Histol Histopathol (2001) 16: 1091-1095

http://www.ehu.es/histol-histopathol

Histology and Histopathology

Cellular and Molecular Biology

Comparative study of the acute toxicity of anionic surfactans alkyl benzene sulphonate (ABS) and sodium dodecyl sulphate (SDS) on gilthead, *Sparus aurata* L., eggs

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Summary. In the present work we have evaluated the acute toxicity of two anionic surfactants, alkyl benzene sulphonate (ABS) and sodium dodecyl sulphate (SDS) to eggs of gilthead *Sparus aurata*. At each surfactant concentration, we determined the exposure time required for 50% mortality of the eggs (LT50), surface tension and volume of oil globule in gilthead eggs.

Clear dose-response relationships for mortality of gilthead eggs was observed for both toxicants; at 30 mg/L 50% mortality took place at 45 minutes for ABS and 8 minutes for SDS. At this concentration, SDS was almost six times more toxic than ABS (LT50 is compared). However, at 0.3 mg/L 50% mortality occurred after exposures of 535 minutes to ABS and 425 minutes to SDS. Descriptively, our results showed SDS was more toxic than ABS at high concentrations whereas at low concentrations their toxicity was very similar. However, statistical analysis demonstrated there were no significant differences in the toxicity of both surfactants to gilthead eggs.

Surface tension value at each concentration of both surfactants was also calculated. We found that these values decreased with increasing concentration of each surfactant, and this trend was more pronounced in solutions of SDS. We also found that the volume of the oil globule of exposed eggs was influenced by surfactants. After exposure, its volume clearly decreased in comparison to controls, mainly in eggs exposed to SDS.

Key words: Pollution, Surfactant, Eggs, *Sparus aurata*

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Introduction

Surfactants are significant components of several consumer products, such as laundry detergents, shampoos, toothpastes and cosmetics (Belanger et al., 1995). They are also an important component of dispersants whose use in oil spill clean-up programs has been continual for many years (Hatcher and Larkum, 1982).

Combined worlwide consumption of surfactants (anionic, cationic and non-ionic) was estimated to be 15 million metric tons in 1989 (Berth and Jeschke, 1989). Among anionic surface-active agents, alkyl benzene sulphonate (ABS) and sodium dodecyl sulphate (SDS) are two of the most widely employed.

These synthetic compounds are easily introduced through wastewater discharges to seas and rivers where certain classes of surfactants can be present at sufficient concentrations to cause toxicity problems to aquatic organisms. For these reasons, detergents have become a problem of concenrn in aquatic systems and their detrimental effects may be clearly demonstrated.

The saltwater teleost gilthead (*Sparus aurata* L.), was used as a test species because it is widely distributed along the southatlantic coast of Spain and for its importance in the fishing industry and aquaculture (Drake et al., 1984). In addition, it is sensitive to any fall in the dissolved oxygen concentration (Arias et al., 1976), which made it a useful test species in the study of pollutants that affect the oxygenation of the water. Eggs were used because earlier life stages of marine organisms have been found to be more sensitive to pollutant stress than adults (Mohan et al., 1983; Kwain and Rose, 1985; Dave and Xiu, 1991) .

Acute toxic effects of anionic surfactants on aquatic species have been studied mainly in juvenile and adult life stages (Okuwosa and Omoregie, 1995; Ribelles et al., 1995a,b; Rosety et al., 2000). The effects of this phenomenon on the early developmental stages have received little attention (Ankley and Burkhard, 1991;

Kusk and Petersen, 1997).

For the reasons already mentioned, this study was conducted to evaluate and compare the acute toxic effects of anionic surfactants ABS and SDS on the eggs of giltheads, *Sparus aurata* L.. The main objective of this work was to determine the exposure time required to cause 50% mortality (LT50) in exposed eggs at the concentrations of: 0.3, 3, 6, 15 and 30 mg/L. In addition, we calculated the surface tension value at each tested concentration and examined the influence of both surfactants on the oil globule volume of gilthead eggs.

Materials and methods

Chemicals

The anionic surfactants used in this experience were alkyl benzene sulphonate (ABS) $R\text{-}CH_2\text{-}C_6H_6\text{-}SO_3Na$ with a purity of 80-85% and sodium dodecyl sulphate (SDS) $CH_3(CH_2)_{11}\text{-}O\text{-}SO_3$ Na with a purity of 99%, both Fluka brand.

Both anionic surfactants were dissolved quickly and completely in de-ionized water to form a stock solution of each one, which was added directly to the seawater to obtain the desired concentrations (0.3, 3, 6, 15 and 30 mg/L of ABS and SDS). It should be mentioned here that test concentrations were adjusted for the purity of the compounds.

Employed seawater proceeded from a well and was filtered through activated carbon and aerated 1 day before use. The physicochemical characteristics of seawater before adding surfactants were as follows: salinity 30‰, pH 7.4, temperature 19.5 °C, dissolved oxygen 8.0-8.6 mg/L, total hardness 100 mg $CaCO_3$ /L, surface tension 72.7 mN/m. Although no chemical disinfectants were used, bacteriological tests (Brilliant green bile broth, Azide dextrose broth and SPS Agar, all MYC Diagnostica brand) did not reveal contamination due to aerobic or anaerobic microorganisms.

To avoid variations in surfactant concentrations by biodegradation, test solutions were changed every 6 hours in those lots where the duration of the experiment was longer (Flores et al., 1980).

Eggs

We used a total of 1,100 gilthead eggs derived from a culture unit at the Institute "Sancti Petri" (San Fernando, Cadiz, Spain). In this experiment, ovulation, spermiation and the consequent fertilization were spontaneous during the physiological reproductive cycle of this species (Arias, 1980; Camus and Koutsikopoulos, 1984).

Gilthead egg, examinated under a light microscope, was spherical, buoyant, tranparent, non-adhesive and colorless. They measured 1.03±0.02 mm (n=20) in diameter and weighed 44.6±2.05 μg (n=20). They also contained a single oil globule with a mean volume of 0.0044±0.0006 μl (n=20). Employed eggs were <2 hours old at the begining of each exposure.

Test method

The acute test consisted of exposing groups of 100 eggs at similar test solutions (0.3, 3, 6, 15 and 30 mg/L) of ABS and SDS in plastic exposure chambers (10x 8x 7.5) which held water volumes of approximately 450 ml each. A control tank with 100 eggs was maintained under identical conditions without the addition of ABS or SDS. All the concentrations and the control were set in triplicate.

We observed the eggs in each tank with a magnifying glass and counted the eggs that lost buoyancy. In agreement with Zanui (1975), eggs that lost buoyancy and tended to lie down on the bottom of the tank were considered dead.

Furthermore, when we examined the eggs lying on the tank bottom, we observed that parts of their content turned opaque and white, which according to Oyen et al. (1991) and Stouthart et al. (1994) clearly indicated that they were dead. It should also be mentioned that dead eggs were immediately removed to prevent fungal growth.

For surface tension analysis, water samples were taken inmediately after 50% mortality of the eggs was observed at each concentration employed. The determination of surface tension was done using a tensiometer (Lauda TE 1 C/2 with SAE + KM3) supported by a computer (Epson).

At the same time, 20 buoyant eggs from each tank were collected to measure the volume of the oil globule. These eggs were stained with Red Oil O and Black Sudan B (Barka and Anderson, 1963) and examined under a light microscope (WILD TYP 355110, 10 x 4 magnification). The volume of the oil globule was calculated using the formula of the sphere: $V = 4/3\pi \times r^3$, in which V is the volume, π is equal to 3.1416 and r is the radius of the oil globule obtained from its diameter measured with a graduated ocular.

Statistical analysis

Data referring to diameter, weight and oil globule volume of gilthead eggs as well as surface tension values were expressed as Means ± (SD). We also calculated the 95% confidence intervals for the LT50's. To predict the toxicity at each surfactant concentration (range 0.3 to 30 mg/L), we carried out a single regression analysis using the method of least squares (Zar, 1996). The percentage of mortality was the variable log transformed to make tire statistical analysis for making biological conclusions. We compared the slopes and after this first approach we compared the two elevations.

Results

The exposure time required for 50% mortality (LT50) of the eggs at different concentrations (0.3, 3, 6, 15 and 30 mg/L) of ABS were 535 (541-530), 370 (374-365), 195 (198-191), 99 (102-97) and 45 (47.3-44) minutes respectively. For SDS, the results were respectively: 425 (429-421.8), 270 (272.5-267), 102 (103.6-100.4), 38 (40-36.9) and 8 (9.1-7) minutes. These results are summarized in figure 1. It shows us that for both surfactants, the LT50's decreased progresively with increasing concentration.

The surface tension value in control water was 72.7 mN/m. At 0.3, 3, 6, 15 and 30 mg/L of ABS surface tension values were respectively 69.8±2.1, 65.5±1.8, 61.8±2.0, 54.6±1.7 and 49.6±1.8 mN/m. For SDS the results were respectively 65.4±1.6, 60.2±1.8, 57.3±1.6, 49.9±1.4 and 44.1±1.7 mN/m. Surface tension values decreased progressively with increasing surfactant concentration.

Another important aspect was to assess the influence of surfactants on the volume of the oil globule of live and buoyant gilthead eggs. Microscopic examination of eggs exposed to ABS showed that the volume of the oil globule decreased from 0.0040±0.0004 μl at test initiation to 0.0034±0.0005 μl at 0.3 and 30 mg/L respectively. For SDS, at 0.3 mg/L it reached as low as 0.0039±0.0003, and at 30 mg/L its volume was 0.0031±0.0004 μl. As ocurred with the LT50 values, the volume of the oil globule decreased progressively when the surfactant concentration was increased.

Discussion

Exposure to anionic surfactants ABS and SDS clearly caused detrimental effects on gilthead eggs. At 0.3 mg/L of both surfactants, negative effects became apparent through elevated mortality, decreased surface tension value and a reduction in oil globule volume. As one might expect, at 30 mg/L all these effects were more pronounced.

Clear dose-response relationship for mortality of eggs was observed for both surfactants. This result was in accordance with those reported previously by Ribelles et al. (1995a,b) and Rosety et al. (1997, 2000) using SDS.

At 30 mg/L ABS, the 50% mortality took place at 45 minutes and in the case of SDS it happened after 8 minutes. At this concentration, SDS seemed to be 6 times more toxic than ABS (based on LT50's). On the other hand, at 0.3 mg/L ABS the LT50 was 525 minutes and for SDS it was 435 minutes. At this concetration both surfactants exhibited similar toxicity.

Descriptively, the results reported above indicated that SDS was more toxic than ABS mainly at high concentrations. However, statistical analysis through the simple regression analysis by the method of least squares concluded that no significant differences in toxicity were observed between the surfactants (t_{exp} = 1.170 and t_{∞} = 3.8; $t_{exp} < t_{\infty}$).

It is well documented that eggs and larvae are much more sensitive than adults to heavy metals (Mohan et al., 1983; Dave and Xiu, 1991; Sayer et al., 1991) and acid stress (Rombough 1982; Kwain and Rose, 1985). Ribelles et al. (1995a,b) reported that the 96 h LT50 of SDS for juvenile giltheads was 6.1 mg/L. In our study, 6 mg/L of SDS killed 50% of the eggs in only about 102 minutes. This result indicates that eggs are more sensitive to surfactants than older life stages.

Related with the toxicity of anionic surfactants on early life stages of fish, Ankley and Burkhard (1991) reported results about the effects of linear alkylbenzene

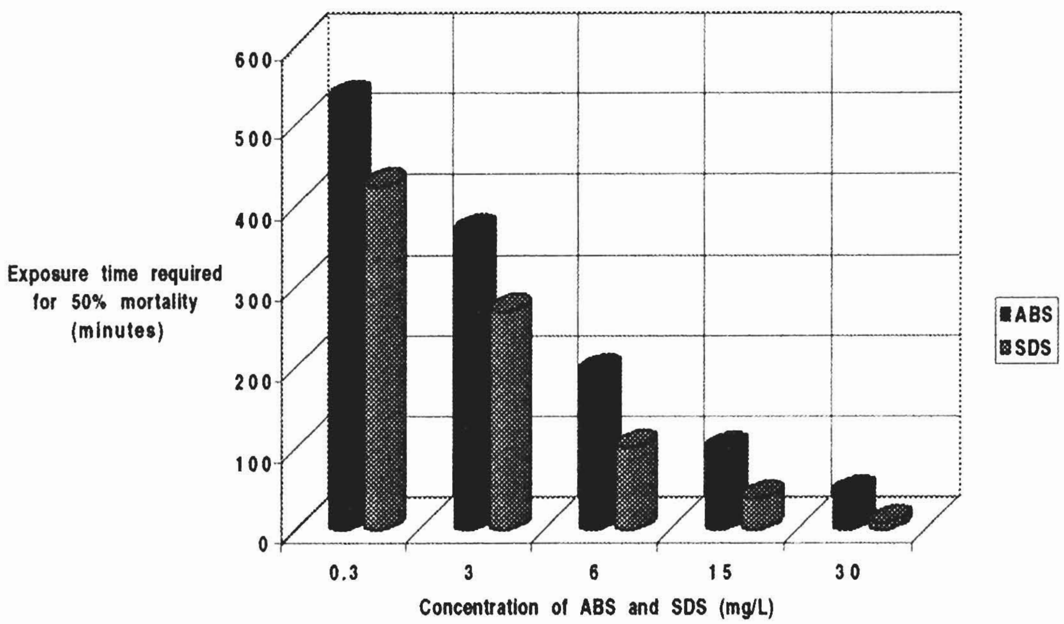

Fig. 1. Effects of different concentrations of anionic surfactants ABS and SDS on gilthead *Sparus aurata*, eggs. Each value is an average of three experiments.

(LAS) on larval stage of *Ceriodaphnia dubia* . The 48 h LT50 calculated using the trimmed Spearman-Krabbe method was 4.62 mg/L. Although differences in experimental conditions require one to proceed with caution, these data suggest that *C. dubia* is less sensitive to surfactants than gilthead eggs. As proposed by Ankley and Burkhard (1991), it appeared to be mainly due to the fact that *C. dunia* was very tolerant to low dissolved oxygen concentrations.

The question arises how surfactants exert their toxic action on the developing embryo. The mechanisms by which surfactants produce their effects have not been well understood (Helenius and Simmons, 1975). It is thought that the fall in surface tension induced by surfactants is the main cause of death (Prat and Giraud, 1964; Mann, 1972). Bock (1965) carried out extensive observations of this phenomenon and concluded that water with surface tension values below 50 mN/m were toxic to 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 decrease of surface tension values with increasing the concentration was in accordance with results of Rosety et al. (1997, 2000). In our experience, it reached as low as 49.6 mN/m and 44.1 mN/m at 30 mg/L of ABS and SDS respectively. Even lower values (42.3 mN/m) have been measured in the Bay of Cadiz, Spain (Flores et al., 1979). The increase in toxicity coincides with decreasing surface tension and therefore it appears to be an important mechanism for the acute toxicity of surfactants.

Thoraugh (1992) reported that surfactants may disrupt biological membranes and subcellular organelles, as well as disturbing the functions of some enzymes. In agreement with Sprague (1976) and Mallat (1985) this study could not confirm a single cause of death. Rather, the decrease in surface tension, destruction of biological membranes and subcellular organelles as well as the alteration of the functions of some enzymes, may have been the main causes of death of gilthead eggs exposed to ABS and SDS.

Although extrapolation from the laboratory to the field requires caution, the results of this experiment suggest that gilthead populations in nature are seriously threatened at ABS and SDS concentrations of 0.3 mg/L. This finding is quite important if we take into account that concentrations of 0.12 mg/L of anionic surfactant LAS have been encountered in the Bay of Cadiz (Spain, 36º30' N, 6º15' W) at a distance of around 5 km from the discharge point of an untreated urban effluent (Gonzalez-Mazo et al., 1997).

For the reasons already mentioned, continual discharges of these pollutants through effluents that either are untreated or receive inadequate secondary treatment may lead to a long term decline or eventual extinction of this species. Moreover, because giltheads are an important link in the food chain, its death via exposure to surfactants may imbalance the littoral ecosystem.

References

Ankley G.T. and Burkhard L.P. (1991). Identification of surfactants as toxicants in a primary effluent. Environ. Toxicol. Chem. 11, 1235-1248.

Arias A.M., Drake P. and Rodriguez R.B. (1976). Los esteros de la salina de San Fernando (Cádiz, España) y el cultivo extensivo de peces marinos. In: L' aquaculture du Bar et des Sparides. INRA. Paris.

Arias A. (1980). Crecimiento, régimen alimentario y reproducción de la dorada *Sparus aurata* L. y del robalo *Dicentrarchus labrax* L. en los esteros de Cádiz. Inv. Pesq. 44, 59-83.

Barka T. And Anderson P. (1963). Histochemistry, theory, practice and bibliography. Hoever Med. Div. Horper and Row Publishing Inc. London.

Belanger S.E., Meiers E.M. and Baush R.G. (1995). Direct and indirect ecotoxicological effects of alkyl sulphate and alkyl ethoxysulphate on macroinvetebrates in stream mesocosms. Aquat. Toxicol. 33, 65-87.

Berth P. and Jeschke P. (1989). Consumption and fields of application of LAS. Surfact. Deterg. 26, 74-79.

Bock J.H. (1965). Uber die wirkung von waschrostoffen auf Fische. Arch. Fish-Wiss. 17, 58-67.

Camus P. and Koutsikopoulos C. (1984). Incubation experimentale et development embryonnaire de la daurade royale, *Sparus aurata* L. a diferentes temperatures. Aquaculture. 42, 117-128.

Dave G. and Xiu R. (1991). Toxicity of mercury, copper, nickel, lead and cobalt to embryos and larvae of zebrafish, *Brachydario rerio*. Arch. Environ. Contam. Toxicol. 21, 126-134.

Drake P., Arias A.M. and Rodriguez R.B. (1984). Cultivo extensivo de peces marinos en los esteros de las salinas de San Fernando (Cadiz) II. Características de la producción de peces. Inf. Tec. Inst. Inv. Pesq. 116, 23.

Flores V., Galán M. and Sales D. (1979). Contaminación de las aguas de la Bahía de Cádiz (II). Ensayos generales de calidad de las aguas. Ing. Quim. 125, 105-109.

Flores V., Galán M. and Sales D. (1980). Contaminación de las aguas de la Bahía de Cádiz (IV). Ensayos de biodegradabilidad con dodecil sulfato sódico. Ing. Quim. 131, 81-111.

Gonzalez-Mazo E., Honing M., Barcelo D. and Gomez-Parra A. (1997). Monitoring long chain intermediate products from the degradation of linear alkylbenzene sulfonates in the marine environmental by solid-phase extraction followed by liquid chromatography/ionspray mass spectrometry. Environ. Sci. Technol. 31, 504-510.

Hatcher A.I. and Larkum A.W.D. (1982). The effects of the short term exposure to Bass Strait crude oil and corexit 8667 on benthic community metabolism in *Posidonia australis* Hook. F. dominated microcosms. Aquatic Botany 12, 219-227.

Helenius A. and Simmons K. (1975). Solubilization of membranes by detergents. Biochem. Biophys. Acta 415, 29-79.

Kusk K.O. and Petersen S. (1997). Acute and chronic toxixity of tributyltin and linear alkylbenzene sulfonate to the marine copepod Acartia tonsa. Environ. Toxicol. Chem. 16, 1629-1633.

Kwain W. and Rose G.A. (1985). Growth of brook trout *Salvelinus fontinalis* subject to sudden reductions of pH during their early life history. Trans. Am. Fish. Soc. 114, 564-570.

Mallat J. (1985). Fish gills structural changes induced by toxicants and other irritants: A statistical review. Can. J. Fish Aqu. Sci. 42, 630-

648.

Mann H.G. (1972). Toxicity and degradation of tensides in sea water. In: Marine pollution and sea life. FAO. Roma.

Mohan C.V., Menon N.R. and Gupta T.R.C. (1983). Effects of cadmium on the early developmental stages of Cirrhina mrigala. Mysore J. Agric. Sci. 17, 378-381.

Muller R. (1980). Fish toxicity and surface tension of non-inic surfactants: investigations and antifoan agents. J. Fish Biol. 16, 585-589.

Okuwosa V.N. and Omoregie E. (1995). Acute toxicity of alkylbenzene sulphonate (ABS) detergent to the toothed carp, *Aphyosemion gairdneri* L.. Aquacult. Res. 26, 755-758.

Oyen F.G.F., Camps L.E.C.M.M. and Wendelar-Bonga S.E. (1991). Effect of acid stress on the embryonic development of the common carp (*Cyprinus carpio*). Aquat. Toxicol. 19, 1-12.

Prat R. and Giraud A. (1964). The pollution of water by detergents. O.E.C.D. Paris.

Ribelles A., Carrasco C., Rosety M. and Aldana M. (1995a). Morphological and histochemical changes caused by sodium dodecyl sulphate in the gills of giltheads, *Sparus aurata* L. Eur. J. Histochem. 39, 141-149.

Ribelles A., Carrasco C., Rosety M. and Aldana M. (1995b). Morphological and histochemical changes on the liver and pancreas of giltheads, *Sparus aurata* L., induced by acute action of the anionic detergent, sodium dodecyl sulphate. Histol. Histopatol. 10, 781-787.

Rosety M., Ribelles A. and Carrasco C. (1997). A morphological study in the kidney and spleen of gilthead, *Sparus aurata* L. caused by sodium dodecyl sulphate. Histol. Histopathol. 12, 925-929.

Rosety M., Ribelles A., Rosety-Rodriguez M., Carrasco M., Ordoñez F.J. and Rosety J.M. (2000). Morpho-histochemical study of the biological effects of sodium dodecyl sulphate on the digestive gland of the Portuguese oyster. Histol. Histopathol. 15, 1137-1143.

Rombough P.J. (1982). Effects of low pH on eyed embryos and alevins of Pacific salmon. Can. J. Fish. Aqu. Sci. 40, 1575-1582.

Sayer M.D.J., Reader J.P. and Morris R. (1991). Embryonic and larval development of brown trout, *Salmo trutta* L.; Exposure to aluminium, copper, lead or zinc in soft, acid water. J. Fish. Biol. 38, 431-455.

Sprague J.B. (1976). The ABC's of pollutant bioassay using fish. In: Biological methods for the assessment of water quality. Cairn J. and Dickson K.L. (eds). American Society for testing and materials, Philadelphia.

Stouthart A.J.H.X., Spanings F.A.T., Lock R.A.C. and Wendelar-Bonga S.E. (1994). Effects of low water pH on lead toxicity to early life stages of the common carp (*Cyprinus carpio*). Aquat. Toxicol. 30, 137-151.

Thoraugh A. (1992). Oil spills in the tropics and subtropics. In: Pollution in tropical aquatic systems. Connel D.W. and Hawker D. (eds). CRC Press. London.

Zanui S. (1975). Desarrollo del huevo y estados larvarios de cabrilla (*Paracentropristis cabrilla* L.). Inv. Pesq. 39, 473-489.

Zar J.H. (1996). Biostatistical analysis. 3rd. Edition. Prentice-Hall Int. New York.

Accepted June 22, 2001