Chemosphere 75 (2009) 405–409

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Effect of perfluorooctane sulfonate on toxicity and cell uptake of other compounds with different hydrophobicity in green alga

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ARTICLE INFO

Article history:
Received 26 July 2008
Received in revised form 27 November 2008
Accepted 30 November 2008
Available online 9 January 2009

Keywords:
Perfluorooctane sulfonate (PFOS)
Combined toxicity
Uptake
Pentachlorophenol
Atrazine
Diuron

ABSTRACT

Perfluorooctane sulfonate (PFOS) was evaluated alone and in binary mixtures with pentachlorophenol, atrazine and diuron, respectively to investigate the effects of interactions between PFOS and other compounds on the growth rate in *Scenedesmus obliquus*. Single application of PFOS showed no inhibition on the growth of *S. obliquus* below 40 mg L^{-1}, whereas PFOS acting with pentachlorophenol resulted in higher algal growth inhibition in comparison with pentachlorophenol alone. A maximum increase of 45% in the growth inhibition was observed at a pentachlorophenol concentration of 2.56 mg L^{-1} together with a PFOS concentration of 40 mg L^{-1}. On the contrary, the algal growth inhibition of atrazine and diuron was depressed by PFOS. Furthermore, cell uptake was examined to gain some insights into the mechanisms of the effects of PFOS on the toxicity of the other compounds. Cell uptake of pentachlorophenol increased while that of atrazine and diuron was reduced in cells that have been exposed to PFOS. The effects of PFOS on the toxicity of pentachlorophenol, atrazine and diuron were possibly related to the influence of PFOS on the cell uptake of these hydrophobic compounds. Results suggested that PFOS influenced the cell uptake and toxicity of structurally different compounds in dissimilar manners and potentially increased the accessibility and toxicity of more hydrophobic compounds to algal cells.

1. Introduction

Perfluorinated compounds (PFCs) are applied as surfactants and surface treatment agents and so forth, both as products and raw material because of their high surface-active effect, thermal and chemical stability, and light transparency (Renner, 2001; Schultz et al., 2003). Their extreme stability makes them practically non-biodegradable and very persistent in the environment (Banks et al., 1994). These chemicals are widespread in the northern hemisphere and are generally in higher concentrations in more populated and industrial regions (Giesy and Kannan, 2001). According to the studies regarding the distributions of PFCs in environmental matrices, perfluorooctane sulfonate (PFOS) has been detected in most samples analyzed and is generally the fluorochemical detected in highest concentrations, since this compound is the stable and extremely persistent end-product of many sulfonated fluorochemicals (Giesy and Kannan, 2001). Furthermore, accumulations of PFOS in biota throughout the world, even in remote areas such as the Arctic, have been described (Kannan et al., 2001; Martin et al., 2004). The determination that PFOS is a persistent, bioaccumulative and toxic (PBT) substance (OECD, 2002a) led to increasing global regulatory concern.

Recent toxicological studies on PFOS mainly concerned mono-substance exposures of PFOS to biological systems, leaving the potential interactive effects of PFOS with other compounds as an area where knowledge is significantly lacking. However, PFOS has been found to co-occur with various kinds of compounds such as herbicides, pharmaceuticals, and alkylphenols in surface water and drinking water (Loos et al., 2007), as well as in the liver of polar bears (Kannan et al., 2005). Hence, the coexistence of PFOS and other pollutants in aquatic environment cause aquatic organisms to be exposed to the mixture of these hydrophobic organic compounds. A significant increase of the toxic effect of tetrachlorodibenzo-*p*-dioxin and estradiol caused by their interaction with PFOS was observed (Hu et al., 2003). In addition, PFOS was reported to increase the genotoxicity of cyclophosphamide in mammalian cells (Jernbro et al., 2007). The researchers suggested that PFOS enhanced the toxicity of these compounds by increasing cell membrane permeability due to its surface activity. Previous study has shown that PFOS increased algal cell membrane permeability (Liu et al., 2008). Therefore, the PFOS-induced enhancement of cell membrane permeability to other pollutants, some more toxic than PFCs, could pose a serious risk to single-celled alga that is directly

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doi:10.1016/j.chemosphere.2008.11.084

exposed to pollutants in the environment. The updated knowledge about the combining effects of PFOS and other organic compounds are still very limited and more research was needed concerning the interactions between PFOS and different compounds, and various target organisms.

In the present study, toxic effects of PFOS on freshwater algae in combination with another organic compound, testing compounds with different hydrophobicity. Pentachlorophenol, atrazine and diuron were evaluated. Furthermore, the effect of PFOS on the cell uptake of these compounds was investigated to gain an insight into the mechanism of the interactions between PFOS and these organic contaminants. These three compounds were selected in light of their extensive applications, frequently observed occurrence in the aquatic environment, and toxicity to the aquatic organisms (Muir and Eduljee, 1999; Delorenzo et al., 2001; Repetto et al., 2001; Alvarez and Fuiman, 2005; Tran et al., 2007).

2. Materials and methods

2.1. Reagents

Perfluorooctane sulfonate (PFOS, $CF_3(CF_2)_7SO_3H$, ⩾98% purity) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Pentachlorophenol (C_6HCl_5O, ⩾98% purity) was obtained from Tianjin NanKai Chemical Company (China). Atrazine ($C_8H_{14}ClN_5$, ⩾98% purity) was purchased from Chem Service (West Chester, PA, USA). Diuron ($C_9H_{10}Cl_2N_2O$, 97.7% purity) was supplied by Labor Dr. Ehrenstorfer-Schäfers (Augsburg, Germany).

2.2. Alga culture

Scenedesmus obliquus were obtained from the Freshwater Algae Culture Collection, Institute of Hydrobiology, Chinese Academy of Sciences (Beijing). Algal medium was prepared according to Organization for Economic Cooperation and Development guidelines (OECD, 2002b) using deionized water and analytically pure chemicals, adjusted to pH 7.5 ± 0.2. The culture medium was used after autoclave sterilization. The growth chamber was maintained at 22 ± 1 °C, and illumination was provided by cool-white fluorescent lights (6000 lux) at 14:10 h light:dark cycle.

2.3. Toxicity tests

Toxicity testing was conducted according to Organization for Economic Cooperation and Development guidelines (OECD, 2002b). Cells in the exponential phase of growth were collected from the stock cultures and used as the inocula for experiments. The stock solution of the test compounds was prepared in methanol and the working solution was obtained by 1000 times of dilution of the stock solution into algal culture medium. Identical volume of methanol was used as control in the toxicity test. The working solution of test compound and alga suspension was mixed to obtain an initial cell density of 10^4 cells mL^{-1} and the designed concentrations of test compound. Twenty mL of toxicity test medium containing the test compound and the algal cells was dispensed into 50-mL flasks. The algal cells were exposed, in triplicate, to serial concentrations of test compounds and control for 72 h. The culture conditions used for toxicity test were consistent with that used for stock culture. Cells were simultaneously exposed to binary mixtures with PFOS and any one of these three compounds. The PFOS concentration applied was in the range of 10-40 mg L^{-1}, where PFOS was found to show no growth inhibition but disturb the algal membrane properties in *S. obliquus* (Liu et al., 2008). Test concentrations for pentachlorophenol, atrazine, and diuron were selected based on preliminary range-finding test, where the growth inhibition ranged from 0 to 100%.

The algal growth responses to toxic compounds were investigated by measuring in vitro chlorophyll fluorescence according to the method described by Mayer et al. (1997). Measurement of the response and the data handling were described in details in previous publication (Liu et al., 2008). Briefly, effect–concentration curves of the growth inhibition were calculated and the data were fit to a non-linear regression. The effective concentrations giving 50% growth rate reduction (EC50) was derived from the fit equation.

2.4. Uptake by algae

Additional bioassays were conducted to measure the cell uptake of pentachlorophenol, atrazine and diuron by algae with and without PFOS exposure. To determine whether the influence of PFOS on the algal cell uptake of other compounds is caused by the disruption of the cell membrane and related cell physiological properties, algae were exposed to PFOS alone for 72 h at concentrations of 10–40 mg L^{-1}. Then the PFOS-treated cells were collected by centrifugation and washed by phosphate buffer solution (PBS) and were applied in the uptake test. Based on the design of the experiment, influence of PFOS on the cell uptake of atrazine and diuron due to the ligand competing in the surrounding medium for limited adsorption sites on cell surface was not involved in the results. Algae were collected by centrifugation (3500 rpm, 10 min) after exposure to PFOS at concentrations of 10, 20, 30, 40 mg L^{-1} for 72 h, and washed three times by PBS. Uptake experiments were initiated by inoculating equal cell numbers of 10^6 cells mL^{-1} into flasks containing a total volume of 200 mL of algal cell suspension per flask. Much larger volume and cell density than that used in the toxicity test were used to facilitate the measurements of the low cellular concentration of the toxicants. Pentachlorophenol, atrazine or diuron was added to each flask to achieve a final concentration equal to the growth rate EC50 value derived when the compound acting alone. Samples were incubated for 1 h under the same conditions as stock cultures. A relatively short incubation period was used to avoid the influence of growth dilution on the uptake (Axelman et al., 1997), since the hydrophobic organic compounds seemed to be uptaked quickly into the algal cells (Jabusch and Swackhamer, 2004; Weiner et al., 2004). Following incubation of 1 h, each sample was filtered through a Whatman GF/F glass fiber filter (Maidstone, England) to collect algal cells. Filters were washed with 50 mL PBS to eliminate the toxicants that loosely adsorbed to the filter. Filters were placed in tubes, followed by addition of 200 μl of 10% sodium hypochlorite to decolorize the sample and twice extracted with 5 mL of methanol and 10 min of sonication. The extraction solution was concentrated down to 1 mL under nitrogen purge. A control sample lacking algal cells was filtered and used to check analytes bound to filters. Cell uptake was expressed as percentage of analytes taken up from total amount solved in solution.

2.5. Chemical analysis of intracellular toxicants

Intracellular concentrations of pentachlorophenol, atrazine and diuron were analyzed by HPLC (PU-1580, UV-1575, Jasco Corporation, Japan) with a Kromasil ODS (5 μm, 4.6 mm × 250 mm) reverse-phase column. For the analysis of pentachlorophenol, the mobile phase was 1.0 mL min^{-1} of methanol and water (1% HAc (v/v)) (V:V = 85:15) and the detector wavelength was set as 254 nm. For the analysis of atrazine and diuron, the mobile phase was 0.7 mL min^{-1} of methanol and water (V:V = 50:20) and the detector wavelength was set as 220 and 251 nm, respectively.