day 5–15 for the green alga. For the blue-green alga, there was a slight inhibitory effect on day 1, a stimulatory effect on day 2–12, and no effect over the day 13–15.

Figures 2d and 3d show the toxicity of MTBE at a concentration of 2 mg/L. A stimulatory effect occurred on the day 2–6 (maximum 74% on the day 4) for the green alga and on the day 3–5 (maximum 27% on the day 4) for the blue-green alga. There was no effect during day 7–15 for the two algae.

The toxicity of MTBE to *C. ellipsoidea* and *A. flos-aquae* is shown in Tables 1 and 2. As for the green alga *C. ellipsoidea* (Table 1), the EC_{50} values gradually increased during day 1–2 from 2.99×10^4 to 4.37×10^4 mg/L, but that gradually reduced in day 3–4 from 9.58×10^3 to 6.65×10^3 mg/L, and follow that increased as far as enormous data when day 5–15. This is owing to MTBE volatilization or/and MTBF was used as carbon resource by algae. Therefore, the most toxicity of MTBE to *C. ellipsoidea* was 6.65×10^3 mg/L after alga were incubated for 5 d. With respect to *A. floc-aquas* (Table 2), EC_{50} were gradually increased when day 1–9 (8.30×10^3–6.04×10^4 mg/L). And later that increased till enormous data when day 10–15. the most toxicity of MTBE to *A. floc-aquas* was 8.30×10^3 mg/L when cyanobacterial incubated time at 1 day.

Table 1 Toxicity of MTBE to *C. ellipsoidea*

Day	Regression equation*	Coefficient correlation	Significant level	EC_{50} (mg/L)
1	$Y = 0.1707X + 1.0991$	0.9241	0.008	2.99×10^4
2	$Y = 0.1528X + 0.9784$	0.9274	0.008	4.37×10^4
3	$Y = 0.2087X + 1.4700$	0.9555	0.003	9.58×10^3
4	$Y = 0.2144X + 1.5387$	0.9925	0.000	7.87×10^3
5	$Y = 0.2593X + 1.8000$	0.9823	0.000	6.65×10^3
6	$Y = 0.1727X + 1.1766$	0.9839	0.000	1.99×10^4
7	$Y = 0.1188X + 0.8636$	0.9884	0.000	4.69×10^4
8	$Y = 0.0378X + 0.2624$	0.8994	0.015	5.37×10^8
9	$Y = 0.0512X + 0.3167$	0.9844	0.000	359×10^7
10	$Y = 0.0602X + 0.3180$	0.9869	0.000	2.06×10^7
11	$Y = 0.0509X + 0.2830$	0.9910	0.000	7.10×10^7
12	$Y = 0.0489X + 0.2496$	0.9829	0.000	1.67×10^8
13	$Y = 0.0408X + 0.1977$	0.9529	0.003	1.65×10^9
14	$Y = 0.0328X + 0.1429$	0.9066	0.013	5.35×10^{10}
15	$Y = 0.0350X + 0.1677$	0.9187	0.010	1.33×10^{10}

* Y and X stand for percent inhibition and natural logarithm of concentration respectively.

Table 2 Toxicity of MTBE to *A. flos-aquas*

Day	Regression equation*	Coefficient correlation	Significant level	EC_{50} (mg/L)
1	$Y = 0.1236X + 1.0923$	0.9884	0.000	8.30×10^3
2	$Y = 0.1489X + 1.1741$	0.9726	0.001	1.08×10^4
3	$Y = 0.1540X + 1.1887$	0.9844	0.000	1.14×10^4
4	$Y = 0.1866X + 1.3582$	0.9839	0.000	1.01×10^4
5	$Y = 0.1878X + 1.2349$	0.9884	0.000	2.00×10^4
6	$Y = 0.1667X + 1.0826$	0.9762	0.001	3.04×10^4
7	$Y = 0.1416X + 0.9099$	0.9980	0.000	5.53×10^4
8	$Y = 0.1604X + 1.0192$	0.9930	0.000	3.93×10^4
9	$Y = 0.1397X + 0.8921$	0.9930	0.000	6.04×10^4
10	$Y = 0.0977X + 0.6099$	0.9220	0.009	3.25×10^5
11	$Y = 0.0887X + 0.4958$	0.9690	0.001	1.05×10^6
12	$Y = 0.0858X + 0.4812$	0.9566	0.003	1.24×10^6
13	$Y = 0.0675X + 0.4123$	0.8860	0.019	3.67×10^6
14	$Y = 0.0512X + 0.2897$	0.8978	0.015	6.08×10^7
15	$Y = 0.0406X + 0.2063$	0.9386	0.006	1.39×10^9

* Y and X stand for percent inhibition and natural logarithm of concentration respectively.

2.3 Comparing sensitivity of green and blue-green algae to MTBE at different concentrations and times

Unexpected variation in the response to MTBE was found for *C. ellipsoidea* and *A. flos-aquae*. When the concentration of MTBE was 1.00×10^4–2.00×10^4 mg/L (Figs. 2a and 3a), *A. flos-aquae* was more sensitive than *C. ellipsoidea* during day 1–3 and during day 7–13, but less sensitive during day 4–6 and reached equal sensitivity during day 14–15.

With the concentration of MTBE was 2.00×10^3–5.00×10^3 mg/L (Figs. 2a and 3a), *A. flos-aquae* was more sensitive than *C. ellipsoidea* during day 1–3 and during 8–9, respectively, but less sensitive during day 4–7 and reached equal sensitivity during day 13–15.

When the concentration of MTBE at 5.00×10^2–1.00×10^3 mg/L (Figs. 2b and 3b), *A. flos-aquae* was more sensitive than *C. ellipsoidea* during day 1–3, but more sensitive during day 4–10 and reached equal sensitivity during day 12–15.

For 100–200 mg/L MTBE (Figs. 2c and 3c), *A. flos-aquae* was less sensitive than *C. ellipsoidea* during day 1–2, but more sensitive during day 3–12 and had similar sensitivity during day 13–15. There was a moderate stimulatory effect on *C. ellipsoidea* (maximum 109% on day 5) and *A. flos-aquae* (maximum 85% on day 3). Moreover, there was a stimulatory effect on *A. flos-aquae*, but an inhibitory effect on *C. ellipsoidea*, reaching equal sensitivity during day 12–15.

For 20–50 mg/L MTBE (Figs. 2c and 3c), *A. flos-aquae* was less sensitive than *C. ellipsoidea* during day 1–6, but more sensitive during day 7–12, with equal sensitivity during day 13–15. Moreover, there was a strong stimulatory effect on *C. ellipsoidea* (maximum 200% on day 3) and on *A. flos-aquae* (maximum 56% on day 3).

For 5–10 mg/L MTBE (Figs. 2d and 3d), *A. flos-aquae* was less sensitive than *C. ellipsoidea* during day 1–3 and 11–12, spectively but more sensitive during day 4–8 and

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equal sensitivity during day 9–10 and 13–15, respectively. Moreover, there was a moderate stimulatory effect on *C. ellipsoidea* (maximum 100% on day 3) and *A. flos-aquae* (maximum 53% on day 4). For 2 mg/L MTBE, *A. flos-aquae* was less sensitive than *C. ellipsoidea* during day 1–6 and equivalent sensitivity during day 7–15.

3 Discussion

The results showed that the toxicity of MTBE to *C. ellipsoidea* and to *A. flos-aquae* was low when the concentration of MTBE was 1.00×10^4–2.00×10^4 mg/L, close to the values reported by Rousch and Sommerfeld (1998), who showed that the growth of three algal species was decreased significantly at MTBE concentrations of 2.40×10^3–9.60×10^3 mg/L. In our study, lower concentrations ($\geqslant 1.00\times10^3$ mg/L) of MTBE could stimulate algal growth, close to the value of 6.00×10^2–2.40×10^3 mg/L used by Rousch and Sommerfeld (1998). Both results were dissimilar to the report by Benkinney *et al*. (1994), who determined 96 h EC_{50} of 184 mg/L for *S. capricornutum*. However, 96 h EC_{50} of 7.87×10^3 mg/L for *C. ellipsoidea* and that of 1.01×10^4 mg/L for *A. spiroides* in our work.

Rousch and Sommerfeld (1998) thought that MTBE might alter the composition of an algal community in a natural environment, based on the ability of individual species to use MTBE and its breakdown products as additional carbon sources, as well as on different lipid content between species. Our results demonstrated that low concentrations of MTBE (1–500 mg/L) stimulate algal growth activity, and the effect was 200% on day 5 for *C. ellipsoidea* when the concentration of MTBE was 20–50 mg/L, and 85% on day 3 for *A. flos-aquae* when the concentration of MTBE was 100 mg/L. This concentration may lead to an algal bloom due to the overabundance of algae, representing an aquatic ecological risk. However, the stimulatory effect occurred only during day 1–5, and decreased to no effect gradually by day 13–15. Moreover, the effective concentrations were higher by orders of magnitude than the concentrations presently in the actual aquatic environment (Schuhmacher *et al*., 2003; Werner *et al*., 2001). In a conclusion, the toxicity of MTBE to *C. ellipsoidea* and *A. flos-aquae* as well as its aquatic ecological risk were generally low.

In environmental risk assessment, the ultimate aim is to provide sufficient information for decision-making with the purpose of protecting the environment from unwanted effects of chemicals (Breitholtz *et al*., 2006). Traditional acute algal tests are normally based on a short-term test, concentrating initially on gathering base-line data from a 72–96 h test with few species as lethal endpoints. As a consequence, crucial information may not be considered in the early stages of a risk assessment process. This could lead to an erroneous risk assessment in worst-case situations (Breitholtz *et al*., 2006). In algal tests, the results from various laboratories were often different, due to factors including algal incubation conditions and the length of time with respect to a full life-cycle of tested organisms. The full life-cycle of *C. ellipsoidea* and *A. flos-aquae* is 10 d in the test. Our results showed that the magnitude of the toxicity was related to time. The toxicity value decreased gradually at low concentrations of MTBE, and the stimulatory effect also decreased gradually at low concentrations of MTBE.

4 Conclusions

The present work was to assess toxicity of MTBE to *C. ellipsoidea* and *A. flos-aquae* during continuous 15 d with MTBE concentration from 2.00×10^4 mg/L to 2 mg/L. The results showed that the toxicity was low when MTBE concentrations were 1.00×10^4–2.00×10^4 mg/L (the highest growth inhibition rate was 70%–71%, occurring on day 1–5). MTBE 500 mg/L stimulated algal growth with the greatest effect of 85%–200% when MTBE was 50–100 mg/L on day 3–5. The concentration of MTBE between 50–100 mg/L may lead to algal bloom owing to the overabundance and present higher aquatic ecological risk. However, the stimulating effect occurred only during day 1–5 and gradually disappeared on day 13–15. The toxicity and aquatic ecological risk of MTBE to algal community structure were low. In conclusion, the toxicity of MTBE varied with time. We recommend that an algal test should be based on the eco-toxicological tests that could provide information from the full life-cycle of individual species, which should be confirmed ahead of a formal test, and the duration of the test should not be less than a half life-cycle.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 20476099).

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