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Oxidative stress and histopathology damage related to the metabolism of dodecylbenzene sulfonate in Senegalese sole

D. Álvarez-Muñoz [a], A. Gómez-Parra [a], J. Blasco [b], C. Sarasquete [b], E. González-Mazo [a,*]

[a] Dpto. Química Física, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, Avd República Saharaui s/n, 11510 Puerto Real, Cádiz, Spain
[b] Instituto de Ciencias Marinas de Andalucía, Consejo Superior de Investigaciones Científicas, Avd República Saharaui s/n, Puerto Real, Cádiz, Spain

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ABSTRACT

Surfactants such as linear alkylbenzene sulfonates (LAS) are widely utilised in the formulation of detergents in commercial products. After use, they pass through waste water treatment plants (WWTP) and are then discharged to aquatic ecosystems, causing risk to aquatic life. The exposure of marine animals to these compounds enhances the production of reactive oxygen species (ROS) with subsequent damage to macromolecules, and produces histological alterations. A flow-through experiment with Senegalese sole (*Solea senegalensis*) has been devised with the object of correlating the metabolism of LAS including sulfophenylcarboxylic acids (SPCs) by fish with their antioxidant defence system (generation of oxyradicals) and histopathological damage. The generation of intermediate degradation products (SPCs) by the organism, the histopathological responses, the antioxidant enzymes (catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR), and glutathione S-transferase (GST)), as well as other kinds of enzyme such as acid and alkaline phosphatases (AcP, ALP), were measured. SPCs from 5ØC$_6$ to 11ØC$_{12}$ were identified and quantified in fish and water; their concentrations differed depending on the sampling moment. In general, the responses found in the enzymes were slight: a decrease in the enzymatic activity in gills and activation in the digestive tract. The evidence of histopathological damage identified was also small; the organism's defensive mechanism against pollutants should enable it to recover easily. A direct relationship was established between biotransformation and the generation of SPCs and ROS. In conclusion, the correct functioning of the antioxidant defence system with absence of large variations, the short-term histopathological damage, and the evidence of SPCs indicate an adequate metabolism of 2-phenyl-C$_{12}$-linear alkylbenzene sulfonates (2ØC$_{12}$LAS) by this specie and non-toxic effects at environmentally realistic levels.

1. Introduction

In recent years, the use of sensitive biomarkers as a tool for monitoring both environmental quality and adaptation of organisms to threats has attracted more attention. Cellular responses identifiable in the different organs have been shown to be useful for characterising the health status of organisms, as well as serving as biomarkers of impact for evaluating environmental parameters (Cajaraville et al., 2000; Sarasquete and Segner, 2000). In addition, the antioxidant defense system has been widely studied because of its function in removing oxyradicals (Zhang et al., 2005), and the system also provides biochemical biomarkers. These oxyradicals, called reactive oxygen species (ROS), are reactive or activated species of O_2, including true oxygen free radicals: the superoxide radical anion (O_2^-) and the hydroxyl radical (OH), and other related species such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$). Several endogenous sources of cellular oxyradical production have been identified (e.g. mitochondrial electron transport) but in toxicology studies oxyradicals are examined chiefly in relation to xenobiotics that enhance their production and the resulting damaging effects.

In the present research the xenobiotic compound evaluated is linear alkyl benzene sulfonate (LAS), the most widely used synthetic anionic surfactant for cleaning products. Commercial LAS is a mixture of homologues of different alkyl chain length and isomers of these homologues, differing in the sulfophenyl group linked to the alkyl chain. About 98% of the total LAS used by the population is removed in sewage treatment plants (Feijtel et al., 2000) and the rest is discharged to aquatic ecosystems. The environmental levels of LAS and its intermediate degradation products, sulfophenylcarboxylic acids (SPCs), found in several Iberian littoral ecosystems (León et al., 2002) take values of some hundreds of μg/L of surfactant near wastewater discharges. Moving downstream, the concentrations of LAS and also of SPCs show a decrease due to dilution, adsorption–precipitation and degradation processes (González-Mazo et al., 1998). Therefore, the concentration falls to a few ppb in water at the mouths of rivers and in the adjacent littoral zones.

* Corresponding author. Tel.: +34 956016159; fax: +34 956016040.
E-mail address: eduardo.gonzalez@uca.es (E. González-Mazo).

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The use of a flow-through regime in laboratory studies to reproduce the real exposure levels of a contaminant and the natural environment is recommended by the Organisation for Economic Co-operation and Development (OECD), the American Society for Testing and Materials (ASTM) and the US Environmental Protection Agency (EPA). Several authors have used this experimental design to study LAS bioconcentration in different aquatic organisms (Tolls et al., 1997, 2000a; Rosen et al., 2001; Sáez et al., 2001; Versteeg and Rawlings, 2003; Álvarez-Muñoz et al., 2007a). The biotransformation has been less extensively researched (Tolls et al., 2000b; Álvarez-Muñoz et al., 2007b) and only the phase I biotransformation has been previously studied in LAS metabolism. This consists of a sequence of reactions: the first step is the oxidation of the terminal methyl group to carboxylic acid, followed by loss of one or two carbon atoms, making the surfactant more hydrophilic and therefore, more easy excreted by the organism. The ω, α, and β oxidations are the reactions involved which are enzyme-catalyzed, by oxidases, mono-oxygenases and dehydrogenases. It has been demonstrated that the biotransformation or metabolism of xenobiotic compounds results in the formation of reactive oxygen species (Peters et al., 1996; Zhang et al., 2005; Monferran et al., 2007). These species may lead to oxidative damage for example, lipid peroxidation, enzyme inactivation, DNA strand breaks, and covalent binding to protein and nucleic acid. Living cells have evolved numerous defence mechanisms to neutralize the harmful effects of oxyradicals. The antioxidant defence system includes enzymes such as catalase (CAT), glutathione peroxidase (GPX), and glutathione reductase (GR) which reduce the ROS levels. CAT is primarily associated with peroxisomes, where it detoxifies H_2O_2. GPX is a cytosolic enzyme that reduces H_2O_2 to H_2O, and lipid peroxides to their corresponding alcohols. GR plays an important antioxidant role by catalyzing the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH) at the expense of NADPH.

The few available studies about the effects of LAS exposure in the activities of enzymes in organisms have employed in static assays: at high exposure levels (Da Ros et al., 1995; Blasco et al., 1999), and at environmentally representative concentrations (Li, 2007), and in flow-through systems at environmental levels (Álvarez-Muñoz et al., 2006, 2007b). There are no studies available that establish a relationship between variations in the activities of enzymes, the appearance or existence of biotransformation and the intermediate degradation products generated.

Concerning the histopathology, fish have previously been exposed to LAS (Hampel et al., 2004, 2008) but, as mentioned before for oxidative stress, the exposure concentrations used were very high and not environmentally realistic. As a consequence of the high exposure concentrations, considerable histological alterations were detected in tissues of *Sparus aurata* and *Solea senegalensis*.

To our knowledge, there are no *in vivo* studies that relate the concentration of the surfactant in an organism and its biotransformation (generation of metabolite products) with the antioxidant defence system and the related histopathological alterations. This is the objective of this research.

2. Material and methods

2.1. Fish

S. senegalensis (Senegalese sole; body weight between 3 and 6 g) were purchased from the Aquaculture facilities of the University of Cadiz. Prior to the experiments, they were acclimated for 2 days. The selected target tissues were the gills (because this is the main uptake site and the most sensitive organ), and the digestive tract (because this tissue is involved in the metabolism and excretion of xenobiotics).

2.2. Surfactant

The compound chosen for this study was the pure homologue $2ØC_{12}$LAS (99% purity and 11.2% active index), supplied by Petroquímica Española S.A. It represents the most adverse situation for the organisms regarding environmental exposure, although its contribution to the commercial LAS mixture is small (5.5%).

2.3. Flow-through regime

An *in vivo* assay was conducted in a continuous flow-through regime (according to the OECD guideline 305), using sea water spiked with $2ØC_{12}$LAS. The regime consisted of an exposure phase (120 h), followed by a depuration stage (72 h) (described in Álvarez-Muñoz et al. (2007a)). The rate of water renewal during both phases was five times per day. A control tank was kept under the same conditions without addition of surfactant. Water was kept oxygen-saturated by aeration, the temperature was maintained at ambient laboratory temperature 18.5 ± 0.5 °C, and the pH was 8.41 ± 0.1.

2.4. Analytical determination

The LAS and SPCs concentrations were determined following the methodology performed by Álvarez-Muñoz et al. (2007c). Briefly, the analysis sequence was: pressurized liquid extraction (100° C) with hexane and MeOH, followed by SPE on a C_{18} minicolumn, and finally determination by Liquid Chromatography Quadrupole (LCQ) ion-trap mass spectrometer equipped with an atmospheric pressure ionization source with electrospray interface (ESI). The quantification was performed using selected ion monitoring (SIM), and external standard solutions were used. The percentage of recovery for LAS was 99.9% ± 3.8 and for total SPCs was 90.1% with a standard deviation of 9.9. The detection limit for $2ØC_{12}$LAS was 22 ng g^{-1}, and between 1 and 58 ng g^{-1} for SPCs.

2.5. Biochemical assays

Five different biomarkers were selected to evaluate subcellular effects: Catalase (CAT), glutathione peroxidase (GPX), Glutathione reductase (GR), Glutathione S-transferase (GST), and acid (AcP) and alkaline phosphatases (ALP).

The fish were dissected and the tissues were stored until needed for processing at −80 °C. About 0.5 g of gill and alimentary tract tissues (pooled sets from three fishes) were homogenised with an Ultraturrax after addition of Henriksson buffer (50% glycerol, Tris–HCl 100 mM, 5 mM β-mercapto-ethanol, 0.5 EDTA, 0.02% BSA and Protease inhibitor) (1:4, w/v). Then the homogenised tissues were centrifuged at 9000g for 15 min at 4° C to obtain the post-mitochondrial fraction for GPX assay, and the second at 50 000g for 2 h at 4 °C for the cytosolic fraction.

The CAT activity was determined by measuring the consumption of H_2O_2 at 240 nm ($\varepsilon = 40\ mM^{-1}\ cm^{-1}$), according to Aebi (1974).

GPX, GR and GST were determined following the methodology of McFarland et al. (1999). Selenium-independent glutathione peroxidase (GPX/HP) and selenium-dependent glutathione peroxidase (GPX/CP) were measured by the loss of NADPH and the substrates for the enzymes were H_2O_2 (GPX/HP) and cumene hydroperoxide (GPX/CP). The GR activity was estimated by NADPH oxidation and the substrate was oxidized glutathione (GSSG). GST activity was estimated using 1-chloro-2,4-dinitrobenzene (CDNB) as a conjugation substrate.

The acid and alkaline phosphatases were determined according the procedure described by Blasco et al. (1993) in which a yellow compound *p*-nitrophenol (PN), liberated from *p*-nitrophenylphosphate (PNP) by phosphatases, is quantified colorimetrically (405 nm). The protein levels were determined by spectrophotometry using the BCA method (Smith, 1985).

The statistical analysis was performed using the SPSS 11.5 for Win computer program. ANOVA and post hoc Tukey test were used to test differences between groups. The differences were regarded as statistically significant when $p < 0.05$.

2.6. Histological analysis

Juvenile specimens of *S. senegalensis* (control and LAS treated) were studied. Digestive tract and gills were fixed in formaldehyde, phosphate buffered 0.1 M (pH 7.2), and processed in a routine paraffin embedding procedure. Sections of 5–6 μm were stained with Haematoxylin–eosin/H-E; Haematoxylin-VOF for histopathological analysis, according to Sarasquete and Gutiérrez (2005).

3. Results

3.1. LAS and SPCs concentrations

At the beginning of the assays (0 h), water and fish samples were analysed and neither LAS nor SPCs were detected. Fig. 1 shows the $2ØC_{12}$LAS concentration measured in the exposure water (a) and in the fish (b) during the experiment. The concentration was determined at 6 h from the beginning of the experiment, and every 24 h until the end of the assay (24 h, 48 h, 72 h, 96 h, 120 h, 144 h, 168 h and 192 h). From 6 h to 120 h the measures correspond to the exposure period, and from 144 h to 192 h to the depuration stage. During the exposure phase, an average LAS concentration in water of 26 ± 11 μg/L was measured. The concentration of $2ØC_{12}$LAS measured in the organism ranged from 3.2 to 7.2 μg/g. It increased from the beginning of the assay up to 48 h of exposure, and then remained constant until the end of the exposure stage (120 h); it decreased in the depuration phase.

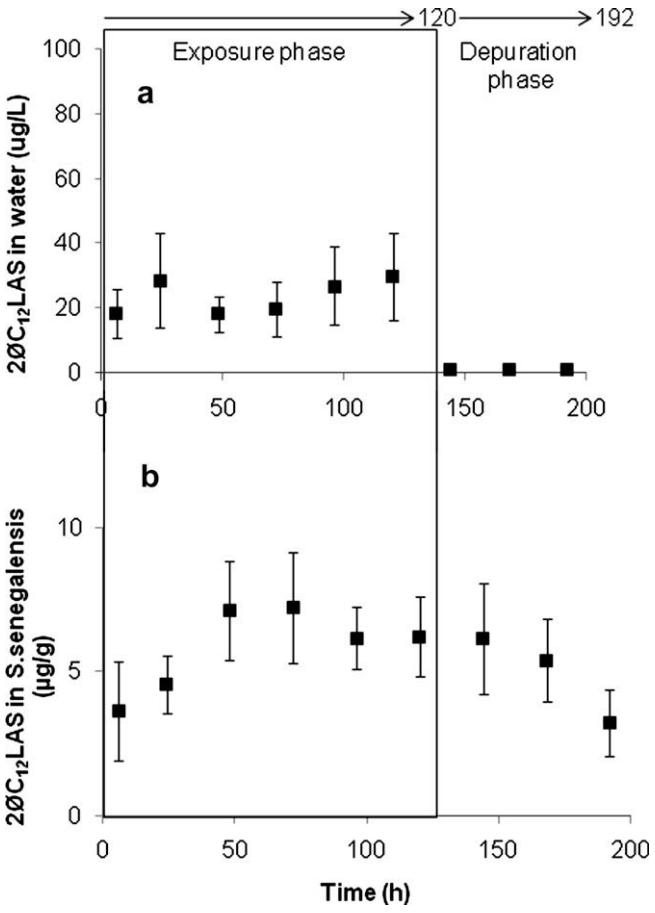

Fig. 1. Concentration of $2ØC_{12}$LAS determined in *S. senegalensis* over time (6, 24, 48, 72, 96, 120 h correspond to the exposure phase, and 144, 168 and 192 h to the depuration phase) (a) in experimental water ($n = 3$) and (b) in the whole fish ($n = 4$).

Over the course of the experiment, homologues of SPC with six to 12 carbon atoms in their carboxylic chain were detected and quantified in the whole body of the organism, as well as in the water of the aquarium. Fig. 2 shows the average levels of the different SPC homologues, in water and in organisms (for triplicate and quadruplicate samples respectively), during the exposure phase (24 h and 120 h), and during the depuration stage when the organisms were transferred to clean water (at 144 h, 168 h and 192 h). In the exposure stage an increase of SPCs concentrations in fish and water is observed between 24 h and 120 h; a similar distribution of SPCs is found between these sampling times for both fish and water (Fig. 2a–d). The only intermediate degradation product detected in Senegalese sole was $10ØC_{11}$SPC while in water the homologues found were SPCs with shorter alkyl chain length.

In the depuration or elimination period, two different situations can be distinguished. At 144 h and 168 h in fish, the SPCs identified and quantified were $5ØC_6$, $6ØC_7$, $7ØC_8$, $10ØC_{11}$, $11ØC_{12}$SPCs, whereas in the water of the aquarium SPCs between $6ØC_7$ and $8ØC_9$ were determined. Between the two sampling times (144 h and 168 h) an increase in the concentration of SPCs was observed in the organism. At 192 h a similar distribution of SPCs as described above for 24 h and 120 h of exposure was found: $10ØC_{11}$SPC was determined in fish, and in water from $5ØC_6$ to $7ØC_8$SPCs. The most abundant SPC in fish during the entire experiment was $10ØC_{11}$. The most abundant SPC in water during the exposure phase and at 192 h was $6ØC_7$SPC, but at 144 h and 168 h $8ØC_9$SPC was the most abundant.

3.2. Enzymatic approach

Fig. 3 shows the values of antioxidant enzymes measured in *S. senegalensis* exposed to $2ØC_{12}$LAS, in the controls (white) and in LAS exposed fish (black), prior to the start of the experiment (0 h) and at the end of the exposure (120 h) and depuration (192 h) phases, for gills and alimentary tract. For CAT a decreased level in gills was observed at the end of the exposure stage, while in the alimentary canal the response found was an activation, at both sampling times (Fig. 3a and b).

The GR activity measured in gills varies in the same way as in the controls, but, as can be seen in Fig. 3d, in the digestive tract, the enzyme was induced at the end of the elimination stage.

Fig. 3e and f shows the modifications of selenium-dependent glutathione peroxidase (GPX/CP). For the gills no differences were found between the controls and the exposed fish but in the alimentary canal a decrease in the enzyme activity was measured at the end of the exposure phase.

The results obtained for selenium-independent glutathione peroxidase (GPX/HP) are shown in Fig. 3g and h. In gills a decrease was observed at the end of the depuration stage while in the digestive tract the opposite effect, an increase, was found.

Changes in GST and phosphatase activity in the target tissues chosen are shown in Fig. 4, before the assay and after the two experimental stages. For GST activity, a slight increase is shown in the alimentary tract at the end of the experiment (192 h) (Fig. 4b) while in the gills a decrease is observed (Fig. 4a). The results for phosphatases do not show any particular tendency.

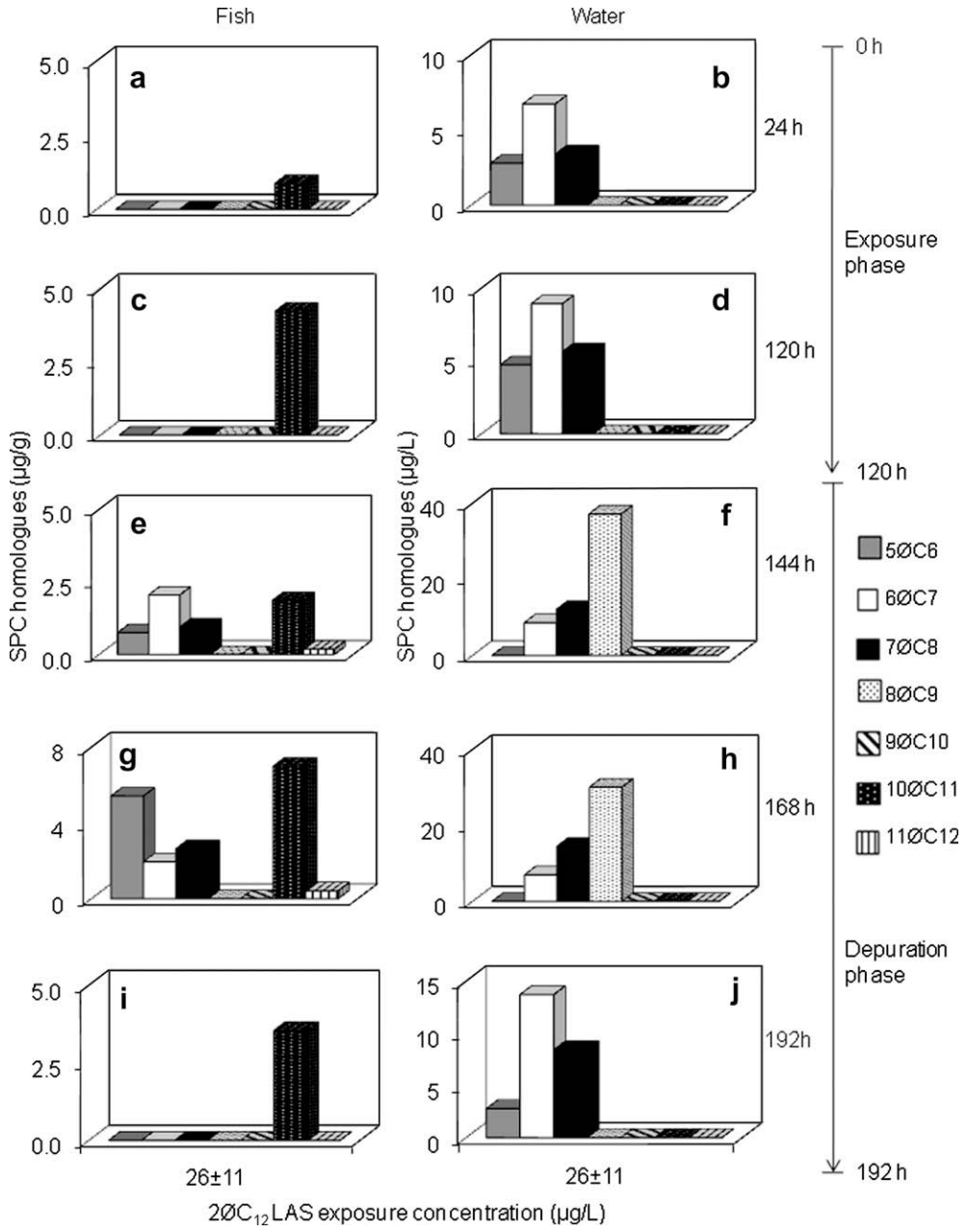

Fig. 2. Distribution of sulfophenyl carboxylic acids by homologues of $2ØC_{12}$ linear alkylbenzene sulfonate, in *S. senegalensis* at 24 h (a), 120 h (c), 144 h (e), 168 h (g) and 192 h (i), and in water at 24 h (b), 120 h (d), 144 h (f), 168 h (h), and 192 h (j).

In general the variations observed are small and no significant differences were obtained with the *post hoc* Tukey test but there is a tendency for the activity of the enzymes to increase in the alimentary tract (related to the biotransformation of the surfactant) (Figs.3b,d,f,h and 4b), whereas in the gills a decrease in the enzyme activity is more common (Figs. 3a,g and 4a).

3.3. Histological approach

The histopathological responses found in *S. senegalensis* control, exposed and depurated fish are shown in Table 1. The results (numeric values) are expressed as semi-quantitative assessment of damage; zero means that the effect was not observed and the increase or decrease of the numerical value indicates the level of alteration detected, in digestive tract and gill tissues. In gills the effect observed was an increase for all the histopathological responses found in the exposed fish, compared to the controls, and a decrease in the depurated fish, with values returning to the control situation for lifting and blood stagnation. For all the alterations detected in the intestine there is also an increase for the exposed fish compared to the controls. In the depurated specimens a decrease is observed for the first and last pathologies; however, the epithelial desquamation is maintained and an increase is found for the lipid droplets. No alterations were identified in the stomach.

Some of these alterations are illustrated in Fig. 5. Control, exposed and depurated tissues are shown; Fig. 5a,c and e correspond to the gills, and Fig. 5b, d, and f to the digestive tract. Gills from control specimens show regular disposition of primary filaments and secondary lamellae, where respiratory gas exchange takes place. In all treated specimens blood stagnation of gill vessels, as well as lamellar fusion and lifting were detected.

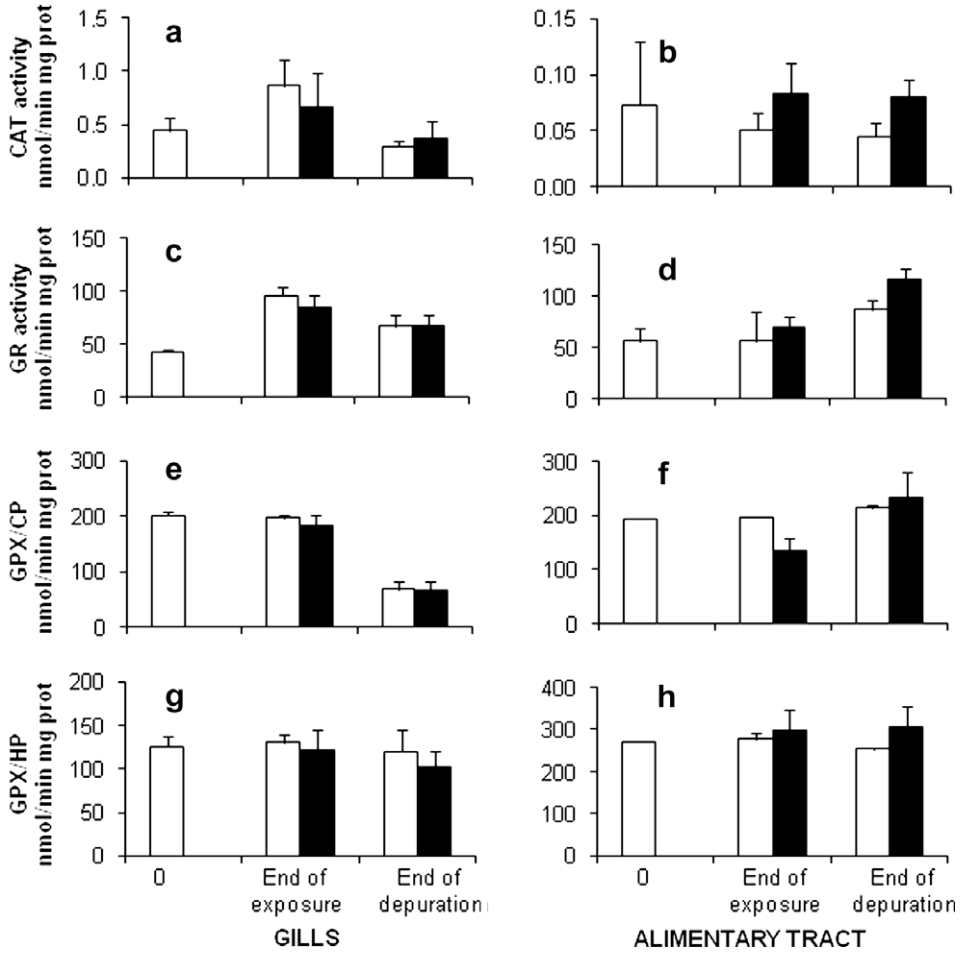

Fig. 3. Antioxidant enzyme activity in gills: (a) CAT activity, (b) GR, (c) GPX/CP, (d) GPX/HP, and in alimentary tract: (e) CAT activity, (f) GR, (g) GPX/CP, (h) GPX/HP, in controls (white) and in LAS-exposed fish (black), before the experiment (day 0), after the exposure phase (day 5) and at the end of the depuration stage (day 8). The values are expressed as mean ± SD (n = 4).

In the digestive tract of control organisms, intestinal folds present a uniform disposition, forming a simple epithelium of cylindrical cells with numerous goblet cells and scarce lipid droplets within the cytoplasm of enterocytes. In treated fish, increased numbers of mucous cells and lipid droplets in the cytoplasm of enterocytes were detected, as well as weak epithelial desquamation and a decrease of connective supporting tissue. The mucosa of the stomach is composed of a simple epithelium of cubic cells with numerous gastric glands located in the submucosa. No cellular response was detected in the stomach of treated fish.

4. Discussion

In the experiment the LAS exposure concentration was within the same range as the LAS concentrations determined in natural environments (Sancti Petri channel (González-Mazo et al., 1998) and Almeria Bay (Petrovic et al., 2002). During the depuration phase the LAS determined in water was below one μg/L because there was no inflow of surfactant in this stage. The increase of LAS concentration in the organism, *S. senegalensis*, from the beginning of the experiment to 48 h corresponds to the uptake. The decrease of LAS concentration in fish during the depuration phase is due to the biotransformation and excretion of the metabolites, and the diffusive elimination of the parent compound from the organism (through gills or skin) to water. After this process the metabolites are excreted actively by urine or faeces, where the digestive tract has an important role.

The detection and quantification in fish of 11ØC$_{12}$SPC, the degradation product with the same numbers of carbon atoms in its alkyl chain as the parent compound (2ØC$_{12}$LAS), confirms that the phase I biotransformation by the organisms starts with the ω-oxidation; this is the oxidation of the terminal methyl group to carboxylic acid. Moreover, the identification of SPCs with even and odd numbers of carbons atoms in their alkyl chains shows that the α–β-oxidations were also taking place together with ω-oxidation. In α and β-oxidations the alkylic chain is shortened by one and two carbons fragments, respectively. Homologues of SPCs with fewer carbon atoms, like 4ØC$_5$SPC, were not detected in fish, because of the cleavage of the aromatic ring prior to mineralization.

In the depuration phase a period of time was identified (from 144 h to 168 h) when all three types of oxidative reaction (ω, α, and β-oxidations) were taking place together; the effect of this is to make the biotransformation by the organism more efficient. The highest concentration of total SPCs in fish (11.8 ± 4.5 μg/g of SPCs) was determined in this period, and a shortening of the alkyl chain is observed in the SPC homologues detected.

At the beginning of the experiment (24 h, Fig. 2a) 10ØC$_{11}$SPC was identified and quantified in Senegalese sole; this shows that the phase I biotransformation was taking place in the organism from 24 h of the experiment at the environmental level of exposure. At the end of the assay this homologue was still present (Fig. 2i) which confirms that the biotransformation process had been continuing at least until this time. In contrast, in a previous research study conducted with 2ØC$_{10}$LAS (Álvarez-Muñoz et al.,

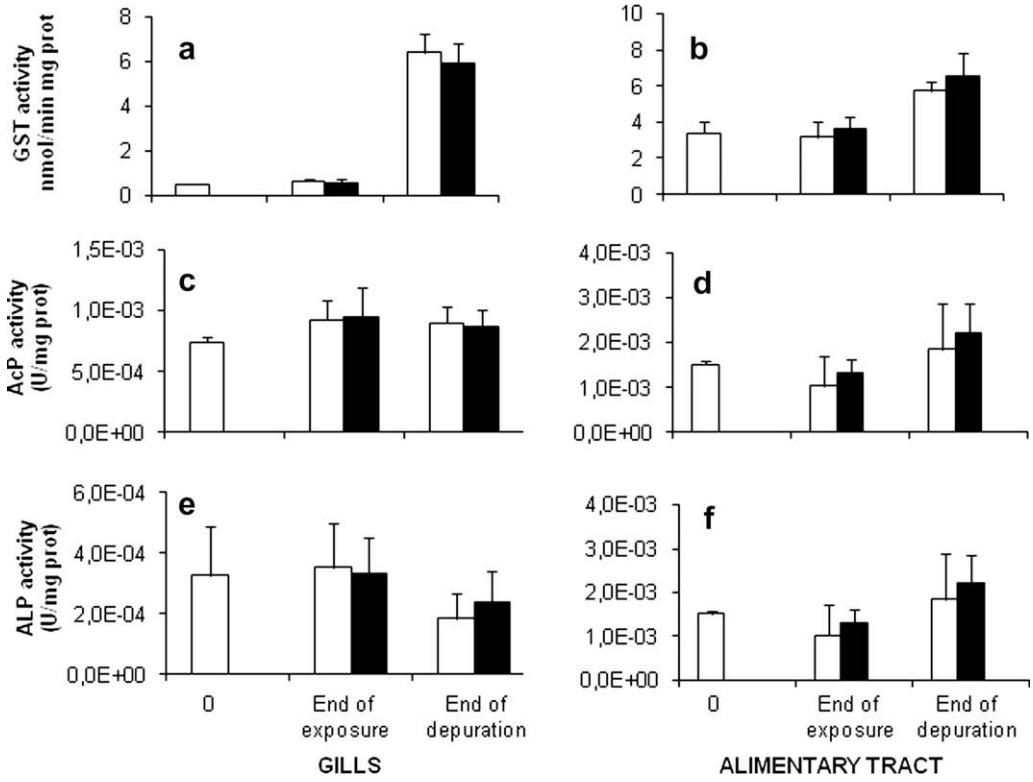

Fig. 4. GST, AcP and ALP enzyme activity in the gills (a–c, respectively) and in the alimentary tract (d–f) in controls (white) and *Solea senegalensis* (black) exposed to C_{12}-2-LAS, prior to the experiment (day 0), after the exposure phase (day 5) and at the end of the depuration stage (day 8). The values are expressed as mean ± SD ($n = 4$).

2007a), at the end of the experiment no SPCs were detected in the fish. This absence can be explained by the stronger lipophilic character of 2ØC_{12}LAS compared with other LAS homologues with shorter alkyl chain, which makes them less bioavailable; therefore, the biotransformation by organisms takes place to a reduced extent.

The SPC homologues detected in water during the exposure phase were more hydrophilic, corresponding to compounds of shorter alkyl chain length (5ØC_6, 6ØC_7 and 7ØC_8, Fig. 2b and d). These were generated by the microbial degradation of the parent compound in the water of the aquarium and by excretion from fish after the biotransformation. However, in the depuration phase, no LAS or SPCs were introduced into the water; consequently the SPCs detected could only have come from the biotransformation, excretion and diffusive elimination of the more polar intermediate compounds. The highest concentrations of SPCs were measured in this phase (at 144 h and 168 h), which coincides with the period of most efficient biotransformation by *S. senegalensis*. The SPC homologue with the highest concentration found in water in this period was 8ØC_9SPC (Fig. 2f); its concentration decreased during the depuration phase and at 192 h it disappeared due to the reduced elimination process by the organism. However, homologues with shorter alkyl chain showed increased concentrations at this sampling time, probably due to the degradation of 8ØC_9SPC in the water of the aquarium and the excretion by fish.

Table 1
Histopathological responses found in specimens of Senegalese sole.

	Control	Exposed	Depurated
Gills			
Lamellar fusion	1	3	2
Lifting	1	3	1
Blood stagnation	1	3	1
Intestine			
Increase of mucous cells	0	3	1
Increase of lipid droplets	1	2	3
Epithelial desquamation	1	2	2
Loss of connective tissue	0	1	0
Stomach	No	Cellular	Response

The generation of reactive oxygen species during the experiment was small (producing only slight variations in the antioxidant defence system) which indicates an adequate metabolism of LAS by the fish, with no apparent toxicity being caused. It is clear that there is a direct relationship between biotransformation, producing SPCs, and the generation of ROS. In the alimentary tract a tendency was observed for the activity of CAT, GR, GPX/HP and GST to increase. This is because the biotransformation of LAS (together with the excretion) takes place in this tissue, and therefore, the oxidases, mono-oxygenases and dehydrogenases involved in the ω, α, and β oxidations are working. As a consequence of the LAS metabolism more ROS (subproducts) are produced and therefore, the antioxidant defence system needs to be activated to remove these potentially harmful species. In the gills the effect observed was a decrease for CAT, GPX/HP and GST activity. In this tissue considerably less biotransformation of the surfactant takes place and therefore less ROS are generated. Furthermore, the amphiphilic character of the surfactant means that it is retained more, allowing the gills to support a higher exposure concentration than the organism's internal tissues, and it has a more toxic effect. In a previous study Tolls et al. (2000a) found the highest BCFs in the gills and skin of rainbow trout exposed to LAS, compared other internal tissues.

In respect of the histological and histochemical characteristics of *S. senegalensis* exposed to 2ØC_{12}LAS, some tissue disturbances were detected, such as: lifting and fusion of gill lamellae; stagnation of gill vessels; an increased number of mucous cells and lipid droplets in the alimentary canal, and a decrease of supporting intestinal connective tissue, with epithelial desquamation.

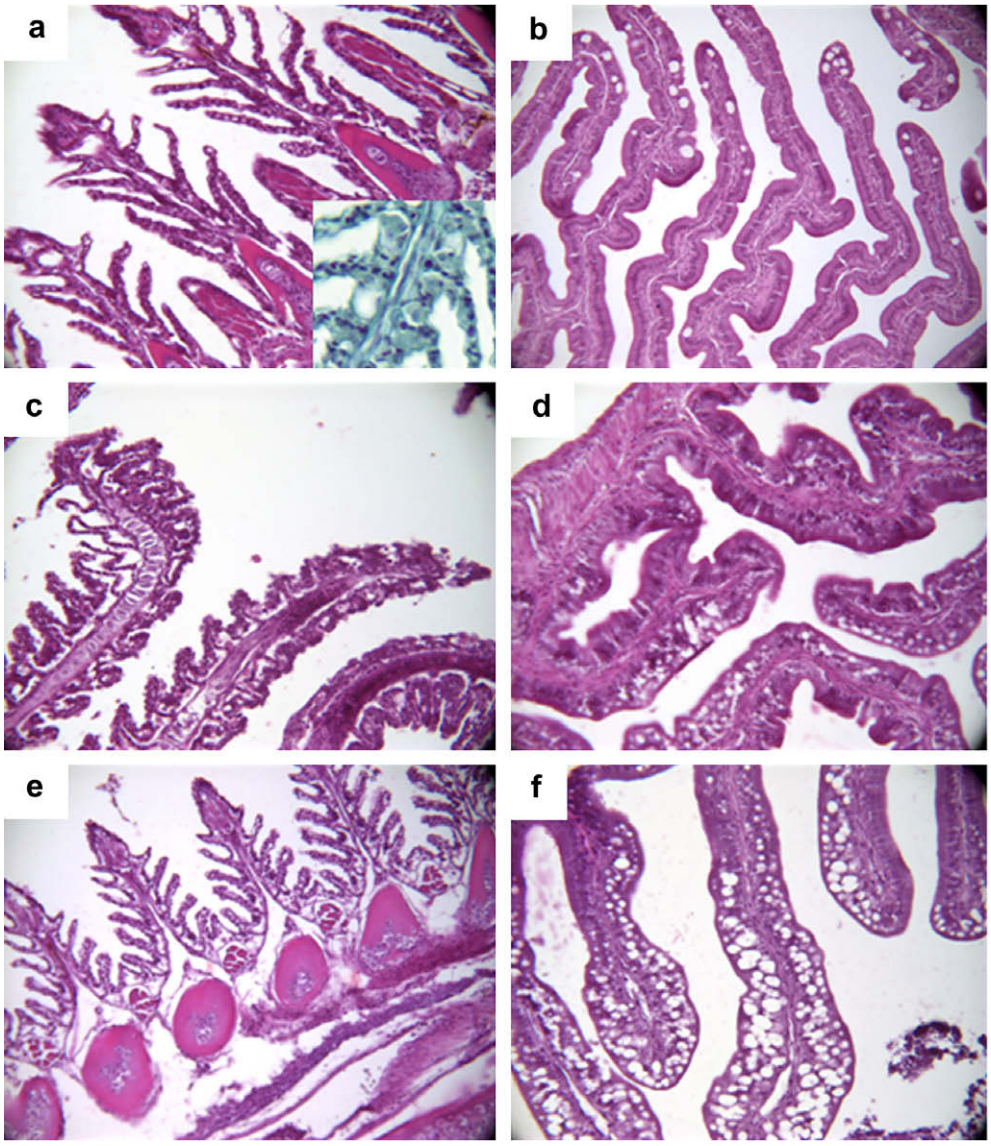

Fig. 5. Histological sections of the gills and intestine from control, exposed and depurated *Solea senegalensis* juvenile specimens. Haematoxylin–eosin staining. (a) Normal structure of gills of control specimens showing gill arches, and primary and secondary lamellae, showing basic features of the primary and secondary lamellae with typical pillar or respiratory, chloride and mucous cells in the epithelium. ×**40.** (b) Intestinal mucosa from control fish showing typical microridges in the single layered columnar epithelium containing absorptive cells or enterocytes, mucous cells and scarce lipid goblets in the enterocytes. ×**25**. (c) Histological section of gills from exposed fish showing lifting in the base and tips of the secondary lamellae and fusion of adjacent secondary lamellae. ×**40**. (d) Section of intestinal mucosa from exposed fish showing a desquamation of epithelium and slight increases of mucous cells lipid droplets within enterocytes. ×**40**. (e) Normal histological characteristics of gills from depurated fish showing the typical structure of primary and secondary lamellae. ×**25**. (f) Intestinal mucosa from depurated fish with a considerable increase of lipids in the enterocytes and mucous cells in the epithelium. ×**40**.

The epithelial lifting in the secondary lamellae is a typical inflammatory reaction against pollutants, but it is unclear whether such reactions are the consequence of a general weakening of the fish caused by some kind of stress factor, or whether they are the result of direct contact with toxicants. Moreover, lifting can diminish the area of gill surface vulnerable to toxicants (Cerqueira and Fernandes, 2002; Alvarado et al., 2006). Gill lamellar fusion cannot be considered a specific response because it has been observed after exposure to different toxicants and irritants (Hampel et al., 2004; Ortiz-Delgado and Sarasquete, 2004; Alvarado et al., 2006). Recently, Ortiz-Delgado et al. (2008) indicated that blood stagnation was a non-specific response, because healthy and treated fish present blood congestions in different tissues.

In summary, the histological cellular changes detected could be considered as a general stress response to LAS concentrations and a general adaptative mechanism; moreover the various types of histopathological damage are short-term responses from which the cells can recover.

5. Conclusion

This study reveals that, although the phase I biotransformation takes place in the organism from the start of the experiment, there is a period of time (in the depuration phase) where this process is more efficient because ω–α, and β-oxidations are happening at the same time. While the biotransformation is taking place in the organism, SPCs, with ROS as byproducts, are being generated, thus establishing a direct relationship between them.

The activation of various enzymes in the alimentary tract was measured. Because this tissue is one of the main sites for the

biotransformation and excretion of these compounds, the generation of oxyradicals increases as a consequence of these processes. However, in the gills the effect observed was a decreased generation because this tissue can tolerate exposure to higher concentrations than the organism's internal tissues, although the compound is also metabolised in gills this occur less than in the alimentary tract.

The histopathological damages found include lamellar fusion, lifting, and blood stagnation in gills, increased numbers of mucous cells and lipid droplets, epithelial desquamation and loss of connective tissue in intestine. These are general stress responses and the organism can recover from them.

In conclusion, the correct functioning of the antioxidant defence system with the absence of large variations, the short-term histopathological damage from which the organism can recover, and the evidence of SPCs indicate an adequate metabolism of $2ØC_{12}$LAS by this specie, and non-toxic effects at environmentally realistic levels.

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