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Morpho-histochemical Changes in the Gills of Turbot, *Scophthalmus maximus* L., Induced by Sodium Dodecyl Sulfate

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The present article reports the effect on survival as well as morpho-histochemical changes in the gills of juvenile turbot *Scophthalmus maximus* L., induced by acute action of the anionic surfactant sodium dodecyl sulfate (SDS). First, LC_{50} at 96 h was found to be 7.5 mg/L of SDS. Second, lots with 20 individuals were exposed to SDS concentrations of 3, 5, 7, and 10 mg/L in order to obtain the exposure time required for 50% mortality of the specimens (384, 190, 12, and 4 h) and surface tension values (60.2, 56, 54.9, and 53.3 mN/m), respectively. Finally, histopathological lesions (clubbing and fusion of the secondary lamellae, hyperplasia and posterior rupture of the respiratory epithelium, destruction and shortening of gill filaments, and the presence of hemorrhagic foci) and histochemical alterations in the distribution of carbohydrates and proteins in the gills of treated specimens were noted. These morpho-histochemical changes in the gills provoked functional disorders (i.e. asphyxia and the loss of osmotic and ionic regulation) that may ultimately play an important role in the mortality of turbots exposed to SDS. © 2002 Elsevier Science (USA)

Key Words: **contamination; sodium dodecyl sulfate; turbot (*Scophthalmus maximus*); gills; histopathology; histochemistry.**

INTRODUCTION

Sodium dodecyl sulfate (SDS) is the most widely used anionic alkyl sulfate surfactant. Its surface active properties make it important in hundreds of household and industrial cleaners, personal care products, and cosmetics. It is also used in several types of industrial manufacturing processes, as a delivery aid in pharmaceuticals, and in biochemical research involving electrophoresis (Singer and Tjeerdema, 1993). In addition, anionic surfactants are important components of dispersants, whose use in oil-spill clean-up programs has been the subject of contentions for many years (Hatcher and Larkum, 1982). Thus, these compounds are extremely ubiquitous, and in seas and rivers, which are the final resting places for almost all pollutants, surfactants have become a severe problem for aquatic organisms. In this connection, it is of great importance to evaluate the effects of pollution on fish both for environmental protection and for socioeconomic reasons (Lin and Hwang, 1998).

Turbot, *Scophtalmus maximus* L. was used as test species because it represents a widespread species along the Atlantic coast of Europe (from the Scandinavian Coast and to the south) with important commercial value in the fishing industry. Also, it is considered economically important to the pisciculture industry (Drake *et al*., 1984).

This study focused on gills because they are the main target for many aquatic pollutants in general and surfactants in particular (Kikuchi *et al*., 1978), as well as the most seriously affected organ due to direct contact with the aquatic environment (Mishra *et al*., 1985). Also, the complex and interconnected functional–structural features of the gill epithelium make it an excellent model system for examining the effects of dissolved substances on tissues (Evans, 1987).

Acute toxic effects of surfactants on branchial structure have been studied, mainly in the pelagic species (Fukuda, 1982; Mishra *et al*., 1985; Roy, 1988; Ribelles *et al*., 1995a). On the other, the effects of this phenomenon on the gills of benthic species have received little attention. Therefore, research on the influence of surface active agents (SDS) on benthic turbots was particularly appropriate to compare its results with those obtained in pelagic species such as gilthead *Sparus aurata* L.

For these reasons, this experimental design was conducted to assess the effects of acute exposure of turbots to the anionic surfactant SDS. The aims of this work were to determine the LC_{50} at 96 h and to estimate the exposure time required for 50% mortality of the specimens as well as the surface tension value at each employed concentration. The final aim was to examine histopathological lesions and histochemical alterations (lamellar epithelium and filamental cartilage) in the gills of the saltwater teleost *Scophtalmus maximus* L. induced by SDS.

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MATERIALS AND METHODS

The fish used in this investigation were 120 juvenile turbot (*S. maximus* L.) 4 months old, 2.5 cm long, and weighing from 10 to 12 mg. They were born and raised on a fish farm. Twenty specimens were used as controls and the remainder divided into four lots A, B, C, and D, which were exposed, respectively, to 3, 5, 7, and 10 mg/L SDS ($(CH_3–(CH_2)_{11}–O–SO_3–Na$) with a purity greater than 99%, Fluka brand). A control tank was maintained under identical conditions without the addition of SDS.

Each group, containing 20 specimens, was maintained in a PVC tank with a capacity of 100 L seawater, the characteristics of which were the following: salinity 30‰, pH 7.4, temperature 16–18°C, surface tension 72.7 mN/m, dissolved oxygen 8–8.6 mg/L, hardness 100 mg CO_3Ca/L, and absence of heavy metals. To avoid variations in surfactant concentration, test solutions were changed every 12 h. Biodegradation occurring in this time frame is less than 10% of the initial concentration (Flores *et al*., 1980).

The LC_{50} causing death in a period of 96 h 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 after reaching at 50% mortality of the specimens in each lot. Once exposure time required for 50% mortality of the specimens at each concentration was noted, viable specimens from the same lot were chosen to examine histopathological and histochemical changes that appeared at this concentration. They were killed by decapitation and, after that, fixed in 10% v/v formol buffered with 0.1 M phosphate buffer, pH 7.2, dehydrated in increasing concentrations of alcohol (50°, 70°, and 96 and Absolute), 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 hematoxylin and acetic eosin and Harris's hematoxylin–VOF (Gutierrez, 1967) were employed as general stains. Histochemical reactions on carbohydrates and proteins in the gills (lamellar epithelium and filamental cartilage) of juvenile turbot exposed to different concentrations of SDS are shown in Tables 1 and 2, respectively. It should be noted that histochemical results were expressed as semiquantitative assessment of color intensities by independent scores of three investigators.

RESULTS

The 96-h LC_{50}, or the concentration of SDS that is lethal to 50% of the specimens in 4 days, 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 were, respectively, 384, 190, 12, and 4 h. No mortality occurred in the control lot. Surface tension values at concentrations employed (3, 5, 7, and 10 mg/L) were found to be 60.2, 56, 54.9, and 53.3 mN/m, respectively. These results are listed in Table 3.

Controls

The sections obtained from untreated specimens revealed normal histological and histochemical patterns of the gills,

TABLE 1
Histochemical Reactions on Carbohydrates in the Gill (Lamellar Epithelium and Filamental Cartilage) of Juvenile Turbot *Scophthalmus maximus* L., Exposed to Different Concentrations of Sodium Dodecyl Sulfate (SDS)

	Lamellar epithelium					Filamental cartilage				
Reaction	CT	A	B	C	D	CT	A	B	C	D
PAS (McManus, 1948)										
Adjacent hydroxyl groups	1	1	2	1	1	3	1	3	4	3
Alpha-Almilase–PAS (Lillie and Greco, 1947)										
Neutral mucosubstances and/or glycoproteins, except glycogen	1	1	2	1	1	3	1	2	2	3
Diastase-PAS (Lillie and Greco, 1947)										
Neutral mucosubstances and/or glycoproteins, except glycogen	1	1	2	1	1	3	1	2	2	3
Alcian-blue, pH 2.5 (Martoja and Martoja-Pierson, 1970)										
Carboxyl-rich glycoconjugates, sulfated or not	0	0	0	0	1	3	3	2	2	1
Alcian-blue, pH 1 (Martoja and Martoja-Pierson, 1970)										
Sulfate glycoconjugates	0	0	0	0	0	3	3	2	2	1
Alcian-blue, pH 0.4 (Martoja and Martoja-Pierson, 1970)										
Highly sulfated glycoconjugates	0	0	0	0	0	4	4	3	1	0
Toluidine blue (Martoja and Martoja-Pierson, 1970)										
Metachromasia, acid mucopolysaccharides	N	N	N	N	N	Y	Y	Y	Y	Y

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); Y, yes; N, no.

TABLE 2
Histochemical Reactions on Proteins in the Gill (Lamellar Epithelium and Filamental Cartilage) of Juvenile Turbot *Scophthalmus maximus* L., Exposed to Different Concentrations of Sodium Dodecyl Sulfate (SDS)

	Lamellar epithelium					Filamental cartilage				
Reaction	CT	A	B	C	D	CT	A	B	C	D
Bromphenol blue-Hg (Chapman, 1971)										
Proteins in general	3	3	3	4	3	0	0	0	0	0
Hartig Zacharias (Martoja and Martoja-Pierson, 1970)										
Siderophile proteins	3	2	2	2	3	3	2	3	3	3
1,2-Naphthoquinone-4-sulfonic acid sodium salt (NQS) (Lillie *et al*., 1971)										
Proteins rich in arginine	1	1	1	1	1	1	1	1	1	1
Potassium ferricyanide-Fe(III) (Chevremont and Frederic, 1943)										
Proteins rich in SH groups	0	0	0	0	0	0	0	0	0	0
Thioglcolate potassium Ferricyanide-Fe(III)										
(Chevremont and Frederic, 1943) Proteins rich in S–S groups	0	0	0	0	0	0	0	0	0	0

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).

the organization of which was similar to that described for other teleostean gills (Laurent and Dunel 1980; Hughes, 1984). Each gill was made up of filaments or primary lamellae, arranged in double rows along the bone. Secondary gill lamellae originate from the filaments and are disposed perpendicular to the inferior and superior margins of each filament. The filaments serve more for support of the secondary lamellae than for respiration. Each secondary lamella contains a thin-walled gill sinusoid that allows for the continuous exchange of respiratory gases such as oxygen and soluble metabolic waste such as carbon dioxide and ammonia. In addition the respiratory epithelium of the secondary lamellae contains specialized chloride cells that assist with osmoregulation by excreting chloride, potassium, and sodium ions.

Epithelial cells. Epithelial cells reached a very weak reactivity to PAS that was not modified after enzymatic digestion with alpha-amilase–PAS or diastase–PAS. In relation to proteins, moderate reactivity to bromphenol blue and the Hartig Zacharias method was observed, indicating moderate presence of protein in general and siderophile proteins, respectively. Also observed was a very weak presence of proteins rich in arginine.

Filamental cartilage. Filamental cartilage presented moderate reactivity to PAS that did not change after enzymatic digestion with alpha-amilase–PAS or diastase–PAS. Alcian blue at pH 2.5 and 1 was moderately positive, indicating the moderate presence of carboxyl-rich glycoconjugates and acid glycoconjugates (sulfated), respectively. At pH 0.4 Alcian blue was strongly positive suggesting a high presence of very sulfated glycoconjugates. Toluidine blue revealed metachromasia. Histochemical reactions on proteins indicated a moderate presence of siderophile proteins and a very weak presence of proteins rich in arginine.

TABLE 3
Exposure Time Required for 50% Mortality of Juvenile Turbot *Scophthalmus maximus* L., and Surface Tension Values Resulting at Each Concentration of Sodium Dodecyl Sulfate (SDS)

SDS concentration (mg/L)	Exposure time for 50% mortality (h)	Surface tension (mN/m)
3	384	60.2
5	190	56
7	12	54.9
10	4	53.3

Lot A (3 mg/L)

The concentration for lot A revealed only the tendency of some secondary lamellae to fuse.

Epithelial cells. For epithelial cells at this concentration, histochemical reactions on carbohydrates and proteins had results similar to those obtained in controls except that those with the Hartig Zacharias method decreased slightly.

Filamental cartilag. For filamental cartilage the reactivity to PAS decreased to very weak and it did not change after enzymatic digestion. In relation to proteins, Hartig Zacharias reactivity decreased to weakly positive, indicating a weak presence of siderophile proteins.

Lot B (5 mg//L)

Histological examination of the gills revealed the clubbing and fusion of secondary lamellae. A further

point of interest was the hyperplasia of the respiratory epithelium.

Epithelial cells. Epithelial cells for lot B presented a weak reactivity to PAS that was not altered after enzymatic digestion with alpha-amilase–PAS and diastase–PAS. Histochemical reactions on proteins presented results similar to those observed in the previous lot.

Filamental cartilage. For lot B filamental cartilage, PAS reactivity was moderately positive, becoming weakly positive after enzymatic digestion with alpha-amilase–PAS and diastase–PAS. Alcian blue at pH 2.5, 1, and 0.4 decreased slightly in comparison to controls. With regard to proteins, the level of siderophile proteins increased to moderately positive.

Lot C (7 mg/L)

Histopathological features seen before were more generalized and severe at the 7 mg/L concentration.

Epithelial cells. In this lot, epithelial cells presented a very weak reactivity to PAS that did not change after enzymatic digestion with alpha-amilase–PAS or diastase–PAS. In relation to proteins, bromphenol blue was strongly positive, whereas Hartig Zacharias staining remained weakly positive.

Filamental cartilage. For lot C, filamental cartilage PAS reactivity was strongly positive, becoming weakly positive after enzymatic digestion with alpha-amilase–PAS or diastase–PAS. There was weak staining with Alcian blue at pH 2.5 and 1, whereas at pH 0.4 it was very weak. Histochemical reactions on proteins presented results similar to those obtained in lot B.

Lot D (10 mg/L)

For lot D, a noticeable feature was the destruction of some filaments, which resulted in their shortening. Also observed was the loss of lamellar and filamental epithelial cells, which left only the filamental cartilage, as well as the presence of blood cells among the gill filaments.

Epithelial cells. For epithelial cells of lot D, histochemical reactions on carbohydrates revealed results similar to those obtained in lot C. In relation to proteins, bromphenol blue and the Hartig Zacharias method were both moderately positive.

Filamental cartilage. For lot D filamental cartilage, PAS reactivity was moderate and did not change after enzymatic digestion with alpha-amilase–PAS or diastase–PAS. Staining with Alcian blue (pH 2.5, 1, and 0.4) increased slightly in comparison to the previous lot. In relation to proteins results similar to those found for lot B were observed.

DISCUSSION

The use of flatfish as biomonitors of heavy metal pollution (Arellano *et al*., 1999) has been well established. Also studied has been the impact that organochlorine pesticides and polychlorinatyed biphenyls (Falandysz, 1985) and aliphatic hydrocarbons (Alvarez-Pineiro *et al*., 1996) produced in these specimens. However, there has been no information regarding the effects of anionic surfactants on turbot in the literature. For this reason, this experimental design was conducted to assess the effects of acute exposure of the turbot *S. maximus* L. to anionic surfactant SDS.

With regard to the effects of anionic surfactants SDS on fish survival, 96-h LC_{50} values of 6.1 mg/L have been reported for *S. aurata L.* (Ribelles *et al*., 1995a,b), 4.5 mg/L for mummichog *Fundulus heteroclitus* (Laroche *et al*., 1972), and 2.19 mg/L for mullet *Mugil curema* (Gomez *et al*., 1984). The LC_{50} at 96 h found for juvenile turbot in the current study was 7.5 mg/L SDS. Although differences in experimental conditions require proceeding with caution, these data suggest that turbot is slightly more resistant to SDS than the other mentioned species.

The results of this investigation also reveal a close inverse relationship between surfactant concentration and time required for 50% mortality of the specimens. According to Ribelles *et al*. (1995a,b) and Rosety *et al*. (2000, 2001), the higher the concentration of anionic surfactant the less is the exposure time required for 50% mortality of treated individuals.

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, because 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 indicate that the surface tension values decrease at increasing detergent concentration, as was also reported by Ribelles *et al*. (1995a,b).

The present experimental design demonstrated that following acute exposure one can find a number of histopathological and histochemical changes in the gills of treated specimens. Furthermore, these modifications were directly correlated with surfactant concentration. This relationship was also noted by Ribelles *et al*. (1995a,b) and Rosety *et al*. (2001) from toxicity assessments on juvenile giltheads using anionic surfactants.

The present article reports evidence about the alterations that SDS exerts on the distribution of carbohydrates and proteins. In this respect, a noticeable feature was that metachromasia in mature cartilage was not altered after exposure to this surfactant. Histopathological gill damage

consisted mainly of clubbing and fusion of the secondary lamellae, hyperplasia and posterior rupture of the respiratory epithelium, destruction and shortening of gill filaments and the presence of hemorrhagic foci. In general terms, the gill lesions observed in juvenile turbot agree with those described previously by Ribelles *et al.* (1995a) in juvenile giltheads exposed to SDS. As a consequence, it appears that the toxicological effects of SDS are not influenced by the benthic or pelagic habit of treated species.

In agreement with Mallat (1985), lamellar fusion could be protective in that it disminishes the amount of vulnerable gill surface area. On the other hand, this lesion reduces the surface area for gaseous exchange (Hemalatha and Banerjee, 1997). Hyperplasia of the lamellar epithelium, as seen in this study, could serve as a defensive function because it increases the distance across which waterborne irritants must diffuse to reach the bloodstream (Erkem and Kolankaya, 2000). The rupture of the branchial epithelium is believed to reflect the direct deleterious effects of irritants on gills (Temmink *et al*., 1983). Similar to the findings Okuwosa and Omoregie (1995), the current histopathological study found hemorrhaging of the gill filaments.

It can be concluded that following acute exposure one can find a number of morpho-histochemical changes in the gill that may result in functional disorders of this organ, and these may ultimately play an important role in the mortality of treated turbot. The results of this study also suggest that histology and histochemistry are successful tools capable of revealing sensitively and selectively the effects of surfactants on the aquatic biota.

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 concentrations around 3 mg/L. As a consequence, the presence of these pollutants at toxic concentrations in the environment (i.e., close to effluents that either are untreated or receive inadequate secondary treatment) represents a serious problem for the maintenance of turbot populations in nature, and this may lead to a long-term decline of this species around these polluted areas.

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