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Bioaccumulation and effects of novel chlorinated polyfluorinated ether sulfonate in freshwater alga *Scenedesmus obliquus*☆

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ABSTRACT

Chlorinated polyfluorinated ether sulfonate (Cl-PFESA) is a novel alternative compound for perfluorooctane sulfonate (PFOS), with its environmental risk not well known. The bioaccumulation and toxic effects of Cl-PFESA in the freshwater alga is crucial for the understanding of its potential hazards to the aquatic environment. *Scenedesmus obliquus* was exposed to Cl-PFESA at ng L^{-1} to mg L^{-1}, with the exposure regime beginning at the environmentally relevant level. The total log BAF of Cl-PFESA in *S. obliquus* was 4.66, higher than the reported log BAF of PFOS in the freshwater plankton (2.2–3.2). Cl-PFESA adsorbed to the cell surface accounted for 33.5–68.3% of the total concentrations. The IC50 of Cl-PFESA to algal growth was estimated to be 40.3 mg L^{-1}. Significant changes in algal growth rate and chlorophyll *a*/b contents were observed at 11.6 mg L^{-1} and 13.4 mg L^{-1} of Cl-PFESA, respectively. The sample cell membrane permeability, measured by the fluorescein diacetate hydrolyzation, was increased by Cl-PFESA at 5.42 mg L^{-1}. The mitochondrial membrane potential, measured by Rh123 staining, was also increased, indicating the hyperpolarization induced by Cl-PFESA. The increasing ROS and MDA contents, along with the enhanced SOD, CAT activity, and GSH contents, suggested that Cl-PFESA caused oxidative damage in the algal cells. It is less possible that current Cl-PFESA pollution in surface water posed obvious toxic effects on the green algae. However, the bioaccumulation of Cl-PFESA in algae would contribute to its biomagnification in the aquatic food chain and its effects on membrane property could potentially increase the accessibility and toxicity of other coexisting pollutants.

1. Introduction

Chlorinated polyfluoroalkyl ether sulfonate (Cl-PFESA, Fig. 1), with trade name F-53B, is a novel substitute for perfluorooctane sulfonate (PFOS). China has been using Cl-PFESA as a mist suppressant in the electrolytic process of metal plating since the 1970s, even before the introduction of PFOS products, and is the only country with a documented usage (Wang et al., 2013). However, the environmental risk of Cl-PFESA has been overlooked for decades until the first report about its occurrence in the wastewater effluent and surface water in 2013 (Wang et al., 2013). Many countries have stopped or limited the production and application of PFOS. As the major producer of PFOS-related substances, the Chinese government also has announced that the amendment of the Stockholm convention to include PFOS came into force in China on Mar. 26, 2014. After the phasing out of PFOS, Cl-PFESA might extend its application from the industries that use PFOS currently. Cl-PFESA concentrations increased from 2007 to 2014 (140-722 pg m^{-3}) in atmospheric particles from the city of Dalian, China (Liu et al., 2017).

Evidences suggesting the bioaccumulation of Cl-PFESA, as well as its potential long-range transportation and hazardous effects are increasing, receiving extensive concerns. The 28 day closed bottle test for aerobic biodegradation according to the OECD guideline 301D classified Cl-PFESA as not readily degradable, with theoretical oxygen demand <44% (Wang et al., 2013). Cl-PFESA was detected at relatively high concentrations of 43 μg L^{-1} to 78 μg L^{-1}, in the wastewater effluent from the chrome plating industry in Wenzhou city, China (Wang et al., 2013). In municipal sewage sludge samples collected from different areas in China, Cl-PFESA was detected at comparable or even higher concentrations (geometric mean: 2.15 ng g^{-1} dry weight) than PFOS (geometric mean: 3.19 ng g^{-1} dry weight) (Ruan et al., 2015). Cl-PFESA was detectable in 51% of

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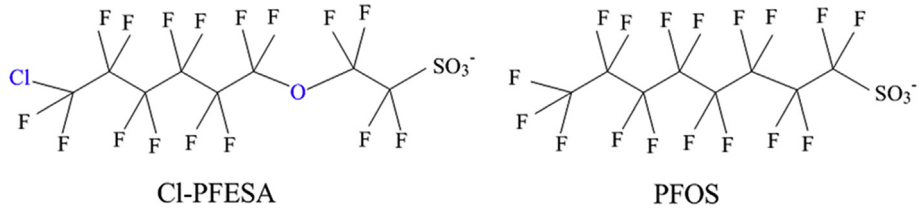

Fig. 1. Chemical structures of Cl-PFESA and PFOS.

surface water samples taken at river mouths of 19 rivers in China, at comparable concentrations (<0.56–78.3 ng L^{-1}) with PFOS (0.4–55.0 ng L^{-1}) (Wang et al., 2016). Moreover, Cl-PFESA was found to bioaccumulate in crucian carp (*Carassius carassius*), with whole body bioaccumulation factors (median log BAF: 4.1–4.3) exceeding the regulatory bioaccumulation criterion and significantly higher than PFOS (median log BAF: 3.3–3.4) (Shi et al., 2015). Cl-PFESA was even detected in the livers from the ringed seals (*Pusa hispida*, 0.045 ± 0.004 ng g^{-1}), polar bears (*Ursus maritimus*, 0.27 ± 0.04 ng g^{-1}) and killer whales (*Orcinus orca*, 0.023 ± 0.009 ng g^{-1}) in Greenland, albeit at concentrations approximately four orders of magnitude lower than PFOS (Gebbink et al., 2016). With a low production volume, estimated to ~30 tons $year^{-1}$ in China, Cl-PFESA can also cause significant human exposure on a regional scale (Shi et al., 2016). Estimated half-lives for renal clearance (median 280 years; range 7.1–4230 years) and total elimination (median 15.3 years; range 10.1–56.4 years) suggested that Cl-PFESA is the most biopersistent per- and polyfluoroalkyl substance (PFAS) in humans reported to date (Shi et al., 2016). Pan et al. (2017) found that Cl-PFESA was detectable in >99% of the paired human maternal and cord sera samples at relatively high concentrations following PFOS and PFOA. It was suggested that Cl-PFESA induces embryo toxicity and disrupts cardiac development in zebrafish embryos at 1.5–12 mg L^{-1} (Shi et al., 2017). Our preliminary study found comparable neurotoxic potency of Cl-PFESA with PFOS in rats (Zhang et al., 2016). Studying both the environmental existence and the hazardous effects of this alternative fluorosurfactant is therefore of importance.

Knowledge of the bioaccumulation and biological effects of Cl-PFESA is crucial for the understanding of its potential hazards to the aquatic ecosystem. The present study evaluated the adverse effects of Cl-PFESA on the freshwater green alga, *Scenedesmus obliquus*, which is ecologically relevant as primary producers. Phytoplankton also accounts for the first step of PFASs transfer to aquatic food webs, and plays a key role on the cycling of these pollutants in the water column (Casal et al., 2017; Nizzetto et al., 2012; Galban-Malagon et al., 2012). Membranes are one of the most typical targets for PFAS, because these chemicals present themselves as similar to fatty acids in their interactions with membranes (Hu et al., 2003; Liu et al., 2008; Butenhoff and Joseph, 2015). Although the bioaccumulation and toxicity of Cl-PFESA in fishes have been reported, the present study appears to be the first to address its accumulation, toxicity and mechanisms in microalgae.

2. Materials and methods

2.1. Reagents

Because no commercial Cl-PFESA standard was available at the time of the study, F-53B product ($Cl(CF_2)_6O(CF_2)_2SO_3K$,CAS number 73606-19-6) was obtained from Shanghai Synica, with a purity of >98%. The detailed characterization of the contents and impurities was reported by Ruan et al. (2015) and Shi et al. (2016). The technical product contained 91%, 7%, and 0.3% C8, C10, and C12 Cl-PFESA, respectively (Shi et al., 2016). Methanol and acetonitrile of high performance liquid chromatography (HPLC) grade was purchased from Fisher Scientific (Pittsburgh, PA, USA). Ammonium acetate (NH_4Ac) and ammonium hydroxide (NH_4OH, 28-30% NH_3 in H_2O) were purchased from Sigma-Aldrich. Glass-fiber filter membranes (GF/F, 47 mm) and Oasis WAX solid-phase extraction cartridges were purchased from Waters (Milford, MA, USA).

2.2. Algae cultures and toxicity test

The microalgae *Scenedesmus obliquus* were supplied by Freshwater Algae Culture Collection of the Institute of Hydrobiology (Wuhan, China). The algae culture and toxicity test were conducted in accordance with the OECD 201 protocol (OECD, 2011). The algae were cultivated in Erlenmeyer flasks (50 mL) which were capped by air-permeable gauze at 25 ± 1 °C, pH 7.5 ± 0.3 and 16:8 h light/dark photoperiod with cool-white light at 7000 lux. The Erlenmeyer flasks were shaken by hand three times per day. The algae were pre-cultured to exponentially growing state before being exposed to Cl-PFESA for 72 h. The stock solution of 200 mg L^{-1} Cl-PFESA, was prepared in culture medium and no evidence of micellar formation was observed. For the exposure experiment, the stock solution was serially diluted to nominal concentrations in the range of 0.05 μg L^{-1}-60 mg L^{-1} in the medium. According to the reported concentrations of Cl-PFESA in surface water and wastewater effluent (Wang et al., 2013, 2016), three concentrations (0.05, 0.5, 5 μg L^{-1}) were chosen to estimate the bioaccumulation factor of Cl-PFESA. The toxicity was tested at 5-60 mg L^{-1}, with the highest concentration causing approximately 100% growth inhibition in the preliminary test. No significant toxic effects were observed below 5 mg L^{-1} of Cl-PFESA. For the bioaccumulation and toxicity tests, 3-5 concentrations were used for each test.

The initial algae density in the toxicity test was adjusted to 3×10^4 cells mL^{-1}. Both the control and the treatment groups were prepared in triplicate. The algae biomass was measured every 24 h via pigment fluorescence (Liu et al., 2008). The concentration of Cl-PFESA leading to a 50% inhibition of algae growth (IC50) after 72 h of exposure was fitted using the followed model (Liu et al., 2008),

$$y = y_0/[1+(x/IC50)^b] \quad (1)$$

where y represents the relative inhibition of algae growth (%), x represents the concentration of Cl-PFESA, parameters y_0 and b are constants in the equation. The Cl-PFESA concentrations which did not cause significant growth inhibition were selected for the studies on membrane property and oxidative stress.

2.3. Cl-PFESA analysis in exposure solution and in algae

In the results presented, treatment concentrations of Cl-PFESA in the culture medium were measured values. Because the change in the exposure concentrations of Cl-PFESA are mainly caused by sorption to the flask surface, the exposure media with the