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Derivation of predicted no effect concentrations (PNEC) for marine environmental risk assessment: Application of different approaches to the model contaminant Linear Alkylbenzene Sulphonates (LAS) in a site-specific environment

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Abstract

Four sediment-dwelling marine organisms were exposed to sediments spiked with increasing concentrations of Linear Alkylbenzene Sulphonate (LAS). The selected endpoint mortality was reported daily and acute LC_{50} (96 h), as well as final LC_{10} values were calculated for the derivation of environmentally safe predicted no effect concentrations (PNEC) for the sediment compartment. PNECs were estimated by both application of assessment factors (AF) and the equilibrium partitioning method (EPM) as proposed by the EU TGD. Finally, environmental risk assessment in a site-specific environment, the Sancti Petri Channel, South Iberian Peninsula, was carried out at three different sampling stations with known environmental LAS concentrations. PNECs obtained by the assessment factor approach with acute toxicity data were one to two orders of magnitude lower than those from the equilibrium partitioning method. On the other hand, when applying lower AFs to the estimated LC_{10} values, the PNECs obtained by both approaches were more similar. Environmental risk assessment carried out with the estimated PNECs in a site specific environment with known sediment LAS concentrations revealed that PNECs obtained with acute toxicity data were over conservative whereas those obtained with AF = 10 on LC_{10} data and EPM produced more realistic results in accordance with field observations carried out in the study area.

Keywords: Linear Alkylbenzene Sulphonate (LAS); Sediment; Lethal concentration; LC50; LC10; Predicted no effect concentration (PNEC); Marine risk assessment; Equilibrium partitioning method; Assessment factor

1. Introduction

The probability of ecological risk due to a certain contaminant can be determined by risk assessment. This is based on the evaluation of the ratio between estimated (PEC: predicted environmental concentration) or analysed exposure concentrations and predicted no effect concentrations (PNEC). When this ratio PEC/PNEC is >1 a risk management is required for the studied chemical which includes refinement of the assessment and/or remediation, whereas when PEC/PNEC is <1 no hazard is foreseen. For the sediment compartment, the EU TGD (2003) proposes different procedures for the derivation of PNECs, depending on the availability of toxicity data and the physico-chemical properties of the compound. PNECs may be derived from laboratory-based toxicity tests using well-defined protocols on a limited number of species (Lam and Gray, 2001). Outputs of these kinds of assays are different effect concentrations such as LC_{50} (concentration of a chemical that causes death of 50% of the exposed organisms) or NOEC (no observed effect concentration), which allow the quantification of observed and previously established effect criteria. If a limited number (1–7) of acute (LC_{50}) or chronic (NOEC) data are available, assessment factors (AF) should be applied to extrapolate from single species toxicity data to the ecosystem (EU TGD, 2003). These factors are higher in the case of LC_{50}

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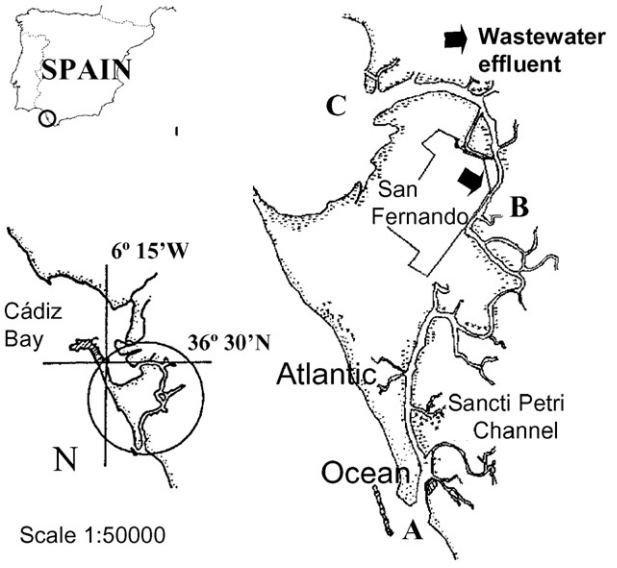

Fig. 1. Map of the studied area, the Sancti Petri Channel, San Fernando (South West Spain) showing the corresponding sampling points A, B and C. The arrow indicates the position of the effluent discharge point.

data and lower when working with NOECs. For results from short-term tests with sediment-dwelling organisms, an assessment factor of 1000 is applied to the lowest LC_{50} value, whereas when chronic toxicity data are available, lower assessment factors of 100, 50 or 10 are proposed, depending on the number of species tested.

In the absence of any ecotoxicological data for sediment-dwelling organisms, the $PNEC_{sed}$ may be provisionally calculated using the equilibrium partitioning method (EPM) for compounds with $K_{ow}>3$, which uses the $PNEC_{water}$ for aquatic organisms and the sediment/water partitioning coefficient as inputs (OECD, 1992; Di Toro et al., 1991):

$$PNEC = \frac{K_{susp-water}}{RHO_{susp}} PNEC_{water} \cdot 1000$$

where $K_{susp-water}$ is the partition coefficient between suspended matter and water, RHO_{susp} the bulk density of wet suspended matter in $kg \cdot m^{-3}$ and $PNEC_{water}$ the predicted no effect concentration for the aqueous compartment in $mg \cdot L^{-1}$. The K_{ow} for commercial LAS is 3.36 and can be therefore considered for EPM. For this aim, the $PNEC_{water}$ estimated by Temara et al. (2001) was employed for potential risk characterisation and posterior comparison of both procedures. In order to take uptake via ingestion of sediment into account, in the subsequent hazard evaluation, the $PEC_{sed}/PNEC_{sed}$ ratio is increased by a factor of 10.

In recent years, another procedure, the so called species sensitivity distributions (SSD) are employed for the derivation of environmentally safe contaminant concentrations (Aldenberg and Slob, 1993; Solomon et al., 1996). In this case, single-species data (e.g., LC_{50} or NOEC values) for many species are fit to a distribution such as the lognormal or log-logistic. From this distribution of species sensitivities, a hazardous concentration (HCp) is identified at which a certain percentage (p) of all species is assumed to be affected (Newman et al., 2000). However, in our case, there is not enough toxicity data about the effects of LAS on marine benthic organisms available for this technique to be performed.

Linear Alkylbenzene Sulphonate (LAS) is an anionic surfactant employed in the formulation of household and personal cleaning products, with a global production rate of 4 million metric tons (Tolls et al., 2000). Once discharged into receiving environments it tends to be sorbed onto suspended solids, which accumulate finally in bottom sediments. Coastal environments may present wide LAS concentration ranges in both water column and sediments, depending fundamentally on population density and the presence or not of waste water treatment facilities. Some sediment quality values have been derived previously for this compound (Bressan et al., 1989; Pittinger et al., 1989; Holmstrup and Krogh, 1996; HERA-LAS, 2004) presenting considerable differences depending on the species tested. Due to its widespread global use, LAS was chosen to study its effects on 4 sediment dwelling organisms and to characterise the environmental risk due to its presence in the sediments in a site specific environment. Marine $PNECs_{sed}$ for LAS are determined by both EPM and AF application on acute LC_{50} (96 h) and final LC_{10} values obtained in toxicity tests with LAS spiked sediments. The selected organisms were the prosobranch mudsnail, *Hydrobia ulvae*, the clam, *Ruditapes philippinarum*, the sole, *Solea senegalensisa* and the crab, *Uca tangeri*, which are representative sediment dwelling species of the zone. The obtained parameters were then employed for potential risk evaluation in a benthic environment presenting a wide LAS concentration range. Results were compared with field observations for the evaluation of the adequacy of the proposed factors.

2. Materials and methods

2.1. Spiking of sediments and analysis of final exposure concentrations

Sediments were collected in of the Bay of Cádiz, in an area far from urban and industrial discharge points, and sieved through a 0.6-mm mesh into a tank in order to remove any associated macrofauna and large sediment particles. The sediment was characterised in terms of organic carbon (%), elemental analysis (C, H, N), grain size composition (–% fine sand–) and pH previous to the experimentation and maintained at 4 °C until spiking. No later than 48 h, the sediment was spiked with LAS as described by Casellato et al. (1992). The employed surfactant was a commercial LAS mixture (CAS Nr. 68411-30-3, supplied by Petroquímica Española S.A., PETRESA) with an average alkyl chain length of 11.6 carbon atoms and homologue distribution of C10 to C14 of 10.9; 35.3; 30.4; 21.2 and 1.1%, respectively. After spiking, the substrate was washed with distilled water, dried at 70 °C and homogenised with a Planetary Mono mill (Pulverisette 6, Fritsch). LAS concentration in the original sediment was 1.59 mg kg^{-1} dry weight. Final exposure concentrations in each test vessel were determined by high performance liquid chromatography (HPLC, HP 1050) with fluorescence detector (λ_{ex}=225 nm, λ_{em}=295 nm) as described by León et al. (2000).

Table 1
Organic Carbon (%), grain size composition (–% fine sand–), elemental analysis (C, H, N) and pH in the reference sediment employed for the bioassays

O.C. (%)	%<63 μm	C (%)	H (%)	N (%)	pH
0.618±0.015	22	1.19±0.42	1.06±0.13	0.06±0.01	7.5±0.1

Table 2
Estimated LC_{50} (96 h) and LC_{10} values from the mortality data obtained in the exposure assays

Organism	LC_{50} (96 h) [mg·kg^{-1} dry wt]	LC_{10} [mg·kg^{-1} dry wt]
H. ulvae	140.65	48.81
S. senegalensis	2179.68	362.99
R. philippinarum	–	560.53

LC_{50} (96 h) for *R. philippinarum* could not be estimated as mortality was greater than the highest tested concentration.

2.2. Study area

The study area is a tidal channel, the Sancti Petri Channel, of about 18 km, which connects the southern part of the Bay of Cádiz, South Iberian Peninsula, with the Atlantic Ocean (Fig. 1). The sampling stations were: one at the discharge point of urban and industrial waste water of the city of San Fernando of about 100 000 inhabitants (B), the second where the channel opens into the inner, southern part of the Bay of Cádiz (C) and finally the third where the channel connects with the open sea (A). Environmental LAS concentrations in sediments from the three sampling stations along the channel were: (A) 0.8±0.2; (B) 138.6±14 and (C) 16.4±8.0.2 (León et al., 2000).

2.3. Exposure of organisms

The organisms employed in the assays were obtained in the field (*H. ulvae*, *U. tangeri*) or supplied by different aquaculture facilities (*R. philippinarum*: Amalthea S.A. Chiclana, Spain, *S. senegalensisa senegalensis*: CICEM el Toruño, El Puerto de Santa Maria, Spain). Prior to the exposure assays, all organisms were maintained under controlled laboratory conditions for acclimation. All assays were performed in triplicates and negative control experiments with untreated sediment were carried out simultaneously. Increasing LAS concentrations were achieved by mixing different proportions of LAS spiked and untreated sediment (ASTM, 1997).

2.3.1. H. ulvae

Organisms (n=30) were exposed during 10 d in Petri dishes to experimental sediments in a thermostatic chamber at 22 °C and 12 h light–darkness photoperiod. Exposure concentrations were: 0.51; 74.50; 139.81; 275.88; 548.36 mg kg^{-1} dry weight. Organisms were fed daily with between 3 and 5 µg of lipophilized *Ulva ulvae* and mortality control was performed transferring the organisms into clean seawater. Dead individuals were removed from the dish.

2.3.2. R. philippinarum, S. senegalensisa senegalensis, U. tangeri

Organisms were exposed during 30 d in aquariums under continuous flow through conditions to sediments presenting the following LAS concentrations: *S. senegalensis*: 0.40; 98.24; 278.92; 697.93; 1343.20; 2103.17 mg LAS kg^{-1}; *R. philippinarum*: 0.68; 328.67; 605.79; 912.52; 1219.86 mg LAS kg^{-1} and *U. tangeri*: 0.50; 228.44; 808.11; 1892.23; 3117.13; 3431.94 mg LAS kg^{-1}, dry weight in all cases. Water supply was about 18 L h^{-1} of clean seawater. Before placing the organisms into the test devices, the system was maintained for 24 h without organisms in order to eliminate possible excesses of LAS. The numbers of exposed individuals in each experiment were: *R. philippinarum*: 60 seeds (approx. 1-year-old), *S. senegalensis*: 30 juveniles (approx. 45-day-old) and *U. tangeri*: 10. Organisms were fed daily corresponding to their special feeding habits: *R. philippinarum*: a mixture of two cultivated algae, *Isochrysis* Aff. *galbana* (*T. Iso*) and *Chaetoceros gracilis* (*C. gracilis*), *S. senegalensis*: *Artemia salina* cysts, crab: *Mytilus edulis*. Mortality control was performed daily and dead organisms were removed from the experimental devices.

Table 3
Risk characterisation for the three sampling stations in the Sancti Petri Channel by EPM

Station	PEC	PEC/PNEC	(PEC/PNEC)·10
B	138.6	5.37	53.66
C	16.4	0.63*	6.34
A	0.8	0.03*	0.31*

Table 4
LC_{50} (96 h) and final LC_{10} values, as well as PNECs obtained by application of proposed assessment factors on acute (96 h) and subchronic (*H. ulvae*: 9d, *S. senegalensis* and *R. philippinarum*: 30 d) toxicity data

Organism	LC_{50} (96 h)	Final LC_{10}	PNEC		
			LC_{50} (96 h)	Final LC_{10}	Final LC_{10}
			1000	100	10
H. ulvae	140.65	48.82	0.14	0.49	4.88
S. senegalensis	2179.68	362.99	2.18	3.63	36.30
R. philippinarum	–	560.53	–	5.61	56.05

LC_{50} (96 h) for *R. philippinarum* could not be estimated and therefore no PNEC could be derived.

2.4. Estimation of critical effect concentrations and risk evaluation

Mortality data for each assay were adjusted employing generalised linear models (GLM), and acute LC_{50} (96 h), as well as final LC_{10} values (concentration of a chemical that causes death of 10% of the exposed organisms) were calculated as described by Kerr and Meador (1996). AF=1000 was applied on acute LC_{50} values, and chronic AFs=100 and 10 on LC_{10} values for $PNEC_{sed}$ calculation and compared with the $PNEC_{sed}$ obtained from $PNEC_{water}$ for LAS by EPM (Temara et al., 2001). Finally, PEC/PNEC ratios were obtained for the three mentioned sample stations for the evaluation of potential environmental risk due to LAS presence.

3. Results

3.1. Derivation of toxicity parameters

The sediment characterising parameters are provided in Table 1. Obtained acute LC_{50} (96) and final LC_{10} values presented wide concentration ranges depending on the tested species (Table 2). Acute LC_{50} (96 h) values for *H. ulvae* and *S. senegalensis*, were 140.65 mg kg^{-1} and 2179.68 mg kg^{-1} dry weight, respectively whereas for *R. philippinarum* this parameter could not be estimated as mortality along the whole assayed concentration range was greater than the highest value tested and therefore the data could not be adjusted. LC_{10} values were 48.8 mg kg^{-1} in the case of *H. ulvae*, 362.99 mg kg^{-1} for *S. senegalensis* and 560.53 mg kg^{-1} dry weight for *R. philippinarum*. No significant mortality was detected in the reference sediments and survival in all the replicates matches the acceptance criteria for this kind of test (survival higher than 90%). The results obtained for the crab, *U. tangeri* were not included in the risk assessment procedure, as mortality reported in one of the controls was 30% and do not fulfil the reliability criteria proposed by the OECD (1992).

Table 5
Risk characterisation in the Sancti Petri Channel with PNEC obtained by extrapolation with a factor 1000 from acute (96 h) toxicity data

Organism	PNEC	Station B	Station C	Station A
	LC_{50} (96 h)	138.6 mg kg^{-1}	16.4 mg kg^{-1}	0.8 mg kg^{-1}
	1000	PNEC	PNEC	PNEC
H. ulvae	0.14	985.42	116.60	5.69
S. senegalensis	2.18	63.59	7.52	0.37*
R. philippinarum	–	–	–	–

Table 6
Risk characterisation in the Sancti Petri Channel with PNEC obtained by extrapolation with a factor 10 from subchronic (*H. ulvae*: 9d, *S. senegalensis* and *R. philippinarum*: 30 d) toxicity data

Organism	PNEC	Station B	Station C	Station A
	Final LC_{10}	138.6 mg kg^{-1}	16.4 mg kg^{-1}	0.8 mg kg^{-1}
	10	PNEC	PNEC	PNEC
H. ulvae	4.88	28.39	3.36	0.16*
S. senegalensis	36.30	3.82	0.45*	0.02*
R. philippinarum	56.05	2.47	0.29*	0.01*

3.2. ERA based on $PNEC_{sed}$ derived by equilibrium partitioning method from $PNEC_{water}$

$PNEC_{sed}$ was calculated employing the $PNEC_{water}$ of 31 μg LAS L^{-1} (Temara et al., 2001), a RHO of 1200 kg m^{-3} (CEFIC: European Chemical Industry Council, 2006) and a $K_{sed-water}$ of 1000. The sediment–water partitioning coefficient was taken as median approximation of the values obtained by González-Mazo et al. (1997) which determined partitioning coefficients for LAS in the Bay of Cádiz ranging between 100 and 2216. This averaged $PNEC_{sed}$ was 25.83 mg LAS kg^{-1} being the values comprised between 2.58 and 57.25 mg LAS kg^{-1}. Employing this $PNEC_{sed}$, the resulting ratio PEC/PNEC, as well as the one increased by a factor 10 to take into account the additional exposure due to ingestion of the substrate, for the three sampling stations are presented in Table 3. This approach identifies hazard due to the existing LAS concentrations at Stations B and C, the discharge point of untreated urban wastewaters and the point where the channel opens into the Bay of Cádiz, respectively. At more distance, the ratio PEC/PNEC decreases, being at the Stations A < 1, indicating that no potential risk is expected.

3.3. ERA based on $PNEC_{sed}$ obtained by assessment factor application on acute LC_{50} (96) and final LC_{10} values

For sublethal toxicity data, AF = 100, 50 and 10, are proposed depending on the number of chronic toxicity values. When data from at least three tests is available, the EU TGD suggests the application of AF = 10, whereas in those cases when data for only one test species are available, a factor of 100 is proposed. In our tests, no sublethal effect criteria were evaluated, but obtained LC_{10} values at the end of the assays were employed for the application of the factors 100 and 10 on the obtained parameter. Even if the document indicates to carry out the risk characterisation with the lowest LC_{50}/LC_{10} value obtained, we have applied the factors to LC_{50} and LC_{10} values of each of the tested organisms in order to detect differential degrees of potential risk, depending on the tested species.

Table 7
Risk characterisation in the Sancti Petri Channel with PNEC obtained by extrapolation with a factor 100 from subchronic (*H. ulvae*: 9d, *S. senegalensis* and *R. philippinarum*: 30d) toxicity data

Organism	PNEC	Station B	Station C	Station A
	Final LC_{10}	138.6 mg kg^{-1}	16.4 mg kg^{-1}	0.8 mg kg^{-1}
	100	PNEC	PNEC	PNEC
H. ulvae	0.49	283.88	33.59	1.64
S. senegalensis	3.63	38.18	4.52	0.22*
R. philippinarum	5.61	24.73	2.93	0.14*

Table 8
Potential risk and no risk scenarios for the different sampling stations and approaches

Station	Approach			
	EPM	AF=1000(LC_{50})	AF=100(LC_{10})	AF=10(LC_{10})
B				
C				
A				

Potential risk.

Table 4 shows the derived $PNEC_{sed}$ by AF application 1000 (LC_{50} (96)), as well as factors 100 and 10 (LC_{10}), respectively. When extrapolating from acute toxicity data (AF = 1000), the ratio between environmental LAS concentrations at Stations A, B and C, PEC/PNEC is >1 for *H. ulva* and *S. senegalensis* at all stations indicating the existence of environmental potential risk (Table 5) except for *S. senegalensis* at Station A with the lowest LAS concentration in the sediment (0.8 mg kg^{-1} dry weight). In contrast, applying AF = 10 on LC_{10} values, the ratios PEC/PNEC indicate that the surfactant load along the Sancti Petri Channel does not represent the same level of potential risk as with the acute data, being no hazard expected at Station A for all organisms, as well as for *S. senegalensis* and *R. philippinarum* at Station C (Table 6). At this latter station, potential risk would be expected for *H. ulva* where PEC/PNEC > 1. At the wastewater discharge point (Station B), potential risk is expected for all tested organisms. With AF = 100 on LC_{10} values (Table 7), similar results are obtained as with LC_{50} (96 h) values (Table 4), indicating hazard at all sampling stations for all the tested species except *S. senegalensis* and *R. philippinarum* at Station A.

Taking into account that risk evaluation should be carried out with the most sensitive test species which in our case is *H. ulva*, the risk identification for the three stations is shown in Table 8, with AFs 1000 and 100 identifying potential risk at all stations and EPM and AF 10 only at Stations B and C.

4. Discussion

Aquatic environmental risk assessment with LAS has been carried out on freshwater organisms by several authors (Fendinger et al., 1994; van de Plassche et al., 1999; Versteeg et al., 1999), where its impact at environmentally realistic concentrations has been characterised as low. However, very little information is available about marine environments (Temara et al., 2001; HERA-LAS, 2004). In their work, Temara et al. (2001) used toxicity data from mainly pelagic species or life-stages and estimated a PNEC for the aquatic compartment of 31 μg LAS L^{-1}. Compared with estimated environmental LAS concentrations this also revealed a reduced potential risk for LAS for the study site, the North Sea. In this case, PNEC was obtained by SSD approach. In 2004, the HERA (Human and Environmental Risk Assessment) on LAS (HERA-LAS, 2004) published a PNEC for LAS in the sediment compartment of 26 mg kg^{-1}, obtained by EPM and which is in excellent agreement with the $PNEC_{sed}$ obtained by EPM in this study. However, in the same document, $PNEC_{sed}$ was also calculated by application of AF = 10 from available toxicity data and was with 8.1 mg kg^{-1} significantly lower than the one obtained by

EPM. In our study, the PNEC obtained by application of AF = 10 on the most sensitive test species, *H. ulvae*, was 4.9 mg kg^{-1} and comparable with the one obtained by HERA.

According to the toxicity parameters obtained in our exposure assays, *H. ulvae* is the most sensitive species towards sediment sorbed LAS, with LC_{50} and LC_{10} values one order of magnitude lower than those obtained for *S. senegalensis* and *R. philippinarum*. The interaction with the sediment in those latter species is based on the simple contact with the substrate, and feeding occurs only from the overlying water column. In contrast, *H. ulvae* also ingests the substrate and the associated contaminants, which pass through its intestine assimilating the microorganisms attached to mineral and detritus particles (Fenchel et al., 1975). This kind of feeding habit facilitates the access of the contaminant to target organs and provides therefore a higher exposure level as the simple contact with the substrate. Thus, for risk assessment purposes, the toxicity parameters derived from toxicity data with *H. ulvae* should be taken as reference in our case in agreement with the EU TGD.

The $PNEC_{sed}$ obtained by EPM of 25.83 mg kg^{-1} is similar to the one obtained with AF = 10 extrapolation from LC_{10} values for *R. philippinarum*, and is also in the same order of magnitude as the one obtained for *S. senegalensis*. The $PNEC_{sed}$ for *H. ulvae* obtained by extrapolating from LC_{10} values with AF = 10 is with 4.9 mg kg^{-1} one order of magnitude lower. However, including the factor 10 in the ratio PEC/PNEC with EPM, similar results in risk identification between EPM and AF = 10 with *H. ulvae* are obtained. On the other hand, extrapolating from acute toxicity data (LC_{50} (96 h)) with the assessment factor 1000, the obtained $PNEC_{sed}$ for *H. ulvae* and *S. senegalensis* are lower than those obtained by extrapolation from subchronic toxicity data. The existence of a higher extrapolation factor for acute data is meant to reproduce the reduction of the toxicity parameter when chronic instead of acute effects are evaluated. A higher AF results in lower PNECs which are more protective for ERA purposes. However, taking into account the similarity with the results obtained by EPM after factor 10 application due to sediment ingestion and the agreement with the results obtained in the HERA document, the assessment factor of 1000 to be applied on acute data seems overestimated. Similar results than with the acute data are obtained by extrapolating with factor 100, which is actually not the recommended one as in our study data from three test species were available, which implies an AF of 10.

The risk characterisation carried out with the PNEC obtained by EPM, identified a potential risk for the population due to sediment LAS load at Stations B and C. The same risk characterisation is obtained with AF = 10. Station B received at the time of this study the direct input of urban and industrial wastewater of a town of approximately 100 000 inhabitants discharged without previous treatment into the Sancti Petri Channel. Station C is located in the inner part of the Bay of Cádiz where restricted water circulation conditions facilitate the accumulation of suspended solids that are transported with the tides from inside the channel where LAS concentrations are relatively higher. On the other hand, Station A with LAS concentrations of 0.8 mg kg^{-1} dry weight was identified with no potential risk. In contrast, extrapolation with AF = 1000 (LC_{50}(96)) and AF = 100 (LC_{10}) identified potential risk at all three sampling stations. However, when considering field observations performed by Drake et al. (1999) these factors seem over conservative, as at this point representatives of all the evaluated species were present in bottom sediments. Further studies should be carried out in order to understand the relationship between acute and chronic effect levels in marine sediments to enable realistic extrapolation from acute toxicity data and thus reduce toxicity testing to acute levels which may increase significantly time- and cost effectiveness of the studies.

5. Conclusions

1. PNECs obtained by EPM are generally one magnitude higher as those obtained by AF approach. However, this underestimation of effects produced by the contaminant is afterwards compensated by the introduction of a factor 10 in the evaluation of the PEC/PNEC ratio.
2. The employment of AF = 1000 on acute toxicity data is considered to be over conservative for this site-specific environment.
3. The results for the risk characterisation EPM and application of AF = 10 are similar with Station A identified with no potential risk due to its LAS load. Field observations carried out at the study site confirm the existence of the most sensitive test species at Station A and therefore the reliability of these approaches.
4. To be able to compare both approaches, the employed test organisms should be carefully selected. *H. ulvae* ingesting the substrate has demonstrated to be much more sensitive to the sorbed contaminant than *S. senegalensis* and *R. philippinarum*. If only results from *S. senegalensis* and *R. philippinarum* had been available, the risk assessment would have identified hazardous sites as not hazardous which for more sensitive organisms, and therefore for the ecosystem, could have important consequences.

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References

Aldenberg T, Slob W. Confidence limits for hazardous concentrations based on logistically distributed NOEC toxicity data. Ecotoxicol Environ Saf 1993;25:48–63.

ASTM (American Society for Testing and Materials). E1367-92* Standard Guide for Conducting 10-day Static Sediment Toxicity Tests with Marine and Estuarine Amphipods; 1997.

Bressan M, Brunetti R, Cesellato S, Fava GC, Giro P, Marin M, et al. Effects of Linear Alkylbenzene Sulfonate (LAS) on benthic organisms. Tenside Surfactants Deterg 1989;26:147–58.

Casellato S, Aiello R, Negrisolo PA, Seno M. Long-term experiment on *Branchiura sowerbyi* Beddard (*Oligochaeta, Tubificidae*) using sediment treated with LAS (Linear Alkylbenzene Sulphonate). Hydrobiologia 1992;232:169–73.

Cefic: European Chemical Industry Council. Information about chemical industry and chemistry applications, health, safety and environmental aspects. Substance data sheet: Anthracene, first draft; 2006.

Di Toro DM, Zarba CS, Hansen DJ, Berry WJ, Schwarz RC, Cowan CE, et al. Technical basis of establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. Environ Toxicol Chem 1991;10: 1541–83.

Drake P, Baldo F, Saenz V, Arias AM. Macrobenthic community structure in estuarine pollution assessment on the Gulf of Cádiz (SW Spain): is the Phylum-level meta-analysis approach applicable? Mar Pollut Bull 1999;38:1038–47.

European Union. Technical guidance document on risk assessment in support of the commission directive 93/67/EEC on risk assessment for new notified substances Commission Regulation (EC) No 1488/94 on risk assessment for existing substances directive 98/8/EC of the European Parliament and of the council concerning the placing of biocidal products on the market. Environmental Chemicals Bureau, Ispra, Italy; 2003.

Fenchel T, Kofoed LH, Lappalainen A. Particle size selection of two deposit feeders: the amphipod *Corophium volutator* and the prosobranch *Hydrobia ulvae*. Mar Biol 1975;15:326–34.

Fendinger NJ, Versteeg DJ, Weeg E, Dyer SD, Rapaport RA. Environmental behaviour and fate of anionic surfactants. In: Baker LA, editor. Environmental Chemistry of Lakes and Reservoirs, Advances in Chemistry Series No 237. Washington DC: American Chemical Society; 1994. p. 527–57.

González-Mazo E, Quiroga JM, Sales D, Gómez-Parra A. Levels of linear alkyl benzene sulfonate (LAS) in waters and sediments of the coastal ecosystems of the Gulf of Cádiz. Toxicol Environ Chem 1997;59:77–87.

HERA-LAS (2004). Human and Environmental Risk Assessment LAS CAS No 68411-30-3. Version No. 9. (http://www.heraproject.com/Index.cfm). A.I.S.E. Association Internationale de la Savonnerie, de la Détergence et des Produits d'Entretien (www.aise-net.org), Cefic. European Chemical Industry Council (www.cefic.org).

Holmstrup M, Krogh P. Effects of anionic surfactant, linear alkyl benzene sulphonate, on survival, reproduction and growth of the soil-living collembolan *Folsomia Fimetaria*. Environ Toxicol Chem 1996;15:1745–8.

Kerr DR, Meador JP. Modelling dose–response using generalized linear models. Environ ToxicolChem 1996;15(3):395–401.

Lam PKS, Gray JS. Predicting effects of toxic chemicals in the marine environment. Mar Pollut Bull 2001;42(3):169–73.

León VM, González-Mazo E, Gómez-Parra A. Handling of marine and estuarine samples for the determination of linear alkylbenzene sulfonates and sulfophenylcarboxylic acids. J Chromatogr A 2000;889:211–9.

Newman MC, Ownby DR, Me' Zin LCA, Powell DC, Christensen TRL, Lerberg SB, et al. Applying species sensitivity distributions in ecological risk assessment: assumptions of distribution type and sufficient numbers of species. Environ Toxicol Chem 2000;19(2):508–15.

OECD (Organisation for Economic Co-operation and Development). Report of the OECD Workshop on Effects Assessment of Chemicals in Sediment. Organisation for Economic Cooperation and Development (OECD), OECD Environment Monographs No. 60, Paris; 1992.

Pittinger CA, Woltering DM, Masters JA. Bioavailability of sediment-sorbed and aqueous surfactants to *Chironomus riparius* (midge). Environ Toxicol Chem 1989;8:1023–33.

Solomon KR, Baker DB, Richards RP, Dixon KR, Klaine SJ, La Point TW, et al. Ecological risk assessment of Atrazine in North American surface waters. Environ Toxicol Chem 1996;15(1):31–76.

Temara A, Carr G, Webb S, Versteeg D, Feijtel T. Marine risk assessment: linear alkylbenzenesulphonates (LAS) in the North Sea. Mar Pollut Bull 2001;42(8): 635–42.

Tolls J, Haller M, Seinen W. Sijm DTHM. LAS bioconcentration: tissue distribution and effect of hardness-implication for processes. Environ Sci Technol 2000;34:304–10.

van de Plassche EJ, Brujin de JHM, Stephenson RR, Marshall SJ, Feijtel TCM, Belanger SE. Predicted no-effect concentrations and risk characterization of four surfactants. Linear alkyl benzene sulphonate, alcohol ethoxylates, alcohol ethoxylated sulfates, and soap. Environ Toxicol Chem 1999;18: 2653–63.

Versteeg DJ, Belanger SE, Carr GJ. Understanding single species and model ecosystem sensitivity. A data based comparison. Environ Toxicol Chem 1999;18:1329–46.