SUMMARY

In the present study we have assessed the effect on the survival and the morpho-histochemical changes in the liver and intestine of young gilthead (fish-nursery), Sparus aurata, L., induced by acute action of the anionic tensioactive alkylbenzene sulphonate (ABS). Firstly, the LC50 of ABS at 96 hours was found to be 0.6 mg/L. Secondly, lots with 50 young gilheads (fish-nursery) were exposed to ABS concentrations of 0.5, 1, 3 and 5 mg/L, to obtain the surface tension value and exposure time required for 50% mortality of the specimens at each tested concentration. Exposure to ABS caused several forms of histopathological damage in the liver (the radial arrangement of hepatocytes was lost) and intestine (destruction of the structure of villi and increase in thickness of the other three layers). In addition, changes in bio-macromolecule components (proteins in general, siderophile proteins, neutral mucopolysaccharides, glycogen and acid mucopolysaccharides) were observed. The degree of these alterations was dependent upon the ABS concentration. These changes could have detrimental effects on the growth and survival of the species.

INTRODUCTION

Marine pollution is intimately related to socio-industrial development (Prat and Giraud, 1964; Swedmark et al., 1971). Tensioactives are significant components of several consumer products, such as laundry detergents, shampoos, toothpastes and cosmetics. About 70% of industrial production of tensioactives corresponds to anionic tensioactives (García Dominguez, 1986), the group to which ABS (alkylbenzene sulphonate) belongs.

Little is known about the damaging effect of detergents on marine fauna (Mahajan and Singh, 1972; Abel, 1974), in particular about histopathological and histochemical aspects. The aim of this work was firstly to determine the LC50 at 96 h and secondly to estimate the surface tension value and exposure time required for 50% mortality of the specimens resulting at each concentration of ABS. Finally, to examine histopathological alterations as well as the histochemical distribution of proteins (proteins in general, siderophile proteins) and carbohydrates (neutral mucopolysaccharides, glycogen and acid mucopolysaccharides) in two
anatomical structures: liver and intestine. Selection of intestine was based on its direct contact with the tensioactive while the liver was chosen due to its indirect contact via the blood.

Research on the influence of the anionic surfactant ABS on gilthead Sparus aurata, L. was particularly appropriate because of its importance in the fishing industry and in fish farming (Arias et al., 1984). Besides, this species was very sensitive to any fall in the concentration of dissolved oxygen (Arias, 1976), which make it a useful tool in the study of pollutants that affect the oxygenation of the water.

In the present work we have chosen specimens younger than those employed by other authors (Ribelles et al., 1995 a, b, c; Rosety et al., 1997), in order to contribute to the limited knowledge about the effect of surfactants on the early life stages of gilthead Sparus aurata L.

Literature concerning the histopathology in aquatic species induced by detergents are circumscribed to gills in freshwater species (Schmid and Mann, 1961; Roy, 1988), as well as gills, intestine and liver-pancreas (Ribelles et al., 1995 a, b, c), and kidney and spleen (Rosety et al., 1997) in sea water species. Conventional histochemical techniques (PAS, alcian blue, bromophenol blue) are powerful and reliable tools to investigate the characteristics and distribution of carbohydrates and proteins in the digestive system of fishes (Elbal and Agulleiro, 1986; Domeneghini et al., 1998).

MATERIALS AND METHODS

250 young healthy gilheads (fish-nursery), fifteen days old, 1.8 - 2 cm long and weighing 0.5 g, coming from a fish farm, were used in this study. Fifty specimens were used as controls and the remainder were divided into four lots, A, B, C and D. The four latter lots were exposed, respectively, to concentrations of 0.5, 1, 3 and 5 mg/L of ABS (R-CH₂-C₆H₄-SO₃Na with a purity of 80-85%; Merck, Spain). Control fish were maintained under identical conditions without the addition of toxins. Each group, consisting of fifty specimens, was maintained in a PVC tank containing 100 litres of sea water whose characteristics were: salinity 30%, pH 7.4, temperature 16-18°C, surface tension 72.7mN/m, dissolved oxygen 8-8.6 mg/L, hardness 100 mg Ca C0₃/l, and absence of heavy metals and contamination due to aerobic and anaerobic microorganisms.

The highest concentration we employed was lower than the one used by other authors (Okwuosa and Omorogie, 1995; Ribelles et al., 1995 a, b, c) but similar to the one those allowed by Spanish legislation in industrial toxic wastes. The lowest concentration we tested was similar to that allowed in drinking water for human consumption (Hernandez, 1992). To avoid variations in detergent concentrations, test solutions were changed every 12 hours. The bio-degradation occurring in this time is less than 10% of the initial concentration (Flores et al., 1980).

The LC50 causing death in a period of 96 hours was determined according to Loomis (1982) and Sprague (1976). Surface tension was measured using a Lauda TE 1 C/2 with SAE+KM3 tensiometer.

Once we noted the exposure time required for 50% mortality of the specimens at each concentration, we chose viable specimens from the same lot to examine histopathological and histochemical changes that appeared at this concentration. The fish killed by decapitation and then fixed in 10% v/v formol buffered with 0.1 M phosphate buffer, pH 7.2, dehydrated in increasing concentrations of alcohol, cleared with benzol and embedded in semisynthetic paraffin wax with a mean fusion point of 54-56°C. Sections were cut at 5 µm.

Harris’s haematoxylin and acetic eosin, Harris’s haematoxylin-VOF (Gutierrez, 1967) and Gridley’s reticulum stain (Gridley, 1951) were employed as general stains. The histochemical reactions made on carbohydrates and proteins in the liver (hepatocytes) and intestine (enterocytes and intestinal goblet cells) of young Sparus aurata, L. exposed to different concentrations of ABS are shown in Tables II, III and IV, respectively. It should be noted that the histochemical results are expressed as semiquantitative assessment of color intensities by independent scores of three investigators.

RESULTS

The LC50 of ABS at 96 h was found to be 0.6 mg/L. In Table I, the exposure times required for 50% mortality of the specimens (120; 38; 16; 9) expressed in hours, and the surface tension values
(68.9; 67.4; 65.5; 62.4) expressed in mN/m were calculated at 0.5, 1, 3 and 5 mg/L of ABS, respectively. No mortality occurred in the control lot. Histopathological and histochemical changes observed were as follows.

**Liver**

Control: The sections obtained from untreated specimens revealed the normal histological and histochemical patterns of the digestive gland. Hepatocytes were cuboidal in form with well-defined limits and a central nucleus. They were arranged in radial strings around a central vein. Within these hepatocyte strings the biliary canaliculi could be seen. PAS reaction was moderately positive although it became negative after enzymatic digestion with α-amylase PAS and diastase PAS, indicating the presence of glycogen. Levels of proteins in general were very weak while siderophile proteins were weakly positive.

Lot A (0.5 mg/L ABS): Hepatocytes were retracted and their radial arrangement was altered. They showed a moderate reactivity to PAS, similar to control, becoming negative after enzymatic digestion with α-amylase PAS or diastase-PAS. The bromphenol blue technique was moderately positive, and siderophile proteins were weakly positive.

Lot B (1 mg/L ABS): Histopathological changes were very similar to those found at 0.5 mg/L. PAS

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### Table I

Exposure time required for 50% mortality of the young *Sparus aurata*, L. and surface tension value resulting at each concentration of alkyl benzene sulphonate (ABS)

<table>
<thead>
<tr>
<th>ABS concentration (mg/L)</th>
<th>exposure time for 50% mortality (hour)</th>
<th>surface tension (mN/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>120</td>
<td>68.9</td>
</tr>
<tr>
<td>1</td>
<td>38</td>
<td>67.4</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>65.5</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>62.4</td>
</tr>
</tbody>
</table>

---

### Table II

Histochemical reactions on carbohydrates and proteins in the liver of young *Sparus aurata*, L. exposed to different concentrations of alkyl benzene sulphonate (ABS)

<table>
<thead>
<tr>
<th>REACTIONS</th>
<th>CONTROL</th>
<th>LOT A</th>
<th>LOT B</th>
<th>LOT C</th>
<th>LOT D</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCHIFF (Pearse, 1960)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Free aldehydes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAS (McManus, 1948)</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Adjacent hydroxyl groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIASTASE-PAS (Lillie and Greco, 1947)</td>
<td>0</td>
<td>0</td>
<td>NP</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Glycogen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALPHA-AMYLASE-PAS (Lillie and Greco, 1947)</td>
<td>0</td>
<td>0</td>
<td>NP</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Neutral mucosubstances and/or glycoproteins, excepting glycogen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BROMOPHENOL BLUE-Hg (Chapman, 1971)</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Proteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HARTIG ZACHARIAS (Martoja and Martoja-Pierson, 1970)</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>SIDEROPHILE PROTEINS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Lot A (0.5 mg/L ABS); Lot B (1 mg/L ABS); Lot C (3 mg/L ABS) and Lot D (5 mg/L ABS). Results are expressed as semiquantitative assessment of color intensities by independent scores of three investigators. Estimated scale: 0 (negative); 1 (very weak); 2 (weak); 3 (moderate); 4 (strong) and NP (has no been performed).
reaction was negative. Proteins in general and siderophile proteins were similar to lot A. Lot C (3 mg/L ABS): Histopathological features were similar to lots A and B. PAS reaction was

Table III
Histochemical reactions on carbohydrates and proteins in the intestine (enterocytes) of young Sparus aurata, L. exposed to different concentrations of alkyl benzene sulphonate (ABS)

<table>
<thead>
<tr>
<th>REACTIONS</th>
<th>CONTROL</th>
<th>LOT A</th>
<th>LOT B</th>
<th>LOT C</th>
<th>LOT D</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCHIFF (Pearse, 1960)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free aldehydes</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PAS (McManus, 1948)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjacent hydroxyl groups</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>DIASTASE-PAS (Lillie and Greco, 1947)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycogen</td>
<td>0</td>
<td>NP</td>
<td>NP</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ALPHA-AMILASE-PAS (Lillie and Greco, 1947)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutral mucosubstances and/or glycoproteins, excepting glycogen</td>
<td>0</td>
<td>NP</td>
<td>NP</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BROMOPHENOL BLUE-Hg (Chapman, 1971)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteins</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>HARTIG ZACHARIAS (Martoja and Martoja-Pierson, 1970)</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

SIDEROPHILE PROTEINS

Note: Lot A (0.5 mg/L ABS); Lot B (1 mg/L ABS); Lot C (3 mg/L ABS) and Lot D (5 mg/L ABS). Results are expressed as semiquantitative assessment of color intensities by independent scores of three investigators. Estimated scale: 0 (negative); 1 (very weak); 2 (weak); 3 (moderate); 4 (strong) and NP (has not been performed).

Table IV
Histochemical reactions on carbohydrates and proteins in the intestine (goblet cells) of young Sparus aurata, L. exposed to different concentrations of alkyl benzene sulphonate (ABS)

<table>
<thead>
<tr>
<th>REACTIONS</th>
<th>CONTROL</th>
<th>LOT A</th>
<th>LOT B</th>
<th>LOT C</th>
<th>LOT D</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCHIFF (Pearse, 1960)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free aldehydes</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PAS (McManus, 1948)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjacent hydroxyl groups</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>DIASTASE-PAS (Lillie and Greco, 1947)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycogen</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>ALPHA-AMILASE-PAS (Lillie and Greco, 1947)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutral mucosubstances and/or glycoproteins, excepting glycogen</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>ALCIAN – BLUE pH 2.5 (Martoja and Martoja-Pierson, 1970)</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Carboxylic-rich glycoconjugates, sulphated or not</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALCIAN – BLUE pH 0.4 (Martoja and Martoja-Pierson, 1970)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulphated glycoproteins, strongly ionized</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>TOLUIDINE BLUE (Martoja and Martoja-Pierson, 1970)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Note: Lot A (0.5 mg/L ABS); Lot B (1 mg/L ABS); Lot C (3 mg/L ABS) and Lot D (5 mg/L ABS). Results are expressed as semiquantitative assessment of color intensities by independent scores of three investigators. Estimated scale: 0 (negative); 1 (very weak); 2 (weak); 3 (moderate); 4 (strong) and + (positive).
strongly positive, becoming negative after enzymatic digestion. Proteins in general and siderophile proteins were very weak.

Lot D (5 mg/L ABS): The radial arrangement of hepatocytes was completely lost and they were retracted. We also observed several cellular damages with wall breakings and lack of parenchyma. PAS reaction was strongly positive becoming negative after enzymatic digestion. Proteins in general were very weak and siderophile proteins were negative.

**Intestine**

For practical purposes, we considered intestine as the medium part of the digestive tract (Cataldi et al., 1987).

Control: The wall of the digestive tract was composed of mucosa, submucosa, muscularis and serosa. Intestinal mucosa was composed of the epithelial layer, the lamina propria and the stratum compactum. Mucosal folds consisted of connective tissue cores covered by intestinal epithelium. Columnar cells or enterocytes were the more numerous of the epithelial lining cells and closely resemble those of higher vertebrates. These tall and cylindrical cells had striated, free borders (brush border or microvilli) and contained oval nuclei which were situated either centrally or toward the bases of the cells.

Intestinal mucous-secreting cells or goblet cells were interspersed among the columnar cells, being more numerous along the sides rather than on the crests or at the bases of the mucosal folds. They assumed the shape of a goblet due to the expansion of the distal region by their secretion. Goblet cell nuclei were found in the narrow basal portions of the cells.

Enterocytes showed a weak reactivity to PAS, becoming negative after enzymatic digestion with $\alpha$-amylase PAS or diastase PAS, suggesting the presence of glycogen. The bromophenol blue technique showed moderate presence of proteins in general. The Hartig Zacharias method showed weak presence of siderophile proteins.

Intestinal goblet cells showed a weak reactivity to PAS, which decreased to very weak after diastase-PAS treatment, suggesting the presence of glycogen and neutral mucopolysaccharides. Alcian blue at pH 2.5 showed a weak presence of carboxylated acidic mucopolysaccharides. Alcian blue at pH 0.4 showed a very weak presence of sulphated, strongly ionized mucopolysaccharides. Toluidine blue revealed metachromasia, indicating the presence of sulphated acidic mucopolysaccharides.

Lot A (0.5 mg/L ABS): Villi were found to anastomose with each other forming a compact mass and losing their individual outline, while lamina propria could not be discerned clearly. Some areas of epithelium become detached. Submucosa was hypertrophied and muscular layer was thickened.

Enterocytes: PAS reaction was negative. Proteins in general were lower in comparison to control and siderophile proteins increased slightly with respect to control.

Intestinal goblet cells: PAS reactivity increased slightly with respect to control, although it decreased to very weak after enzymatic digestion. Alcian blue (pH 2.5 and pH 0.4) stains increased slightly with respect to control. Toluidine blue revealed metachromasia.

Lot B (1 mg/L ABS): Morphological changes are similar to those found at 0.5 mg/L.

Enterocytes: PAS reaction was negative. Proteins in general were similar to control and siderophile proteins increased slightly with respect to control.

Intestinal goblet cells: PAS reaction increased slightly respect to control, becoming very weak after enzymatic digestion. Alcian blue (pH 2.5 and pH 0.4) stains also increased slightly respect to control. Toluidine Blue revealed metachromasia.

Lot C (3 mg/L ABS): There was a complete detachment of epithelium in some areas. Submucosa was infiltrated by lymphocytes.

Enterocytes: PAS reaction increased slightly with respect to control, becoming negative after enzymatic digestion. Proteins in general were similar to control and siderophile proteins were negative.

Intestinal goblet cells: PAS reaction increased slightly with respect to control, becoming very weak after enzymatic digestion. Strong staining with Alcian Blue (pH 2.5 and pH 0.4). Toluidine blue revealed metachromasia.

Lot D (5 mg/L ABS): Histopathological features observed before were more pronounced. According to this, whole blocks of epithelia became detached and the mucosal cells were found in a highly desintegrated condition. We observed a more complete destruction of all the layers.

Enterocytes: PAS reaction increased strongly with respect to control, becoming negative after enzymatic digestion. Proteins in general were similar to control and siderophile proteins were negative.
Intestinal goblet cells: PAS reaction increased slightly with respect to control, becoming negative after enzymatic digestion. They showed a strong reactivity to Alcian blue (pH 2.5 and pH 0.4) stains. Toluidine blue revealed metachromasia.

**DISCUSSION**

Information regarding the effect of tensioactives on fish survival is very limited. The LC50 at 96 h obtained by Okwuosa and Omorogie (1995) in toothed carp, *Aphyosemion gairdneri, L.* using ABS was 25.11 ± 8.4 mg/L and by Ribelles (1995 a, b, c) in 6 months old gilthead using sodium dodecyl sulphate (SDS) in the same structures of gilheads (*Sparus aurata, L.*). In general terms, histopathological lesions as well as histochemical alterations described by this author coincided with ours, although he used a different anionic tensioactive, higher concentrations and older specimens.

The mechanisms by which tensioactives produce their effects have not been well understood (Hele-nius and Simmons, 1975). In agreement with Sprague (1976) and Mallat (1985), this work could not confirm a single cause of death. Rather, the decrease in surface tension, destruction of tissue, and effects at the organ levels may be co-contributors to the death of gilthead exposed to ABS.

We also conclude that using concentrations allowed by Spanish legislation, which are lower than those found in tests in the South Atlantic coast of Europe, severe histopathological lesions and histochemical alterations were produced in the liver and intestine of young gilthead (fish-nursery). This is quite important since *Sparus aurata, L.* constitutes an important link in the food chain and its death via exposure to ABS may cause imbalance in the aquatic ecosystem.

**REFERENCES**


Cairns J., and Scheier A.: The acute and chronic effects of sodium alkylbenzene sulphonate upon the pumpkiseed sun-


