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

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

Roberto Rosal[2*], Ismael Rodea-Palomares[1], Karina Boltes[2], Francisca Fernández-Piñas[1], Francisco Leganés[1], Alice Petre[2]

[1] Departamento de Biología, Facultad de Ciencias, Universidad Autónoma de Madrid. 28049 Madrid, Spain.

[2] Departamento de Ingeniería Química, Universidad de Alcalá, E-28871, Alcalá de Henares, Madrid, Spain.

* Corresponding author: roberto.rosal@uah.es

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 *Pseudokirchneriella 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.

Keywords: Combination index; Synergism; Antagonism; Perfluorinated surfactants; Anabaena sp.; Vibrio fischeri; Pseudokirchneriella subcapitata.

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. 3M Company introduced in 2003 the shorter-chain compound perfluorobutane sulfonate (PFBS) under the trade name 3M's Novec^{TM}. 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 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 298 ng/L (PFOA). In drinking water they have also been frequently reported. Ericson 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 (Haig, 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. (2010) 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%), and docusate sodium (98%) triclosan (> 97%), TCP (98%), were obtained from Sigma-Aldrich. PFBS (98.2%) and Polyfox 656 (PF-656) were kindly provided by the 3M 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. Other details are given elsewhere (Rosal et al., 2010b). 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 (ISO, 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 (*Vibrio 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 ºC 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 (*Pseudokirchneriella 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):

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

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 or EC_{50}). 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:

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

where $^{n}(CI)_x$ 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 equation 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 as shown in Table 2. Individual compounds and all combinations plus a control were tested in at least three independent experiments with replicate samples performed simultaneously.

3. Results and discussion

3.1. Toxicity of individual compounds

Table 1 lists the results of toxicity tests for the surfactants and chlorinated compounds tested in this work together with their 95% confidence intervals. Confidence intervals were determined using a linear interpolation method that did not assume any particular dose-effect model (USEPA, 2002). PFOS could be classified as harmful to *Pseudokirchneriella subcapitata* (10 mg/L < EC_{50} <100 mg/L), EC_{50} values were considerably larger for *Anabaena* CPB4337 and, particularly, for *Vibrio fischeri*. Concerning literature data for PFOS, Boudreau et al., (2003) obtained EC_{50} values for the 96 h growth inhibition test on *Pseudokirchneriella subcapitata* of 78.2 mg/L (cell density) and 59.2 mg/L (chlorophyll A), not very different to those reported here although obtained for a different growth time. The toxicity of PFOA was also low, with the lower EC_{50} for *Anabaena* CPB4337. Mulkiewicz et al. (2007) measured a EC_{50} of 571.6 ± 57.5 mg/L for *Vibrio fischeri*, in agreement with our value 524 (505-538) mg/L. No previous data on PFOS and PFOA toxicity is available for cyanobacteria Literature data for technical surfactants are scarce. A report from NICNAS (2005) indicated for PFBS EC_{50} values of 5733 mg/L (96 h growth inhibition) and 5733 mg/L (96 h biomass), both for *Pseudokirchneriella subcapitata*. In our work, we found a much lower toxicity, with only a 37% growth inhibition at 20250 mg/L. For the other microorganisms, the toxicity of PFBS was also very low. For the case of PF-656, luminescence inhibition of *Vibrio fischeri* and *Anabaena* CPB4337 was very low for concentrations < 100 mg/L, but the inhibition of algal growth reached 50% at 43.4 mg/L; so, PF-656 could also be classified as harmful to the green alga. The data showed that the sulfosuccinate docusate sodium exhibited a considerable acute toxicity for all three organisms and could also be classified as "harmful to aquatic organisms"; this is the first report of docusate sodium toxicity to aquatic organisms as no

Table 1. Toxicity of surfactants and chlorinated compounds expressed as EC_{50} values (mg/L) with confidence limits (95% probability) towards *Pseudokirchneriella subcapitata*, *Vibrio fischeri*, and *Anabaena* CPB4337.

	Pseudokirchneriella subcapitata		*Vibrio fischeri (15 min)*		Anabaena CPB4337	
	EC_{50} (mg/L)	C.I. 95%	EC_{50} (mg/L)	C.I. 95%	EC_{50} (mg/L)	C.I. 95%
Docusate sodium	39.5	38.1-40.8	74.5	70.6-77.1	43.0	36.6-50.1
PFOA	96.2	88.6-113.7	524	505-538	72.3	57.96-82.9
PFOS	35.0	34.2-35.5	> 500*	-	143.27	120.3-155.9
PF-656	43.0	41.1-44.9	> 250**	-	> 250***	-
PFBS	> 20250****	-	17520	16850-18200	8386	7752-8693
Triclosan	0.037	0.036-0.038	0.95	0.91-0.99	1.15	0.86-1.46
TCP	0.061	0.058-0.062	18.4	17.5-19.2	0.37	0.32-0.64

* 12% luminescence inhibition at 500 mg/L
** 15% luminescence inhibition at 250 mg/L
*** Luminescence between 100-250% that of the control
**** 37% growth inhibition at 20250 mg/L

toxicity values have been reported in the scientific literature except for the value of 36 mg/L for *Daphnia magna* (Carlsson et al., 2006).

The data for TCP and triclosan are in agreement with previously reported value for *Vibrio fischeri* (Hoffmann et al., 2003), being highly toxic towards *Pseudokirchneriella subcapitata* and *Anabaena* CPB4337. Concerning triclosan we also obtained high toxicity in line with other values reported for *Pseudokirchneriella subcapitata* and *Vibrio fischeri* (Orvos et al., 2002; Tatarazako et al., 2004; de Lorenzo et al., 2008). All toxicants fitted well to the median-effect equation except docusate sodium in *Vibrio fischeri* that clearly deviated from the common sigmoidal or hyperbolic monotonic shape exhibiting a biphasic dose-response curve (Fig. 1). The logarithmic form of Eq. 1 shows a broken line with a turning point at a dose of about 55 mg/L. For the computation of CI (Eq. 2), we used as reference for docusate sodium acting individually the biphasic response as shown in the inset of Fig. 1. We also determined that the inflection is not related to critical micelle concentration, that appears somewhat below 200 mg/L in the presence of 2% NaCl in solution (*Vibrio fischeri* medium).

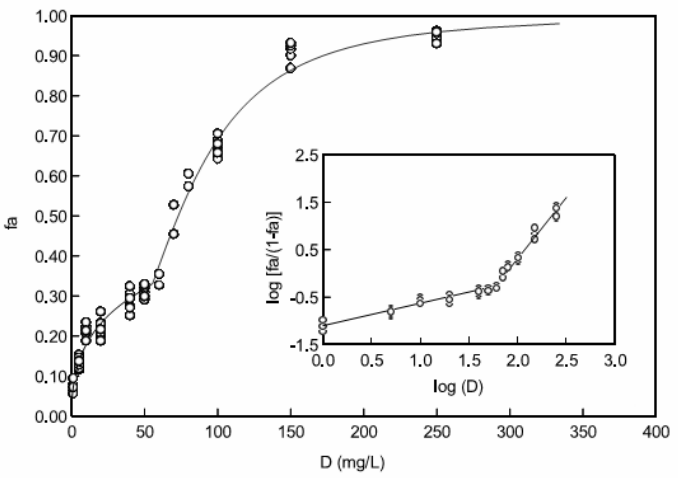

Figure 1. Dose-effect curve and logarithmic median-effect plot for docusate sodium in *Vibrio fischeri* ecotoxicity test (15 min).

3.2. Toxicity of binary and ternary mixtures

From the tested surfactants, the sulfosuccinate surfactant docusate sodium proved to be the most toxic to the three organisms; we used the combination index method to determine the nature of its interaction with TCP and triclosan that were also toxic or very toxic for the three microorganisms tested in this work. Table 2 shows the dose-effect curve parameters from Eq. 1, namely, D_{50}, m and the linear correlation coefficient corresponding to the data in logarithmic form, r (Chou, 2006). These values are listed together with the combination index (CI) for all combinations and three degrees of effect levels (EC_{10}, EC_{50} and EC_{90}). The linear correlation coefficient was > 0.97 in all cases indicating the conformity of the data to the median-effect principle. The data from single toxicants was used to quantify synergism or antagonism by applying sequential deletion analysis as indicated elsewhere (Chou and Martin, 2005). The responses for the three microorganisms are represented in Figs. 2 to 4 as a function of the fraction affected from f_a = 0.05 to 0.95 with explicit indication of their 95% confidence intervals as error bars. Some representative results for every mixture are also shown in Table 2. In the *Pseudokirchneriella subcapitata* test, the binary mixtures containing docusate sodium exhibited a strong antagonism, being their toxicity apparently marked by docusate sodium with low or negligible contribution of TCP and triclosan, compounds individually much more toxic to the alga (Fig. 2). This effect was not observed either for *Anabaena* CPB4337 or for *Vibrio fischeri* for which the difference in individual toxicity of the chlorinated compounds with respect to sodium docusate was less intense. The binary mixture of triclosan and TCP were also antagonistic at all *fa* levels in the alga.

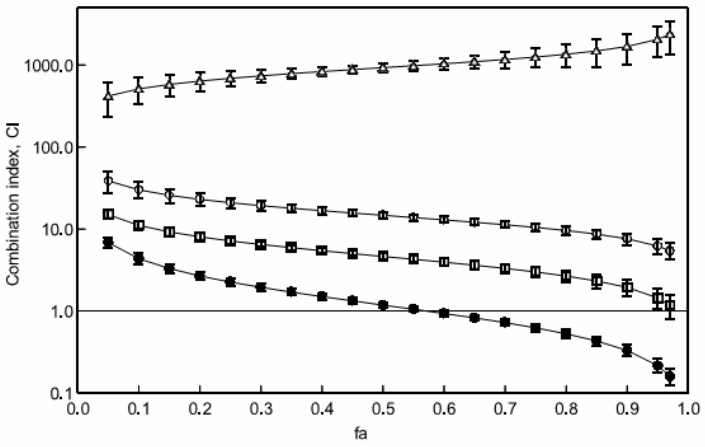

Figure 2. Combination index plot for binary and ternary combinations of docusate sodium, triclosan and TCP for the *Pseudokirchneriella subcapitata* growth test: TCP + triclosan (-□-), docusate + TCP (-Δ-), docusate + triclosan (-○-) and the ternary mixture (-●-). The line at CI = 1 represents additivity.

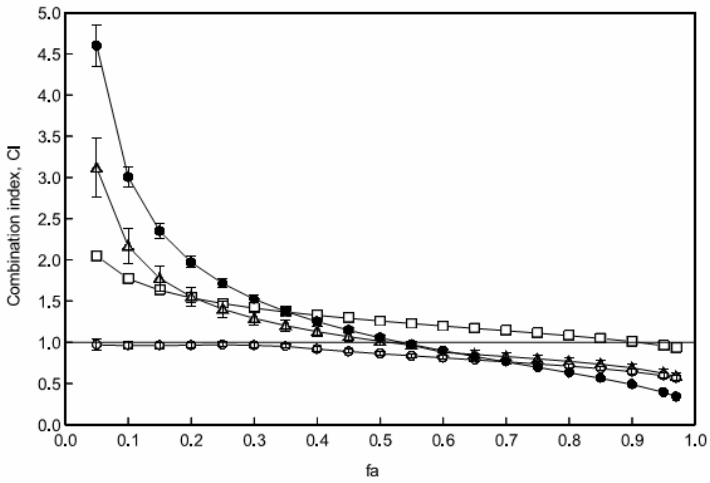

Figure 3. Combination index plot for binary and ternary combinations of docusate sodium, triclosan and TCP for the *Vibrio fischeri* test: docusate + TCP (-Δ-), docusate + triclosan (-○-), TCP + triclosan (-□-) and the ternary mixture (-●-). The line at CI = 1 represents additivity.

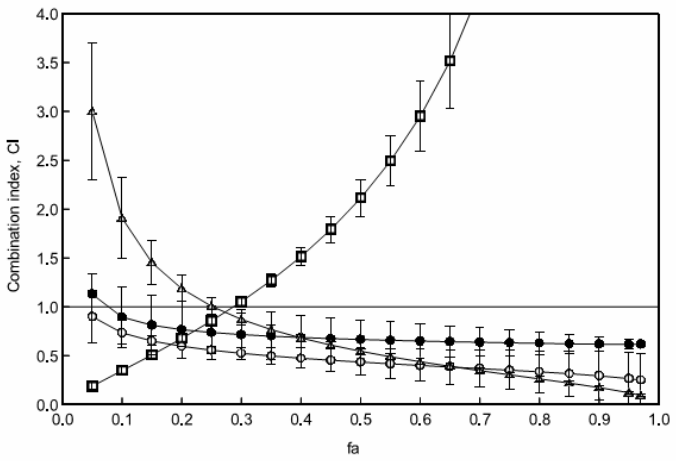

Figure 4. Combination index plot for binary and ternary combinations of docusate sodium, triclosan and TCP for the Anabaena CPB4337 test: docusate + TCP (-Δ-), docusate + triclosan (-○-), TCP + triclosan (-□-) and the ternary mixture (-●-). The line at CI = 1 represents additivity.

The results for *Vibrio fischeri* and *Anabaena* CPB4337 tests exhibited certain similarities. The binary combination of docusate sodium and TCP showed a relatively strong antagonism at low effect levels to approach an additive or synergistic effect at the highest f_a levels. The combination of docusate sodium and triclosan also showed a certain synergism at high f_a values, the same behaviour was observed for the ternary mixture. Except for the strong antagonism exhibited by the binary mixtures of triclosan and TCP at $f_a > 0.3$, the toxicity pattern obtained for *Vibrio fischeri* and *Anabaena* CPB4337 were similar, with a tendency to synergism at higher levels of effect. The presence of sodium docusate in the ternary mixture resulted in a synergistic behaviour for the three organisms. In the case of the green alga, synergism predominated only at high *fa* levels (*fa* > 0.6); in *Vibrio fischeri*, synergism was found at *fa* levels greater than 0.5 (Fig. 3) whilst in *Anabaena* CPB4337, synergism of the ternary mixture was evident almost throughout the whole *fa* range as shown in Fig. 4. A correlation analysis between CI values suggests that the binary interactions of mixtures containing docusate sodium predominated in the three component mixture both for *Anabaena* CPB4337 and *Vibrio fischeri*. For the case of *Pseudokirchneriella subcapitata* the result was opposite, with a higher correlation with the mixture of triclosan and TCP.

Risk assessment tools are usually based on single component data, a procedure which may lead to a

Table 2. Dose-effect relationship parameters and mean combination index (CI) values of docusate sodium, triclosan and TCP in their binary and ternary combinations for *Pseudokirchneriella subcapitata*, *Vibrio fischeri* and *Anabaena* CPB4337 tests. (Synergism emphasized in bold).

			Anabaena CPB4337			
	Dose effect parameters			*CI values*		
	EC_{50} *(mg/L)*	*m*	*r*	EC_{10}	EC_{50}	EC_{90}
Docusate+Triclosan	12.7	4.85	0.987	**0.73 ± 0.12**	**0.43 ± 0.11**	**0.29 ± 0.24**
Docusate+TCP	8.54	3.44	0.971	1.53 ± 0.59	**0.47 ± 0.10**	**0.17 ± 0.01**
Triclosan+TCP	1.65	0.72	0.975	**0.34 ± 0.04**	2.11 ± 0.18	12.8 ± 3.76
Docusate+Triclosan+TCP	7.87	2.09	0.974	0.89 ± 0.30	**0.66 ± 0.19**	**0.61 ± 0.07**
			Pseudokirchneriella subacpitata			
	Dose effect parameters			*CI values*		
	EC_{50} *(mg/L)*	*m*	*r*	EC_{10}	EC_{50}	EC_{90}
Docusate+Triclosan	39.9	1.32	0.980	30.3 ± 6.9	14.7 ± 1.4	7.6 ± 1.2
Docusate+TCP	46.2	0.86	0.997	523 ± 209	933 ± 114	1724 ± 768
Triclosan+TCP	0.23	2.01	0.975	11.1 ± 1.9	4.60 ± 0.36	1.92 ± 0.43
Docusate+Triclosan+TCP	0.54	3.00	0.972	4.3± 0.7	1.17± 0.09	**0.33 ± 0.05**
			Vibrio fischeri			
	Dose effect parameters			*CI values*		
	EC_{50} *(mg/L)*	*m*	*r*	EC_{10}	EC_{50}	EC_{90}
Docusate+Triclosan	6.85	3.15	0.978	2.16 ± 0.16	1.01 ± 0.05	**0.69 ± 0.03**
Docusate+TCP	22.5	1.13	0.990	0.96 ± 0.06	1.01± 0.03	**0.64 ± 0.06**
Triclosan+TCP	8.56	2.41	0.991	1.77 ± 0.05	1.26 ± 0.02	1.01 ± 0.02
Docusate+Triclosan+TCP	12.8	4.46	0.987	3.00 ± 0.13	1.05 ± 0.03	**0.52 ± 0.02**

The parameters *m*, EC_{50} and *r* are the antilog of *x*-intercept, the slope and the linear correlation coefficient of the median-effect plot, which signifies the shape of the dose-effect curve, the potency (EC_{50}), and conformity of the data to the mass-action law, respectively (Chou and Talalay, 1984; Chou, 2006).EC_{50} and *m* are used for calculating the CI values (equation 3); CI < 1, CI = 1, and CI > 1 indicate synergism (Syn), additive effect (Add), and antagonism (Ant), respectively. EC_{10}, EC_{50} and EC_{90}, are the doses required to reach a response inhibition of 10%, 50% and 90%, respectively.

misestimation of the actual risk associated with complex mixtures. The classic models for the prediction of mixture toxicity, concentration addition and independent action, are based on simple assumptions on the mode of toxic action (Teuschler, 2007). The idea is misleading as the mode of action have proved irrelevant after being demonstrated that toxicological interactions, namely synergism or antagonism, can occur irrespective of the primary mode of action (Chou, 2006). Moreover, the mode of action of a substance as toxic in environmentally relevant conditions is largely unknown and different for different organisms and trophic levels. Cleuvers (2003) noted that, even for substances such as drugs, whose biochemical mechanism is known, the action in a non-target organism, once released in the environments is essentially unknown. Liu et al (2008) have reported that PFOS may increase the membrane fluidity and permeability to hydrophobic substances. If this was the case, a synergistic effect would be expected as general rule in binary mixtures PFOS-organic compounds; however, Liu et al. (2009) reported a synergistic effect PFOS-PCP but antagonism in the binary mixtures PFOS-diuron and PFOS- atrazine in the green alga *Scenedesmus obliquus*; the authors indicated that PFOS may differentially affect the toxicity of structurally different compounds stressing the effect of hydrophobicity between the compounds they tested. In this work we present data on the interactive effects of one surfactant, docusate sodium whose toxicity to aquatic organisms has been tested for the first time in this work, with two chlorinated compounds; we have made binary and, ternary mixtures, finding that particularly in the ternary mixture, docusate sodium increased the toxicity of both chlorinated compounds in the three tested organisms with the difference that synergism was present in a wider range of effect levels in prokaryotes (particularly *Anabaena* CPB4337) than in the eukaryotic green alga; the observed synergism indicated a potential toxicological risk associated with the co-existence of docusate sodium and other organic pollutants in aquatic environments. It has been stated that the interaction of surfactants and chemicals, including other surfactants, affects different functions and multiple cellular response targets. Such interaction generates a complex cascade of events in biological systems that cannot be summarized in a simple pattern (Wei et al., 2009). As a consequence, synergism or antagonism may occur independently of a similar or dissimilar mode of action and, with the current knowledge of toxicity mechanisms cannot be predicted. On the other hand, ecotoxicity studies conducted on several species and trophic levels may show a completely different response to the same toxicant mixture. Moreover, the nature of the interaction may depend on the effect level, but we found is a general tendency of mixtures to exhibit synergistic responses as they become more complex. In a preceding work we proved that mixtures of several fibrates in wastewater showed a synergistic behaviour practically all over the f_a range, while the same compounds in pure water were antagonistic in binary mixtures (Rodea-Palomares et al., 2010).

4. Conclusions

We derived the median effect dose of the perfluorinated surfactants PFOS and PFOA as well as those of several alternative surfactants that including docusate sodium and the fluorinated substances 3M's PF-656 and PFBS. Docusate sodium showed the highest toxicity to *Anabaena* CPB4337 and *Vibrio fischeri* and exhibited a similar toxicity than PFOS and PF-656 towards algal growth. Both PFOS and PF-656 were, however, not toxic to *Anabaena* CPB4337 and *Vibrio fischeri.* PFBS exhibited significant toxicity only towards the algae *Pseudokirchneriella subcapitata***.** The data indicated that docusate sodium can be classified as "harmful to aquatic organisms" for the three species tested in this work. The chlorinated compounds triclosan and TCP were highly toxic to *Pseudokirchneriella subcapitata.*

The application of the combination index (CI) isobologram method to mixtures of docusate, triclosan and TCP, showed that they behaved antagonistically for most binary mixtures that turned into synergistic in ternary mixtures for the three organisms and at least for a certain range of effect. For *Anabaena* CPB4337, also the mixtures of docusate sodium and triclosan were synergistic all over the range of effect levels. The toxicity pattern obtained for *Vibrio fischeri* and *Anabaena* tests were similar due most probably to the prokaryotic nature of both. The increased toxicity of the ternary mixtures containing sodium docusate suggested a potential risk associated to the co-occurrence of this surfactant with other xenobiotics in the same environments that needs further research. This displacement from antagonistic to synergistic behaviour in complex mixtures may led to an important underestimation of mixture toxicity when assuming additive behaviour from single component data.

Acknowledgements

The research was funded by the Spanish Ministry of Education through grant CSD2006-00044 and the Comunidad de Madrid, grants 0505/AMB-0395, 0505/MB/0321 and S-2009/AMB/1511.

References

Boudreau, T.M., Sibley, P.K., Mabury, S.A., Muir, D.C.G., Solomon, K.R., 2003. Laboratory evaluation of the toxicity of perfluorooctane sulfonate (PFOS) on *Selenastrum capricornutum*, *Chlorella vulgaris*, *Lemna gibba*, *Daphnia magna* and *Daphnia pulicaria*. Arch. Environ. Contam. Toxicol. 44, 307–313.

Carlsson, C, Johansson, A.K., Alvan, G., Bergman, K., Kühler, T., 2009. Are pharmaceuticals potent environmental pollutants? Part II: Environmental risk assessments of Perfluorocarboxylates. Environ. Sci. Technol. 43, 5565–5576.

Chambliss, W.G., Cleary, R.W., Fischer, R., Jones, A.B., Skierkowski, P., Nicholes, W., Kibbe, A.H., 1981. Effect of docusate sodium on drug release from a controlled-release dosage form. J Pharm Sci. 70, 1248–1251.

Chou, T.C., 2006. Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. Pharmacol. Rev. 58, 621–681.

Chou, T.C., Talalay, P., 1984. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. Adv. Enzyme Regul. 22, 27–55.

Chou, T.C., Martin, N., 2005. CompuSyn for Drug Combinations: PC Software and User's Guide: A Computer Program for Quantification of Synergism and Antagonism in Drug Combinations and the Determination of IC_{50} and ED_{50} and LD_{50} Values. ComboSyn, Inc., Paramus, NJ.

Cleuvers, M., 2003. Aquatic ecotoxicity of pharmaceuticals including the assessment of combination effects. Toxicol. Lett. 142, 185–194.

Correa, J., Domínguez, V.M., Martínez, M., Vidal, G., 2003. Aerobic degradation of 2,4,6-TCP content in ECF bleached effluent. Environ. Int. 29, 459–465.

DeLorenzo, M.E., Keller, J.M., Arthur, C.D., Finnegan, M.C., Harper, H.E., Winder, V.L., Zdankiewicz, D.L., 2008. Toxicity of the Antimicrobial Compound Triclosan and Formation of the Metabolite Methyl-Triclosan in Estuarine Systems. Environ. Tox. 23, 224–232.

Ericson, I., Domingo, J.L., Nadal, M., Bigas, E., Llebaria, X., van Bavel, B., Lindström, G., 2009. Levels of Perfluorinated Chemicals in Municipal Drinking Water from Catalonia, Spain: Public Health Implications. Arch. Environ. Contam. Toxicol. 57, 631–638.

Guo, R., Sim, W.J., Lee, E.S., Lee, J.H., Oh, J.E., 2010. Evaluation of the fate of perfluoroalkyl compounds in wastewater treatment plants. Water Res., 44, 3476–3486.

Haigh, S.D., 1996. A review of the interaction of surfactants with organic contaminants in soil. Sci. Total Environ. 185, 161–170.

Hoffmann, C., Sales, D., Christofi, N., 2003. Combination ecotoxicity and testing of common chemical discharges to sewer using the Vibrio fischeri luminescence bioassay. Int. Microbiol. 6, 41–47.

International Organization for Standardization, 2007. Water quality-determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test). ISO 11348-3 revised version, Geneva, Switzerland.

Jin,Y.E., Liu, W., Sato, I., Nakayama, S.F., Sasaki, K., Saito, N., Tsuda, S., 2009. PFOS and PFOA in environmental and tap water in China. Chemosphere 77, 605–611.

Liu, W., Chen, S., Quan, X., Jin, Y.H., 2008. Toxic effect of serial perfluorosulfonic and perfluorocarboxylic acids on the membrane system of a freshwater alga measured by flow cytometry, Environ. Toxicol. Chem. 27, 1597–1604.

Liu, W., Zhang, Y.B., Quan, X., Jin, Y.H., Chen, S., 2009. Effect of perfluorooctane sulfonate on toxicity and cell uptake of other compounds with different hydrophobicity in green alga. Chemosphere 75, 405–409.

Loganathan, B.G., Sajwan, K.S., Sinclair, E., Kumar, K.S., Kannan, K., 2007. Perfluoroalkyl sulfonates and perfluorocarboxylates in two wastewater treatment facilities in Kentucky and Georgia. Water Res., 41, 4611–4620.

Mulkiewicz, E., Jastorff, B., Składanowski, A.C., Kleszczynski, K., Stepnowski, P., 2007. Evaluation of the acute toxicity of perfluorinated carboxylic acids using eukaryotic cell lines, bacteria and enzymatic assays. Environ. Toxicol. Pharmacol. 23, 279–285.

Murakami, M., Shinohara, H., Takada, H., 2009. Evaluation of wastewater and street runoff as sources of perfluorinated surfactants (PFSs). Chemosphere 74, 487–493.

National Industrial Chemicals Notification and Assessment Scheme (NICNAS), 2005. Potassium perfluorobutane sulfonate report, ISBN 0–9758470–07.

Okumura T, Nishikawa Y., 1996. Gas chromatography-mass spectrometry determination of triclosans in water sediment and fish samples via methylation with diazomethane. Analytica Chimica Acta 325, 175–184.

OECD, 2008. Guidelines for the Testing of Chemicals, No. 23: Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures, PDF Edition (ISSN 1607-310X), 18th Addendu.

Orvos, D.R., Versteeg, D.J., Inauen, J., Capdevielle, M., Rothenstein, A., Cunningham, V., 2002. Aquatic Toxicity of Triclosan. Environ. Toxicol. Chem. 21, 1338–1349.

Rodea–Palomares, I., González-García, C., Leganés, F., Fernández-Piñas, F., 2009. Effect of pH, EDTA, and anions on heavy metal toxicity toward a bioluminescent cyanobacterial bioreporter. Arch. Environ. Contam. Toxicol. 57, 477–487.

Rodea-Palomares, I., Petre, A.L., Boltes, K., Leganés, F., Perdigón-Melón, J.A., Rosal, R., Fernández-Piñas, F., 2010. Application of the combination index (CI)–isobologram equation to study the toxicological interactions of lipid regulators in two aquatic bioluminescent organisms. Water Res. 44, 427–438.

Rosal, R., Rodríguez, A., Perdigón-Melón, J.A., Petre, A., García-Calvo, E., Gómez, M.J., Agüera, A., Fernández-Alba, A.R., 2010a. Occurrence of emerging pollutants in urban wastewater and their removal through biological treatment followed by ozonation. Water Res., 44, 578–588.

Rosal, R., Rodea-Palomares, I., Boltes, K., Fernández-Piñas, F., Leganés, F., Gonzalo, S., Petre, A, 2010b. Ecotoxicity assessment of lipid regulators in water and biologically treated wastewater using three aquatic organisms, Environ. Sci. Pollut. Res., 17(1), 135-144.

Tatarazako, N., Ishibashi, H., Teshima, K., Kishi, K., Arizono, K., 2004. Effects of Triclosan on arious Aquatic Organsims. Environ. Sci., 11, 133–140.

Teuschler, L.K., 2007. Deciding which chemical mixtures risk assessment methods work best for what mixtures. Toxicol. Appl. Pharmacol. 223, 139–147.

USEPA, 2002. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, 5th Edition. US Environmental Protection Agency, Washington DC EPA-812-R02-012.

Wei, Y., Shi, X., Zhang, H., Wang, J., Zhou, B., Dai, J., 2009. Combined effects of polyfluorinated and perfluorinated compounds on primary cultured hepatocytes from rare minnow (*Gobiocypris rarus*) using toxicogenomic analysis. Aquat. Toxicol. 95, 27–36.