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Ecotoxicological assessment of surfactants in the aquatic environment: Combined toxicity of docusate sodium with chlorinated pollutants

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ABSTRACT

The toxicity of perfluorinated surfactants perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorobutane sulfonate (PFBS) and PF-656 as well as the sulfosuccinate surfactant docusate sodium has been examined using two bioluminescence inhibition assays based on the marine bacterium *Vibrio fischeri* and the self-luminescent cyanobacterial recombinant strain *Anabaena* CPB4337. We also determined multigenerational toxicity towards the growth of the algae *Pseudokirchneriella subcapitata*. With EC_{50} values in the 43–75 mg/L range, docusate sodium exhibited a higher toxicity towards the three organisms than PFOS, PFOA, PF-656 and PFBS. We investigated the toxicological interactions of the most toxic surfactant, docusate sodium, with two chlorinated compounds, triclosan and 2,4,6-trichlorophenol (TCP), in their binary and ternary mixtures using the method of the combination index based on the median-effect equation. In general, the binary mixture of the chlorinated compounds triclosan and TCP exhibited antagonism, which was stronger for the growth test using *P. subcapitata*. Except for the green alga, the binary mixtures of docusate sodium with TCP or triclosan showed synergism at medium to high effect levels; the synergistic behaviour predominating in the ternary mixture and in the three tested species. This result highlights the potential toxicological risk associated with the co-occurrence of this surfactant with other pollutants.

1. Introduction

The dissemination of anthropogenic pollutants in the aquatic environment takes place either by point-sources associated to the local discharges or from a large variety of activities, the main point-source being the effluents of sewage treatment plants. Xenobiotics are a source of concern not only due to their specific physical and chemical properties, but because they are released in large and increasing quantities and in complex mixtures whose properties are largely unknown. Surfactants are synthetic chemicals used in large amounts in varieties of industrial cleansing processes as well as in consumer products. Spent surfactants, either from domestic or industrial use, reach biological treatment units and, eventually, are discharged to the environment.

Perfluorinated surfactants such as perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA) and their salts find use in formulating paints or cleaning agents as well as in the production of water impermeable products. The environmental concern about these compounds is due to the fact that they are persistent and bioaccumulative. PFOS has been banned in Europe by the Directive 2006/122/EC and has recently been added to Annex B of the Stockholm Convention on Persistent Organic Pollutants. It has been suggested that PFOA can be generated from certain precursors during biological wastewater treatment (Murakami et al., 2009). The potential substitutes to replace PFOS and PFOA are still mainly perfluoroalkyl based surfactants due to the polarity properties given by the carbon–fluorine bond. 3 M Company introduced in 2003 the shorter-chain compound perfluorobutane sulfonate (PFBS) under the trade name 3 M's Novec™. PolyFox PF-656 is a fluorinated and hydroxylated polyether produced by Omnova Solutions Inc. Several companies market products based on sulfosuccinate derivatives which can be an alternative to fluorinated surfactants. Docusate sodium, bis(2-ethylhexyl) sodium sulfosuccinate, is an anionic surfactant, potentially bioaccumulative and widely used in pharmaceutical formulations. Perfluorinated surfactants have been detected in the effluent of wastewater treatment plants at levels of hundreds of nanograms per liter (Loganathan et al., 2007; Guo et al., 2010). In surface water they appear in highly populated and industrialized areas such as Yangtze River for which Jin et al. (2009) reported a median concentration of 4.2 ng/L for PFOS and 5.4 ng/L for PFOA with peaks as high as 298 ng/L (PFOA). In drinking water they have also been frequently reported. Ericson

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et al. (2009) found up to 58.1 ng/L (PFOS), 57.4 ng/L (PFOA) and 69.4 ng/L (PFBS) in municipal drinking water from Catalonia (Spain). The toxicity of PFBS, PF-656 and docusate sodium to aquatic organisms has been seldom reported with no data for aquatic microorganisms prior to this work except a value of 36 mg/L of docusate sodium for a 48 h *Daphnia magna* test attributed to CYTEC Industries and included in the IUCLID Dataset (Carlsson et al., 2006) and a report from NICNAS (2005) indicating for PFBS a EC_{50} value of 5733 mg/L for 96 h algal growth inhibition.

Perfluorinated/sulfosuccinate surfactants may interact with other xenobiotics with an additional cause for concern due to their ability to solubilize non-polar compounds (Haigh, 1996). There are very few studies of the toxicological interactions of these surfactants with other organic compounds and those reported deal mainly with PFOS (Liu et al., 2008). Chlorinated organic pollutants have been a subject of extensive research, many of them having been banned in different regulatory schemes. Of particular concern are chlorophenols such as 2,4,6-trichlorophenol (TCP) which can originate in the disinfection of water with chlorine or chlorinated compounds (Correa et al., 2003). Triclosan, 5-chloro-2-(2,4-dichlorophenoxy)-phenol, is an emergent pollutant widely used in consumer and professional health care products as disinfecting agent. It has been repeatedly reported in natural water and wastewater from the early detection of 50–150 ng/L of Okumura and Nishikawa (1996) to the recent work of Rosal et al. (2010a) who measured an average concentration of 219 ng/L in the effluent of an activated sludge sewage treatment plant.

The objective of this study was to evaluate the individual toxicity of the perfluorinated surfactants PFOS, PFOA, PFBS, PF-656 and docusate sodium towards three aquatic organisms. In addition, we aimed to assess the toxicological interaction of the most toxic of the surfactants, docusate sodium, with two environmentally relevant chlorinated pollutants, TCP and triclosan. For it, we used the method of the combination index (CI)-isobologram equation; a method that we have previously used to assess the nature of interactions of lipid regulators in non-target organisms (Rodea-Palomares et al., 2010).

2. Material and methods

2.1. Materials

Perfluorooctane sulfonate (PFOS) potassium salt (98%) was purchased from Fluka. PFOA (96%), docusate sodium (98%) triclosan (>97%) and TCP (98%), were obtained from Sigma–Aldrich. PFBS (98.2%) and Polyfox 656 (PF-656) were kindly provided by the 3 M Company and Omnova respectively. We avoided the use of solvents and for the cases in which we reached the solubility limit at the pH of the bioassay this value has been stated as lower boundary.

2.2. Toxicity bioassays

The chronic toxicity was determined following the algal growth inhibition test following OECD TG 201 *Pseudokirchneriella subcapitata* open system using 96-well microplates in which the algae was cultured in a total volume of 200 μL. The results showed that nominal and measured exposure concentrations did not show significant deviations Bioassays with the photo-luminescent bacteria *Vibrio fischeri* were performed according to ISO 11348-3 standard protocol (International Organization for Standardization, 2007). This bioassay measures the decrease in bioluminescence induced in the cell metabolism due to the presence of a toxic substance. The incubation period used in this work was 15 min in all cases. The bacterial assay used the commercially available Biofix Lumi test (Macherey–Nagel, Germany) in which the bacterial reagent is supplied freeze-dried (*V. fischeri* NRRL-B 11177), reconstituted and incubated at 3 °C for 5 min before use. The analysis media was 0.34 M NaCl (2% w/v) and tests were performed at 18 °C and the measurements of light were made using a microplate luminometer. The bioassays using the recombinant bioluminescent cyanobacterium *Anabaena* CPB4337 were based on the inhibition of constitutive luminescence caused by the presence of any toxic substance (Rodea-Palomares et al., 2009). *Anabaena* CPB4337 was routinely grown at 28 °C in the light, ca. 65 mmol photons m^2 s^{-1} on a rotary shaker in 50 mL AA/8 supplemented with nitrate (5 mM) in 125 ml Erlenmeyer flasks and 10 mg/mL of neomycin sulphate (Nm). Details are given elsewhere (Rodea-Palomares et al., 2010). The stability of target compounds under chronic bioassay conditions was assessed according to OCDE Guidance (OECD, 2008). In this work, analyses have been performed at the start and at the end of tests lasting 72 h (*P. subcapitata*) for the compounds studied in mixtures. The test has been carried out for the higher concentration and for a concentration near EC_{50} for each compound using an HPLC-Diode Array Liquid Chromatograph as indicated elsewhere. The stability of chemicals in short acute assays was not examined in view of results published elsewhere (Rosal et al., 2010b).

2.3. Median effect and combination index (CI) equations for determining individual and combined toxicities

The response to toxic exposure in the three microorganisms was estimated using the median-effect equation based on the mass-action law as derived by Chou and Talalay (1984):

$$\frac{f_a}{1-f_a} = \left(\frac{D}{EC_{50}}\right)^m \tag{1}$$

where f_a represents the fraction of the population/system affected by a certain dose, D, expressed as concentration of toxicant. EC_{50} is the median effect–dose or the concentration required to inhibit or affect a system by 50% (e.g., 50% inhibition of bioluminescence or growth). The power, m, identifies the shape of the dose–effect relationship curve, that is hyperbolic, sigmoidal and negative sigmoidal if $m = 1$, $m > 1$, and $m < 1$ respectively (Chou, 2006).

The quantification of synergism or antagonism for a combination of a set of n substances (i.e., sodium docusate, triclosan and TCP) is given by a combination index, CI:

$$^n(\mathrm{CI})_x = \sum_{j=1}^{n} \frac{(D)_j}{(D_x)_j} = \sum_{j=1}^{n} \frac{(D_x)_{1-n}\frac{D_j}{\sum_1^n [D]}}{(D_m)_j\left[\frac{(f_{ax})_j}{1-(f_{ax})_j}\right]^{1/mj}} \tag{2}$$

where $^n(\mathrm{CI})_x$ is the combination index for n chemicals at x% inhibition (e.g., bioluminescence/growth inhibition); $(D_x)_{1-n}$ is the sum of the dose of n chemicals that exerts x% inhibition in combination, $D_j/\sum_1^n[D]$ is the proportionality of the dose of each of n chemicals that exerts x% inhibition in combination; and $(D_m)_j$ $\{(f_{ax})_j/[1-(f_{ax})_j]^{1/mj}$ is the dose of each drug alone that exerts x% inhibition. From Eq. (2), CI < 1, CI = 1 and CI > 1 indicates synergism, additive effect and antagonism, respectively.

Combination index for different f_a values can be determined from the preceding equations together with the experimental data from toxicant mixtures (Chou, 2006). The experimental design for sodium docusate-chlorinated compounds combinations was carried out at a fixed constant ratio (1:1) based on the individual EC_{50} values with five levels using a serial dilution factor of 2. Individual compounds and all combinations plus a control were tested in at least three independent experiments with replicate samples performed simultaneously.