Bull. Environ. Contam. Toxicol. (1995) 55:179–186

Environmental Contamination and Toxicology

Acute Toxicity of Some Synthetic Cationic and Zwitterionic Surfactants to Freshwater Amphipod *Echinogammarus tibaldii*

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Received: 1 July 1994/Accepted: 14 January 1994

Quaternary ammonium compounds (QACs) are an important class of industrial chemicals with a wide range of commercial and consumer uses as detergents, antistatic and softening agents, biocides, germicides, deodorizers and emulsifiers. These substances are now increasingly used all over the world and their annual rates of production and consumption are considerable.

Data from scientific literature point out their potential threat towards many aquatic organisms, acute toxicity ranging in the order of mg/L and lower (Boethling 1984). It is possible that, as stated for cationic polyelectrolites by Biesinger et al. (1976) and Biesinger and Stokes (1986), QACs may act on aquatic organisms by altering the gill membranes and thus interfering with respiratory process and ionic balance.

The study of Neufahrt et al. (1978) on the uptake and tissue distribution of $^{14}CDA_{18}DMAC$ (dioctadecyldimethylammonium chloride) mixed with food, pointed out that this radiolabeled surfactant was concentrated, in the highest degree, in the intestinal tract and gall bladder of common carp (*Cyprinus carpio*). Knezovich et al. (1989) showed that, in three aquatic species exposed to sublethal water concentration of ^{14}C labeled cetylpyridinium bromide (CPBr), radiolabel was mainly concentrated in the gills, thus suggesting a low bioaccumulation potential.

The length of the hydrophobic chain and the presence and position of particular chemical groups seems to be of great significance for the toxicity of anionic and nonionic surfactants (Gafa 1974; Kimerle et al. 1977; Macek and Krzeminski 1975). However, much less information on the influence of the structure on toxicity is available for cationic and zwitterionic surfactants.

The following study was therefore undertaken to evaluate the effect of molecular structure changes on the acute toxicity of cationic and zwitterionic surfactants to *Echinogammarus tibaldii*, a benthic shredder amphipod with an important role in the detritus chain of rivers and streams.

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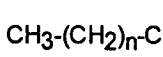
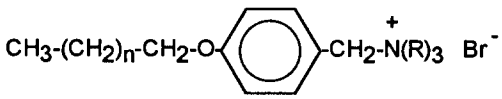
MATERIALS AND METHODS

Cationic ammonium surfactants selected for testing were different as regards chain length:



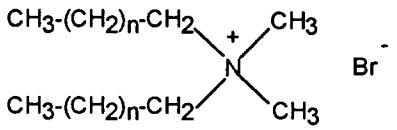
n = 10, dodecyltrimethylammonium bromide (DTABr)
n = 12, tetradecyltrimethylammonium bromide (TTABr)
n = 14, cetyltrimethylammonium bromide (CTABr)

and, compared with these, to hydrocarbon chain type and length:



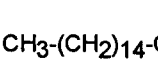
n = 6, R = Me, p-octyloxybenzyltrimethylammonium bromide (pOOTABr)
n = 10, R = Me, p-dodecyloxybenzyltrimethylammonium bromide (pDOTABr)
n = 6, R = n-Bu, p-octyloxybenzyltributylammonium bromide (pOOTBABr),

number and length of hydrocarbon tails:

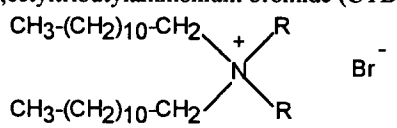


n = 10, didodecyldimethylammonium bromide (DDDABr)
n = 12, ditetradecyldimethylammonium bromide (DDTABr),

head group hydrophobicity:

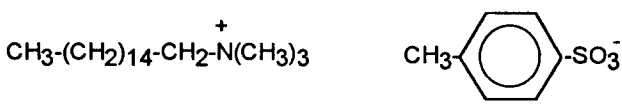


R=Et, cetyltriethylammonium bromide (CTEABr)
R=n-Pr,cetyltripropylammonium bromide (CTPABr)
R=n-Bu,cetyltributylammonium bromide (CTBABr)



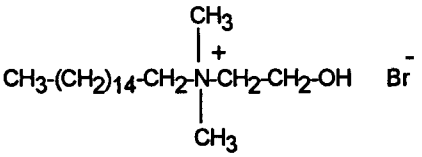
R=Et, didodecyldiethylammonium bromide (DDDEABr)
R=n-Pr, didodecyldipropylammonium bromide (DDDPABr)
R=n-Bu, didodecyldibutylammonium bromide (DDDBABr)

and to counterion type, by substituting a more hydrophobic toluene-p-sulphonate to bromide:

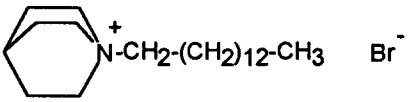


cetyltrimethylammonium toluene-p-sulphonate (CTATos).

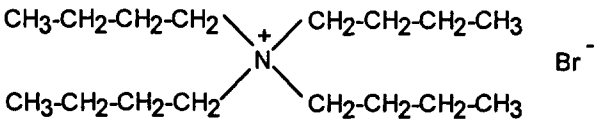
In addition we have considered (i) cetyldimethylethanolammonium bromide (CDEABr) in which one methyl group of CTABr is substituted with an ethanolic residue:



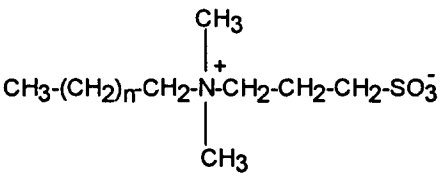
(ii) tetradecylquinuclidinium bromide (TDQBr) having a head group like CTPABr with alkyl groups bound together, to form a quinuclidinium ring:



and (iii) tetrabutylammonium bromide (TBABr), a substance with no surfactant properties owing to the presence of four butyl groups bound to ammonium:



We have furthermore investigated the acute toxicity of some zwitterionic surfactants, sulphobetaines, in order to evaluate the effect that the charge variation of the head group may exert on acute toxicity to *E. tibaldii*. These surfactants, uncharged on the whole, have on the head group a positive charged quaternary ammonium and a negative charged sulphonate group, with interposed three methylene groups:



n = 10, dodecyldimethylammonium propanesulphonate (C12BS)
n = 12, tetradecyldimethylammonium propanesulphonate (C14BS)
n = 14, cetyldimethylammonium propanesulphonate (C16BS).

Some surfactants, purchased from Fluka (DTABr, CTABr, C12BS, C14BS, C16BS), Sigma (TTABr) and Aldrich (TBABr), were purified by crystallization (Germani 1987; Di Profio 1990), all the others were synthesized and purified by following published procedures (Bunton and Ionescu 1973; Bunton et al. 1978; Cipiciani et al. 1987; Germani 1987; Bacaloglu et al. 1988; Germani et al. 1989). Purity control was performed, for single chain surfactants, by surface tension measurements and for twin-chain by potentiometric titration of counterions. All surfactants were ≥ 99% pure.

Organisms of the species *E. tibaldii* Pink & Stock (Crustacea, Amphipoda) were collected near the spring of the river Vera (L'Aquila, Italy), a high quality natural environment with a water chemistry which does not reveal the presence of any xenobiotic substance (Ghetti et al. 1989). The animals were screened from mayor detritus, transported to the laboratory under continuous aeration and held for acclimation in cool, aerated water in 20-L aquaria. Mature adult male individuals obtained from precopula pairs and kept for about 3 d in cool "reconstituted water" (Organization for Economic Cooperation and Development 1981),

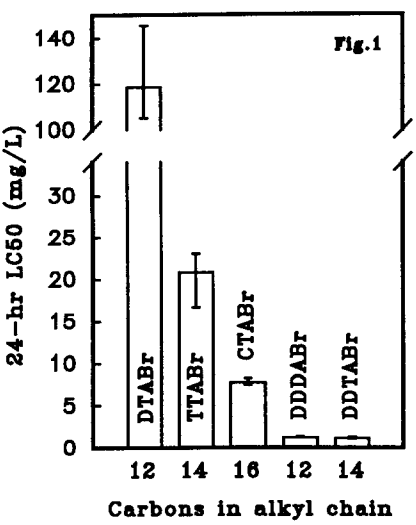
received artificial oxygenation and were fed on dry poplar leaves previously soaked in spring water in order to enrich them with fungi and bacteria. Feeding was suspended 24 hr before experiments. "Reconstituted water" hardness and alkalinity were 240 mg/L and 55 mg/L as $CaCO_3$, respectively, and pH was 7.9±0.5. After range finding preliminary tests, stock solutions of the chemicals were prepared in order to obtain six concentrations at the same common logarithmic intervals, plus the control, and the definitive tests were carried out in duplicate by adding 20 animals in each 1-L glass jar containing 250 mL of each precooled and aerated solution. Those individuals which after 24 hr showed no movements of the pleopodes when gently prodded with a spatula, were considered dead. During the test, carried out in static conditions and without oxygenation, the animals were not fed, and like in all handling and rearing operations, the temperature was kept at 8 ± 0.5°C. Experimental data were analysed through probits (Finney 1971) and the LC50 values were expressed in mg/L.

RESULTS AND DISCUSSION

Data in Table 1 make it possible to underline that many QACs we studied are potentially very toxic to *E. tibaldii*, having 24-hr LC50 values lower than 10 mg/L and, in some cases, 1 mg/L. TBABr, a molecule without long hydrocarbon chains and therefore without surfactant properties, is virtually atoxic (LC50>2 g/L).

Table 1. Values of 24-hr LC50 (mg/L) with 95% confidence intervals (95% C.I.) and probit equations of the cationic and zwitterionic surfactants to *E. tibaldii*.
Significant value of $\chi^2 = 9.488$ ($p \leq 0.05$). x= surfactant concentration (mg/L).

Substance	LC50 (95% C.I.)	Probit equation	χ^2
TBABr	>2000		
DTABr	118 (105-145)	y=4.57 logx-4.47	2.293
TTABr	21 (17-23)	y=4.45 logx-0.86	2.538
CTABr	7.7 (7.4-8.2)	y=8.52 logx-2.57	1.214
CTEABr	3.8 (3.5-4.1)	y=7.89 logx+0.40	7.215
CTPABr	2.1 (1.9-2.3)	y=5.04 logx+3.34	1.390
CTBABr	0.50 (0.46-0.55)	y=5.18 logx+6.57	2.282
DDDABr	1.2 (1.2-1.3)	y=9.42 logx+4.16	1.539
DDDEABr	0.66 (0.58-0.72)	y=4.30 logx+5.77	1.605
DDDPABr	0.69 (0.65-0.73)	y=8.00 logx+6.28	3.190
DDDBABr	0.96 (0.90-0.97)	y=11.2 logx+5.33	7.112
DDTABr	1.1 (1.0-1.3)	y=4.58 logx+4.73	5.926
pOOTABr	147 (136-166)	y=4.62 logx-5.02	2.140
pDOTABr	5.1 (4.8-5.4)	y=8.41 logx-0.98	1.240
pOOTBABr	4.0 (3.5-4.8)	y=2.74 logx+3.33	5.492
C12BS	431 (423-437)	y=31.4 logx-77.8	5.638
C14BS	51 (50-52)	y=21.6 logx-32.0	2.825
C16BS	7.4 (7.1-7.8)	y=9.23 logx-3.04	5.997
CTATos	5.4 (5.1-5.8)	y=7.51 logx-0.53	1.691
CDEABr	7.7 (7.2-8.4)	y=6.16 logx-0.48	6.205
TDQBr	7.5 (6.9-8.0)	y=6.47 logx-0.66	3.676



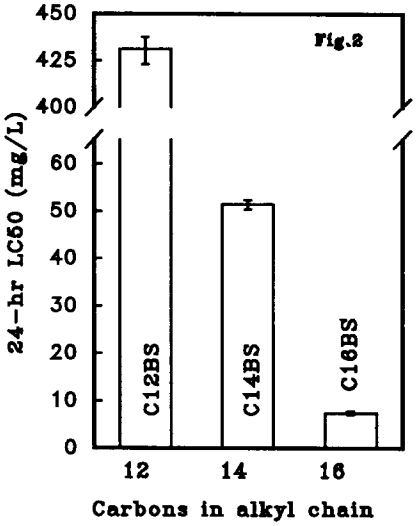


Figure 1. Acute toxicity of cationic single and twin-chain surfactants vs hydrocarbon chain length. Vertical bars indicate 95% C.I.

Figure 2. Acute toxicity of zwitterionic surfactants vs hydrocarbon chain length.Vertical bars indicate 95% C.I.

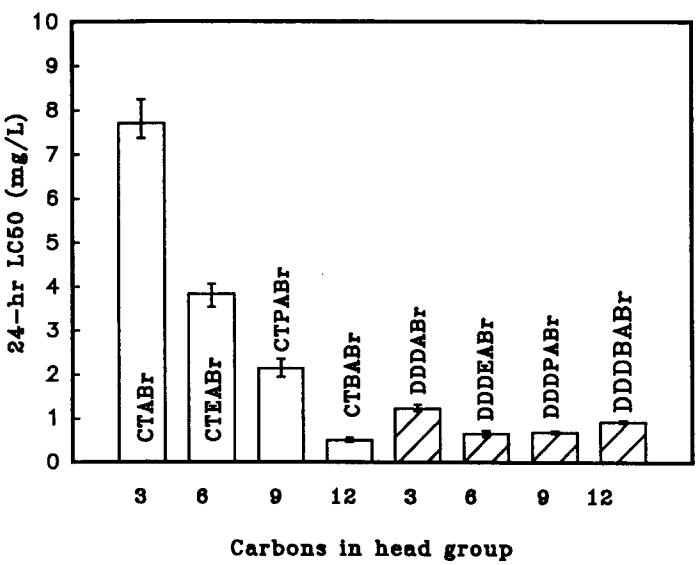


Figure 3. Acute toxicity of cationic single and twin-chain surfactants vs head group size.
Empty= single-chain surfactants.
Diagonal = twin-chain surfactants.
Vertical bars indicate 95% C.I.

These results allow us to correlate structural characteristics of monomeric surfactants with their acute toxicity:

1. Acute toxicity (mg/L) increases with increasing hydrocarbon chain length both for cationic and zwitterionic single-chain surfactants (Figure 1 and 2):

DTABr (118) TTABr (21) CTABr (7.7)
C12BS (431) C14BS (51) C16BS (7.4).

2. For single-chain surfactants, which are forming in water classical micelles, an increase of head group size and hydrophobicity clearly increases toxicity (Figure 3):

CTABr (7.7) CTEABr (3.8) CTPABr (2.1) CTBABr (0.50).

However if only one methyl group is substituted with a small hydrophilic residue as an ethanolic residue, the toxicity remains unchanged.

3. Twin-chain surfactants, which usually form vesicles or bilayers in water solutions, are much more toxic compared with single-chain surfactants (Figure 1).

4. Increasing the dimension of head group in twin-chain surfactants does not seem to affect significantly their toxicity (Figure 3).

5. If the counterion of CTABr is changed by substituing bromide with the more "hydrophobic" toluene-p-sulphonate, the LC50 value changes from 7.7 to 5.4 mg/L.

In order to obtain further information about the effect produced by hydrocarbon chain length and head group size, we studied pOOTABr, a surfactant with a benzyl group and an ether function in the hydrophobic portion of the molecule, and, moreover, two other surfactants: pOOTBABr and pDOTABr. The former is similar to pOOTABr, but the dimension of its head group is greater, since it has three butyl groups instead of three methyl groups; the latter has an hydrocarbon chain of twelve methylene groups compared with eight groups chain of pOOTABr. The LC50 values are 4.0 mg/L for pOOTBABr and 5.1 mg/L for pDOTABr compared with 147 mg/L for pOOTABr. The hydrophobic tails of TDQBr and TTABr are of the same length, but the former has a more hydrophobic head group like CTEABr or CTPABr: the LC50 value of TDQBr (7.5 mg/L) is lower than TTABr value (21 mg/L), but higher than CTEABr (3.8 mg/L) and CTPABr (2.1 mg/L).

These findings indicate that hydrophobic chain length and head group dimension affect the acute toxicity of cationic and zwitterionic surfactants. For single-chain surfactants, toxicity increases as a result of an increase in alkyl chain length and head group hydrophobicity; in contrast, the LC50 values for twin-chain surfactants are considerably lower, and changes in their molecular structure do not apparently affect these values in a significant way. The increase of toxicity with increasing chain length also occurs in LAS (Linear Alkyl Sulphonate), a class of anionic surfactants largely used for domestic purposes. A LAS molecule seems to be toxic to the highest degree when its hydrocarbon tail has 16 C, being therefore sufficiently long to interact in the best way with the cellular membrane. When the tail is longer, the toxicity decreases again owing to possible folding of this portion of the molecule, no more able to effectively pass through the cellular membrane (Lundhal and Cabridenc 1978).

Comparison of data of cationic versus zwitterionic single-chain surfactants makes it possible to underline that, the length of the hydrophobic chain being the same, the "positive" effect due to amphiionic head group on the compounds with a tail of 12 C is cancelled in those with 16 C. The zwitterionic / cationic LC50 ratio is in fact about 3.5 for

dodecyl, 2.5 for tetradecyl and 1 for esadecyl surfactants. It follows that also small structural variations could modify the toxicity of these substances; in the same way, the combined action of the structural modifications as a whole may modulate the above toxicity.

Our results, in accordance with those from the literature, clearly point out that QACs may represent, under certain conditions, a threat to animal and vegetal populations of a water body when present in a significant concentration. We must take into account, however, that under natural conditions the fate and therefore the actual toxicity of pollutants, besides of their own toxicity, depends on many other factors. As regard the surfactants, biodegradation seems to be very important; these substances are in fact more or less rapidly degraded by naturally occurring bacteria according to their molecular structure and to bacterial populations present in a water body (Ruiz Cruz 1979). Last but not least, another factor which must be taken into account in toxicity screening of QACs, is the adsorption by negative charged organic and inorganic suspended particles, which can lead to a dramatic decrease of their actual concentration, particularly for cationic surfactants, under natural conditions, and ultimately to their substantial detoxification.

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