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Note

Bioconcentration of the anionic surfactant linear alkylbenzene sulfonate (LAS) in the marine shrimp *Palaemonetes varians*: A radiotracer study

Florent Renaud [a,b], Michel Warnau [a], François Oberhänsli [a], Jean-Louis Teyssié [a], Ali Temara [c], Claude Rouleau [d], Marc Metian [a,*]

[a] International Atomic Energy Agency – Environment Laboratories (IAEA-EL), 4 Quai Antoine Ier, MC-98000 Principality of Monaco, Monaco
[b] Littoral Environnement et Sociétés (LIENSs), UMRi 7266 CNRS-Université La Rochelle, 2 rue Olympe de Gouges, F-17000 La Rochelle, France
[c] Procter & Gamble, The Procter & Gamble Company, Temselaan 100, 1853 Strombeek-Bever, Belgium
[d] Institut Maurice-Lamontagne, Pêches et Océans Canada, 850, Route de la Mer, C.P. 1000, Mont-Joli G5H 3Z4, Québec, Canada

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ABSTRACT

Uptake and depuration kinetics of dissolved [^{14}C]C_{12}-6-linear alkylbenzene sulfonate (LAS) were determined in the shrimp *Palaemonetes varians* using environmentally relevant exposure concentration. The shrimp concentrated LAS from seawater with a mean BCF value of 120 L kg^{-1} after a 7-day exposure. Uptake biokinetics were best described by a saturation model, with an estimated BCF_{ss}, of 159 ± 34 L kg^{-1}, reached after 11.5 days. Shrimp weight influenced significantly BCF value with smaller individuals presenting higher affinity to LAS. To the light of a whole body autoradiography, major accumulation of LAS occurred in the cephalothorax circulatory system (gills, heart, hepatopancreas) and ocular peduncle, but not in the flesh, limiting potential transfer to human consumers. LAS depuration rate constant value of the shrimp was 1.18 ± 0.08 d^{-1} leading to less than 1% of remaining LAS in its tissues after 8 days of depuration.

1. Introduction

The presence of linear alkylbenzene sulfonate (LAS) in many commonly used household detergents since de 60s has given rise to a continuous consumption and subsequent discharge of these compounds with urban effluents in receiving waters (HERA, 2013). Despite the fact that LAS are efficiently retained by wastewater secondary treatment (⩾99%), LAS traces are found in the environment impacted by effluent discharge, especially in marine coastal waters In most cases, marine organisms are exposed to concentrations typically below 1 μg L^{-1} that is far below acute and chronic toxic thresholds (Temara et al., 2001). In acute exposure regimes, LAS have been assessed to act as a polar narcotic (Hwang et al., 2003) and exposures to LAS in the 10–100 mg L^{-1} range will lead to bio-membrane disruption (Hansen et al., 1997). Such toxicity rather depends on the accumulation efficiency, exposure concentration and exposure duration at the target site (Hwang et al., 2003). The wide dispersive use of LAS, its surface-activity and bio-membrane effects, as well as its discharge to coastal waters often without waste water treatment are all relevant reasons to study its toxico-kinetics and distribution in marine organisms. This assessment represents a key step to derive BCF values, estimate NOEC and critical body burden values useful in risk assessments (Nendza et al., 1997).

Therefore, the present work investigated uptake and depuration kinetics of LAS in the marine shrimp *Palaemonetes varians* (Leach, 1814) exposed via seawater. The use of highly sensitive radiotracer techniques and C_{12}-6-LAS congener allowed studying bioaccumulation mechanisms at realistic levels encountered in the field. Complementary objectives of the present study were to determine the organotropism of LAS using autoradiography and to investigate the possible effect of body size/weight of *P. varians* on the capacity of the shrimp to bioconcentrate LAS.

2. Materials and methods

2.1. Organisms

Shrimps, supplied by "Les Salins du Midi" (France), were maintained in flow-through, 20-L aquarium with controlled conditions (water renewal rate: 20 L h^{-1}, temperature: 20 ± 0.2 °C, 12 h/12 h

* Corresponding author. Address: Radioecology Laboratory, IAEA Environment Laboratories, 4 Quai Antoine 1er, MC-98000 Principality of Monaco, Monaco. Tel.: +377 97 97 72 17.
E-mail address: m.metian@iaea.org (M. Metian).

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light cycle and salinity: 38 psu) for at least 2 weeks prior to LAS experiment. They were fed daily on fresh mussel tissues.

2.2. Exposure phase

Forty shrimps (0.30 ± 0.15 g wet weight) were selected and placed in a 20-L aquarium filled with 0.2-μm filtered seawater and spiked with 5 Bq mL^{-1} of [^{14}C]C_{12}-6-LAS (isomer with specific activity of 477.3 MBq $mmol^{-1}$; Procter & Gamble). Due to the specific activity of the radiotracer, this spike corresponds to an addition of 4 μg LAS L^{-1}, *viz.* a concentration that is actually found in coastal area subjected to urban effluent discharge (Marcomini et al., 2000). The seawater was changed and the radiotracer spike was renewed daily to maintain [^{14}C]C_{12}-6-LAS activity as constant as possible. Radioactivity in the water was measured before and after seawater renewal in order to determine the time-integrated radiotracer activity (Rodriguez y Baena et al., 2006). After 0.4, 1, 2, 3, 4 and 7 d, three shrimps were gently collected, dried on soft tissue, weighed and ground in methanol. After evaporation and elution in acidified (formic acid, 1%; Merck) deionised water, the samples were placed in scintillation vials with 10 mL of scintillation cocktail (UltimaGold XR, Perkin Elmer) and counted using a liquid scintillation counting (LSC) analyser (Tri-Carb 2900 TR, Perkin Elmer). Basic parameters (temperature, salinity, pH) and [^{14}C] radioactivity in aqueous phase were controlled at each sampling time. No variation in [^{14}C]C_{12}-LAS concentration was observed in the water and sorption to the aquarium glass-wall was negligible. Biodegradation in seawater was not expected during the 24-h period of experiment performed with filtered seawater (Terzic et al., 1992). Therefore 100% of the measured [^{14}C] activity was assumed to represent the spiked congener, C_{12}-6-LAS. LAS concentration in the samples was determined by comparison with standards of known activities and measurements were corrected for counting efficiency, physical radioactive decay and quenching effects (Hédouin et al., 2007).

Uptake of dissolved LAS was expressed in terms of bioconcentration factors (BCF, L/kg; *viz.* ratio between LAS activity in the shrimp – Bq kg^{-1} wet wt – and LAS concentration in the aqueous phase – Bq L^{-1}) over time (Metian and Warnau, 2008). A first-order saturation exponential kinetic model was used to describe the time course of the LAS bioconcentration factor in shrimps, BCF_t (L kg^{-1}, Eq. (1)), as a function of time. The bioconcentration factor at steady state (BCF_{ss}, L kg^{-1}) and the depuration rate constant (λ, d^{-1}) were determined by iterative adjustment using the non-linear estimation module of Statistica®6.1 (StatSoft, Inc., 1998) with the quasi-Newton method for calculating least squares. BCF_{ss} is equal to the ratio between the uptake rate constant (k_u) and depuration rate constant (λ).

$$BCF_t = BCF_{ss}(1 - e^{-\lambda t}) \quad (1)$$

In order to identify size effect on LAS bioconcentration, individual BCF values were plotted against specific shrimps weight after 9 h, 48 h, 96 h and 7 days.

2.3. Whole-body autoradiography

At the end of the exposure period, two shrimps were embedded in a 2.5% carboxymethylcellulose gel and flash-frozen in a slurry of dry ice in hexane in order to perform a whole-body autoradiography study (Rouleau et al., 2003). From each frozen shrimp, 20-μm thick, sagittal sections were cut with a specially designed cryomicrotome (Leica CM3600). Sections were then freeze-dried and placed on phosphorus screens (Perkin–Elmer) for 4–7 d. After exposure, the screens were scanned with a Cyclone Phosphor Imager (Perkin–Elmer) and ^{14}C activity distribution among shrimp tissues was identified.

2.4. Depuration phase

Following the exposure period (7 days), the remaining shrimps (n = 20) were transferred to a new aquarium containing flowing 0.2-μm filtered natural seawater (open circuit; flux: 20 L h^{-1}; constantly aerated; salinity, temperature and light conditions as described above) and let depurate for 8 days. Three shrimps were sampled after 1, 2, 3, 4 and 8 days to determine C_{12}-6-LAS depuration biokinetics. The same LSC protocol, as described before, was adopted to measure radioactivity in shrimp.

The depuration kinetics were best described by a one component exponential model (Eq. (2)), where A_t and A_0 are the remaining activities (%) at time t (d) and t_0, respectively. λ is the biological depuration rate constant (d^{-1}) and allows the calculation of the biological half-life ($T_{b1/2} = \ln(2)/\lambda$).

$$A_t = A_0 e^{-\lambda t} \quad (2)$$

3. Results and discussion

3.1. Bioconcentration

P. varians readily concentrated C_{12}-6-LAS congener, reaching a mean BCF value of 120 ± 90 L kg^{-1} after only 7 days of exposure. Uptake biokinetics were best described by a first-order saturation model (Fig. 1A), which was characterized by an estimated BCF_{ss}, of 159 ± 34 L kg^{-1} ($p < 0.01$). From this model, the uptake rate constant value was 33 ± 4 L kg^{-1} d^{-1} ($p < 0.01$). Results are similar to BCF values observed for freshwater fish exposed to C_{12}-6-LAS (168 L kg^{-1}; Tolls et al., 2000b), fit in the range of C_{12}-LAS BCF values observed for invertebrates (164–316 L kg^{-1}; Hwang et al., 2003), suggesting no significant impact of salinity. The BCF value reported in this study is lower than the value in marine fish exposed to C_{12}-2-LAS (387 L kg^{-1}; Álvarez-Muñoz et al., 2007), confirming that the hydrophobicity of LAS isomers is critical for the bioaccumulation in aquatic organisms.

Based on BCF results, LAS body residue in shrimps exposed to 4 μg C_{12}-6-LAS L^{-1} reached 0.64 μg C_{12}-6-LAS g^{-1} wet weight (=0.002 mmol kg^{-1}) in shrimp tissues. Such body residue fall in the lower part of the range of LAS concentration measured in marine organisms (0.35–8.76 μg g^{-1}; Sáez, 2002). This body residue is at least 40 times below critical body burden assessed for polar narcotic associated to acute (0.8 mmol kg^{-1}) or chronic (0.08 mmol kg^{-1}) exposure. It can therefore be estimated that LAS concentrations in coastal waters do not present a risk for marine shrimps exposed through the dissolved phase.

3.2. Body weight effect on BCF

BCFs displayed wide variability at each sampling time during the exposure phase (Fig. 1A). Among factors that could explain this variability, size/age is known to be of primary importance (Boyden, 1974; Hédouin et al., 2006; Warnau et al., 1995). The allometry of bioconcentration was confirmed in this study for LAS in shrimps. Notwithstanding the limited number of individuals (n = 3 in each point), BCFs are influenced by shrimp body weights: smaller shrimps displayed higher affinity to LAS (Fig. 1B). Negative relationships were observed between BCF and weights at each sampling time during the accumulation process. This relation continues to apply when the individual BCFs are normalized (using the ratio $BCF_{individual}/BCF_{average}$) and are plotted against the corresponding body weight of each individual (data not shown). The

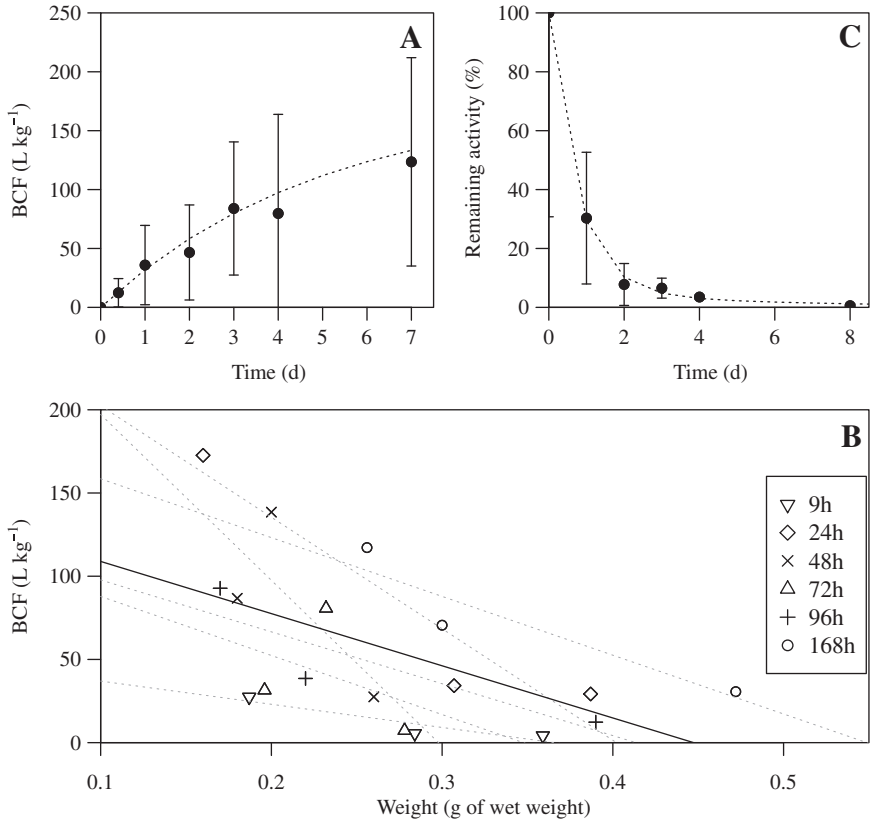

Fig. 1. *Palaemonetes varians*. (A) Uptake of C_{12}-6-LAS by the shrimp *P. varians* ($n = 3$) exposed to 4 μg L^{-1} for 0.4, 1, 2, 3, 4 and 7 days. The data best described an exponential model. (B) C_{12}-6-LAS BCF_{ind}/BCF_{mean} plotted against individual weight (in the range 0.15–0.45 g) by the shrimp *P. varians* exposed to 4 μg L^{-1} for 0.4, 1, 2, 3, 4 and 7 days ($n = 18$). (C) Loss of C_{12}-6-LAS by the shrimp *P. varians* ($n = 3$).

data were best described by a power function ($BCF_{ind}/BCF_{mean} = a * \text{weight}^b$), with $a = 0.04$ g^{-1} and $b = -2.2$ ($R^2 = 0.65$). In addition, the analysis of covariance confirmed that body weight statistically influenced the LAS bioconcentration ($n = 21$; pANCOVA <0.01; time as covariable).

3.3. Organotropism

After 7 days of exposure to 4 μg LAS L^{-1}, C_{12}-6-LAS was mainly concentrated in the cephalothorax circulatory system and the ocular peduncle of the shrimps (Fig. 2). The large surface areas of the gills are the typical entrance pathway of dissolved compounds; movements of the maxillulae (mouthparts) for breathing and swimming movement continuously renew the water layer adjacent to these tissues (Tolls et al., 2000a). Due to its interfacial activity, LAS can be adsorbed onto the surface of these organs. In addition, the autoradiography study demonstrated actual absorption of LAS in the hepatopancreas, the heart, the pericardium and possibly the sub-rostral/supra-stomacal venous sinuses (Fig. 2). LAS probably reach these internal organs through the gills, and subsequent transfer through the hemolymph circulation to the most highly irrigated organs (Tolls et al., 2000a).

3.4. Depuration

Depuration kinetics were best fitted by a one-component exponential equation (Fig. 1C), characterized by a rapid depuration rate constant (1.18 ± 0.08 d^{-1}). Estimated biological half-life value is

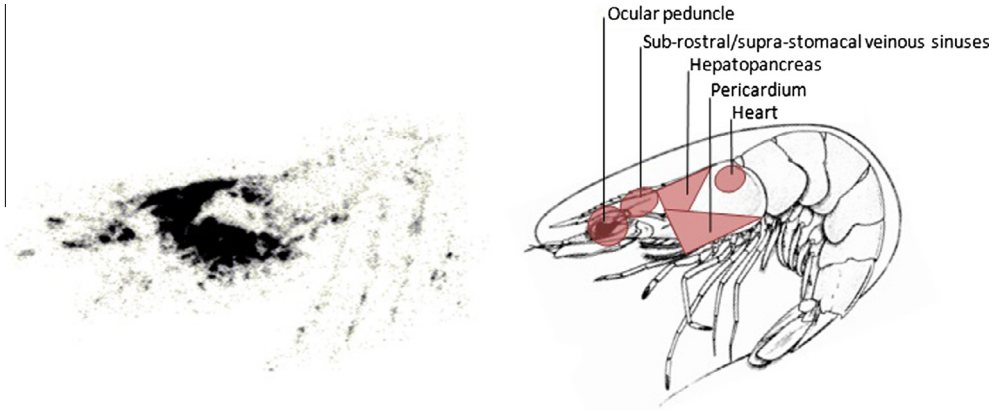

Fig. 2. *Palaemonetes varians*. Autoradiogram of the marine shrimp exposed to dissolved C12-6-LAS.

14 h. This value is similar to those determined for freshwater species (Hwang et al., 2003). After 8 days of depuration, LAS remaining in the tissues represents less than 1% of initial body burden. Such rapid and efficient elimination of LAS suggests a low risk of chronic toxicity and a limited transfer through the food chain in areas exposed to urban effluent discharge. This hypothesis nevertheless needs to be confirmed with dietary exposure of the shrimp since accumulation through food can be substantial for some classes of contaminants (Metian et al., 2008).

4. Conclusion

In this work, we assessed C_{12}-6-LAS availability for accumulation in the shrimp *P. varians*. C_{12}-6-LAS enters the body most probably through the gills and then distributes mainly in the cephalothorax circulatory system. The estimated BCF_{ss} was 159 L kg^{-1}, reached within 11.5 days and BCF was inversely related to shrimp body weight. Depuration was rapid, with less than 1% of initial LAS remaining after 8 days.

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