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Acute effects of sodium dodecyl sulphate on the survival and on morpho-histochemical characteristics of the trunk kidney of juvenile turbot *Scophthalmus maximus L.*

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SUMMARY

The present article reports the effect on survival as well as on the morpho-histochemical changes in the trunk kidney of juvenile turbot *Scophthalmus maximus*, L. induced by acute action of the anionic surfactant, sodium dodecyl sulphate (SDS). The LC50 of SDS at 96 hours was found to be 7.5 mg/l. Lots of 20 juvenile turbots were exposed to SDS concentrations of 3, 5, 7 and 10 mg/l: the exposure time required for 50% mortality of the specimens was 384,190,12 and 4 hours and surface tension values were 60.2, 56, 54.9, and 53.3 mN/m, respectively. It should be mentioned that there was a relatively high resistance to SDS of turbot compared to other teleost species, which may be related to its benthic habit. Histopathological lesions and histochemical changes that appeared in the trunk kidney of specimens exposed to the concentrations mentioned were examined. The abnormalities observed in this organ included vacuolation and desquamation of epithelial cells and degeneration of glomeruli and tubules. At the histochemical level, we appreciated some changes in the normal distribution of carbohydrates and proteins. Although the precise mechanism by which the renal tissue injury occurs is unknown, the function of this vital organ was seriously affected and this fact may ultimately play an important role in the mortality of turbots exposed to SDS.

INTRODUCTION

The coastal waters receive a variety of pollutants from anthropogenic activities (Jaffe *et al.* 1995). Among them, domestic surfactants deserve particular attention because of their specificity and large abundance in municipal wastes (Chalaux *et al.* 1992).

Surfactants are significant components of several consumer products, such as laundry detergents, shampoos, toothpastes and cosmetics (Belenger *et al.* 1995). The volume of surfactants produced in the United States was estimated to exceed seven billion pounds for 1989 (Greek and Layman, 1989). Among the anionic surfactants, sodium dodecyl sulphate (SDS) is one of the most widely employed.

Faunal and chemical monitoring has frequently been used to assess environmental quality (Martin and Richardson, 1995). The use of biota as an indicator of pollution is advantageous over chemical analysis as it produces results that are ecologically more realistic (Pocklington and Wells, 1992). In

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this connection, it is of great importance to evaluate the effects of pollution on fish and molluscs both for environmental protection and for socio-economic reasons (Lin and Hwang, 1998).

Turbot, *Scophthalmus maximus, L.* was used as a test species because it represents a widespread benthic species in the Atlantic Coast of Europe (from Scandinavian Coasts in the north, to Spanish Coasts in the south). Besides, it is considered economically important in the pisciculture industry (Drake *et al.*, 1984).

This study focused on the kidney, because it is one of the most important excretory organs of teleost fish. This vital organ maintains a delicate ionic and osmotic balance between the fish and the environment. In particular, the contribution of the kidney to the regulation of the body fluid composition is obtained by regulating the glomerular filtration rate and the amount of tubular water and salt reabsorbed/secreted.

Wood (1960) suggested histological examination as a means for determining the substances responsible for fish mortality in polluted water. In this respect, the scientific literature detailing the histopathological and histochemical changes induced by surfactants in teleosts is limited mainly to pelagic ones (Okuwosa and Omoregie, 1995; Ribelles *et al.* 1995 a,b,c; Rosety *et al.* 1997; Rosety *et al.*, 2001). To date, however, the evaluation of these changes on benthic teleosts has received little attention.

This study was conducted to evaluate the acute toxicity of the anionic surfactant SDS on the benthic teleost, *Scophthalmus maximus, L.* The objectives of the present investigation were firstly to determine the LC50 at 96 h, and secondly, to estimate the exposure time required for 50% mortality of the specimens as well as the surface tension value at each employed concentration (3, 5, 7 and 10 mg/l SDS). Finally, to examine the histopathological lesions and histochemical alterations which appeared in the kidney of saltwater teleost *Scophthalmus maximus, L.* induced by the acute action of SDS.

MATERIALS AND METHODS

The anionic surfactant SDS (CH_3-$(CH_2)_{11}$-O-SO_3-Na) with a purity greater than 99% was purchased from Fluka. The SDS was dissolved in deionized water to form a stock solution, which was added directly to the seawater to obtain the desired concentrations.

The test organisms used in the bioassay were 100 juvenile four months old turbots (*Scophthalmus maximus, L.*), 2.5 cm long and weighing from 10 to 12 mg. They all were born and raised on a fish farm.

Twenty specimens were used as controls and the remainder was divided into four lots A, B, C and D, which were exposed respectively to 3, 5, 7 and 10 mg/L of SDS. Control fishes were maintained under similar conditions without surfactant in the medium.

Each group of twenty specimens was maintained in a PVC tank with a capacity of 100 liters of seawater with the following physico-chemical characteristics: salinity 33‰, pH 7.4, temperature 16-18ºC, dissolved oxygen 8-8.6 mg/L, total hardness 100 mg $CaCO_3$/L, surface tension 72.7 mN/m and absence of heavy metals. All containers were aerated by mean of air pumps (Maxima A805) inside the chambers.

To maintain concentrations at a constant level throughout the experimental period, test solutions were renewed every 12 hours. The bio-degradation occurring in this time was less than 10% of the initial concentration (Flores *et al.* 1980). In addition, the media were kept free of detritus.

The LC50 causing death in a period of 96 hours was determined according to Sprague (1976) and Loomis (1982). Surface tension values at each concentration were calculated using a Lauda TE 1C /2 with SAE +KM3 tensiometer when 50% mortality of the specimens in each lot took place.

Once we had noted the exposure time required for 50% mortality of the specimens at each concentration, we examined surviving specimens from the same lot to examine the histopathological and histochemical changes in the kidney that appeared at each concentration. The fish were killed by decapitation and their kidneys were fixed in 10% v/v formol buffered in 0.1 M phosphate buffer (pH7.2), dehydrated in increasing concentrations of alcohol, cleared with benzol and embedded in semisynthetic paraffin wax with a melting point of 58-60ºC. Paraffin sections were cut at 5 μm.

Harris's haematoxylin and acetic eosin and Harris's haematoxylin-VOF (Gutierrez, 1967) were employed as general stains. Histochemical reactions on carbohydrates and proteins in the kidney (tubular cells and interstitial cells) are shown in Tables I and II. The histochemical results were

Table I
Histochemical reactions on carbohydrates in the kidney (tubular cells and interstitial cells) of juvenile *turbot Scophthalmus maximus, L.* exposed to different SDS concentrations

REACTIONS	TUBULAR CELLS					INTERSTITIAL CELLS				
	CT	A	B	C	D	CT	A	B	C	D
PAS (McManus, 1948) Adjacent hydroxyl groups	2	1	3	3	1	1	1	3	3	1
ALPHA-AMYLASE-PAS (Lillie and Greco, 1947) Neutral mucosubstances and/or glycoproteins, excepting glycogen	2	1	3	3	1	1	1	3	3	1
DIASTASE PAS (Lillie and Greco, 1947) Neutral mucosubstances and/or glycoproteins, excepting glycogen	2	1	3	3	1	1	1	3	3	1
ALCIAN-BLUE pH 2.5 (Martoja and Martoja-Pierson, 1970) Carboxyl-rich glycoconjugates, sulphated or not	1	1	0	0	1	1	1	0	0	0
ALCIAN-BLUE pH 1 (Martoja and Martoja-Pierson, 1970) Sulphate glycoconjugates	0	0	0	0	0	0	0	0	0	0
ALCIAN-BLUE pH 0.4 (Martoja and Martoja-Pierson, 1970) Very sulphated glycoconjugates	0	0	0	0	0	0	0	0	0	0

Note: CT: control group; A: 3mg/L SDS; B: 5mg/L SDS; C: 7mg/L SDS; D: 10mg/L SDS.
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).

expressed as semiquantitative assessment of color intensities by independent scores of three investigators according to Arellano *et al.* (1999).

RESULTS

The 96h-LC50 was found to be 7.5 mg/L. The exposure time required for 50% mortality of the specimens at 3, 5, 7 and 10 mg/L of SDS was, respectively, 384, 190, 12 and 4 hours.

The surface tension values at the concentrations employed (3, 5, 7 and 10 mg/L) were found to be 60.2, 56, 54.9 and 53.3 mN/m, respectively.

It is known that there is a functional division in the kidney of teleost, the head kidney being mostly endocrine in function while the trunk kidney is the excretory part. The sections obtained from untreated specimens showed the normal histological and histochemical patterns of the trunk kidney. At the histological level, it is composed by identical nephrons, forming the functional excretory units of the kidney. Each nephron is made up of a renal corpuscle and a well-developed renal tubule. The renal corpuscle contains a vascular capillary glomerulus enclosed by Bowman's capsule. This capsule contains an inner layer of visceral epithelium and an outer layer of parietal epithelium separating the renal corpuscle from the rest of the kidney. Tubules can be divided into different regions: neck segment, proximal tubule, distal tubule and collecting duct. The proximal and distal tubules are characterized by columnar cells with brush borders only in the proximal one. The collecting ducts consist of cuboidal cells with a centrally placed nucleus. Interstitial tissue, surrounded by reticular fibers, is present in the intertubular space (Ogawa, 1962).

The histochemical study of the kidney was focused on tubular and interstitial cells. The tubular cells showed weak reactivity to PAS which was resistant to enzymatic digestion with α-amylase or diastase. Staining with Alcian blue (pH 2.5 and 1) was weak whereas at pH 0.4 it was very weak. In relation to proteins, bromophenol blue and the Hartig Zacharias method were moderately positive. We also noticed the moderate presence of proteins rich in arginine and proteins rich in S-S groups

Table II
Histochemical reactions on proteins in the kidney (tubular cells and interstitial cells) of juvenile *turbot Scophthalmus maximus, L.* exposed to different SDS concentrations

REACTIONS	TUBULAR CELLS					INTERSTITIAL CELLS				
	CT	A	B	C	D	CT	A	B	C	D
BROMOPHENOL BLUE-Hg (Chapman, 1971) Proteins in general	3	2	3	3	1	2	1	2	2	2
HARTIG ZACHARIAS (Martoja and Martoja-Pierson, 1970) Siderophile proteins	3	2	0	3	4	2	2	3	3	4
NQS (Lillie *et al.*, 1971) Proteins rich in arginine	3	0	0	0	0	0	0	0	0	0
POTASSIUM FERRICYANIDE-Fe(III)(Chevremont and Frederic, 1943) Proteins rich in SH groups	2	2	2	2	2	2	2	2	2	2
THIOGLYCOLATE K-FERRICYANIDE-Fe(III) (Chevremont and Frederic, 1943) Proteins rich in S-S groups	3	2	2	2	2	2	2	2	2	2

Note: CT: control group; A: 3 mg/L SDS; B: 5 mg/L SDS; C: 7 mg/L SDS; D: 10 mg/L SDS. 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).

(cystine). Besides, we observed a weak presence of proteins rich in S-H groups (cysteine).

In the interstitial cells, PAS reactivity was very weak and resistant to enzymatic digestion with α-amylase or diastase. Staining with Alcian blue at pH 2.5 was also very weak. Bromophenol blue and the Hartig Zacharias method showed weak reactivity indicating the presence of low levels of proteins, in particular siderophilic proteins. Proteins rich in S-S groups (cystine) and proteins rich in S-H groups (cysteine) were also found.

The histopathological and histochemical changes in the kidney of exposed turbots were as follows:

Lot A (3 mg/L): at the histological level, tubules and renal corpuscles appeared slightly retracted. We also appreciated that the interstitial space was infiltrated by leukocytes.

Tubular cells: PAS reactivity was very weak and did not change after enzymatic digestion with α-amylase or diastase. With regard to proteins, the reactivity to bromophenol blue and the Hartig Zacharias method decreased slightly with respect to the controls. Levels of proteins rich in S-H and proteins rich in S-S groups were both weakly positive.

Interstitial cells: histochemical reactions on carbohydrates showed similar results to those observed in controls. With respect to proteins, the most conspicuous change was the very weak reactivity to bromophenol blue.

Lot B (5 mg/L): glomerular and tubular retraction was multifocal and more severe. Tubular cells lost their regular shape and their cytoplasm appeared slightly vacuolated. The interstitial tissue showed numerous leukocytes.

Tubular cells: PAS reactivity was moderately positive and resistant to α-amylase or diastase. With regard to proteins, bromophenol blue reactivity was moderate whereas the reaction with the Hartig Zacharias method was negative.

Interstitial cells: PAS reactivity was moderately positive and resistant to α-amylase or diastase. Staining with Alcian blue at pH 2.5 decreased to negative. Staining with bromophenol blue and the Hartig Zacharias method increased slightly with respect to lot A.

Lot C (7 mg/L): histopathological characteristics were similar to those observed in the previous lot.

Tubular cells: histochemical results were similar to those of lot B. With respect to proteins, bromophenol blue and the Hartig Zacharias method were both moderately positive.

Interstitial cells: histochemical reactions on carbohydrates and proteins were similar to those obtained in the previous lot .

Lot D (10 mg/L): glomeruli totally lost their normal structure and became fragmented. Renal tubules were in a collapsed condition and some of their cells were desquamated and vacuolated. Another point of interest was the rupture of some

capillaries and the presence of hemorrhagic foci.

Tubular cells: PAS reaction was very weak and resistant to α-amylase or diastase. Staining with Alcian blue at pH 2.5 was weakly positive and negative at pH 1 and 0.4. With respect to proteins, bromophenol blue reactivity decreased to very weak whereas the Hartig Zacharias method increased strongly. In addition, the presence of proteins rich in S-S groups increased to moderate.

Interstitial cells: PAS reaction was very weak and resistant to α-amylase or diastase. The Hartig Zacharias method was strongly positive.

DISCUSSION

It is well documented that surfactants have hazardous effects on teleost fish. With regard to the effects of the anionic surfactant SDS on fish survival, 96h-LC50 values of 6.1 mg/L have been reported for *Sparus aurata L.* (Ribelles *et al.* 1995 a,b,c), 4.5 mg/L for *Fundulus heteroclitus* (Laroche *et al.* 1972), and 2.19 mg/L for *Mugil curema* (Gomez *et al.* 1984).The LC50 of SDS at 96h that we found for juvenile *Scophthalmus maximus* was 7.5 mg/L. Although differences in experimental conditions urge us to proceed with caution, the data suggest that turbot is more resistant to SDS than the other mentioned species.

The results of our investigations also show a close inverse relationship between surfactant concentration and time required for 50% mortality of the specimens. This is in line with observations by Ribelles *et al.* (1995 a,b,c) and Rosety *et al.* (1997)., who observed that the higher the concentration of the anionic surfactant, the shorter is the exposure time required for 50% mortality of the specimens.

In our experimental design, we demonstrated that following acute exposure one can find a number of histopathological and histochemical changes in the kidney. Furthermore, these modifications were directly correlated with surfactant concentration. This finding was also noticed by Ribelles *et al.* (1995 a,b,c) and Rosety *et al.* (1997) from their toxicity assessment on juvenile giltheads using SDS.

To date, the information regarding the histopathological changes in the kidney of teleosts exposed to surfactants is very limited. In general terms, the renal lesions we observed in juvenile turbots agree with those described previously by Rosety *et al.* (1997) in juvenile giltheads exposed to SDS, despite their pelagic habit. It should be noted here that the authors cited above did not examine renal histochemical changes in the normal distribution of carbohydrates and proteins induced by surfactants.

The question arises how surfactants exert their toxic action on aquatic organisms. It has been suggested that the fall in surface tension induced by surfactants is the main cause of death since, under such conditions, the access of dissolved oxygen is limited (Prat and Giraud, 1964). In addition, Bock (1965) reported that surface tension values of 50 mN/m were very dangerous for the normal development of marine fauna. However, it has also been claimed that surface tension has little to do with the toxic effects of detergents on fishes (Muller, 1980). The results of this work have shown that the surface tension values decrease at increasing detergent concentration, as was also reported by Ribelles *et al.* (1995 a,b,c).

It should be mentioned that the kidney is a highly dynamic organ in most vertebrates and that the two kidneys together receive about 20% of the cardiac output. Thus, chemical substances in the systemic circulation are delivered in relatively high amounts to this organ (Banerjee and Bhattacharya, 1994). This fact may very well be related to the marked abnormalities that appeared in the kidneys of treated specimens. However, the precise mechanism by which the renal tissue injury is produced remains unclear.

In agreement with conclusions of Sprague (1976) and Mallat (1985), from our work no single cause of death can be deduced. Rather, both the decrease in surface tension and the effects at organ levels may be the main causes of death of fish exposed to SDS.

It can be concluded that following acute exposure one can find a number of histopathological and histochemical changes in the kidney that may ultimately lead to functional disorders in the affected organ. Consequently, these may play an important role in the mortality of the exposed turbots.

Although extrapolation from the laboratory to the field requires caution, the results of this work suggest that turbot populations in nature are seriously threatened at levels of SDS around 3 mg/L. Thus, environmental effects of surfactants on turbot will be expected close to sites of toxicant discharges that either are untreated or receive inadequate secondary treatment, where these pollutants may lead to a long term decline and a partial extinction of the turbot fishery, which traditionally is very important.

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