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Subcellular distribution of fluoranthene in *Chlorella vulgaris* with the presence of cetyltrimethylammonium chloride

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HIGHLIGHTS

- The joint action of CTAC and Flu on *Chlorella vulgaris* changed from synergism to antagonism.
- The uptake of Flu by algae was enhanced with the presence of CTAC.
- The highest amount of Flu bound to the cytosol when synergism was observed.
- The highest amount of Flu bound to the cellular debris when antagonism was displayed.
- Subcellular distribution could elucidate the possible mechanism of joint toxicity.

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ABSTRACT

This study explored the possible mechanism of the joint toxicity of binary mixtures of cetyltrimethylammonium chloride (CTAC) and fluoranthene (Flu) to the green alga *Chlorella vulgaris* by examining the subcellular distribution of Flu within the alga. The joint action of CTAC (100 μg L^{-1}) and Flu (0–200 μg L^{-1}) on the algae changed from a synergetic effect (0–50 μg L^{-1}) to an antagonistic effect (50–200 μg L^{-1}) with an increase of the Flu concentration. The Flu uptake was enhanced by the presence of CTAC through the intracellular detection of Flu. Furthermore, the highest amount of Flu bound to the cytosol, whereas the least amount bound to the cellular debris when synergistic effect was observed at 2.5 μg L^{-1} Flu. However, the highest amount of Flu bound to the cellular debris, whereas the least amount bound to the organelles when antagonistic effect was displayed at 200 μg L^{-1} Flu. The different subcellular distribution of Flu may affect the uptake of the highly toxic CTAC by the algae in the binary mixture, and consequently lead to a different level of CTAC toxicity. The abovementioned results indicate that the subcellular distribution of chemicals can be used to elucidate possible mechanisms for the joint toxicity of their binary mixtures to aquatic organisms.

1. Introduction

The combined pollution of xenobiotics in aquatic ecosystems represents an emerging environmental issue due to insufficient information on the reciprocal interactions between chemicals and their toxic mechanisms. Therefore, understanding the joint action of multi-pollutants is important for the ecological risk assessment of contaminants in aquatic systems. Over the past decades, considerable attention has been given to the joint toxicity of metals and/or organic matter to aquatic organisms (Mehler et al., 2008; Schuler et al., 2009; Chen et al., 2010; Lister et al., 2011).

Surfactants, an ubiquitous class of organic contaminants in aquatic environments, have been recently produced in increasing amounts, and over 7.3 million tones were used in China in 2010 (Wang and Chen, 2011). Quaternary ammonium compounds (QACs) are one type of the typical cationic surfactants, which contain at least one hydrophobic long alkyl chain attached to a positively charged nitrogen atom (Ash and Ash, 2000; Sütterlin et al., 2008). QACs represent a prodigious risk for aquatic ecosystems due to their biocidal activity (Andrew et al., 2004; Tezel et al., 2006). Xu et al. (2011) demonstrated that cetyltrimethylammonium chloride (CTAC) had significant inhibitory effects on *Chlorella vulgaris* biomass. In aquatic biota, QACs usually coexist with other contaminants, such as aromatic hydrocarbons, pesticides, and hea-

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vy metals. Aromatic hydrocarbons have carcinogenic potential, thus, their toxicities on aquatic organisms are of critical concern (Djomo et al., 2004; Echeveste et al., 2010). Previous investigations have revealed that QACs may interact with aromatic hydrocarbons by electrostatic, hydrophobic, and/or cation–π bonding in the aquatic phase (Jaynes and Boyd, 1991; Qu et al., 2008). Our previous study demonstrated that the toxicity of CTAC can be enhanced by low concentrations of aromatic hydrocarbons in the binary mixtures (Ge et al., 2010). However, the mechanisms relating to the enhanced toxicity of QACs and aromatic hydrocarbons in the binary system need further investigation.

In the past decades, different approaches have been used to explore the interactions between binary mixtures and organisms, such as biomass measurement, sorption of contaminants into organisms, zeta potential measurement, enzymatic activity, and determination of gene expression (Xiao et al., 2007; Qian et al., 2009; Schuler et al., 2009; Pokora and Tukaj, 2010). These approaches, which have been used in attempts to explain the mechanism of the joint action of contaminants in organisms, are not straightforward because they cannot directly predict the detailed distribution of the organic contaminants in those organisms.

Recently, the subcellular distribution of chemicals, which takes into account their cellular fates, has been shown to be an effective approach for elucidating the toxicity of chemicals in aquatic organisms. This approach uses a differential centrifugation technique to separate the organism homogenates into individual subcellular fractions including cellular debris, organelles, and cytosol (Wallace et al., 2003). A few studies have been conducted on organisms to test whether potential correlations exist between the toxicity and subcellular distribution of contaminants (Chang and Reinfelder, 2000; Bach et al., 2005; Miao and Wang, 2007). Bach et al. (2005) found that the differences in the biotransformation capability of fluoranthene (Flu) were reflected in the different subcellular distribution of Flu among four fractions (i.e., debris, mitochondria, microsomes and cytosol) of *Capitella* sp. I and *C.* sp. S, resulting in different levels of tolerance to Flu toxicity. A more recent study on the accumulation and subcellular distribution of copper (Cu) in the marine diatom *Thalassiosira weissflogii* under different nutrient conditions suggested that Cu toxicity can be better predicted using the intra-Cu concentration and its subcellular distribution (Miao and Wang, 2007). Previous studies mainly focused on the subcellular distribution of individual contaminants in organisms. However, to the best of our knowledge, there have been few experiments on the joint toxicity mechanisms of binary mixtures in freshwater alga using subcellular fractionation.

Based on the abovementioned background information, CTAC and Flu were chosen as the representatives of QACs and aromatic hydrocarbons, respectively. *C. vulgaris*, a unicellular alga, is generally regarded as a model aquatic organism for its high sensitivity to contaminants (Wilson et al., 2003; Qian et al., 2008). In contrast to CTAC, Flu can be detected accurately by fluorescence analysis with a minimum detectable concentration of 0.0015 μg L^{-1} (Hong et al., 2008). Therefore, Flu was selected as the objective chemical in this investigation on the subcellular distribution of the binary mixtures. The objectives of this study are (1) to investigate the intracellular concentration of Flu and its subcellular distribution in algal cells; and (2) to explore the possible mechanism for the joint toxicity of binary mixtures through the subcellular distribution of Flu.

2. Materials and methods

2.1. Chemicals

Flu (purity > 98%) was purchased from Sigma Chemical Co., ACROS, Belgium. CTAC (analytical grade) was purchased from Robiot Co., Ltd., Nanjing, China. Selected physicochemical properties of Flu and CTAC were listed in Table 1.

2.2. C. vulgaris cultures

C. vulgaris was obtained from the Institute of Hydrobiology, Chinese Academy of Sciences, and maintained in OECD algal test medium (OECD, 2006), which has been autoclaved at 120 °C for 15 min and stored in darkness at 4 °C. The algae were cultured at 25 ± 0.5 °C in Erlenmeyer flasks (250 mL) with fluorescent lamp under a light intensity of 2500 lx for a daily light: dark cycle of 14 h:10 h. The pH of the medium was adjusted to 8.0 with NaOH or HCl. Algal cells in the exponential growth phase were used for all experiments, and the initial cell density for each experiment was about 3.5×10^5 cells mL^{-1}. The Erlenmeyer flasks containing the medium were manually shaken three times per day.

2.3. Toxicity measurements

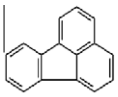
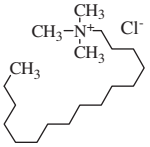
The concentration gradient of Flu was determined in our previous research based on its acute toxicity to *C. vulgaris* (Ge et al., 2010). In the individual toxicity test, either Flu (0–200 μg L^{-1}) or CTAC (0–300 μg L^{-1}) was added to the culture medium. In the binary mixture toxicity test, *C. vulgaris* was placed in the binary system containing 100 μg L^{-1} of CTAC and varying concentrations (0, 1, 2.5, 5, 50, 100, and 200 μg L^{-1}) of Flu. The control groups, which contained the algal cells without Flu and CTAC, were carried out under the same conditions previously discussed. Three replicates were prepared for each treatment. The algal biomass was quantified with a spectrophotometer (522S, Shanghai Analytical Instrument Co. Ltd, CN) at 680 nm, and the yield was calculated as the biomass at the end of 96 h minus the starting biomass for each control and treatment.

The accumulation of Flu and its subcellular distribution in algal cells were determined individually and in binary system. The term "Flu accumulation" used later in the text refers to the intracellular concentration of Flu, and does not include Flu adsorbed to the surface of the cells. The individual system was spiked with Flu (2.5, 50, 200 μg L^{-1} in treatments A–C, respectively) alone. The binary system was spiked with both CTAC (100 μg L^{-1}) and Flu (2.5, 50, 200 μg L^{-1} in treatments D–F, respectively). All experiments were performed in sextuplicate. Algal samples were collected after 24, 48, 72 and 96 h culture, respectively.

2.4. Flu concentration at intracellular and subcellular fractionation

At the end of each exposure, the intracellular concentration of Flu and its subcellular distribution were measured in treatments A–F. A 100 mL sample from each replicate was first centrifuged (4000*g*, 15 min). After that the resulting algal pellets were resus-

Table 1
Selected physicochemical properties of Flu and CTAC.

Substance	CAS	Structure	HLB	MW	CMC (mol L^{-1})
Flu	206-44-0		5.22	202.26	[a]
CTAC	112-02-7		12.2	319.95	1.3×10^{-3}

[a] The CMC value of Flu was not reported.