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A new index to assess chemicals increasing the greenhouse effect based on their toxicity to algae

Ting Wang[a], Xiaoxian Zhang[a], Dayong Tian[d], Ya Gao[a], Zhifen Lin[a,b,*], Ying Liu[c], Lingyun Kong[a]

[a] State Key Laboratory of Pollution Control and Resource Reuse, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China
[b] Collaborative Innovation Center for Regional Environmental Quality, China
[c] Shanghai Key Lab of Chemical Assessment and Sustainability, Shanghai, China
[d] Department of Chemical and Environmental Engineering, Anyang Institute of Technology, Anyang 455000, China

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ABSTRACT

CO_2, as the typical greenhouse gas causing the greenhouse effect, is a major global environmental problem and has attracted increasing attention from governments. Using algae to eliminate CO_2, which has been proposed as an effective way to reduce the greenhouse effect in the past decades, can be disturbed by a growing number of artificial chemicals. Thus, seven types of chemicals and *Selenastrum capricornutum* (algae) were examined in this study, and the good consistency between the toxicity of artificial chemicals to algae and the disturbance of carbon fixation by the chemicals was revealed. This consistency showed that the disturbance of an increasing number of artificial chemicals to the carbon fixation of algae might be a "malware" worsening the global greenhouse effect. Therefore, this study proposes an original, promising index to assess the risk of deepening the greenhouse effect by artificial chemicals before they are produced and marketed.

CO_2, which is blamed for the greenhouse effect as a greenhouse gas, has drawn increasing attention from governments and scientists in recent decades. Scientists are finding ways to eliminate greenhouse gases, and meanwhile, they suggest measures to reduce greenhouse gas emissions (Arent et al., 2014). Among these current measures, using photosynthetic organisms such as plants and algae to take up carbon dioxide is a well-accepted one (Falkowski, 2012). It was reported in *Nature* that an early experiment involving adding iron to the ocean confirmed that algae could absorb CO_2 and thus eliminate the effect of global warming (Boyd et al., 2000).

However, the absorbance of CO_2 by algae can be disturbed by many factors, such as artificial chemicals. A number of studies have reported that certain artificial chemicals are toxic to algae and have developed some toxicological indexes, such as effect concentrations at median inhibition (EC_{50}), to evaluate the toxicity of chemicals to algae. For instance, Sabater and Carrasco (1997) reported that the EC_{50} values of carboxin for *Scenedesmus* and *Chlorella vulgaris* were 0.22 mg/L and 54.0 mg/L, respectively. Further, scientists found that the toxic effects of compounds on algae were achieved by interfering with the carbon fixation of algae (Mishra and Pandey, 1989). In terms of these side effects of artificial chemicals, increasing numbers of researchers have directed their attention to them due to the flourishing international chemical industry. The United Nations Environment Programme (UNEP) noted that global chemical output (produced and shipped) increased markedly from US$171 billion in 1970 to $4.12 trillion by 2010 in a report named Global Chemicals Outlook in 2012 (UNEP, 2012), in which it was predicted that global chemical sales would grow by approximately 3% per year before 2050 (UNEP, 2012).

Consequently, the question arises whether, in addition to the combustion of fossil fuels (coal, gas and oil), the disturbance of CO_2 absorption by algae due to the growing number of chemicals is another powerful cause of worsening greenhouse effects. If so, can we propose a novel index to assess the disturbance of chemicals to the carbon fixation of algae, which will be beneficial to the design of new chemicals? This question is the main concern of this study.

Therefore, this study is conducted to reveal the exact relationship between the toxicity of artificial chemicals to algae and their disturbance of carbon fixation and then answer the questions above. The aims of this study are (1) to determine the toxicity of

* Corresponding author at: State Key Laboratory of Pollution Control and Resource Reuse, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China.
E-mail address: lzhifen@tongji.edu.cn (Z. Lin).

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Table 1
Growth inhibition of typical chemicals to algae and their disturbance on carbon fixation.

No.	Chemical	CAS number	MW (g/mol)	Disturbance [log(1/EC_{50}-CSD), mol/L]	Growth inhibition [log(1/EC_{50}-toxicity),mol/L]
1#	Prometryn	7287-19-6	241.36	7.31	7.09
2#	Chlortoluron	15545-48-9	212.68	6.64	6.75
3#	Prometon	1610-18-0	225.29	6.11	6.00
4#	Diuron	330-54-1	233.09	7.63	7.45
5#	2,4-Dinitrotoluene	121-14-2	182.13	5.42	5.23
6#	2,4-Nitrochlorobenzene	97-00-7	202.55	5.78	5.83
7#	$ZnCl_2$	7646-85-7	136.30	6.08	6.17
8#	$CuCl_2$	10125-13-0	170.48	6.25	6.2
9#	Nano Pr_6O_{11}	12037-29-5	329.81	6.10	6.11
10#	Nano Nd_2O_3	1313-97-9	336.48	5.10	5.06
11#	Glyphosate	1071-83-6	169.07	4.10	4.14
12#	Phoxim	14816-18-3	298.30	5.28	5.18

artificial chemicals and their disturbance of the carbon fixation of algae, (2) to reveal the relationship between the toxicity of artificial chemicals to algae and their disturbance to carbon fixation of algae, and (3) to propose a novel index for assessing the disturbance of chemicals to carbon fixation based on the toxicological index to provide a reference for the assessment of artificial chemicals.

1. Materials and methods

1.1. Chemicals and algae

All chemicals were obtained from the Sigma–Aldrich Company (St Louis, MO, USA), except that 2,4-nitrochlorobenzene was purchased from Shanghai Fenke Chemical Reagent Company (Shanghai, China), and glyphosate and phoxim were purchased from the Aladdin Chemical Reagent Company (Shanghai, China). *S. capricornutum* was obtained from the Freshwater Algae Culture Collection at the Institute of Hydrobiology, Chinese Academy of Sciences. Detailed information on the chemicals used in this study is shown in Table 1.

1.2. Toxicity test of chemicals to S. capricornutum

The culture was maintained in *f*/2 marine medium (pH 7.5 ± 0.1) at 22 ± 1 °C, with a 12:12 h light/dark cycle of 3500–4000 lux. For the twelve chemicals individually studied, six concentrations in a geometric progression ranging from no effect to 100% growth inhibition concentration were planned after preliminary experiments. The algae in the logarithmic growing period were inoculated into 100-mL Erlenmeyer flasks containing 40 mL culture media and test chemicals. The culture media without test chemicals served as a control. The initial concentration of the cell inoculum of each species was 2×10^4 cells/mL in both the treated and control flasks. All experiments were repeated three times. Data were expressed as effective concentration and the 95% confidence intervals at 50% response. Growth inhibition was determined after 96 h using a counting chamber (Agency, 1996) under a microscope. The median effective concentration of a chemical towards the algae ($EC_{50\text{-toxicity}}$) was calculated based on the decrease in algal biomass.

1.3. Disturbances of chemicals to the carbon fixation of S. capricornutum

During the toxicity tests of chemicals against *S. capricornutum*, the disturbance of chemicals to the carbon fixation of *S. capricornutum* was determined simultaneously, and the samples were obtained as follows (Fig. 1):(1) For cultures to which chemicals were added, 10 mL samples of the algae solution were taken at t = 0 h and t = 96 h and added to the sample vial for the determination of total organic carbon using a total organic carbon analyzer (TOC analyzer). The results were named $TOC_i^{t=0}$ and $TOC_i^{t=96}$.(2) For cultures to which chemicals were not added, 10 mL samples of the algae solution were taken at t = 0 h and t = 96 h and placed in the sample vial for the determination of total organic carbon using a total organic carbon analyzer (TOC analyzer). The results were named $TOC_0^{t=0}$ and $TOC_0^{t=96}$.(3) The total organic carbon sequestered between t = 0 h and t = 96 h (ΔTOC_i and ΔTOC_0) could be obtained by comparing the variation of the experimental samples and the control samples using Eqs. (1) and (2).(4) Similarly to the toxicity test, the disturbance of chemicals to the carbon fixation of *S. capricornutum* could be denoted using the effective concentration at median inhibition at 96 h ($EC_{50\text{-CSD}}$, EC_{50} for carbon fixation disturbance). A linear fitting was performed between the logarithm concentration and the relative rate of carbon fixation. $EC_{50\text{-CSD}}$ could thus be obtained based on the results of the linear regression.

1.4. Statistics

All statistical analyses were performed using the SPSS 16.0 software package (SPSS Inc.). The coefficient of determination (r^2),

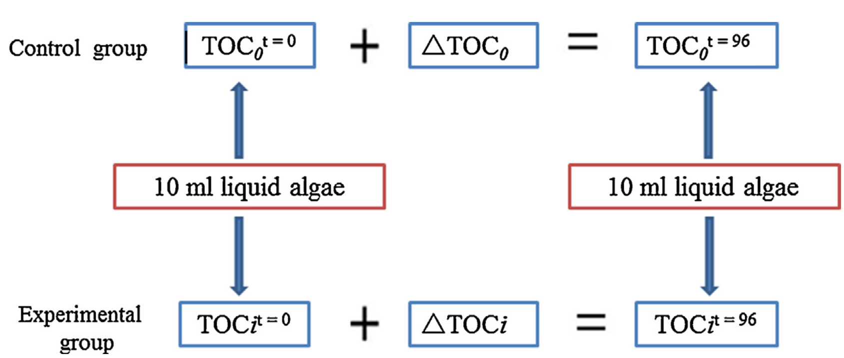

Fig. 1. Schematic of the increase in organic and inorganic carbon fixation by algae at t = 0–96 h.