FATE OF CATIONIC SURFACANTS IN THE MARINE ENVIRONMENT, I.
BIOCONCENTRATION OF LONG-CHAIN ALKYLNITRILES AND TRIALKYLAMINES

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ABSTRACT
Long-chain alkynitriles ($C_{n}H_{2n+1}CN$, where $n=13, 15, 17$) (LANs) and long-chain-trialkylamines ($NC_{m}H_{2m-1}C_{n}H_{2n+1}C_{j}H_{2j}$, where $m,n = 1, 14, 16, 18$ and $j= 14, 16, 18$) (TAMs) were identified for the first time in biota samples at concentrations ranging from 9-574 ng g$^{-1}$ and from 157 to 825 (fresh wt. basis), respectively. In addition, the simultaneous occurrence in the aquatic environment of LANs and TAMs were also reported for the first time. The $K_{b}$ values of both, LANs and TAMs varied according to the different species but their were comparable to those found for PAHs.

INTRODUCTION
Ecotoxicological implications of dumping large amounts of urban wastes into the sea is an issue of major concern, since many deleterious effects to inhabiting organisms have been reported$^1$. However, knowledge of the bioavailability of those contaminants associated to urban wastes is rather limited and prediction models of different levels of complexity have been outlined$^2$-$^3$. Accuracy of those has been largely assessed for polycyclic aromatic hydrocarbons (PAHs) and polychlorinated compounds (OCs), but similar approach has hitherto not been carried out for surfactants and related products despite their abundance$^4$-$^5$, because ionized forms of organic compounds are less able to penetrate biological membranes than the non-ionized forms$^6$. In this regard, we have already identified the linear alkylbenzenes (LABs) in pelagic fishes$^7$, those derived from anionic surfactant residues$^8$. Conversely, the linear alkylbenzene sulphonates (LASs) counterparts have not been found in biological tissues, but have been identified in other environmental compartments$^9$.

Until very recently, the occurrence of cationic surfactants in the environment has not been addressed$^{10}$, although they account for 10% of the total industrial output. Particularly, we have proposed the long-chain alkylamines (TAMs) ($CH_{3}NR_{1}R_{2}$ where $R_{1},R_{2}=C_{14}-C_{18}$) as conservative tracers of this class of surfactants because they have been found widely distributed in the aquatic environment but their ecotoxicological implications still remain unknown$^{11}$.
Consequently, the aim of this work is to assess the bioconcentration of the tracers of cationic surfactants, namely TAMs and long-chain alkylnitriles (LANs) (RCN, where R=C_{13-17} saturated and monounsaturated) which are intermediates in their production and all of them are present as impurities in surfactant formulations. Accordingly, water, benthic and pelagic biota and sediment samples were collected in coastal areas and near a dumping site, all of them were examined for those tracers.

**EXPERIMENTAL**

Sampling Strategy
Samples were collected in coastal areas off Barcelona (Fig. 1) located in front of Besos river mouth (station 1), at the vicinity of Barcelona city sewage disposal site (station 3), close to wastewater outfall (station 4) and a transect parallel to the coast of Barcelona city (station 2).

![Fig. 1. Sampling site locations.](image)

Reagents
All solvents were pesticide grade (SDS, Peypin, France). Silica, neutral alumina (70-230 mesh) and analytical grade sodium hydroxide were supplied by E. Merck (Darmstadt, F.R.G.). The dioctadecylamine was obtained from Pfaltz and Bauer (Waterbury, CT, USA), N,N-dimethyloctadecylamine from Eastman Kodak (Rochester, NY, USA) and hexadecylchloride from Fluka (Buchs, Switzerland).

Synthesis of Standards
Heptadecynitrile was obtained by reaction of hexadecylchloride and potassium cyanide, as described previously^{12}. The N,N-dioctadecylmethylamine was obtained from dioctadecylamine by reductive alkylation with formic acid and formaldehyde in ethanol^{13}. 


The trioctadecylamine was prepared from the N,N-dioctadecylamine and octadecyliodide\textsuperscript{14}, which was obtained from n-octadecanol and P13\textsuperscript{15}.

Sample Handling
Large-volumes of surficial seawater (100-2000L) were extracted \textit{in situ} from the R/V \textit{Invincible} using the sampling assembly described previously\textsuperscript{16}. Marine sediment samples were collected with a box coring device in coastal Barcelona during the R/V \textit{Garcia del Cid} cruises along 1987-88. Samples were cut at each 2 cm, wrapped in aluminium foil and frozen at -20°C until analysis. Polychaete samples were collected at the same locations than sediments, with a Van Veen dredge, filtered through 1 mm rated mesh size, transferred into glass jars and frozen at -20°C until analysis.

Biota homogenates were analyzed as a composite containing at least samples of two cruises. About 10 g of freeze-dried sediments were soxhlet extracted with methylene chloride-methanol (2:1) for 36h. A 5-6 g of biota homogenate was transferred into centrifuge tube and were treated with 15 mL of aqueous NaOH 6N, prextracted with methylene chloride. Tubes were closed with teflon-lined caps and shaken for several minutes and kept at 30°C for 18 h. The hydrolyzed mixture was extracted with diethyl ether (5 x 10 mL). Organic extracts were reduced to small volume by rotary evaporation, adsorbed onto 2 g of 5% deactivated alumina by solvent evaporation under a gentle stream of nitrogen, and transferred on top of a glass column packed with 6 g of 5% deactivated alumina (top) and 8 g of 5% deactivated silica (bottom), previously activated at 350 and 150°C, respectively. LANs and TAMs were recovered at fractions IV and VII, respectively, as described before\textsuperscript{17}.

Instrumental analysis
The LANs and TAMs fractions were rotary evaporated and analyzed in a Mega 5300 series gas chromatograph (Carlo Erba, Milan, Italy) equipped with flame ionization detector (FID) and nitrogen-phosphorus selective (NPD) detectors at 370 and 310°C, respectively. Fused silica capillary columns of 25 m x 0.32 mm i.d. coated with 0.10 um of SE-54 (HP-5) and 10 m x 0.25 mm i.d. coated with 0.15 um of OV-1 (Rescom) were used. On column injection was performed at 95°C, and the oven temperature was programmed to 200°C at 15°C min\textsuperscript{-1}, and then to 370°C at 10°C min\textsuperscript{-1}. A 3 m x 0.32 mm i.d. deactivated fused silica tube was used as a retention gap, which was connected to the analytical column \textit{via} press-fit connector.

Capillary gas chromatography(CGC)-mass spectrometry analyses were performed on a Hewlett-Packard 5995 instrument interfaced to a 9825A data system. Multiple ion and selected ion monitoring were acquired in the electron impact ionization mode. Transfer line, ion source and analyzer temperatures were held at 300, 200 and 230°C, respectively. Samples were injected using the splitless mode at 300°C in the injector port and oven temperature was programmed from 60 to 90 at 15°C min\textsuperscript{-1} and then to 300°C at 4°C min\textsuperscript{-1}. Helium at 30 cm sec\textsuperscript{-1} of average linear velocity was used as carrier gas. Quantitation was performed by CGC coupled to FID or NPD detectors and/or CGC-MS in SIM mode, using
heptadecynitrile, N,N-dimethyloctadecylamine, N,N-dioctadecylmethylamine, and trioctadecylamine as external standards.

RESULTS and DISCUSSION

The composition of polychaetes was completely different depending on the sampling site. Whereas at station 1 located in the vicinity of Besos river mouth only *Capitella capitata* which is characteristic of heavily polluted areas, was found, several species were identified at station 4, namely *Spiochaetopterus costarum* and *Prionospio cirrifera*. Conversely, neither of them were present in the station 3 located in the vicinity of the disposal sewage site (Fig 1), where only pelagic and hemipelagic organisms were collected (Table 1).

LANs were adequately detected by NPD, exhibiting in all the environmental compartments a fatty acid distribution with even-odd carbon number predominance of saturated homologs ranging from n-C14 to n-C18 with a maximum in concentration in the C16 and monounsaturated series with a maximum in the C18 (Fig. 2). Although the characterization of TAMs can also be accomplished by CGC coupled to FID or NPD (Figs 3A-4), it was performed by CGC-MS in the selected ion monitoring detection mode in case of remarkable matrix complexity found in pelagic fishes (Fig 3B). It is worth to mention, that despite several interferences were detected, they were absent in the retention time of TAMs, allowing their accurate quantitation. Moreover, high temperature CGC was required for the elution of the three-long chain alkyl substituents (Fig 4), those were identified for the first time in polychaete sp and sediment samples as well. Futhermore, the distribution of the alkyl substituents was similar to that found for LANs with a similar remarkable even-odd carbon number predominance ranging from C14 to C18, but unsaturated alkyl-chain moieties were not present. In case of CH$_3$NR$_1$R$_2$ a significant increase in bioconcentration factor (Kb) was detected paralleling with an increase of alkyl-chain moieties (Fig 3 and 4), probably associated to their different Kow partition coefficients.

![CGC-NPD gas chromatogram of fraction IV isolated from coastal sediment (station 1). The carbon number of LANs is indicated.](image)
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**注释**

*采样地点如图 1 所示。*
The quantitative data obtained for LANs and TAMs is listed in Table 1. The concentrations of LANs and TAMs in polychaetes were obtained from the total original organism since, as it was reported previously, the burden of contaminants contained in the sediments ingested into the gut of polychaetes is negligible and not relevant for quantitation purposes. Whereas the highest concentrations were found in the mono- and dialkyl long-chain substituted TAMs, which were detected in most of biota samples analyzed, the three long-chain substituted TAMs and LANs demonstrated a selective occurrence found only in polychaete sp.

Fig. 3 Distribution of TAMs in: A) seawater (station 2) and B) Macropipus depurator (station 3) obtained by GC-MS under multiple ion and SIM detection, respectively (the follow ions were monitored: m/z = 58 from 2 to 25 min; m/z = 240, 268, and 296 from 40 to 60 min.). The carbon number of R1 and R2 in CH3NR1R2 is depicted.
Fig. 4 High temperature CGC-FID gas chromatograms of fraction VII isolated from A) coastal sediment and B) polychaete sp collected at station 1. The carbon number moieties of $NR_1R_2R_3$ are depicted ($R_1$ = CH$_3$ unless indicated).
The correlation between concentration of LANs found in sediments and polychaetes is remarkable, since higher concentrations are found in the proximity of Besos river mouth. Nevertheless, a similar correlation was not followed for TAMs identified in biota and sediments collected in the same area. Recent studies on the biouptake of chlorinated hydrocarbons from laboratory-spiked and field sediments by olygochaetes suggested that body burden of chemicals come mainly from the pore water rather than from ingestion of contaminated sediment particules19,20. Nevertheless, the selective distribution of the three long-chain TAMs in polychaete sp, and taking into account the molecular weight of these compounds exceeds 600 daltons which is the upper limit of the permeability of biological membranes, these compounds should be taken up by ingestion21. The parallelism between sediment and polychaete distributions of those compounds might confirm this assumption (Fig 4).

![Fig. 5. Bioconcentration factors (Kb) of several contaminants (see legends) in different biota species: 1, Polychaete sp, 2, Capitella capitata, 3, Macropipus depurator, 4, Sardinella aurita, 5, Sepia officinalis, and 6, Solea solea.](image)

In order to estimate the relative level of bioconcentration of cationic surfactant derived products, the Kb values of different pollutant classes were calculated from surficial or bottom seawater depending on the specimen examined (Fig 5). The highest Kb values were obtained for PCBs for all the species examined ranging from 26 to 204 x 10^3 but TAMs and PAHs exhibited intermediate levels of bioconcentration, which demonstrate that bioconcentration of those compounds should be taken futher into account. On the other hand, because LANs exhibited a more restricted occurrence in the aquatic environment, their bioconcentration only deserve interest in limited areas as sampling site 1 (Capitella capitata) where exhibited the highest Kb value.

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REFERENCES


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